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EFFECT OF 24-EPIBRASSINOLIDE ON GROWTH AND PHOTOSYNTHESIS OF YOUNG RICE PLANTS TREATED WITH NaCl

Efecto de la 24-epibrasinólida en el crecimiento y la fotosíntesis de plantas jóvenes de arroz tratadas con NaCl

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ABSTRACT. At present, salinity is one of the most pressing causes of stress, and brassinosteroids are compounds which stimulate plant growth and also, increase the plant tolerance to salt stress. In this paper, the effect of foliar spraying with a natural brassinosteroid (24-epibrassinolide, EBL) in rice seedlings subjected to this kind of stress was evaluated. To do this, rice young plants of J-104 and Gines cultivars were sprayed with EBL (2 μmol L^{-1}), and then, half of them were treated with NaCl (100 mmol L^{-1}) for seven days, and later, they were transferred to nutrient solution to assess recovery for 14 days. Evaluations of growth (length and dry mass of aerial parts and roots) were performed at three and seven days of saline treatment and seven and 14 days of recovery. Some indicators of photosynthesis such as photosynthetic rate, stomatal conductance, transpiration, intercellular CO_2 concentration and photosynthetic pigments (chlorophylls and carotenes) were also determined. The results showed that, in general, foliar spraying with EBL 2 μmol L^{-1} stimulated the length and dry mass of rice seedlings of both cultivars under saline conditions which appear to be associated with increased photosynthesis, gas exchange and chlorophyll concentration.

Key words: brassinosteroids, chlorophylls, *Oryza sativa*, salinity

RESUMEN. La salinidad es una de las causas de estrés más acuciantes en la actualidad y los brasinoesteroides son compuestos que, además, de estimular el crecimiento vegetal aumentan la tolerancia de las plantas al estrés salino. En el presente trabajo, se evaluó el efecto de la aspersión foliar con un brasinoesteroide natural (24-epibrasinólida, EBL) en plántulas de arroz sometidas a este tipo de estrés. Para ello, se asperjaron plantas jóvenes de arroz de los cultivares J-104 y Ginés con EBL (2 μmol L^{-1}), la mitad de ellas se trataron con NaCl (100 mmol L^{-1}) por siete días, y posteriormente, se transfirieron a solución nutritiva para evaluar su recuperación durante 14 días. Las evaluaciones de crecimiento (longitudes de la parte aérea y de las raíces y masas secas de la parte aérea y de las raíces) se realizaron a los tres y siete días del tratamiento salino y a los siete y 14 días de recuperación. También se determinaron algunos indicadores de la fotosíntesis como tasa fotosintética, conductancia estomática, transpiración, concentración interna de CO_2 y pigmentos fotosintéticos (clorofilas y carotenos). Los resultados demostraron que, de forma general, la aspersión foliar con EBL (2 μmol L^{-1}) estimuló la longitud y la masa seca de las plántulas de arroz de ambos cultivares en condiciones salinas, lo que parece estar asociado a un incremento de la fotosíntesis y el intercambio gaseoso, así como a un aumento de la concentración de clorofilas.

Palabras clave: brasinoesteroides, clorofilas, *Oryza sativa*, salinidad

INTRODUCTION

Salinity is one of the most pressing causes of stress today. Inadequate irrigation of soils cause, this phenomenon to reach a global level as well as climate change (1). In Cuba, the agricultural surface is affected by 14 % and another 15 % presents potential salinization hazards.

To face this problem, we work in the search of solutions that allow increasing the productivity of crops under these conditions, for which not only

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[A]Orellana R, Febles J, Ortega F, et al. GEO Cuba. Evaluación del medio ambiente cubano. Santo Domingo. República Dominicana: Centenario SA; 2009. 293 p.

tolerant cultivars could be used; but also, work on the introduction of technologies that minimize the damage that these types of stress cause in plants (2).

One of the technologies for increasing agricultural yields that has been developed in recent years is based on the use of environmentally friendly natural products, such as brassinosteroids (BRs) (3).

These compounds are potent plant growth regulators of a steroidal nature. These hormones have pleiotropic effects such as stimulation of cell elongation and de-differentiation of protoplasts, regeneration of the cell wall, regulation of the differentiation of tracheal elements and increase of biomass and yield (4). In recent years, several authors have studied the protection that BRs give plants to certain types of stress both biotic and abiotic (5-8).

However, as far as is known, it has not been possible to optimize the application of these compounds in the protection of crops subjected to different environmental stresses, mainly due to the lack of knowledge of the mechanism in which they act in plants under these conditions. The knowledge of the mode of action of brassinosteroids in rice cultivation under conditions of salt stress would allow, in the future, a more effective and rational application of these growth regulators. For this reason, the objective of this work was to determine whether foliar spraying with 24-epibrasinolide is able to protect young rice plants from the adverse effects caused by salt stress, as well as to evaluate some physiological indicators associated with this response.

MATERIALS AND METHODS

The experiments were carried out in the Molecular Biology laboratory of the Biology Faculty of the University of Alberta in Canada. Rice seeds (*Oryza sativa* L.) of cultivars Jucarito-104 (J-104), susceptible to saline[B] stress and Ginés, tolerant (9) were used. The seeds were disinfected with commercial sodium hypochlorite for five minutes; subsequently, they were washed six times with sterile distilled water and then immersed in water for 24 hours.

After this time, they were placed in Petri dishes, at room temperature in the dark, to promote germination. After 48 hours, the germinated seeds were transplanted into 250 mL plastic pots containing vermiculite.

60 pots were used in total (each with four growing plants) and placed in plastic trays containing Hoagland nutrient solution diluted 1: 1v/v. The trays were placed inside a growth chamber with controlled conditions of temperature (28 ºC), humidity (70 %) and photoperiod (12 light hours/12 hours of darkness).

