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COINOCULATION WITH *Bradyrhizobium* AND *Trichoderma* ALLEVIATES THE EFFECTS OF SALT STRESS IN COWPEA[1]

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ABSTRACT - The deleterious effects of salt stress can be mitigated by the use of beneficial microorganisms. The aims of this study were to evaluate whether coinoculation with *Bradyrhizobium* and *Trichoderma asperelloides* alleviates salt stress in cowpea. The experiment was conducted in a greenhouse using pots filled with sterile soil. Seeds were sown and inoculated with *Bradyrhizobium* or coinoculated with *Bradyrhizobium* and *T. asperelloides*. At 15 days after sowing (DAS), the nitrogen-free nutritive solution was supplemented with 50 or 100 mmol L^{-1} sodium chloride (NaCl) to induce salinity. Uninoculated plants and irrigated with solution without NaCl were used as absolute control. At 35 DAS, plants were collected and nodules were excised for use in the determinations. The absolute controls did not show root nodules. Salt stress decreased plant biomass and growth, especially in cowpea inoculated with *Bradyrhizobium*. The stem diameter increased in cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides*, mainly in plants subjected to salt stress at 100 mmol L^{-1} NaCl. Cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides* maintained a higher content of free ammonia and organic compounds in its nodules even under salt stress. We concluded that the coinoculation of cowpea with *Bradyrhizobium* and *T. asperelloides* induces an increase in the concentration of organic solutes in the root nodules, especially when cowpeas are cultivated under salinity. Therefore, the use of coinoculation with *Bradyrhizobium* and *T. asperelloides* alleviates the negative effects of salt stress in cowpea.

Keywords: Salinity. Osmoprotectants. Plant growth-promoting fungi.

COINOCULAÇÃO COM *Bradyrhizobium* E *Trichoderma* ALIVIA OS EFEITOS DO ESTRESSE SALINO EM FEIJÃO-CAUPI

RESUMO - Os efeitos deletérios do estresse salino podem ser mitigados pelo uso de microrganismos benéficos. O objetivo foi avaliar se a inoculação com *Bradyrhizobium* e *Trichoderma asperelloides* alivia o estresse salino em feijão-caupi. O experimento foi conduzido em casa de vegetação utilizando vasos preenchidos com solo estéril. As sementes foram semeadas e inoculadas com *Bradyrhizobium* ou coinoculadas com *Bradyrhizobium* e *T. asperelloides*. Aos 15 dias após a semeadura (DAS), a solução nutritiva isenta de nitrogênio foi suplementada com 50 ou 100 mmol L^{-1} de cloreto de sódio (NaCl) para induzir a salinidade. Plantas não inoculadas e irrigadas com solução sem NaCl foram utilizadas como controle absoluto. Aos 35 DAS, as plantas foram coletadas e os nódulos excisados para uso nas determinações. O controle absoluto não apresentou nódulos radiculares. O estresse salino diminuiu a biomassa e o crescimento das plantas, especialmente no feijão-caupi inoculado com *Bradyrhizobium*. O diâmetro do caule aumentou no feijão-caupi coinoculado com *Bradyrhizobium* e *T. asperelloides*, principalmente nas plantas submetidas a estresse salino com 100 mmol L^{-1} de NaCl. O feijão-caupi coinoculado com *Bradyrhizobium* e *T. asperelloides* manteve um conteúdo mais alto de amônia livre e compostos orgânicos em seus nódulos, mesmo sob estresse salino. Nós concluímos que a coinoculação do feijão-caupi com *Bradyrhizobium* e *T. asperelloides* induz um aumento na concentração de solutos orgânicos nos nódulos radiculares, especialmente quando o feijão-caupi foi cultivado sob salinidade. Portanto, o uso da coinoculação com *T. asperelloides* alivia os efeitos negativos do estresse salino em feijão-caupi.

Palavras-chave: Salinidade. Osmoprotetores. Fungos promotores de crescimento de plantas.

