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Full Length Research Paper

24-Epibrassinolid in the biometry of acclimatization to salinity in two cultivars of *Vigna unguiculata* (L.) Walp.

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In order to evaluate the influence of application of brassinosteroid phytohormone in mitigating the effects of salt stress at the height, root growth, leaf area, dry mass of leaf and root, stomatal conductance and transpiration, plants of cowpea bean [*Vigna unguiculata* (L.)Walp, cultivars BR3 Guariba and BRS Tracuateua, they were sown in a greenhouse in the absence and presence of brassinosteroid (24-epibrassinolid) in concentrations (0.2 and 0.4 µM), under diferent concentrations of NaCl (50 mM and 100 mM). The highest concentration of NaCl was 100 mM, affects plant growth. This treatment reduced by 46% the length of the BRS Tracuateua root, and 82 and 50% stomatal conductance and transpiration of BR3 Guariba respectively. However, the effects of salinity have been attenuated by supplementation as phytohormone. Under effect of treatment interaction Br of 0.4 µM and NaCl of 100 mM, root length and dry mass of leaves was increased by 87 and 37% in Guariba and Tracuateua cultivars concomitantly compared to those under stress by NaCl to 100 mM. For the same concentration of NaCl and Br 0.2 µM, there increases of 88% in stomatal conductance BR3 Guariba. It is suggested a possible regulation of 24-epibrassinolid on photosynthetic mechanisms of cowpea plants, in order to change made promoted stomatal conductance, which may have induced the greatest uptake and sequestration of CO_2 thereby allowing the growth processes in plants.

Key words: Brassinosteroids, expansion and development, *Vigna unguiculata* (L.) Walp.

INTRODUCTION

The caupi bean [*Vigna unguiculata* (L.) Walp.] belongs to the legume family (Fabaceae), subfamily Papilionoidea Faboideae). It features rustic genotypes adapted to soils with low fertility and drought, but still express significant

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(production standards, which is an alternative low risk to grain crops in regions with latent edaphoclimatic problems (Correa et al., 2012).

In 2014, world production of this species amounted to approximately 25 million tonnes (FAO, 2016). In the same period, the area harvested in Brazil totaled 3.1 million hectares in the North and Northeast of the country, stand out as the top producers. For the same year, in the state of Pará, the area planted with cowpea amounted to 41,300 ha (IBGE, 2016).

However, the productivity of the culture is affected by various biotic and abiotic factors. Among the abiotic factors, salinity is what promotes the greatest metabolic and nutritional disorders (Hasegawa, 2013). According to Maas (1986), although this species is classified as moderately tolerant to salinity by presenting a threshold of saturation 4.9 dS^{-1}, excess of Na^+ cytosolic affects the activity of enzymes and proteins, which may impair the transport of water and minerals to the plants resulting from the lower osmotic potential of the soil, potentially toxic ions, particularly Na^+ and Cl^- interfere with the assimilation of nutrients and plant growth, in view of the effect of competition by the same transport membrane between these essential ions and essential elements (Aragão et al., 2011; Calvet et al., 2013).

These ions promote decrease in the potential of turgor of the plant, which is essential for plant growth. One of the primary effects of salt stress is the reduction of leaf area, due to the ionic or osmotic effect or both, promoted by the excess of salts (Munns and Tester, 2008; Silveira et al., 2010; Hasegawa, 2013). As a result, there is significant reduction in transpiration and stomatal conductance, which appear as a defense mechanism of plants to lower osmotic potential.

As a result of excess salts occur, we decrease in CO_2 assimilation which reflects in lower photosynthetic rates and synthesis of carbohydrates (Calvet et al., 2013). Accordingly, the carbon skeletons to be used for the growth processes, synthesis of organic compounds and photosynthate translocation, are diverted to the power plant maintenance, promoting decreases in leaf and root dry weight and plant height (Bezerra et al., 2010; Lacerda et al., 2011; Silva et al., 2013).