At the time of emergence of the third true leaf, foliar spray was performed with 24-epibrasinolide at the concentration of 2 μmol L^{-1}. A total volume of 60 mL was sprayed for 120 plants. To all solutions, 0,01 % Tween 20 was added as a detergent. The plants were divided into two groups, one was placed in trays to which were added nutrient solution Hoagland diluted 1: 1 v / v (SN) and the other was placed in trays containing diluted Hoagland nutrient solution supplemented with 100 mmol NaCl L^{-1} (SN + NaCl).

After seven days, the plants that underwent saline treatment were placed in nutrient solution Hoagland diluted 1: 1 v/v, for 14 days, for recovery.

The growth evaluations (the aerial part length, length of the roots, dry mass of the aerial part and of the roots) were carried out to 16 plants per treatment, at three and seven days of the saline treatment and at seven and 14 days of recovery. At the same time, evaluations of the photosynthetic rate, stomatal conductance, transpiration and internal CO_2 concentration were carried out, using a portable photosynthesis measuring device (LI-6400, LI-COR, and Lincoln, NE, USA). For the determination of total chlorophylls *a, b* and carotenes, four samples were taken per treatment and extraction was carried out with 80 % acetone for 24 h in the dark (10). After this time, the absorbances were measured at the following wavelengths 440, 649 and 665 nm.

In the experiment a completely randomized design was used. After verifying that the obtained data fulfilled the theoretical assumptions of normality and homogeneity of variance, a simple classification ANOVA was performed. In the cases in which there were significant differences between treatments, a comparison of means was performed using the Tukey test with $p \leq 0{,}05$ (11), using the statistical program STATGRAPHICS Plus 5,1 (12).

RESULTADS AND DISCUSSION

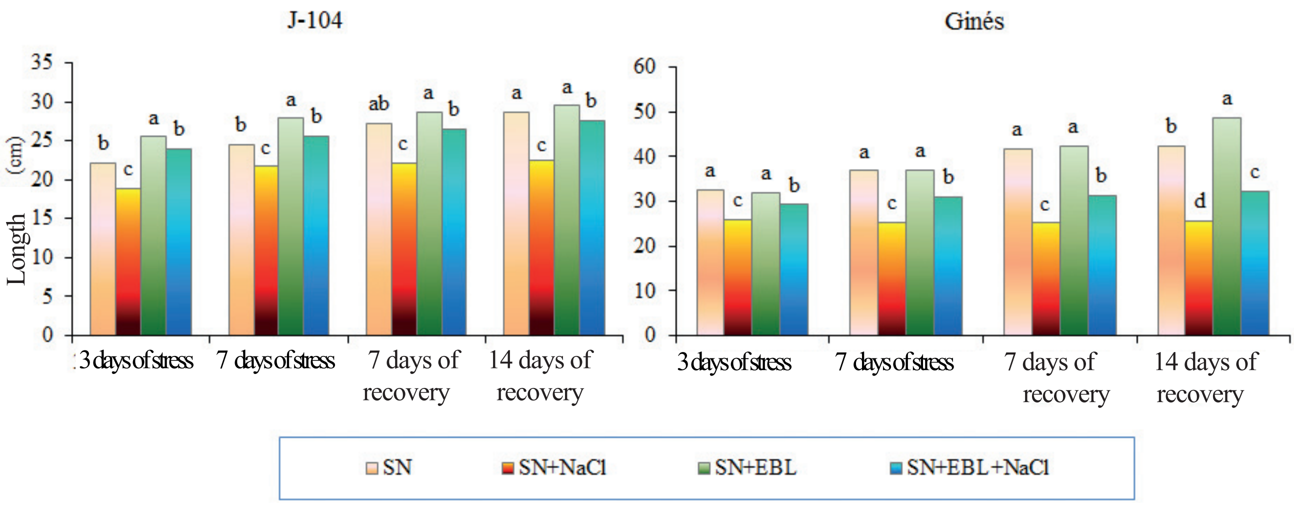
Figure 1 shows the behavior of the aerial part length of the seedlings. As it can be observed, the treatment with NaCl affected this indicator from the three days of stress in both cultivars, while the foliar

[B]Alfonso, R. Determinación de parámetros genético-fisiológicos indicadores del estrés hídrico para su empleo en el mejoramiento genético del arroz (*Oryza sativa* L.) y la estabilidad varietal [Tesis de Doctorado]. IIA; 1998.

spraying with EBL managed to revert this decrease in all the moments evaluated, being more effective in the cultivar ¨J-104¨, where plants treated with EBL and subjected to stress achieved values similar to the control without salt, except 14 days after recovery.

Figure 2 shows the behavior of the length of the roots in both cultivars. It is interesting to note how the salinity affected the J-104 cultivar from the three days of stress, while in the Ginés cultivar this affectation became visible after seven days of stress. The foliar spraying with EBL had a positive influence in all the evaluated moments (Figure 2), highlighting the Ginés cultivar where it recovered totally the radical length, except at 14 days of recovery.

The dry mass of the aerial part diminished significantly from the three days of the NaCl treatment, being this effect more marked in the cultivar ¨J-104¨ than in the Ginés (Figure 3). The foliar spraying with EBL in the case of the cultivar ¨J-104¨ only managed to revert this negative effect to the three days of stress and to the 14 days of recovery; however, in the Ginés cultivar the foliar spray (SN + EBL + NaCl) managed to significantly increase the dry mass of the aerial part, compared to the control treatment (SN+NaCl), from the seven days of stress, maintaining this behavior until the end of the experiment.