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INTRODUCTION

Cowpea is a legume of great importance in Brazilian farming, mainly in the northeastern region, and is considered a subsistence crop that is widely adaptable to local ecological conditions but with low productivity (FREIRE FILHO et al., 2011). The major problems in cowpea cultivation in the northeastern region are related to low nitrogen availability in soils (XAVIER et al., 2006). One of the alternatives to improve cowpea productivity is the use of biological inputs, such as the application of rhizobial inoculants, which can continuously supply nitrogen to this legume (PEREG; MCMILLAN, 2015; O'CALLAGHAN, 2016). Rhizobial inoculation is considered a low-cost economical technology that offers a safe environmental alternative for legume production (RODRIGUES et al., 2015; MORAES et al., 2016). Rhizobia are nitrogen-fixing bacteria that elicit the formation of nodules in legume root systems (FIGUEIREDO et al., 2016; POOLE; RAMACHANDRAN; TERPOLILLI, 2018), where they perform biological nitrogen fixation (BNF) that results in the production of nitrogenous compounds (ureides) exchanged by carbohydrates (sucrose) with the host plant (PÉREZ-MONTAÑO et al., 2014; PEREG; MCMILLAN, 2015; O'CALLAGHAN, 2016; MASSON-BOIVIN; SACHS, 2018).

Abiotic stresses, such as salinity and drought, can negatively influence the ability of plants to establish and maintain symbiosis with rhizobia because they directly affect infection and nodulation, respiration, BNF and other processes that occur inside the nodule (RODRIGUES et al., 2013; O'CALLAGHAN, 2016). Soil salinization is a global problem, mainly in irrigated lands, and causes serious loss of production every year (BYRT et al., 2018). Salinity causes osmotic, ionic and oxidative stress in plants and thus affects important morphological, physiological, and metabolic biological processes of plants, mainly in terms of cell division and elongation rates, nutrient uptake, photosynthesis, and protein synthesis (MUNNS; GILLIHAM, 2015). In search of alternatives that mitigate the deleterious effects of salt stress, the use of microbial inoculum formed by the association between rhizobia and beneficial soil microorganisms (bacteria and/or fungi) has been proposed to improve plant metabolism (FIGUEIREDO et al., 2016; NUMAN et al., 2018).

The results showed that salinity negatively affects plant growth, but coinoculation with rhizobia and plant growth-promoting bacteria (PGPB) can reduce the inhibitory effect of this stress. Egamberdieva et al. (2017) showed that detrimental effects of salinity are alleviated in chickpea by coinoculation with *Mesorhizobium ciceri* IC53 and the PGPB *B. subtilis* NUU4. Chinnaswamy et al. (2018) emphasized that the combination of *Ensifer medicae* and PGPB *B. megaterium* NMp082 is synergistic and alleviates salt stress in alfalfa. Santos et al. (2018) showed that cowpea coinoculated with *Bradyrhizobium* sp. and PGPB *Bacillus* sp. IPACC11 exhibits better symbiotic performance and efficient nitrogen fixation even when subjected to salt stress. In addition to the use of PGPB to induce plant resistance to abiotic stresses, the use of plant growth-promoting fungi (PGPF), nonpathogenic free-living fungi, has attracted a great deal of attention in recent years (HANEY et al., 2015; RUBIO et al., 2017). However, the association between rhizobia and PGPF is poorly documented.

Trichoderma is a rhizosphere fungus that promotes plant growth and possesses the ability to stimulate the plant defense system to suppress attack by phytopathogens (RUBIO et al., 2017; POOLE POOLE; RAMACHANDRAN; TERPOLILLI, 2018). *Trichoderma*-based bioproducts, such as biofungicides or biofertilizers, are largely used worldwide (EGAMBERDIEVA et al., 2017), and it has been reported that *Trichoderma* are effective in alleviating the adverse effects of salt stress (RUBIO et al., 2017). There are some reports on the benefits of the association between rhizobia and *Trichoderma* in some important legume crops (BABU et al. 2015; ALCÁNTARA et al. 2016; MWEETWA et al. 2016; CHAGAS et al. 2017; JAGADEESH et al. 2017); however, the exact mechanism by which these microorganisms contribute to alleviating the detrimental effects of salt stress is not fully understood.

The aims of the present study were to evaluate whether coinoculation with *Bradyrhizobium* sp. BR 3267 and *T. asperelloides* T02 are able to alleviate salt stress in cowpea. In addition, our study intends to provide valuable information about *Trichoderma asperelloides* T02, a type of PGPF, which can contribute to a more efficient symbiosis between *Bradyrhizobium* sp. BR3267 and cowpea plants under salt stress conditions.