In this regard, scientific researches indicate that a number of hormones are involved in modulating plant response to stresses such as those induced by NaCl (Sharma et al., 2007). Thus, brassinosteroids are a new class of phytohormones with esteroídicapolioxigenada structure, with pronounced regulatory activity growth, which seems to be related to its role in antioxidative mechanism of plants, as well as their involvement in the metabolism of carbohydrates (Rao et al, 2002; Zullo and Adam, 2002).

Thus, this research aims to evaluate the changes in the growth biochemistry cowpea plants exposed to increasing levels of NaCl, and analyze the modulation mechanisms of brassinosteroid in which gives the acclimatization of this species to salinity.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse belonging to the Rural Federal University of Amazonia-UFRA, Belém, Pará, located at 01° 28'03 "S; 48° 29'18 "W, in the period July-August 2015, the physiological and biochemical analyzes were performed on Biodiversity Studies Laboratory in Higher Plants (EBPs) belonging to the Institute of Agricultural Sciences (ICA) of the university campus.

In this study, we used two cultivars of cowpea bean [*V. unguiculata* (L.) Walp] being BRS Tracuateua and BR3 Guariba, susceptible and tolerant to salt stress, respectively. The seeds were acquired from the Germplasm Bank of the Brazilian Agricultural Research Corporation - EMBRAPA Eastern Amazon.

Seeding and experiment conduction

Five cowpea seeds were sown in each prolipropilenom cup with volumetric capacity of 300 ml, containing sand autoclaved and moistened with distilled water, and each of them was sown a cultivar. Seven days after sowing (DAS), at which time the plants have launched the first pair of leaves, cowpea seedlings were transplanted cups of polypropylene for vessels with 1000 ml capacity, containing in its base 500 ml of nutrient solution Hoagland and Arnon (1950), with ionic strength ¼.

The nutrient solution was replaced daily during the hours of 8:00 and 17:00, according to the need of absorption of seedlings and the reduction solution by evaporation. The pH of the solution was maintained at a range of 5.5 ± 0.5. At 12 DAS when the cowpea plants released third leaf nutrient solution was changed to ½ of its original concentration and at that time, treatment was initiated with NaCl concentrations (50 and 100 mM) levels, brassinosteroids of the (0.2 and 0.4 µM) and control (without brassinosteroids and NaCl). The plants remained for 12 days under the effect of these treatments.

Collection of plants and growth variables

The collection of plants occurred at 24 DAS to 06:00. At that time, was determined biometrics plants: height, stem diameter, root length, number of leaves, leaflets and leaf area. Gas exchange, transpiration and stomatal conductance were also determined even in the greenhouse through a portable porometer to obtain the data evaluated. The different parts of the plant, leaf, stem and root were collected for the determination of dry matter (DDM).

The biometric variables were determined with the aid of a centimeter ruler, determining plant height and root length. Using a digital caliper ZAAS Precision model obtained if the diameter of the neck of the plant height, number of leaves and leaflets was determined by manual counting.

The dry matter was determined by separation of plant leaves, stem and root, and then were taken to dry in air oven of forced air at a temperature of 65 ± 5 ° C for 48h. After drying, the material was weighed on analytical balance, ground in mill to obtain a fine and stored in falcon tubes powder. The leaf area was determined by scanning the leaves with your area designed by Jimage program.