Common letters do not differ from each other according to Tukey's test (p≤0,05) n=16

Figure 1. Effect of EBL on the aerial part length of the rice seedlings subjected to NaCl

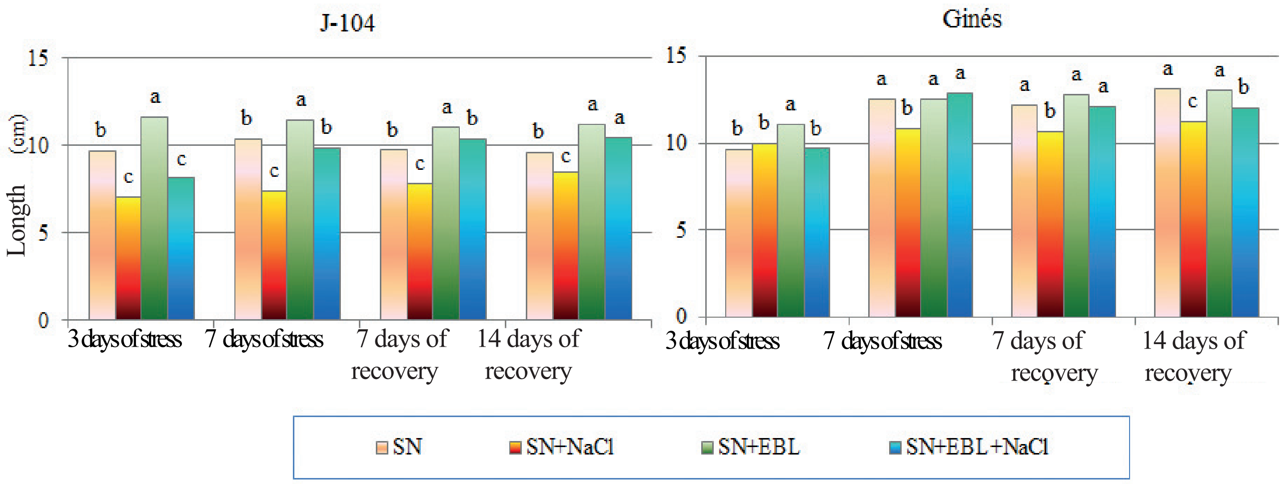


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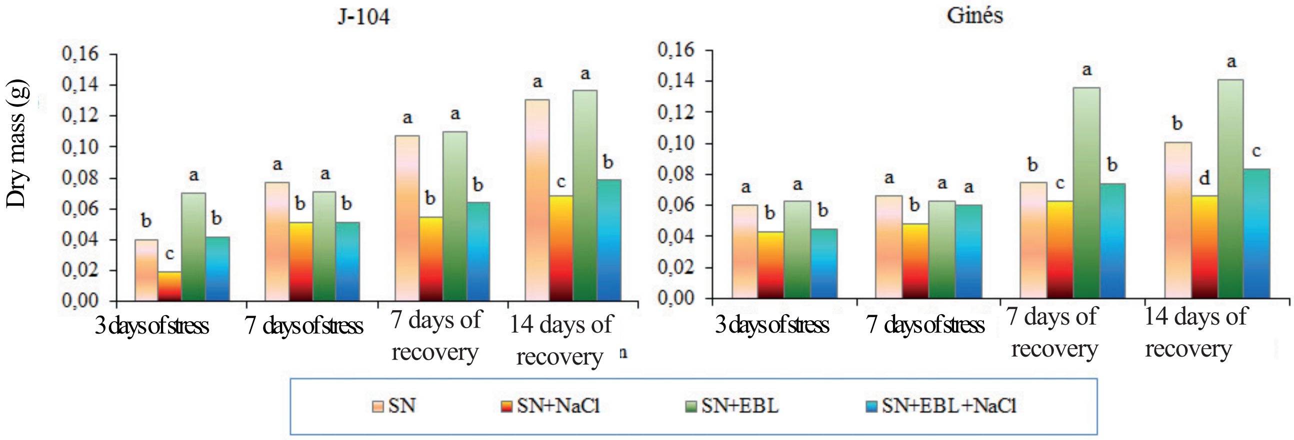
Figure 2. Effect of EBL on root length of the rice seedlings subjected to NaCl



Common letters do not differ from each other according to Tukey's test (p≤0,05) n=16

Figure 3. Effect of EBL on the dry mass of the aerial part of rice seedlings subjected to NaCl

When evaluating the dry mass of the roots it is observed that the saline stress caused a significant decrease that was reversed in the plants sprinkled with EBL but only in the cultivar ¨J-104¨. The behavior in the Ginés cultivar was different since foliar spraying could only improve this indicator after seven days of stress and 14 days of recovery (Figure 4).

Salinity negatively affected the growth indicators in both cultivars; even during the recovery these effects became visible. It is well known that salinity inhibits the growth of plants and in many cases the aerial part is more affected than the roots. This effect of salt stress on growth is widely known, both water stress and the accumulation of ions delay the processes of cell division and differentiation (13). For example, in four citrus cultivars subjected to different levels of salinity both the length and the dry masses of roots and shoots were affected, this effect being more marked in sensitive cultivars (14). Likewise, the decrease in length and fresh mass of mustard seedlings subjected to different concentrations of NaCl has been observed (15). In addition, in *Fraxinus ornus*, the dry mass of roots decreased in plants exposed to salinity (16).

On the other hand, the decrease in the growth of the treated plants, during the recovery, could be due to the fact that the toxic effects of the accumulation of salts take longer to manifest but remain in time, even after passing the recovery period. To corroborate this hypothesis, it has been pointed out that once the stress period was over and the plants were subjected to control treatment for seven days, they did not recover, which demonstrates the irreversible nature of the damage caused (17).