MATERIAL AND METHODS

Microorganisms and inoculant production

The *Bradyrhizobium* sp. (BR 3267 strain) obtained from the National Center for Research in Agrobiology (Seropédica, Rio de Janeiro, Brazil) and the isolate T02 of *Trichoderma asperelloides* acquired from the Culture Collection of JCO Industry and Trade of Fertilizer (Barreiras, Bahia, Brazil) were used in the greenhouse experiments. The *Bradyrhizobium* sp. was purified in yeast mannitol agar (YMA) medium using 0.25% (w/v) Congo red as an indicator and posteriorly multiplied in tubes with YMA medium without the indicator. For the preparation of the bacterial inoculant (10^8 CFU mL^{-1}), *Bradyrhizobium* sp. was inoculated into yeast mannitol (YM) liquid medium and incubated in a rotator shaker at 220 rpm (28 ºC) for 96 h. Isolate T02 of *T. asperelloides* was multiplied on potato dextrose agar (PDA) culture medium for seven days (25 °C). Afterwards, the plates were flooded with sterile distilled water, filtered with two layers of muslin cloth and then used as a fungal inoculant (10^5 conidia mL^{-1}).

Experimental preparation, inoculation and planting

The experiment was conducted in a greenhouse of the Interuniversity Network Development Sector Sugarcane (RIDESA) belonging to the Department of Plant Science (Federal University of Piaui; Teresina, Piaui, Brazil) at a temperature range of 27-36 °C with 60-80% relative humidity and 1200 μmol m^{-2} s^{-1} photosynthetically active radiation. For the greenhouse experiment under axenic conditions, soil (Table 1) was collected (0-20 cm layer), autoclaved (3 times; 120 °C; 101 kPa; 1 h) and used to fill the pots (3.5 kg of sterilized soil). In each pot, the seeds of cowpea cultivar 'Tumucumaque', obtained from the National Center for Research in Semi-Arid (Teresina, Piaui, Brazil), were sown after being disinfected with 70% (v/v) ethanol and 2% (v/v) sodium hypochlorite and washed with sterile distilled water (seven times). At the time of sowing, the seeds were inoculated using 1.0 mL of bacterial suspension (10^8 CFU mL^{-1}) of *Bradyrhizobium* sp. or coinoculated with 1.0 mL of the bacterial suspension of *Bradyrhizobium* sp. and 1.0 mL of the conidial suspension (10^5 conidia mL^{-1}) containing *T. asperelloides* T02. The absolute control consisted of uninoculated plants.

Table 1. Physical-chemical analysis of the soil used in the experiment.

Chemical attributes											Physical attributes		
pH	P	K	Na	Ca^{+2}	Mg^{+2}	Al^{+3}	H+Al	CTC(t)	CTC(T)	SB	Sand	Silt	Clay
(H_2O)	(mg dm^{-3})			(cmolc dm^{-3})				(cmolc dm^{-3})			(%)		
6.8	1.5	19.7	12.3	1.9	0.5	-	1.5	2.5	3.9	2.5	84	11	5

CTC (t) = Effective cation exchange capacity; CTC (T) = Potential cation exchange capacity at pH 7.0; SB = Sum of bases.

Throughout the experiment, all plants were irrigated with a modified nitrogen-free nutritive solution (HOAGLAND; ARNON, 1950; SILVEIRA et al. 1998). Thinning was carried out at seven days after sowing, and two cowpea plants were maintained per pot (experimental unit). On the 15th day after sowing, plants were subjected to salt stress by irrigation with nitrogen-free nutritive solution supplemented with 50 mmol L^{-1} (-0.22 MPa) or 100 mmol L^{-1} (-0.44 MPa) of sodium chloride (NaCl) according to the treatment. The absolute control and parts of the plants that were inoculated or coinoculated continued to be irrigated with a nutritive solution free of nitrogen and NaCl. The salt stress was gradually imposed with the application of 25 mmol L^{-1} NaCl until reaching the desired concentration for each treatment. The substrate was washed with distilled water weekly, and the pH and electrical conductivity (EC) of the drainage were measured to match the pH (6.8) and EC of the soil. The EC values were 0.89 dS m^{-1} for the control, 6.5 dS m^{-1} for the nitrogen-free nutritive solution with 50 mmol L^{-1}, and 12.3 dS m^{-1} for the nitrogen-free nutritive solution with 100 mmol L^{-1}.

