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# BIOBRAS-16 FOLIAR SPRAYING ENHANCES RICE (*Oryza sativa L.*) YOUNG PLANT GROWTH UNDER NaCL TREATMENT

## Aspersión foliar con biobras-16 estimulael crecimiento de plantas jóvenes de arroz (*Oryza sativa* L.) Sometidas a tratamiento con NaCL

Yanelis Reyes Guerrero✉, Lisbel Martínez González and Miriam Núñez Vázquez

**ABSTRACT**. Salinity is one of the most pressing stresses today and brassinosteroids are compounds which, not only stimulate plant growth but also improve plant tolerance to salt stress. In this paper, the effect of foliar spraying with a brassinosteroid analogue (Biobras-16, BB-16) on rice seedlings under this kind of stress was evaluated. For this purpose, rice young plants cv. J-104 were sprayed with BB-16 (0; 0,01; 0,1; 1 µmol $L^{-1}$), half of them was treated with NaCl (100 mmol $L^{-1}$) for seven days and later, these plants were transferred to nutrient solution to assess recovery during fourteen days. The growth evaluations (shoot and root length and dry mass) were made at the beginning and the end of saline treatment and seven and fourteen days after the recovery. From the results of this experiment, the best BB-16 concentration was chosen and a second experiment, following the same methodology, was performed and, where, the growth indicators and some biochemical indicators such as malondialdehyde, proline, chlorophylls and the activities of peroxidase and catalase enzymes were determined. Results demonstrated, in general, that the foliar spraying with BB-16 0,1 µmol $L^{-1}$ increased the length of rice seedlings under saline conditions what appears to be associated with increased antioxidant defenses as well as an increase of the concentration of chlorophylls, chlorophyll *a* fundamentally.

*Key words*: brassinosteroids, catalases, chlorophylls, salt stress, proline

**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 análogo de brasinoesteroides (Biobras-16, BB-16), en plántulas de arroz sometidas a este tipo de estrés. Para ello, se asperjaron plantas jóvenes de arroz del cultivar J-104 con BB-16 (0; 0,01; 0,1; 1 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 catorce 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 al inicio y final del tratamiento salino y a los siete y catorce días de recuperación. Partiendo de los resultados de este experimento, se seleccionó la mejor concentración de BB-16 y se ejecutó un segundo experimento, siguiendo la misma metodología, donde, además, de evaluar los indicadores de crecimiento, se determinaron algunos indicadores bioquímicos como malondialdehído, prolina, clorofilas y las actividades de las enzimas peroxidasa y catalasa. Los resultados demostraron que, de forma general, la aspersión foliar con BB-16 a 0,1µmol $L^{-1}$ estimuló la longitud de las plántulas de arroz en condiciones salinas lo que parece estar asociado a un incremento de las defensas antioxidantes, así como a un aumento de la concentración de clorofilas, fundamentalmente clorofila *a*.

*Palabras clave*: brasinoesteroides, catalasas, clorofilas, estrés salino, prolina

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## INTRODUCTION

Salinity is one of the most pressing stressors today. Inadequate irrigation of soils, as well as climate change, makes this phenomenon reach a global level (1).

In our country, the agricultural area is affected by 14 % and another 15 % has potential hazards of salinization[A].

In order to face this problem, we are working to find solutions that allow us to increase the productivity of crops under these conditions, for which not only tolerant cultivars could be used, but also to work on the introduction of technologies to minimize the damages that these types of stress provoke in plants.

One of the technologies to increase agricultural yields developed in recent years, is based on the use of ecologically safe natural products, such as brassinosteroids (BRs).

These compounds are potent regulators of plant growth of a steroidal nature. These hormones have pleiotropic effects such as: stimulation of cell elongation and protoplast dedifferentiation, regeneration of the cell wall, regulation of differentiation of tracheal elements and increase of biomass and yield (2). In recent years, several authors have studied the protection that BRs confer on plants against certain types of stress, both biotic and abiotic (3, 4).

In Cuba, it investigates the synthesis and biological activity of some formulations, which have as their active ingredients spirostanic analogues of brasinosteroids (5).