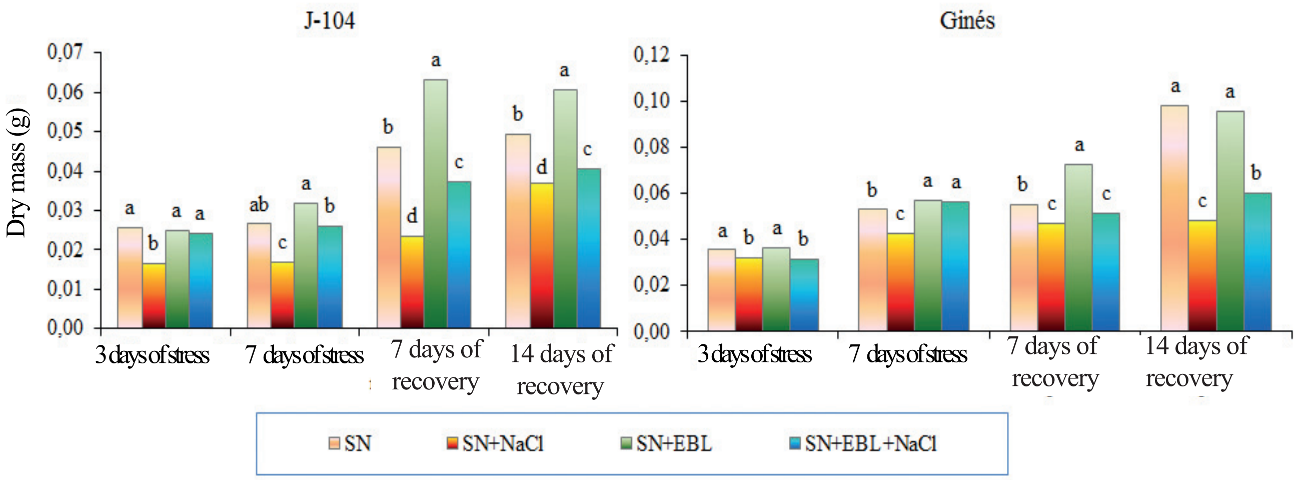
It is noteworthy, as the cultivar ¨Ginés¨ considered tolerant did not behave as such, having reductions in growth similar to the cultivar ¨J-104¨. This could be due to the concentration of salt used in the experiment (100 mmol L^{-1}), equivalent to 9,867dS m^{-1}, considered by the Cuban standards of salinity as very strongly saline (18), which would cause severe damage to the plants. Another explanation could be that the tolerance mechanisms of this cultivar develop in later stages of growth and this allows obtaining a better yield, even when in the first stages it behaves in this way. In the same way, other works where rice cultivars J-104 and INCA LP-7 were used, which, at the field level, respond differently to salt stress (19), were not very contrasting in their responses to concentration. 100 mM NaCl in the initial growth phase (7 days) (20).

On the other hand, leaf spraying with EBL managed to reverse the adverse effects of salt stress on the growth of the two cultivars studied.

Similar results have been reported by other authors, who observed a recovery in the growth of rice seedlings IR-64 variewhose seeds were treated for 24 h with NaCl (150 mmol L^{-1}) and EBL (3 µmol L^{-1}) (21).

On the other hand, the increase in the initial growth of cultivated rice seedlings IR-28 in the presence of salt stress (120 mmol L^{-1}) treated with EBL (3 µmol L-1) was reported (22). In addition, growth recovery has been observed in rice plants sprinkled during the paniculation initiation phase and subjected to salt stress (100 mmol L^{-1}) (23).

More recently, the treatment influence on seeds with various concentrations of 24-epibrasinolide and Biobras-16 (BB-16), an analogue of brassinosteroids, on the growth of the seedlings of two rice cultivars (J-104 and Ginés) in saline medium. Both the EBL and the BB-16 were able to partially reverse the inhibition that in the growth of the seedlings of both rice genotypes caused the presence of NaCl; treatments are more effective in the Ginés tolerant cultivar (24).



Common letters do not differ from each other according to Tukey's test (p≤0,05) n=16

Figure 4. Effect of EBL on the dry mass of the roots of rice seedlings subjected to NaCl

In other crops, EBL has also been able to increase growth under saline stress conditions such as *Vigna radiata* (25), *Lactuca sativa* L. (26) and *Triticum aestivum* L. (27).

The osmotic effect of salt stress produces the closing of stomata with the consequent decrease in photosynthesis due to CO_2 deficiency. For this reason, the photosynthetic rate, stomatal conductance, transpiration and internal CO_2 concentration were evaluated in the experiment.

As can be seen in Figure 5, the photosynthetic rate of the plants decreases with salt stress in both cultivars from the three days of the same placed and this behavior is maintained throughout the experiment, so that the recovery cannot be reversed this effect. Something similar has been reported by other authors who refer to the decrease of photosynthesis in four citrus cultivars at different concentrations of NaCl, with the most affected being sensitive cultivars (14). Likewise, the decrease in the photosynthetic rate has been observed in mustard seedlings subjected to different concentrations of NaCl (15). Also, in *Fraxinus ornus*, the photosynthetic rate decreased in plants exposed to salinity (16).

Foliar spraying with EBL partially reversed the effect of saline stress on photosynthesis, highlighting the Ginés cultivar where it is observed at all times evaluated, not so in cultivar ¨J-104¨.

Other authors have reported the positive influence of the treatment with brassinosteroids in photosynthesis, for example, in eggplant the concentration of 100 nmol L^{-1} of 24-EBL managed to reverse the inhibitory effects of salt stress in photosynthesis (28).

Also in mung beans, the foliar spraying of EBL (10^{-8} mol L^{-1}) increased the photosynthesis in the NaCl treatment at levels similar to the control (25). In addition, other natural brassinosteroids such as 28-homobrasinolide (28-HBL) have also had positive effects on photosynthesis under stress conditions. For example, in *Brassica juncea* the concentration of 10^{-6} mol L^{-1} of 28-HBL was the best result in the increase of photosynthesis in the three concentrations of NaCl evaluated (50,100 and 150 mmol L^{-1}) (29).

Another of the indicators evaluated in the experiment was the stomatal conductance which is a measure of the opening of the stomata for the realization of gas exchange. The regulation of stomatal conductance in the leaves is a key phenomenon both for the absorption of CO_2 and to avoid desiccation under stress conditions (30).

In Figure 6, it is observed that salt stress significantly decreased stomatal conductance in both cultivars, this behavior being more pronounced in cultivar ¨J-104¨ after seven days of stress. Similar results have been demonstrated by other authors in four citrus cultivars at different concentrations of NaCl, the most affected cultivars being the sensitive ones. Likewise, the decrease in stomatal conductance in mustard seedlings subjected to different concentrations of NaCl has been observed. Also, in *Fraxinus ornus* decreased stomatal conductance in plants exposed to salinity (14-16).

The foliar spraying with EBL managed to reverse this effect, significantly increasing the stomatal conductance in both cultivars, in almost all evaluated moments, except for the seven days of recovery in cultivar ¨J-104¨.