At 35 days after sowing (harvest), the height and root length were measured with a metric tape and the stem diameter with a digital pachymeter. The dry weight of the shoots and roots was determined after drying in a forced aeration oven at 65 °C until reaching constant weight. The total dry weight was used to calculate the relative growth rate (EVANS, 1972). Fresh nodules in cowpea roots were mixed with distilled water, heated for 1 h at 95 °C, and aliquots of the extract (supernatant) were used to determine free ammonia (WEATHERBURN, 1967), free amino acids (YEMM; COCKING, 1955), ureides (YOUNG; CONWAY, 1942), free proline (BATES et al., 1973), and total soluble carbohydrates (DUBOIS et al., 1956). The data of these solutes were expressed in µmol g^{-1} fresh weight (FW). Afterward, fresh nodules in cowpea roots were mixed with MCW (methanol, chloroform and water) solution (12:5:3) to determine the sucrose levels (VAN HANDEL, 1968), and data were expressed in mg g^{-1} FW. Reducing sugars were estimated by subtracting the concentration of sucrose from the total soluble carbohydrates. Fresh nodules in cowpea roots were mixed with 100 mM potassium phosphate buffer (pH 7.0) containing 1.0 mM EDTA, and aliquots of the extract (supernatant) were used to determine soluble protein (BRADFORD, 1976). The soluble protein data were expressed in mg g^{-1} FW.

Statistical analysis

The experimental outline was a randomized experimental design with four replications, and the treatments were distributed in a 2 x 3 + 1 factorial arrangement, with two inoculations (only *Bradyrhizobium* and *Bradyrhizobium* plus *T. asperelloides* T02), three salt levels (0, 50 and 100 mM of NaCl) and one absolute control. The absolute control consisted of uninoculated plants. The experimental unit was composed of a pot with two plants. The data were analyzed using the Shapiro-Wilk test to evaluate normality and tested for homogeneity of variance using Bartlett's test. Posteriorly, the means were subjected to analysis of variance (ANOVA) with the F test (p<0.05). Comparison of treatment means was performed using Tukey's test (p<0.05). Dunnett's test (p<0.05) was used to compare all treatments with the absolute control. All statistical analyses described were performed using the free software RStudio version 1.1.456.

RESULTS AND DISCUSSION

Here, we report that salt stress decreased cowpea growth, but the combination of *Bradyrhizobium* and *T. asperelloides* improved the growth of cowpea plants even under stressful conditions. As observed in Figure 1, the growth was significantly impaired in cowpea plants inoculated with *Bradyrhizobium* or coinoculated with *Bradyrhizobium* and *T. asperelloides* and exposed to different levels of salt stress (50 and 100 mmol L^{-1} NaCl) for twenty days compared to the control. Salt stress decreased the relative growth rate (Figure 1A) and height (Figure 1B) of cowpea plants inoculated with *Bradyrhizobium* or coinoculated with *Bradyrhizobium* and *T. asperelloides*, but this reduction was more pronounced in cowpea plants inoculated with *Bradyrhizobium*. Under salt stress treatment with 100 mmol L^{-1} NaCl, cowpea plants coinoculated with *Bradyrhizobium* and *T. asperelloides* exhibited relative growth rates of approximately 19% and 37% higher than that of cowpea plants inoculated with *Bradyrhizobium* only and that of the absolute control, respectively (Figure 1A).

In relation to plants inoculated with *Bradyrhizobium*, cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides* exhibited an increase in height of 180% with no salt additions and when subjected to salt stress with 50 mmol L^{-1} NaCl (Figure 1B). Sharma et al. (2018) also observed that plant height was significantly increased when chickpea plants were inoculated with *Rhizobium* and *Trichoderma*. Similar positive responses were reported in maize and rice plants treated with *T. harzianum*, and the authors affirm that *T. harzianum* mitigates the deleterious effect of salt stress, improving plant growth and biomass production (YASMEEN; SIDDIQUI, 2018). According to Ahmad et al. (2015), the application of *T. harzianum* restored mustard plant height. Additionally, a 15% increase was observed in mustard plants inoculated with *T. harzianum* and exposed to salt stress with 100 mM or 200 mmol L^{-1} NaCl. Zhang et al. (2016) found that *Trichoderma* sp. exerted a growth-promoting effect in soybean seedlings, and their positive effects are probably associated with the production of plant hormones, especially indol-3-acetic acid.