From the practical point of view, the importance of the use of Cuban analogues in place of natural compounds is that the latter are more expensive and their effects are of short duration in plants (6). On the other hand, the analogues, synthesized in Cuba, have proven to be cheaper and more innocuous products with practical usefulness to increase agricultural yields between 10 and 25% in different species, including *Oryza sativa*, a species of great economic importance for our country; for being a staple food for the population (7). However, to the best of our knowledge, the optimization of the application of these compounds in the protection of crops subjected to different environmental stresses has not been achieved, mainly due to the fact that the mechanism in which they act on the plants in these plants is unknown terms. Knowledge of the mode of action of the brasinoesteroids in rice cultivation under conditions of saline stress would allow, in the future, a more effective and rational application of these growth regulators. For this reason, the objective of this work is to determine if the leaf spray with Biobras-16 is able to protect young rice plants from the adverse effects caused by saline stress, as well as to evaluate some biochemical indicators associated with this response.

## MATERIALS AND METHODS

In order to fulfill the proposed objective, rice seeds (*Oryza sativa* L.) of cultivar Jucarito-104 (J-104) considered as susceptible to saline[B] stress were used. The seeds were disinfected with commercial sodium hypochlorite for five minutes; subsequently, 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 to 240 mL plastic vessels containing the Gley Nodular Petroferric Hydromorphic soil (8) from the UCTB of Los Palacios, Pinar del Río. The characteristics of this soil were in the range that allowed the adequate growth of the rice plants (Table I). The determinations were made in the Laboratory of Chemical Analysis of the National Institute of Agricultural Sciences (9).

**Table I. Main chemical characteristics of the Hydromorphic Gley Nodular Petroferric soil used in the experiment**

| Na | K | Ca | Mg | P | OM | pH |
|---|---|---|---|---|---|---|
| | cmol $kg^{-1}$ | | | mg $kg^{-1}$ | % | |
| 0,23 | 0,05 | 9,8 | 3,98 | 150,7 | 0,83 | 7,1 |

Twenty glasses (each with three growing plants) were used and placed in plastic baskets containing running water. The baskets were placed inside shading structure to avoid the effect of the precipitations

At the moment of emergence of the third true leaf, leaf spray with Biobras-16 was performed at concentrations of 0,01; 0,1 and 1 µmol $L^{-1}$. A total volume of 60 mL was sprayed for 120 plants. To all solutions was added 0,01 % Tween 20 as detergent.

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

[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, I.I.A., 1998.

At that time, the plants were divided into two groups, one placed in baskets to which Hoagland nutrient solution diluted 1: 1 v/v (SN) was added and the other was placed in baskets containing diluted Hoagland nutrient solution supplemented with NaCl 100 mmol $L^{-1}$ (SN+NaCl).

At seven days, the plants that underwent saline treatment were placed in Hoagland nutrient solution diluted for 14 days for recovery.

Growth evaluations (shoot length, root length, shoot dry mass and roots) were performed at 12 plants per treatment at the beginning and end of saline treatment and at 7 and 14 days of recovery.

The experiment was carried out in two seasons of the year, the first one started on December 24th, 2009 and the second on May 25th, 2010. In both cases, the minimum, mean and maximum temperatures and the relative humidity of the air were recorded during The following stages: initial seedling growth (from transplantation to application of NaCl treatment), treatment period with NaCl (seven days) and recovery phase (14 days) (Table II). These data were taken from the Tapaste Weather Station located approximately 500 m from the area where the experiments were carried out.

From the results of the previous experiment, a second experiment was executed in April-May 2011, where the same methodology was used as in the previous experiment, with the difference that the leaf spray was performed with Biobras-16 0,1 µmol $L^{-1}$. Growth evaluations (shoot and root lengths, dry shoot and root masses) were performed at 12 plants per treatment at the beginning and end of saline treatment and at 14 days of recovery. In the case of the biochemical evaluations carried out on the leaves of the plants, they were carried out at the end of the saline treatment and at 14 days of recovery. The following indicators were determined: proline according to the method of ninhydrin (10), malondialdehyde (MDA) by thiobarbituric acid method (11) and chlorophyll *a*, *b* and totals by extraction with 80 % acetone (12); as well as the activities of antioxidant enzymes peroxidase (EC 1.11.1.7) (13) and catalase (EC 1.11.1.6) (14). Total proteins were also determined according to the Bradford method (15).