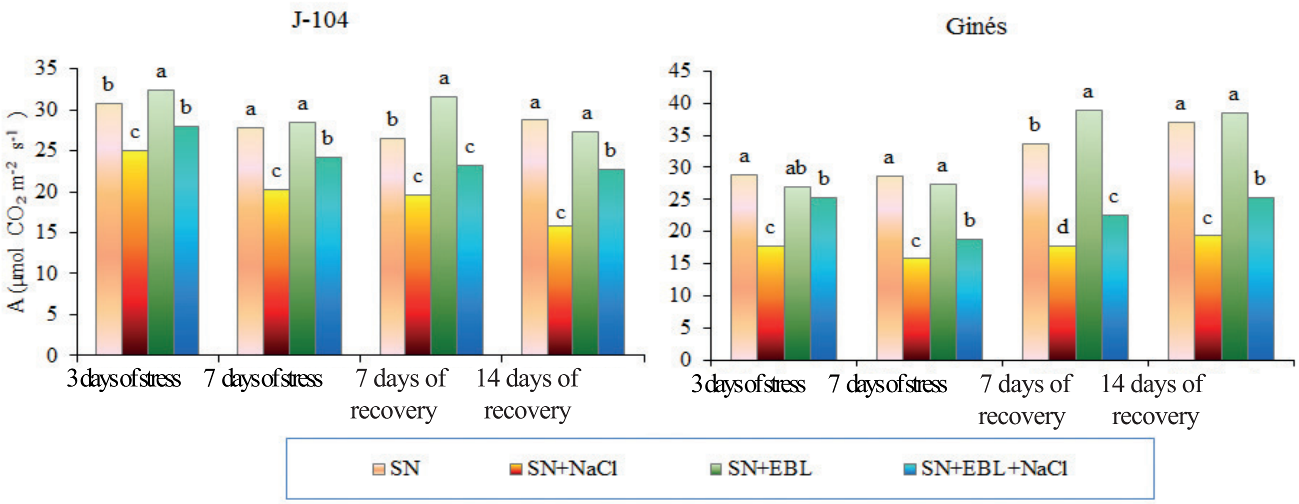


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Figure 5. Effect of EBL on the photosynthetic rate (A) of rice seedlings subjected to NaCl

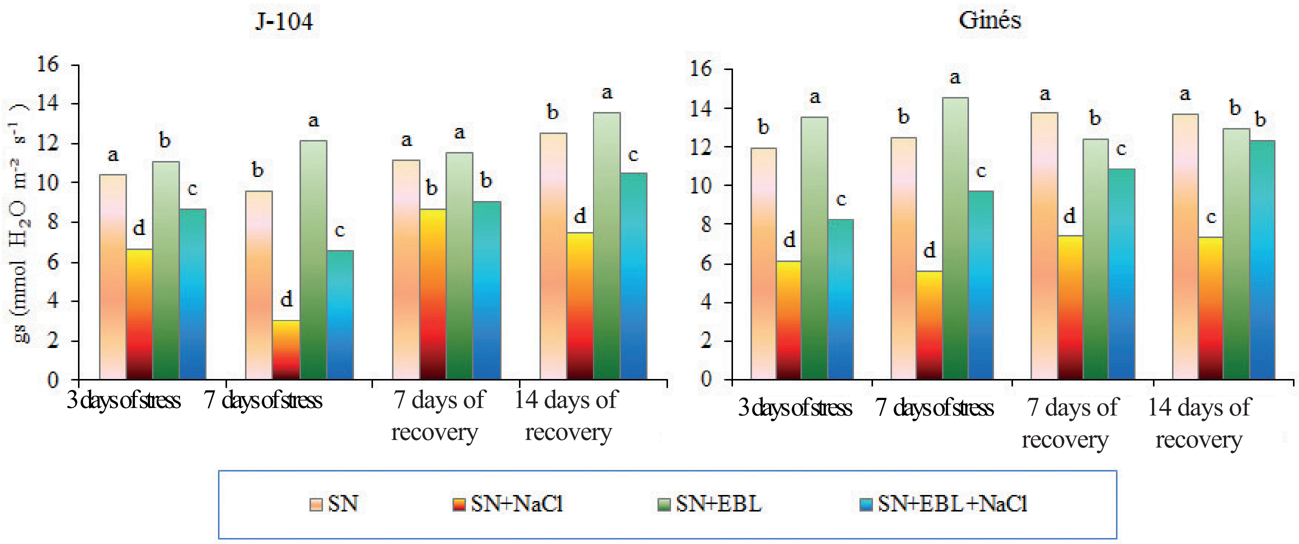

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Figure 6. Effect of EBL on stomatal conductance (gs) of rice seedlings subjected to NaCl

Other authors have obtained increases in the stomatal conductance with the use of the EBL, for example in lettuce the treatment to the seed and the later foliar spraying of three concentrations of EBL (1, 2 and 3 µmol L^{-1}) increased the stomatal conductance in 75 % with respect to the control, the most effective treatment being 3 µmolL^{-1} both at the NaCl concentration of 50 mmol L^{-1} and that of 100 mmol L^{-1} (26). Also in mung beans, the foliar spraying of EBL (10^{-8} mol L^{-1}) increased the stomatal conductance in plants treated with NaCl at levels similar to the control (25). In eggplant, EBL concentrations of 50 and 100 nmol L^{-1} were able to reverse the decrease caused by NaCl at the concentration of 90 mmol L^{-1}; however, EBL concentrations of 200 and 400 nmol L^{-1} potentiated the adverse effect of salinity (28).

Saline stress significantly decreased transpiration in both cultivars, maintaining this behavior during recovery (Figure 7). Something similar was obtained in four citrus cultivars with different concentrations of NaCl, decreasing transpiration more in sensitive cultivars than in tolerant ones (14). Likewise, the decrease in transpiration in mustard seedlings subjected to different concentrations of NaCl has been observed (15). Also in *Fraxinus ornus* this indicator decreased in plants exposed to salinity (16).

