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Changes induced by co-inoculation in nitrogen–carbon metabolism in cowpea under salinity stress

Alexandra de Andrade Santos[a], *Joaquim Albenísio Gomes da Silveira*[b], *Eliezer de Araujo Guilherme*[b], *Aurenivia Bonifacio*[c], *Artenisa Cerqueira Rodrigues*[d], *Márcia do Vale Barreto Figueiredo*[e,*]

[a] *Universidade Federal Rural de Pernambuco, Departamento de Agronomia, Recife, PE, Brazil*
[b] *Universidade Federal do Ceará, Departamento de Bioquímica e Biologia Molecular, Fortaleza, CE, Brazil*
[c] *Universidade Federal do Piauí, Departamento de Biologia, Teresina, PI, Brazil*
[d] *Universidade Federal do Piauí, Departamento de Engenharia Agrícola e Solos, Teresina, PI, Brazil*
[e] *Instituto Agronômico de Pernambuco, Recife, PE, Brazil*

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ABSTRACT

To mitigate the deleterious effects of abiotic stress, the use of plant growth-promoting bacteria (PGPB) along with diazotrophic bacteria has been increasing. The objectives of this study were to investigate the key enzymes related to nitrogen and carbon metabolism in the biological nitrogen fixation process and to elucidate the activities of these enzymes by the synergistic interaction between *Bradyrhizobium* and PGPB in the absence and presence of salt stress. Cowpea plants were cultivated under axenic conditions, inoculated with *Bradyrhizobium* and co-inoculated with *Bradyrhizobium* sp. and *Actinomadura* sp., *Bradyrhizobium* sp. and *Bacillus* sp., *Bradyrhizobium* sp. and *Paenibacillus graminis*, and *Bradyrhizobium* sp. and *Streptomyces* sp.; the plants were also maintained in the absence (control) and presence of salt stress (50 mmol L^{-1} NaCl). Salinity reduced the amino acids, free ammonia, ureides, proteins and total nitrogen content in nodules and increased the levels of sucrose and soluble sugars. The co-inoculations responded differently to the activity of glutamine synthetase enzymes under salt stress, as well as glutamate synthase, glutamate dehydrogenase aminating, and acid invertase in the control and salt stress. Considering the development conditions of this experiment, co-inoculation with *Bradyrhizobium* sp. and *Bacillus* sp. in cowpea provided better symbiotic performance, mitigating the deleterious effects of salt stress.

* *Corresponding author at*: Agronomical Institute of Pernambuco, 50761-000, Brazil.
E-mail: mbarreto@elogica.com.br (M.V. Figueiredo).

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Introduction

Biological nitrogen fixation (BNF) involves a complex interaction with a host plant and represents an environmentally clean nitrogen pathway in agricultural systems.[1] In this way, the inoculation of legumes with rhizobia favors the increase in and the availability of nitrogen in commercial legume production.[2] This process can be affected by physical, chemical and biological factors, and it is more frequent in legumes in symbiosis with fixing bacteria.[3] However, it is necessary to observe the combination and compatibility of the bacterial strains involved.[4] Co-inoculation involving strains of *Bradyrhizobium* sp. with plant growth-promoting bacteria (PGPB) can have positive effects by increasing nutrient mobilization, mainly that of N and C.[5]

PGPB can improve the growth and development of plant species via several mechanisms, such as nitrogen fixation, phosphate solubilization, phytohormone synthesis and increased iron uptake by the production of siderophores.[6] Moreover, PGPB can also benefit plants by exhibiting protective action against pathogens, diseases and environmental stresses.[7] In addition, PGPB can colonize the roots of plant species and create a favorable microenvironment for plant development. Thus, the inoculation of agricultural species with PGPB represents a very promising technique.