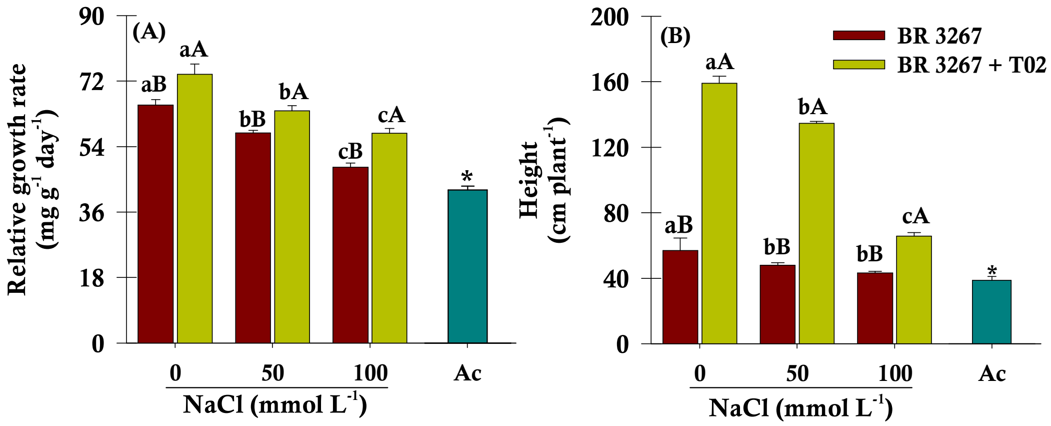

Figure 1. Relative growth rate (A) and plant height (B) of the cowpea plants inoculated with *Bradyrhizobium* sp. (BR 3267) or coinoculated with *Bradyrhizobium* sp. and *T. asperelloides* (BR 3267 + T02) and subjected to control and salt stress conditions with sodium chloride at 50 or 100 mmol L^{-1}. Ac represents absolute control, i.e., uninoculated plants. Different lowercase letters represent significant differences among the salt stress levels, and uppercase letters represent significant differences among the inoculation and coinoculation treatments (Tukey's test; p < 0.05). The asterisk (*) represents significant differences among all treatments and the absolute control (p < 0.05). Data are the mean of four replicates.

Shoot dry weight was increased by 31% in cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides* when compared to plants inoculated with *Bradyrhizobium*, under both control conditions (Figure 2A). Under salt stress at 100 mmol L^{-1} NaCl, the inoculation of cowpea with *Bradyrhizobium* and *T. asperelloides* led to an increase in dry weight of this plant in relation to plants inoculated with *Bradyrhizobium* only, with increases in shoot and root dry weight of 24% and 74%, respectively (Figure 2A and B). Similarly, Chagas et al. (2017) registered increases in the biomass of soybean plants when inoculated with *T. asperellum* at 25 and 50 days after planting. In response to salinity, stem diameter increased linearly in cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides* (Figure 2C). Higher values of stem diameter were observed in cowpea grown in soil subjected to salt stress at 100 mmol L^{-1} NaCl (Figure 2C). The cowpea root length decreased in response to increased soil salinity (Figure 2D). The presence of *Trichoderma* did not influence the root length of cowpea (Figure 2D).