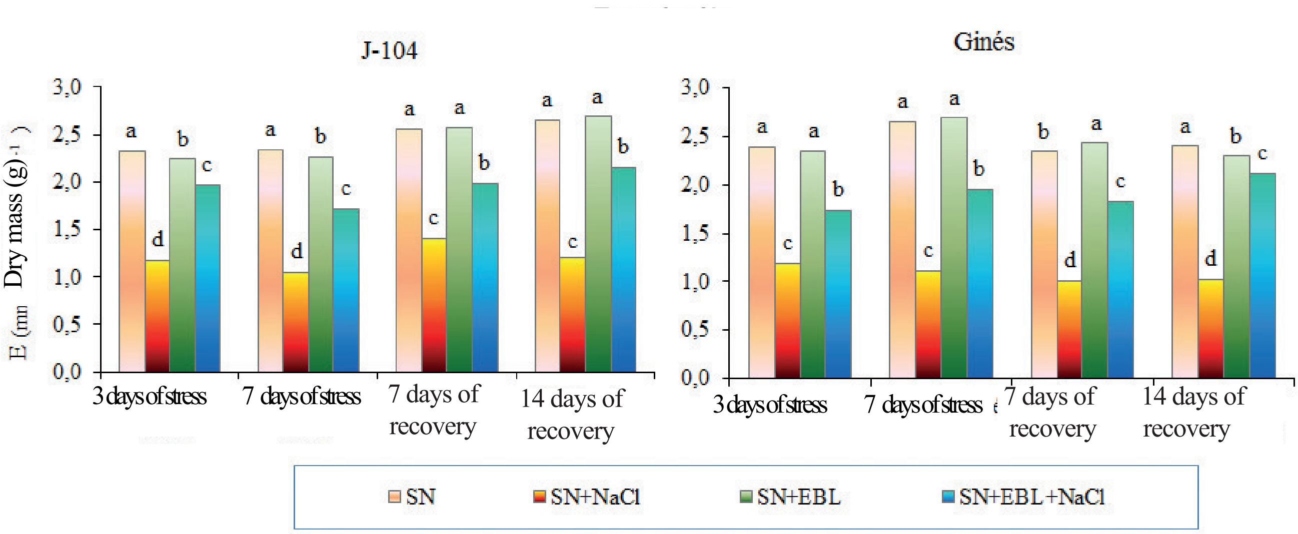

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Figure 7. Effect of EBL on transpiration (E) of rice seedlings subjected to NaCl

The foliar spraying with EBL managed to reverse this effect, significantly increasing the transpiration; in this case no differences were found between the plants of the two cultivars studied, in any of the evaluated moments.

This behavior was similar to that found in water melon, where foliar spraying with EBL (10^{-6} mol L-1) reversed the decrease in perspiration caused by treatment with salinized water (70 mmol L^{-1} NaCl) (31).). Likewise, in grapes, the application of EBL (3 and 6 µmol L^{-1}) was able to restore the transpiration of stressed plants with several levels of NaCl (25, 50, 75 and 100 mmol L^{-1}) (32).

As can be seen, in Figure 8, salt stress significantly decreased the internal concentration of CO_2 in both cultivars, this effect being more marked in the Ginés cultivar. During the recovery this behavior was maintained. Other authors have reported the decrease in *Ci* in different rice cultivars exposed to salt stress for seven days (33).

Foliar spraying with EBL reversed this negative effect of salt, particularly in the Ginés cultivar, where there was a greater increase in *Ci* at all the evaluated moments, while in cultivar ¨J-104¨ the increase was lower and at seven days of recovery no significant differences were found.

This result agrees with that reported by other authors, who observed an increase in *Ci* in mung bean plants sprinkled with EBL (10^{-8} mol L^{-1}) and subjected to stress by NaCl (25). Also, in eggplant, EBL concentrations of 50 and 100 nmol L^{-1} managed to reverse the decrease in *Ci* caused by NaCl at the concentration of 90 mmol L^{-1}; however, the EBL concentrations of 200 and 400 nmol L^{-1} did not differ with the salinized control (28). On the other hand, wheat showed very little increase in this indicator in stressed seedlings with several EBL concentrations (0,052; 0,104; 0,156 µmol L^{-1}) (34).

When analyzing the results on chlorophyll pigments in cultivar ¨J-104¨, after three days of treatment with NaCl, a decrease in chlorophyll *a* and total chlorophylls in stressed seedlings was observed; at the end of the stress period, this decrease also reached chlorophyll *b* and carotenoids. In the recovery, this behavior was maintained in all the pigments evaluated (Figure 9). However, in the Ginés cultivar, the decrease in chlorophylls began from the three days of stress until the end of the experiment, while the carotenes did not change during the stress period, but decreased in recovery (Figure 10).

This result agrees with different authors who have observed the decrease of the concentration of this pigment before saline stress in numerous crops such as: basil, rice, grape and tomato (32,35-37).

The reduction in chlorophyll content in leaves under saline stress conditions has been attributed to several causes: the destruction of chlorophyll pigments by the increase in chlorophyllase activity and the suppression of biosynthesis enzymes such as porphobilinogen desaminase. It can also be caused by the interference of saline ions with the de novo synthesis of proteins and structural components of chlorophyll (30).

Foliar spraying with EBL managed to reverse the adverse effects of salt stress on the chlorophyll concentration from three days of stress until the end of the experiment in both cultivars.