When subjected to abiotic stress such as drought and salt stress, plants develop mechanisms of tolerance or adaptation such as osmotic adjustment; these mechanisms allow plants to maintain their development even under stress conditions.[8] Recent advances in molecular studies have yielded insights into the signaling networks of plant-microbe interactions that contribute to salt tolerance. The beneficial effects of plant growth-promoting rhizobacteria involve boosting key physiological processes including water and nutrient uptake, photosynthesis, and source-sink relationships that promote growth and development.[9]

Osmotic adjustment with organic or inorganic solutes carried out by plant species subjected to salinity allows those plants to maintain a continuous soil water uptake.[10] In addition, plants can use other mechanisms when subjected to salinity stress, such as the control of the absorption of Na^+ by roots or the accumulation and selective compartmentalization of excess ions in the vacuole.[11]

Several studies have shown that co-inoculation of legume species with rhizobia and PGPB has a beneficial effect on the growth and development of plants.[12] In this context, we hypothesized that co-inoculation with *Bradyrhizobium* sp. and PGPB optimizes the development and the BNF of cowpea by mitigating the deleterious effects of salt stress. Metabolites and key enzymes of carbon and nitrogen metabolism were evaluated in cowpea inoculated with *Bradyrhizobium* sp. and co-inoculated with *Bradyrhizobium* sp. and PGPB in the presence and absence of salt stress.

Materials and methods

Experimental design and statistical analysis

The experimental design consisted of a randomized block in a $5 \times 2 + 1$ factorial scheme. There were five bacterial combinations (one inoculation with *Bradyrhizobium* sp. and four co-inoculations with *Bradyrhizobium* sp. and PGPB), two salinity levels (0 and 50 mmol L^{-1} NaCl), and an absolute control (uninoculated plants without nitrogen and without NaCl). Four blocks and two replicates per block were used. The data were subjected to analysis of variance (ANOVA) with a significance level of 5% by the F test, and the means were compared by the Tukey test at 5% probability. A contrast analysis was performed to evaluate the effects of inoculation treatments versus the absolute control in the presence and absence of salinity. All statistical analyses were performed using the SASM-Agri 8.1 program.[13]

Production of microorganisms and the preparation of inoculants

The rhizobia and PGPB strains used in the experiment (Table 1) were multiplied under controlled conditions for the production of inoculants. The following media were used to purify and multiply microorganisms: yeast mannitol agar (YMA) and yeast mannitol (YM)[14] at pH 6.5 for *Bradyrhizobium* sp., dextrose yeast glucose sucrose (DYGS)[15] at pH 6.0 for *Bacillus* sp., trypticase soy agar (TSA) and tryptic soy broth (TSB) at pH 7.3 for *Paenibacillus graminis*, and arginine yeast and agar (AYA)[16] at pH 6.4 for *Actinomadura* sp. and *Streptomyces* sp. The rhizobia inoculants were incubated on a rotary shaker (200 rpm) at 28 °C for 96 h, and the PGPB inoculants were maintained on a rotary shaker (200 rpm) at 30 °C for 48–96 h depending on the bacterial strain. These bacterial strains showed promising results in previous studies, and their behavior was tested in cowpea in the presence and absence of salinity stress.[12]

Plant culture and treatments

The experiment was conducted under axenic conditions in a greenhouse (air temperature of 31 °C, air humidity of 60% and a day length of 9 h). The seeds of cowpea cultivar 'IPA 206' were disinfested[17] and sown in Leonard pots containing washed (pH 6.5) and autoclaved (1 h; 120 °C; 101 kPa) sand. At the time of sowing, the seeds were inoculated using 1.0 mL of the bacterial suspension (10^8 CFU mL^{-1}) with *Bradyrhizobium* sp. or co-inoculated with 1.0 mL of the bacterial suspension containing *Bradyrhizobium* sp. and 1.0 mL of the bacterial suspension containing a strain of PGPB (10^7 CFU mL^{-1}). Co-inoculations were formulated in accordance with the information in Table 1.

Throughout the experiment, the plants were irrigated by capillarity with an N-free nutrient solution,[18] as modified by Silveira et al.[19] Thinning was carried out at four days after germination (DAG), and two plants per pot (experimental unit) were maintained. The plants were subjected to salt stress at 15 DAG, in which 50 mmol L^{-1} sodium chloride (NaCl) was added to the nutrient solution. The nutrient solutions (pH of 6.5) were placed in Leonard jars and changed weekly. At the time, the substrate was washed with distilled water, and the pH and electrical conductivity (EC) of the drainage were measured to match the pH and EC of the vessel; the EC values were 0.99 mS cm^{-1} and 5.6 mS cm^{-1} for the control and stress treatments, respectively.

At 37 DAG, the roots containing nodules were immersed in liquid nitrogen and then kept at -80 °C. For transport, the

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