Determination of physiological variables

The stomatal conductance (gs) and transpiration (E) of cowpea

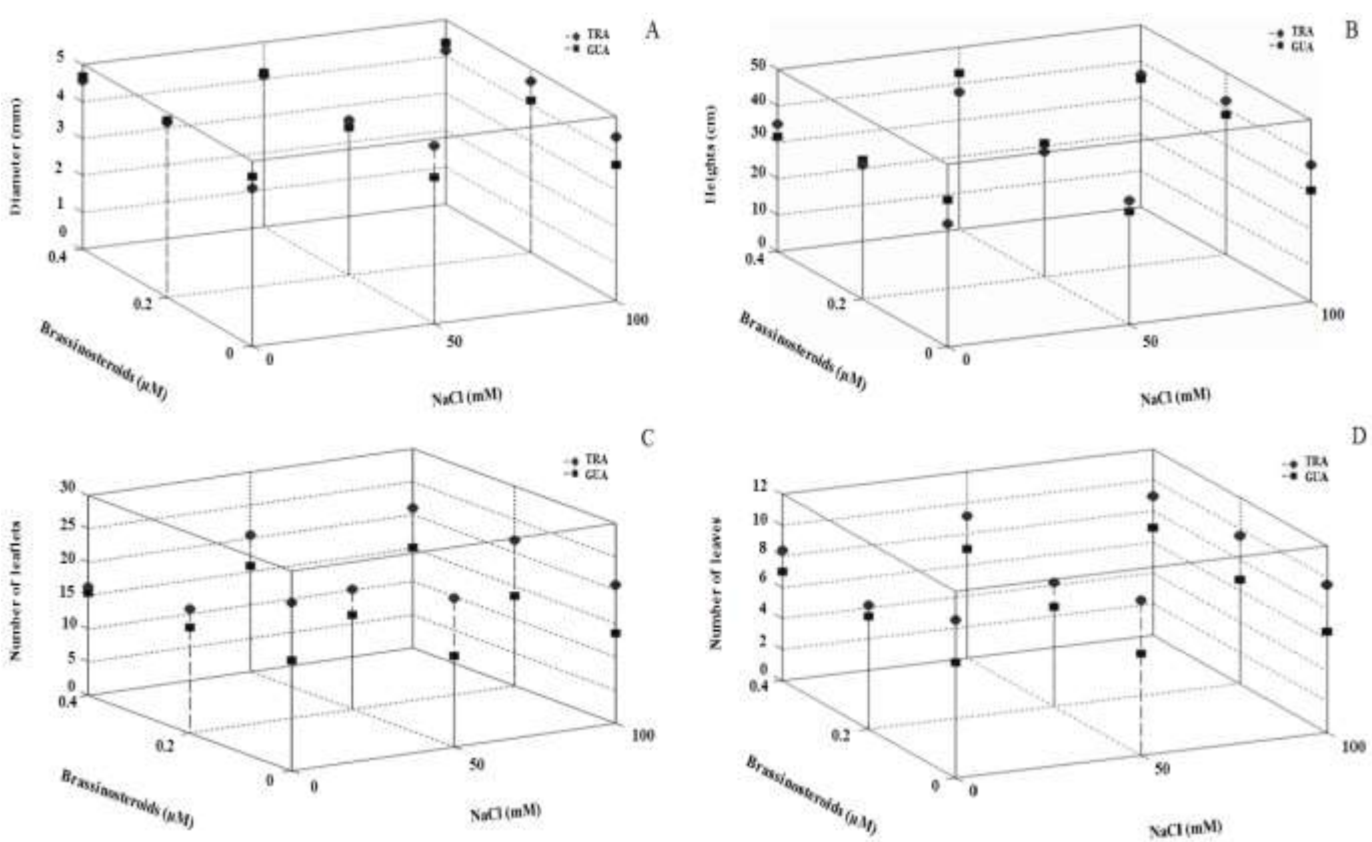

Figure 1. Diameter (A), heights (B), number of leaflets (C) and leaves (D) in cultivars BRS Guariba and BR3 Tracuateua of *Vigna unguiculata* (L.) Walp grown in nutrient solution as a function of increasing concentrations of brassinosteroids (Br) and NaCl.

plants were determined in the morning on time from 9:00 am to 11 am. To obtain the data used a portable porometer dynamic equilibrium (MOD. Li 1600 liquor, Nebraska, USA). Measurements were performed with fully expanded leaves, selected from the third pair of leaves counted from the apex to the base.

Experimental design and statystical analysis

The design was completely randomized (DIC) in factorial scheme 2 x 3 x 3, two cultivars of cowpea (BRS Guariba Tracuateua and BR3), three levels of brassinosteroids with concentrations (0.2 µM and 0.4 µM) and with three levels of salinity concentrations (50 mM and 100 mM) yielding 18 treatments and 4 replicates, totaling 72 experimental units, each containing two plants per pot. Statistical results were submitted to analysis of variance (ANOVA) by Sisvar program version 5.4, where the averages were compared by Tukey test level of 5% probability.