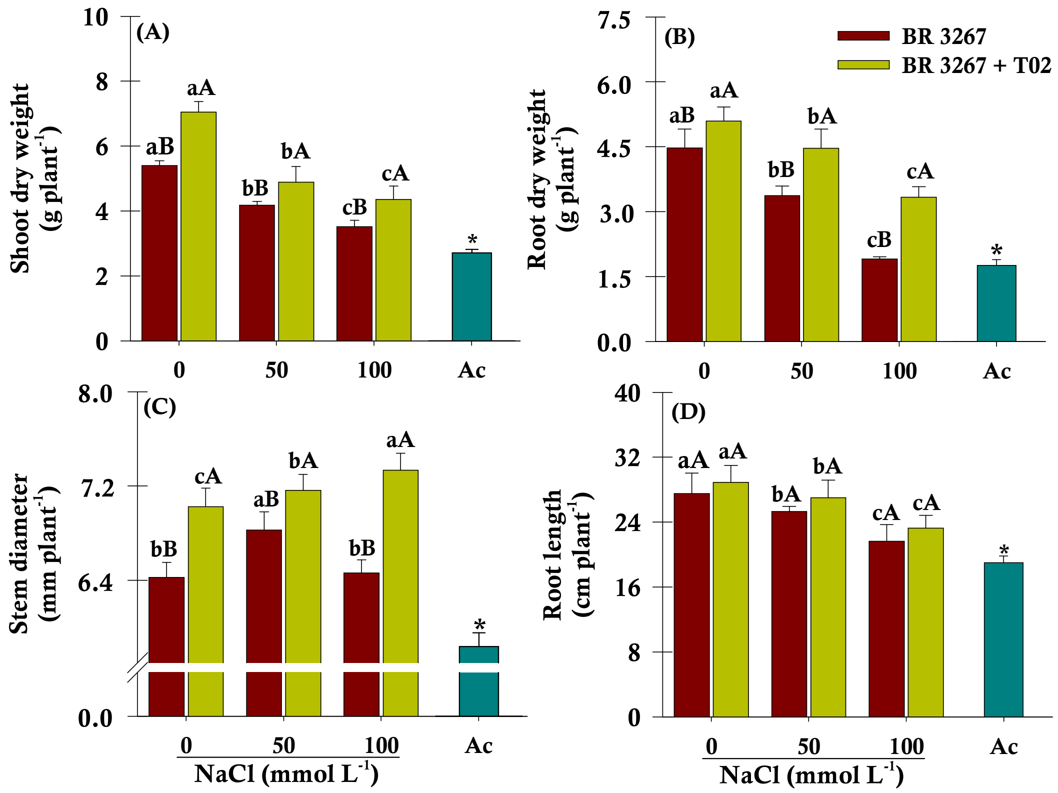

Figure 2. Shoot dry weight (A), root dry weight (B), stem diameter (C) and root length (D) of the cowpea plants inoculated with *Bradyrhizobium* sp. (BR 3267) or coinoculated with *Bradyrhizobium* sp. and *T. asperelloides* (BR 3267 + T02) and subjected to control and salt stress with sodium chloride at 50 or 100 mmol L^{-1}. Ac represents absolute control, i.e., uninoculated plants. Different lowercase letters represent significant differences among the salt stress levels, and uppercase letters represent significant differences among the inoculation and coinoculation treatments (Tukey's test; $p < 0.05$). The asterisk (*) represents significant differences among all treatments and the absolute control ($p < 0.05$). Data are the mean of four replicates.

Cowpea plants inoculated with *Bradyrhizobium* or coinoculated with *Bradyrhizobium* and *T. asperelloides* were analyzed in terms of organic compounds (Figures 3 and 4). Because the absolute control did not present nodules, there is no data regarding the absolute control in Figures 3 and 4. Cowpea plants inoculated or coinoculated with microorganisms showed higher concentrations of total soluble carbohydrates when subjected to salinity in comparison with the control (Figure 3A). There was a reduction in the concentration of total soluble carbohydrates in the cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides* when the salt stress was increased (Figure 3A), similar to the observed reduction in sugars (Figure 3B). Plants have a variety of strategies to combat salt stress with an emphasis on the accumulation of various types of organic and inorganic solutes, which can help balance osmotic pressure and maintain cell turgor (MUNNS; GILLIHAM, 2015; BYRT et al., 2018).