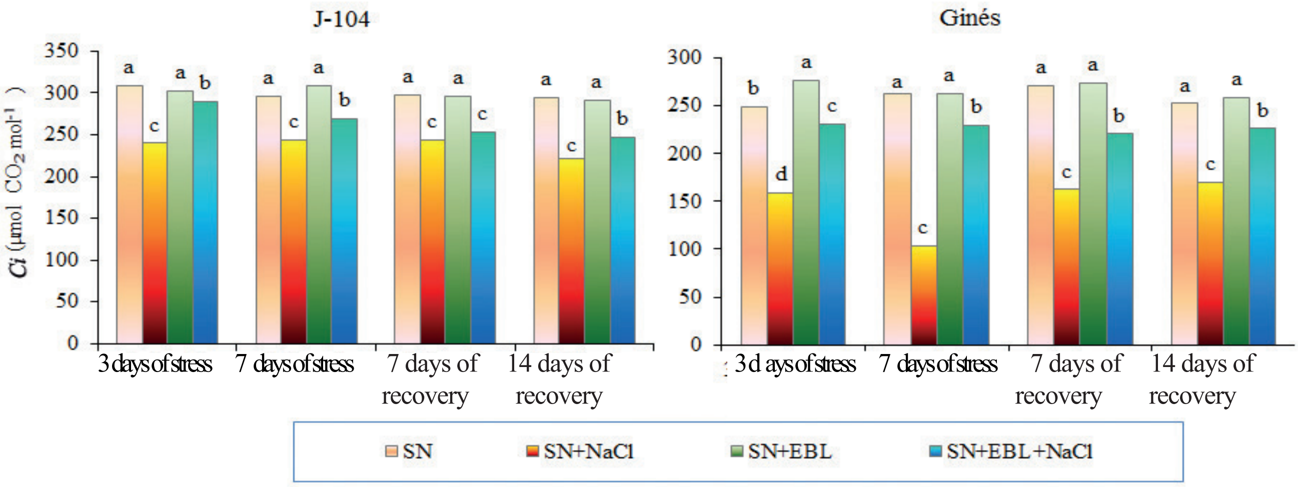

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Figure 8. Effect of the EBL on the in the internal concentration of CO2 (Ci) of rice seedlings subjected to NaCl

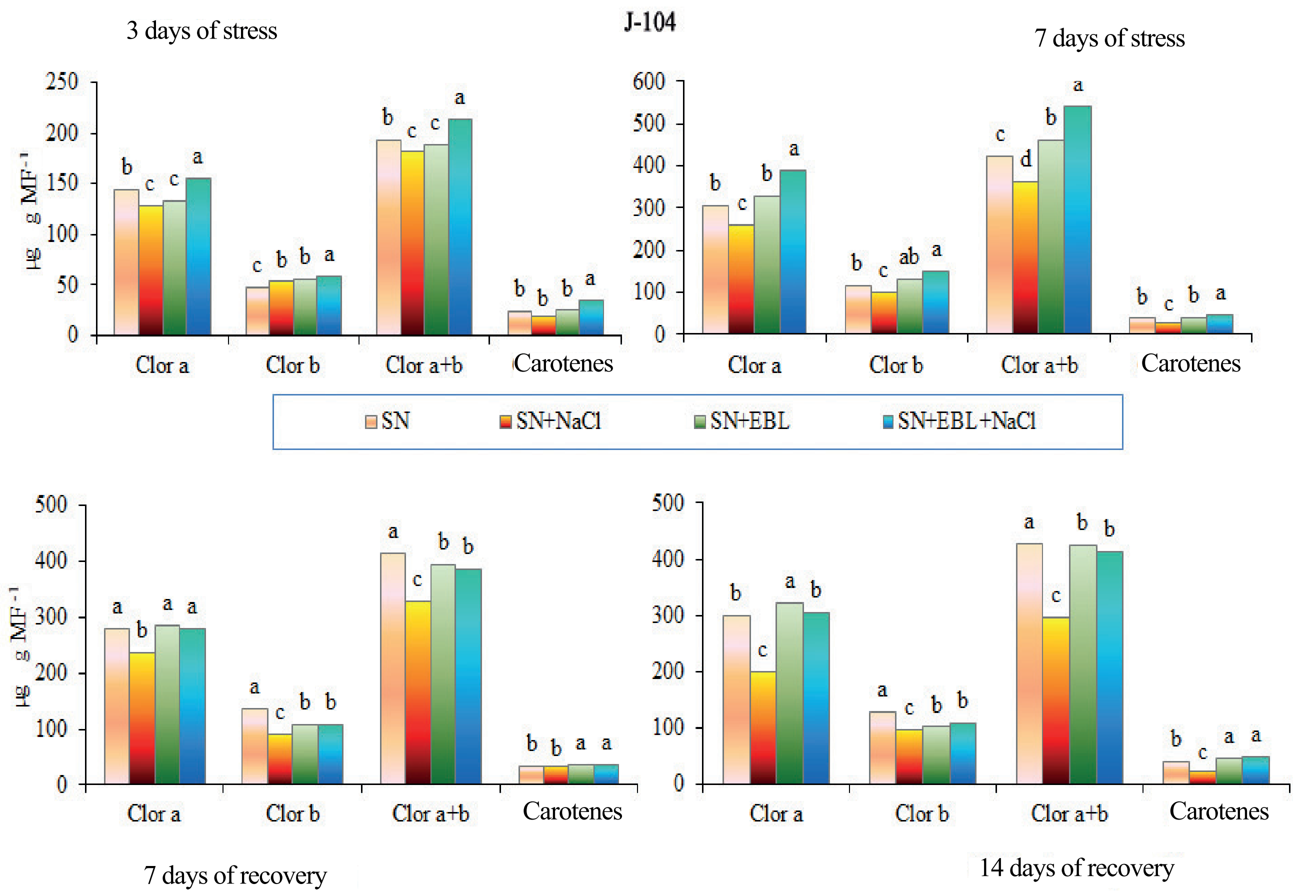

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Figure 9. Effect of EBL on the content of chlorophylls a, b and total and the carotenes of rice seedlings cv. J-104 subjected to NaCl