A completely randomized design was used in all experiments. After verifying that the obtained data fulfilled the theoretical assumptions of normality and homogeneity of variance, a simple classification ANOVA was performed. In cases where there were significant differences between treatments, a means comparison was performed using the Tukey test with $p \leq 0.05$ (16), using the statistical program STATGRAPHICS Plus 5.1 (17).

## RESULTS AND DISCUSSION

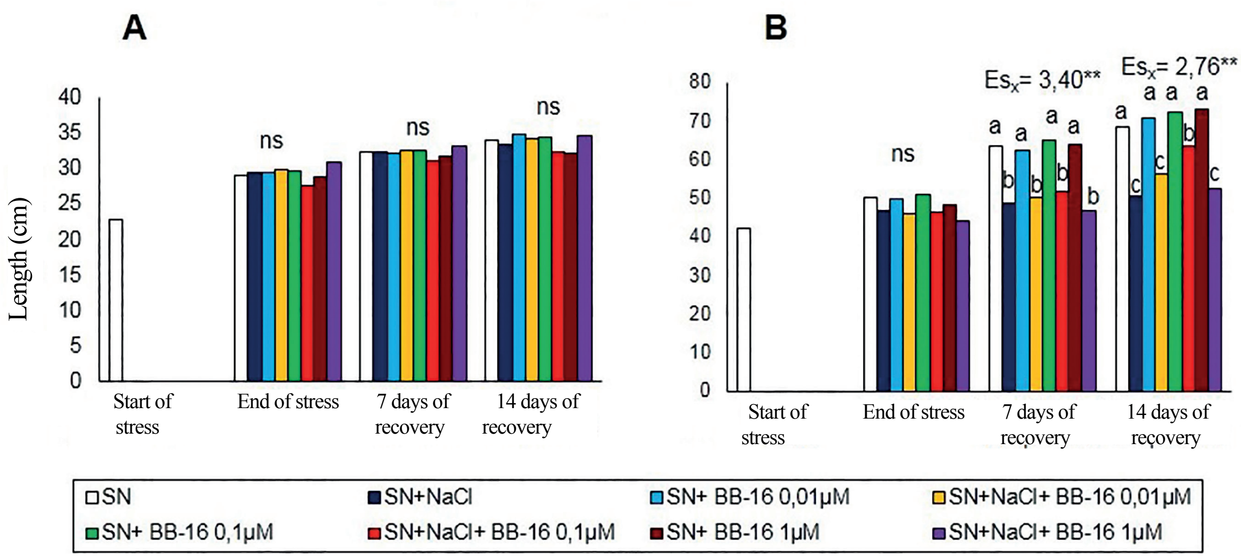
The behavior of the aerial part length of the seedlings is shown in Figure 1. As can be observed, in the first repetition of the experiment, the treatment with NaCl did not affect this indicator in any of the evaluated moments, nor was it observed influence of the leaf spray with BB-16. However, in the second repetition, carried out in the months of May-June, it was observed that the treatment with NaCl produced a significant reduction of the length of the aerial part in all treatments, which was manifested during the recovery. The leaf spray with BB-16 0,1 µmol $L^{-1}$ was able to protect the seedlings, significantly stimulating the shoot length at 14 days of recovery.