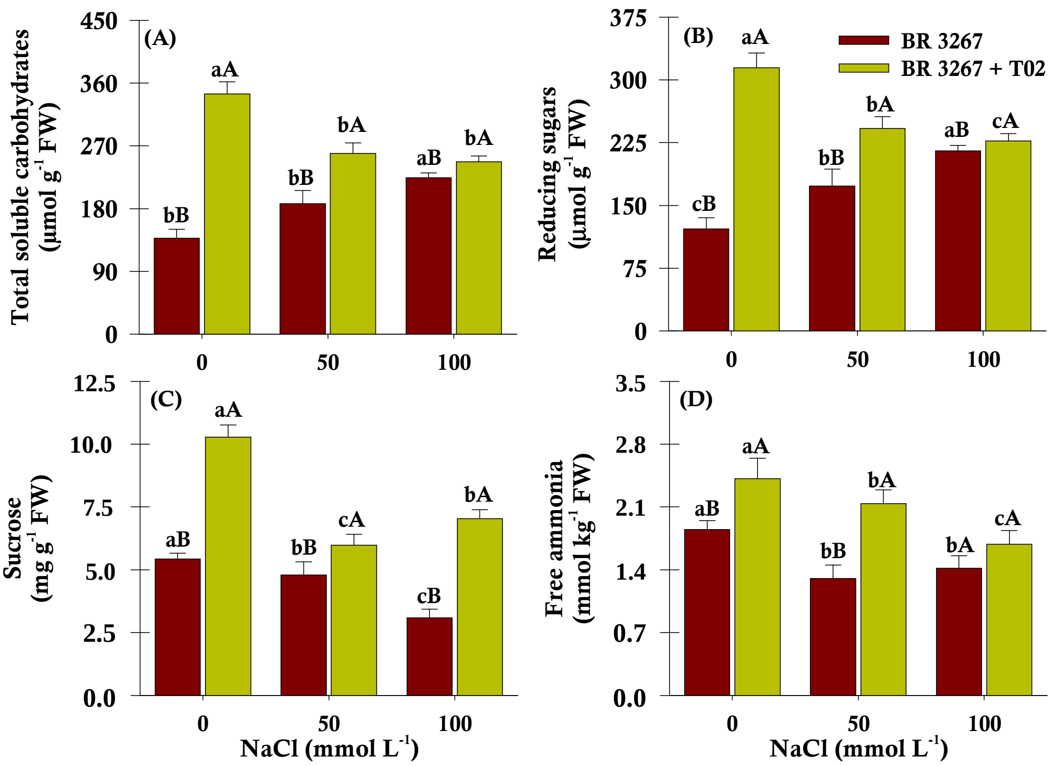

Figure 3. Total soluble carbohydrates (A), reducing sugars (B), sucrose (C) and free ammonia (D) in nodules of cowpea plants inoculated with *Bradyrhizobium* sp. (BR 3267) or coinoculated with *Bradyrhizobium* sp. and *T. asperelloides* (BR 3267 + T02) and subjected to control and salt stress with sodium chloride at 50 or 100 mmol L^{-1}. Different lowercase letters represent significant differences among the salt stress levels, and uppercase letters represent significant differences among the inoculation and coinoculation treatments (Tukey's test; $p < 0.05$). Data are the mean of four replicates.

The sucrose levels were reduced in cowpea plants subjected to salt stress in relation to the control, but cowpea coinoculated with *Bradyrhizobium* and *T. asperelloides* maintained a higher content of sucrose in its nodules even under salt stress (Figure 3C). Free ammonia was reduced in cowpea plants inoculated with *Bradyrhizobium* in response to salinity (Figure 3D). In the plants coinoculated with *Bradyrhizobium* and *T. asperelloides*, the reduction in levels of free ammonia occurred gradually as the salinity was increased. According Rodrigues et al. (2013), the reduction in free ammonia indicates an improved capacity for the use this ion in amino acid and protein synthesis. Furthermore, the nodules of cowpea plants coinoculated with *Bradyrhizobium* and *T. asperelloides* showed higher concentrations of nitrogenous compounds (free amino acids, soluble proline, ureides and free proline) when compared to plants inoculated with *Bradyrhizobium*, even under salt stress (Figure 4).

In general, the concentration of free amino acids was reduced in the nodules of cowpea plants, mainly when these plants were inoculated with *Bradyrhizobium* (Figure 4A). In nodules of the plants coinoculated with *Bradyrhizobium* and *T. asperelloides* and exposed to salt stress at 100 mmol L^{-1} NaCl, we observed levels of free amino acids 58% higher than those in plants inoculated with *Bradyrhizobium* and exposed to the same level of salinity. In nodules of cowpea plants coinoculated with *Bradyrhizobium* and *T. asperelloides* exposed to salt stress with 50 and 100 mmol L^{-1} NaCl, soluble protein was 28% and 20% higher, respectively, when compared to cowpea inoculated with *Bradyrhizobium* under the same saline treatments (Figure 4B). In cowpea plants inoculated with *Bradyrhizobium*, the ureide content was decreased by 32 and 51% when treated with 50 and 100 mmol L^{-1} of NaCl compared to the control, respectively (Figure 4C). Cowpea plants coinoculated with *Bradyrhizobium* and *T. asperelloides* displayed a ureide content 41% superior to that of cowpea plants inoculated with *Bradyrhizobium* when both were subjected to salt stress with 100 mmol L^{-1} NaCl.