RESULTS

Stem diameter, Plant height, number of leaflets and leaves

In this study, we sought to evaluate the effect of brassinosteroids in height, stem diameter, number of leaves and leaflets bean plants cowpea subjected to salt stress and treated with phytohormone (Figure 1). Analysis of variance showed that, in effect interaction between the Br concentrations and NaCl, there was a statistically significant difference ($p < 0.05$) for all the variables mentioned except for stem diameter for which it was not observed significant increases ($p > 0.05$) under the effect of such treatment on both cowpea bean cultivars analyzed (Figure 1).

The height of the cultivars BR3 Guariba and BRS Tracuateua was 52 and 64% concomitantly higher plants under stress by 50 mM NaCl, Br 0.4 µM when treated with saline and the same level (Figure 1 B). However, the isolated treatment with the highest concentration of phytohormone (0.4 µM), did not guarantee greater plant height. Under unique effect of this treatment, the height of Guariba and Tracuateua cultivars was 28 and 13% lower, respectively, those under treatment interaction with the highest dosage of the 24-epibrassinolide and the median concentration of NaCl (50 mM).

The number of leaflets was significantly increased ($p<0.01$) by 69 and 32% in BR3 Guariba and BRS Tracuateua, respectively, when under stress 50 mM NaCl, supplemented with the highest concentration of the phytohormone in relation to plants under only saline treatment at 50 mM (Figure 1C). For the number of

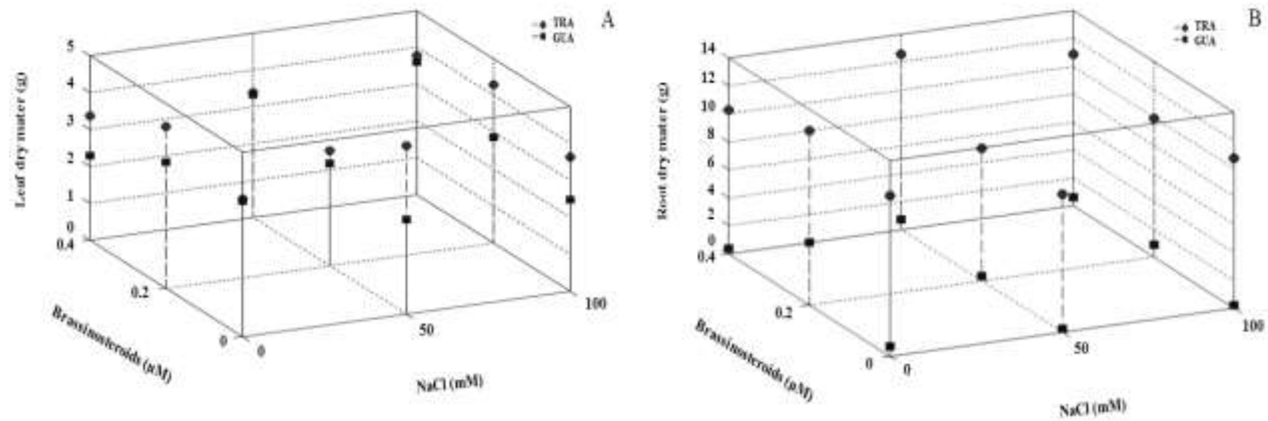

Figure 2. Leaf dry mater (A), Root dry mater (B) in cultivars BRS Guariba and BR3 Tracuateua of *Vigna unguiculata* (L.) Walp grown in nutrient solution as a function of increasing concentrations of brassinosteroids (Br) and NaCl.

sheets, there was a similar increase of 62% in this variable in bean cultivars cowpea under effect of the treatment cited interaction and was no statistical difference ($p>0.05$) between control plants and those supplemented only with Brassinosteóides concentrations of 0.2 µM and 0.4 µM (Figure 1 D).

Dry weight of shoot and root

Salinity promoted significant decrease ($p<0.05$) in the dry matter of shoot and root in cowpea plants. The Guariba and Tracuateua cultivars when treated with 100 mM NaCl, showed a reduction of 49 and 40%, respectively, in dry mass (Figure 2).