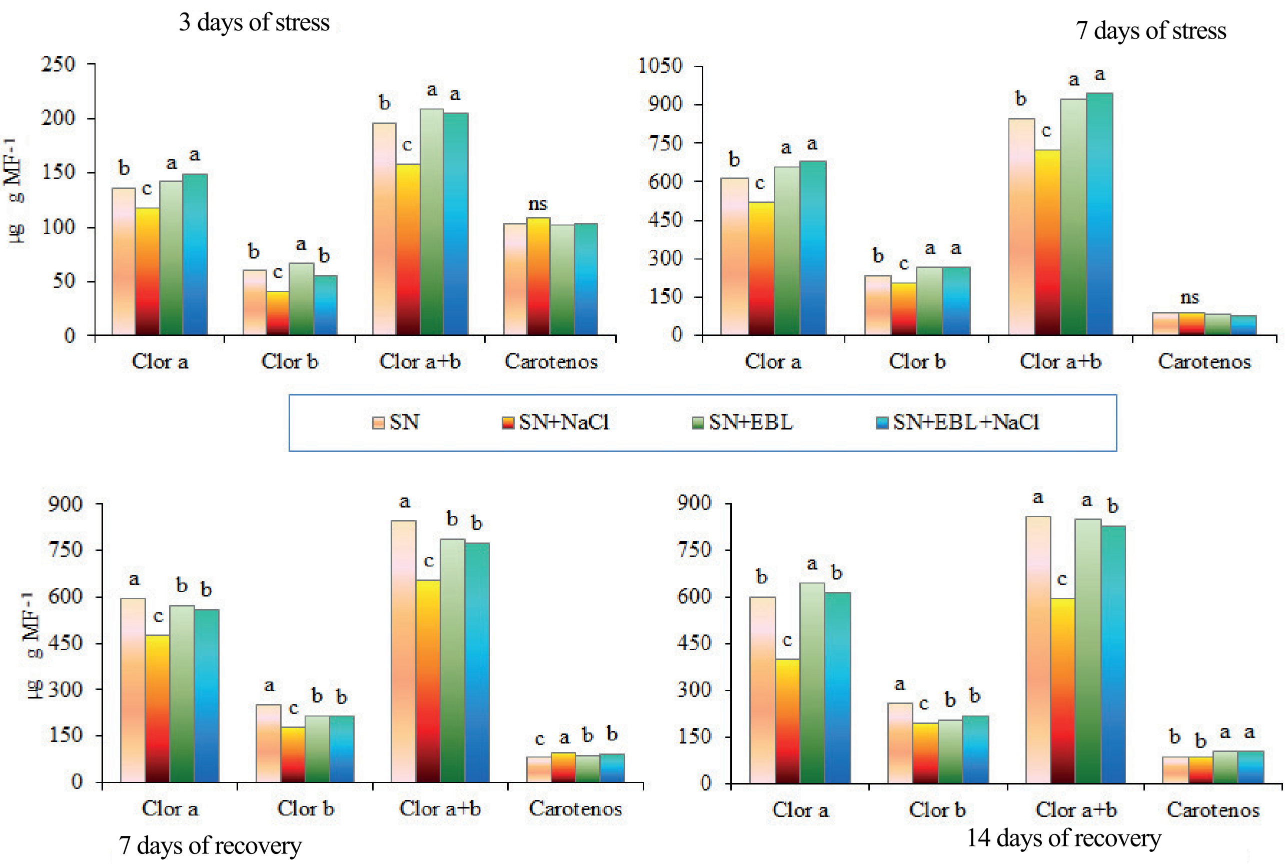

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Figure 10. Effect of EBL on the content of chlorophylls a, b and total and the carotenes of rice seedlings cv. Ginés subjected to NaCl

Regarding the carotenes, the leaf sprinkling of EBL increased the concentration of them in the stressed seedlings in the cultivar ¨J-104¨ at all the evaluated moments. In the Ginés cultivar, foliar spray managed to increase the concentration of carotenes during recovery.

The concentration stimulation of chlorophylls in the leaves of plants sprinkled with EBL and subjected to treatment with NaCl, confirms that obtained by other authors who have reported that the foliar spray with EBL at various concentrations (50, 100, 200 and 400 nmol L^{-1}) decreased the loss of photosynthetic pigments caused by salt stress (NaCl 90 mmol L^{-1}) in eggplant seedlings (28). Also, in *Phaseolus vulgaris* and *Hordeum vulgare* the pre-treatment to the seeds with brassinosteroid (5 μmol L^{-1}) reversed the decrease in chlorophyll content caused by salt stress (150 mmol L^{-1}), reaching levels similar to control plants (38). Similarly, the foliar spraying of EBL in bean plants subjected to salt stress reversed the decrease in chlorophylls caused by this stress (39).

The effect of other BRs on the restitution of chlorophyll molecules in salt stress has also been demonstrated. For example, in *Brassica juncea* the concentration of 10^{-6} mol L^{-1} of 28-HBL was the best result in the increase of the chlorophyll concentration in the three NaCl concentrations evaluated (50,100 and 150 mmol L^{-1}) (29).

In general, the Ginés cultivar responded better to the application of the EBL. These results agree with previous studies where these same cultivars were used and the response of the Ginés cultivar to the application of EBL to the seeds was superior to that of the cultivar ¨J-104¨ (24). On the other hand, several researchers have reported that the response of plants to the exogenous application of brassinosteroids depends on their sensitivity to a certain type of stress; responding better to tolerant cultivars than sensitive cultivars (27,32).

The reduction in growth in the stressed seedlings in the present work apparently is due to a decrease in photosynthesis, which could be due to stomatal factors since the stomatal conductance and perspiration were affected and therefore the *Ci* available for the assimilation of CO_2.

In addition, the work presents evidence that this decrease in photosynthesis could also be associated with non-stomatal factors since chlorophylls also decreased, both *a b*, which could hinder the absorption of light.

The positive effect of leaf spraying of EBL on the growth variables of stressed seedlings, apparently was due to an increase in stomatal conductance, transpiration and *Ci*, which would increase the photosynthetic rate and biomass production. Likewise, the restitution of chlorophyll molecules by EBL treatment could explain the increase in photosynthesis.

CONCLUSIONS

Foliar spraying with EBL stimulates the growth of rice plants subjected to salt stress, which could be associated with an increase in the photosynthetic rate, stomatal conductance, transpiration and internal CO_2 concentration, as well as the increase in chlorophyll concentration. The cultivar ¨Ginés¨ responded better to the exogenous application of the EBL, than the cultivar ¨J-104¨.

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