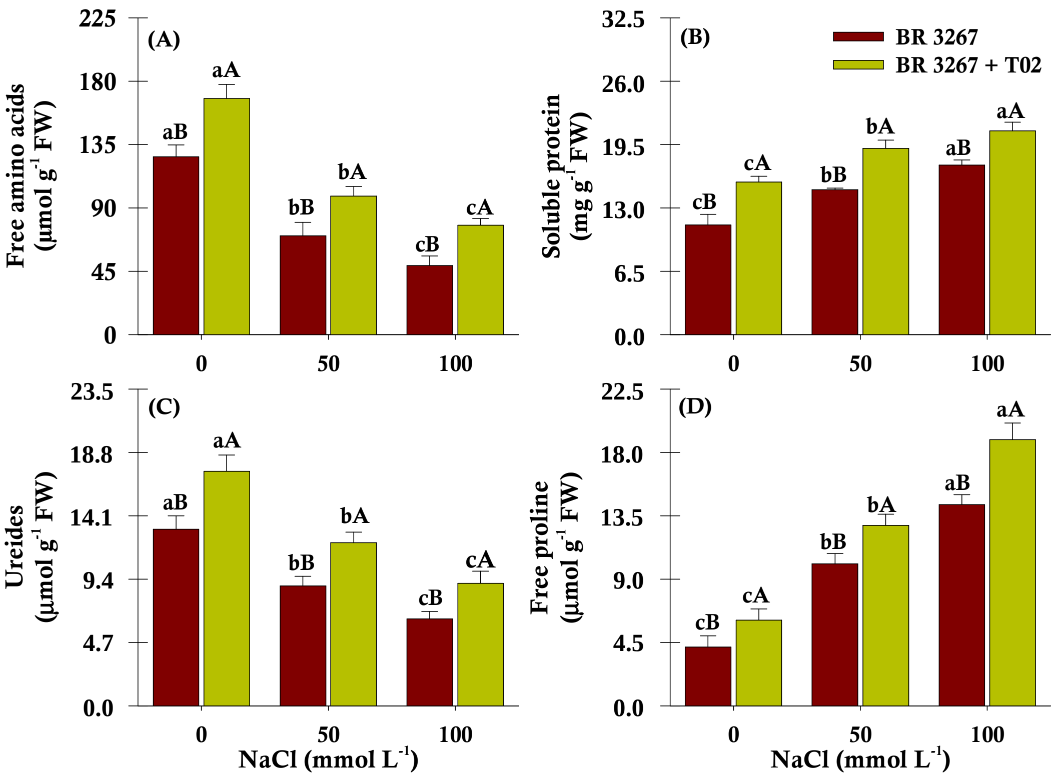

Figure 4. Free amino acids (A), soluble protein (B), ureides (C) and free proline (D) in nodules of cowpea plants inoculated with Bradyrhizobium sp. (BR 3267) or coinoculated with *Bradyrhizobium* sp. and *T. asperelloides* (BR 3267 + T02) and subjected to control and salt stress with sodium chloride at 50 or 100 mmol L^{-1}. Different lowercase letters represent significant differences among the salt stress levels, and uppercase letters represent significant differences among the inoculation and coinoculation treatments (Tukey's test; $p < 0.05$). Data are the mean of four replicates.

The highest free proline content was detected in the nodules of cowpea plants coinoculated with *Bradyrhizobium* and *T. asperelloides* and subjected to salt stress at 100 mmol L^{-1} NaCl (18.9 mmol g^{-1} FW) (Figure 4D). Similar to the results found here, Yasmeen and Siddiqui (2018) reported positive effects of *T. harzianum* in maize and rice plants exposed to 50, 100, and 150 mmol L^{-1} NaCl. Mustard seedlings inoculated with *T. harzianum* and exposed to salt stress exhibit 56% and 70% increases in proline content when subjected to 100 and 200 mmol L^{-1} NaCl, compared to the control, respectively (AHMAD et al., 2015). These authors affirm that the increased accumulation of proline induced in mustard seedlings by *T. harzianum* proves its protective nature against salt stress. According to Egamberdieva et al. (2017), increased proline contents in chickpea coinoculated with *Mesorhizobium ciceri* and *Bacillus subtilis*, compared to uninoculated plants, indicating an alleviation of adverse effects of salt stress. A compatible osmolyte such as proline, glycine, or betaine plays an important role in plant tolerance to stress factors through osmotic adjustment (HASHEM et al., 2015).

CONCLUSION

It is concluded that the coinoculation of cowpea plants with *Bradyrhizobium* and *T. asperelloides* induces an increase in the concentration of organic solutes in the root nodules of these plants, especially when they are subjected to saline stress, indicating that *T. asperelloides* alleviates the adverse effects of salt stress.

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