Under the same salt treatment, the dry root mass of the growing Guariba kept constant, while that for the BRS Tracuateua, that same organ, there was a 20% decrease in the measured variable (Figure 2B). Thus, the latter cultivar sensitive to excess salts, was considerably affected by incremental increases in NaCl concentration. Only in isolated effect with the brassinosteroid 0.2 µM, there was 75% increase in root dry weight of the plant variety Guariba.

However, concentrations of 24 epibrassinolídeos partially reversed negative effects of salinity on the variables under consideration. Under treatment interaction 0.4 µM and 100 mM NaCl, there was a significant increase ($p<0.05$) of 37% and 33% of dry weight of shoot and root for BRS Tracuateua compared to plants under stress by NaCl to 100 mM (Figure 2A, B). However, for this same cultivar, higher increases in root biomass 66% occurred under the effect of interaction treatment 0.2 µM and 50 mM NaCl as compared to saline treatment plants exclusively to 50 mM; thereby indicating that the lower concentration of the hormone and the lowest salt level ensured higher production biomass at the root.

Root length

In this research, it is evident that the greater salt concentration, significantly reduced ($p<0.01$) in 46% of the root length cultivate Guariba, in relation to their control, indicating that this crop was the most affected by salt stress, in relation to BRS Tracuateua. For the latter variety, the stress 100 mM NaCl, did not cause significant changes in root growth compared to those cultured in the absence of NaCl (Figure 3).

The deleterious effects of salinity were partially reversed by concentrations of 24-epibrassinolídeos. There were significant increases ($p<0.05$) 87 and 25% in root length cultivar howler when maintained under stress by 100 mM NaCl, and treated with Br to 0.4 µM to 0.2 µM concurrently in relation those maintained only under stress with the highest salt concentration used in this research.

Surprisingly, the root growth 62% was observed to grow Tracuateua when treated with the lowest dose of the phytohormone 0.2 µM and higher salt level 100 mM grating those treated with 100mM NaCl. The results of this study show that moderate salt levels NaCl to 50 mM, and the isolated treatment with Br concentrations of 0.2 µM and 0.4 µM, kept the root length of both cultivars with values very close to showing that such treatments did not interfere in biochemical processes essential to the growth and development of this species. Thus, as salt concentration, it proved ineffective in promoting negative changes in root length.

Leaf area

Gradual increases in the concentration of brassinosteroids did not promote significant increases ($p>0.05$), leaf area of Guariba cultivar compared to the respective control. Conversely, for BRS Tracuateua there

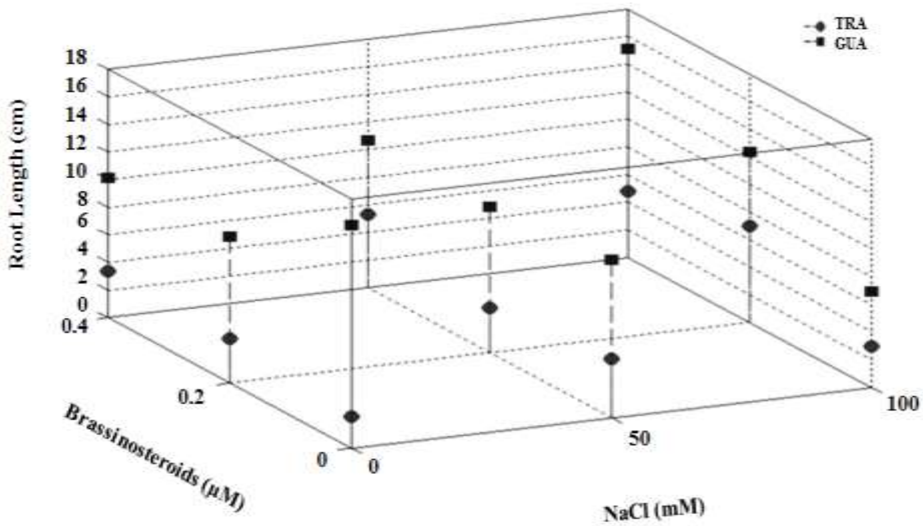

Figure 3. Root length in cultivars BRS Guariba and BR3 Tracuateua of *Vigna unguiculata* (L.) Walp grown in nutrient solution as a function of increasing concentrations of brassinosteroids (Br) and NaCl.

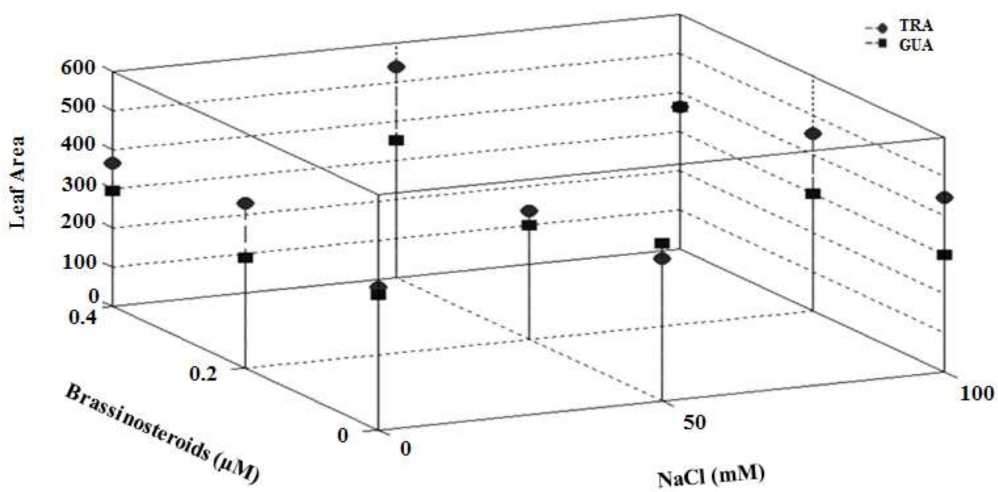

Figure 4. Leaf Area in cultivars BRS Guariba and BR3 Tracuateua of *Vigna unguiculata* (L.) Walp grown in nutrient solution as a function of increasing concentrations of brassinosteroids (Br) and NaCl.

were significant increases (p> 0.01) of 180 and 220% when treated with Br to 0.2 μM and 0.4 μM, respectively, compared to plants cultivated in the absence of this hormone (Figure 4).

However, under 0,4μM treatment interaction effect and 50 mM NaCl leaf area was significantly increased (p <0.01) by 47 and 28% in BR3 Guariba and BRS Tracuateua concurrently from the plants under stress 100 mM NaCl. It is noteworthy that the salt stress did not significantly affect the variable in question, which can be attributed to the time when the plants were subjected to stress.

Isolated concentrations of 50 mM NaCl and treatment of interacting Br 0.2 μM and 50 mM NaCl, induced similar responses in growth variable studied. It is considered in this respect that the low concentration of hormone was not sufficient to promote changes in physiology and biochemistry growth of both cultivars.

Stomatal conductance and transpiration

For physiological variables stomatal conductance and transpiration, there was a significant interaction effect (p

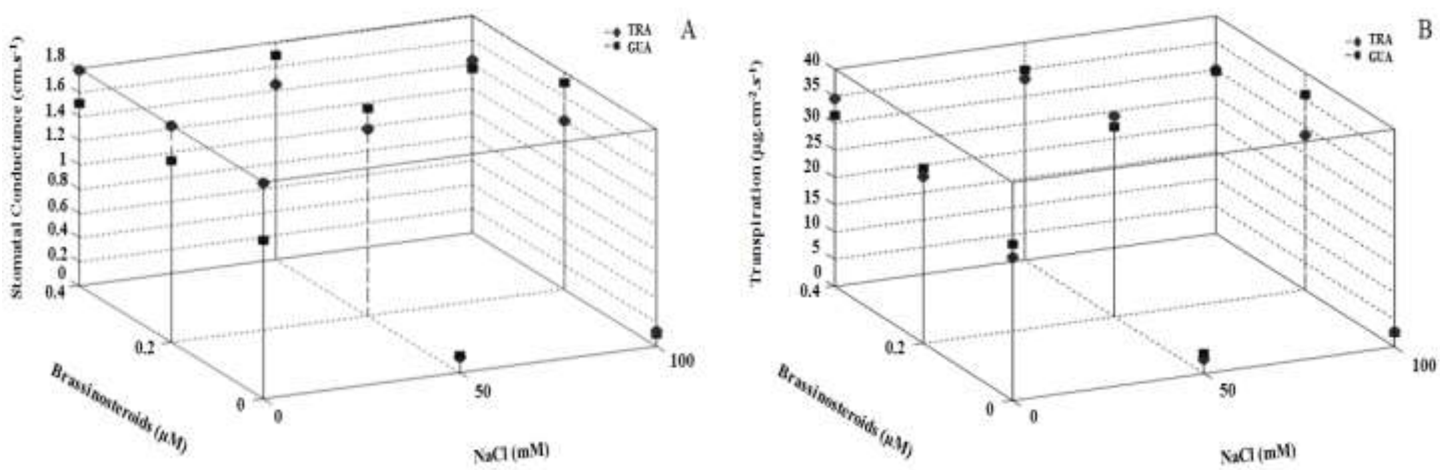

Figure 5. Stomatal conductance (A) and Transpiration (B) in cultivars BRS Guariba and BR3 Tracuateua of *Vigna unguiculata* (L.) Walp grown in nutrient solution as a function of increasing concentrations of brassinosteroids (Br) and NaCl.

<0.01) between the saline levels and phytohormone concentrations used (Figure 5). The higher salt concentration (100 mM), reduced by 66% and 82% stomatal conductance and 37.5% and 50% perspiration of Guariba and Tracuateua cultivars in relation to their control.

However, the dosages of the hormone, mitigated the salt stress, which was evidenced by the increase in the variables analyzed in both cultivars when under treatment effect interaction with NaCl to 100 mM and level of phytohormone Br to 0.2 µM. Under the effect of this treatment, particularly cultivating guariba presented increments of 88% in stomatal conductance and transpiration of 83% compared to plants maintained only with the highest salt concentration (Figure 5B).

For BRS Tracuateua, there was no statistically significant difference (p> 0.05) in stomatal conductance control plants and those under treatment effect interaction analysis. However, for this cultivar, perspiration was increased by 83% in plants under stress 100 mM NaCl and treated with Br to 0.2 µM.

DISCUSSION

Salinity changes the water balance in the plant, which is assigned to its ionic, or both osmotic effect, which results in less water availability in plant tissues. The immediate consequence of this process is to reduce the potential turgor essential to expansion and cell growth (Munns and Tester, 2008; Hasegawa, 2013).

In this research, it became clear that the high salt concentrations negatively affected the growth variables of cowpea plants, especially in regard to height, biomass and root length, which was most evident in the growing Tracuateua, sensitive salt stress.

According to Silva et al. (2009), NaCl stress affects differently the growth variables, suggesting that its deleterious effects are not evenly distributed among the various organs of plants. In fact, in this study, the roots were considerably more affected by salinity.

From the results the high salt concentrations, have promoted ionic changes, resulting in metabolic and nutritional disorders, reflecting in reduced root growth. According to Aragão, et al., (2011) high levels of Na+ inhibit the absorption of K+ ions, which is involved in the activity of cytosolic enzymes that participate, albeit indirectly, in growth processes in plants.

Thus, the competition Na^+ / K^+ for binding sites on the membrane leads to less absorption of the latter root nutrient salt conditions. Thus, it is considered that something similar has occurred in this study, resulting in less root growth of cowpea.

Some studies suggest that brassinosteroids act stimulating root growth, or still may inhibit it (Núñez, 2008; Borcioni and Negrelle, 2012). In fact, it is noted from this research, the effects of salinity were mitigated by concentrations of 24-epibrassinolídeos being observed increases in dry weight of leaves and roots.

To Araujo et al. (2010), reduction of dry mass production, are mainly associated to the toxic effect of ions such as Na^+ and Cl^- to net carbon fixation and consequent production of assimilates. As adaptation to salt stress mechanisms, some species accumulate organic solutes in the vacuole, to balance the salts pressure in the cytosol which ensures the maintenance of the water status of the plant and the efficiency of the photosynthetic apparatus (Willadino et al., 2011; Hasegawa, 2013).

The higher dry matter production both shoot dry mass and root of Guariba and Tracuateua cultivars under stress and supplemented with the phytohormone, is due to a possible involvement of this in osmorreguladores accumulation as free proline in the vacuole.

This assertion appears to be plausible given that, in works developed by Maciel et al. (2012) and Guedes. - Filho et al, (2013), confirm the osmoregulator role of brassinosteroids, stimulating the accumulation and compartmentalization of inorganic solutes in the vacuole and organic solutes in the cytoplasm. This process maintains the salt concentration inside the cells at low levels and, thus, excess salts not interfere with the hydration of proteins and the biochemical metabolism of plants.

Thus it is believed that stomata and photosynthetic pathways procedures may have been changed by the phytohormone inducing greater uptake of CO_2 from which organic compounds are produced essential to plant growth resulting in higher biomass production.

In this respect, it appears that the osmotic adjustment in plants under stress by NaCl, assigned to the 24-epibrassinolídeos restored growth pathways which could be confirmed by increases in dry weight and height of cowpea bean cultivars studied here.

Authors such as Silva et al. (2007) and Monteiro et al. (2014), show that the increase in the concentration of 24-epibrassinolid in plants sensitive to abiotic stress, induce significant increases in growth variables, thus corroborating with the results found in this research.

Several studies claim that inhibition of growth in height by excess NaCl is due to increased power offset for the maintenance (Garcia et al., 2010; Ashraf and Harris, 2013). The decrease in this variable may reflect the metabolic energy cost associated with the adaptation to salinity and reducing the carbon gain (Garcia et al, 2010; Ashraf and Harris, 2013).

Thus, the increments in time arising from 24 epibrassinolids modulation may be due to its probable regulation in carbon metabolism and nitrogen in plants in favor of the latter, which appears to be consistent in order that this study did increases in conductance stomatal plants under stress and treated with the phytohormone, which probably influenced to higher CO_2 assimilation.

Authors like Larré et al. (2011), claim that the action of the hormone in the highest growth induction can in part be explained, too, for their involvement in the modification in the structure and permeability of cell membranes of plants on stress by NaCl.

It is suggested thereby that the phytohormone modulated such changes with a view to their possible involvement in the antioxidant system, working in greater regulation of the photosynthetic machinery of cowpea plants. The exact understanding of what major routes the phytohormone set initially is not well defined, but it can be inferred from this study, supplementation with brassinosteroids accelerated the synthesis of protein and increased net CO_2 assimilation, these processes are essential to plant growth, which could be observed in both legume cultivars analyzed.

Our results show were consistent in that under hormonal effect, leaf area was significantly increased, which is an important growth parameter because it shows the size of the photosynthetic apparatus, which determines the dry matter accumulation, plant metabolism, the ability photosynthetic potential. Thus, justifiable increments observed in plant height, stem diameter and the biomass production under the effect of 24-epibrassinolide.

Conclusion

The deleterious effects of salinity are minimized by the 24-epibrassinolid in cultivating sensitive to salinity. The increases in growth were more significant variables in plants under stress and 100 mM NaCl supplemented with 0.2 µM BR, which may result from the regulation of phytohormone on carbon and nitrogen metabolism in plants in favor of the latter.

The deleterious effects of salinity on the height and dry matter production of shoots and roots, both BR3 Guariba and BRS Tracuateua were reversed by concentrations of 24-epibrassinolid, assuming the involvement of brassinosteroids on photosynthetic matabolism plant, in view to greater stomatal conductance, which can guarantee the highest CO_2 assimilation in inducing these variables increases.

Conflict of Interests

The authors have not declared any conflict of interests.

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