**Table II. Maximum, mean and minimum temperatures and mean relative air humidity during the initial growth phase, treatment period with NaCl and recovery stage of rice plants (means ± standard deviation)**

| Phases | Maximum temperature (ºC) | | Minimum temperature (ºC) | | Mean Temperature (ºC) | | Average relative humidity(%) | |
|---|---|---|---|---|---|---|---|---|
| | *First repetition* | *Second repetition* | *First repetition* | *Second repetition* | *First repetition* | *Second repetition* | *First repetition* | *Second repetition* |
| *Initial stage of growth* | 24,6 ± 1,6 | 33,1 ±0,5 | 13,6 ± 1,7 | 22,0 ±0,9 | 19,4 ± 1,4 | 26,4 ±1,1 | 76,0±3,2 | 79,5± 1,8 |
| *Treatment period with NaCl* | 26,8± 1,4 | 33,4± 0,4 | 15,5± 1,5 | 23,4± 0,6 | 21,3± 1,0 | 27,9± 0,4 | 73,7± 7,7 | 77,1± 3,0 |
| *Recovery Phase* | 25,0± 1,9 | 31,8± 0,4 | 13,9± 1,9 | 22,7± 0,5 | 19,6± 1,4 | 26,2± 0,4 | 71,2± 3,9 | 83,5± 1,4 |

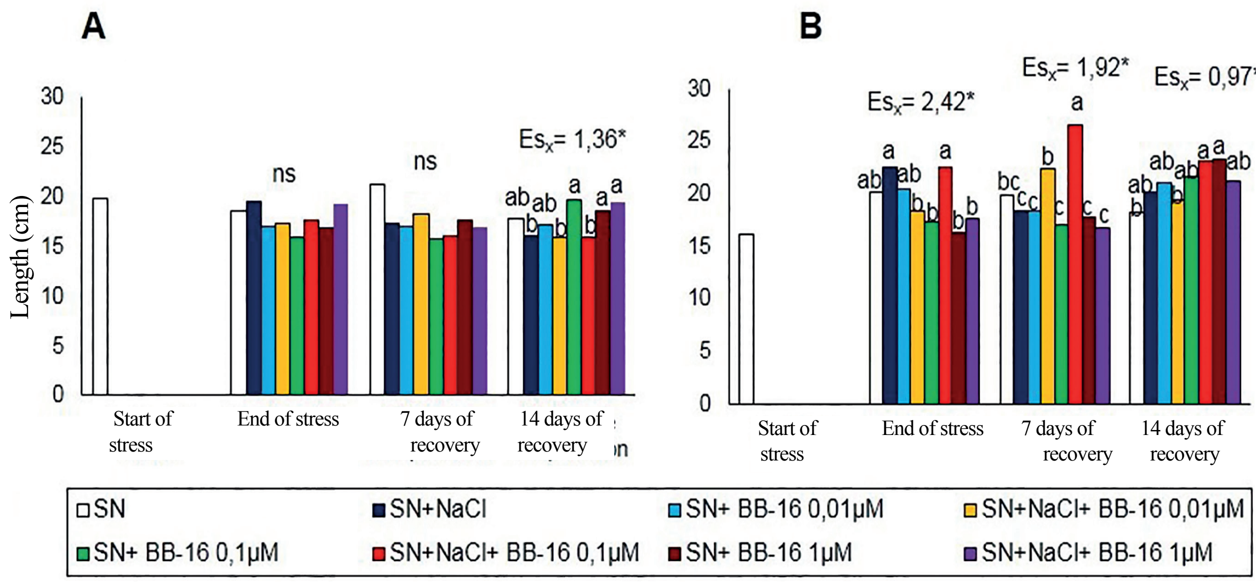
In the length of the roots it is observed that, in the first repetition, in general, the treatment with NaCl did not affect this indicator in any of the treatments or moments evaluated (Figure 2A), while in the second one (Figure 2B) Observed that salinity caused a significant increase of this indicator in the seedlings sprinkled with 0,1 µmol $L^{-1}$ of BB-16, at seven days of recovery.

Regarding the dry mass of the aerial part, no affectations were found in this indicator after the NaCl treatment was finished in neither of the two replicates of the experiment. However, at seven days of recovery in the seedlings with the NaCl treatment, the dry mass of the seedlings increased significantly in the first repetition, and only the concentrations of 0,01 and 1 µmol $L^{-1}$ of BB-16 this effect.




A: First Replay B: Second Replay
Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 1. Effect of foliar spraying with different concentrations of Biobras-16 (BB-16) on the length of aerial part of rice seedlings cv. J-104 treated with 100 mmol L-1 NaCl for seven days**



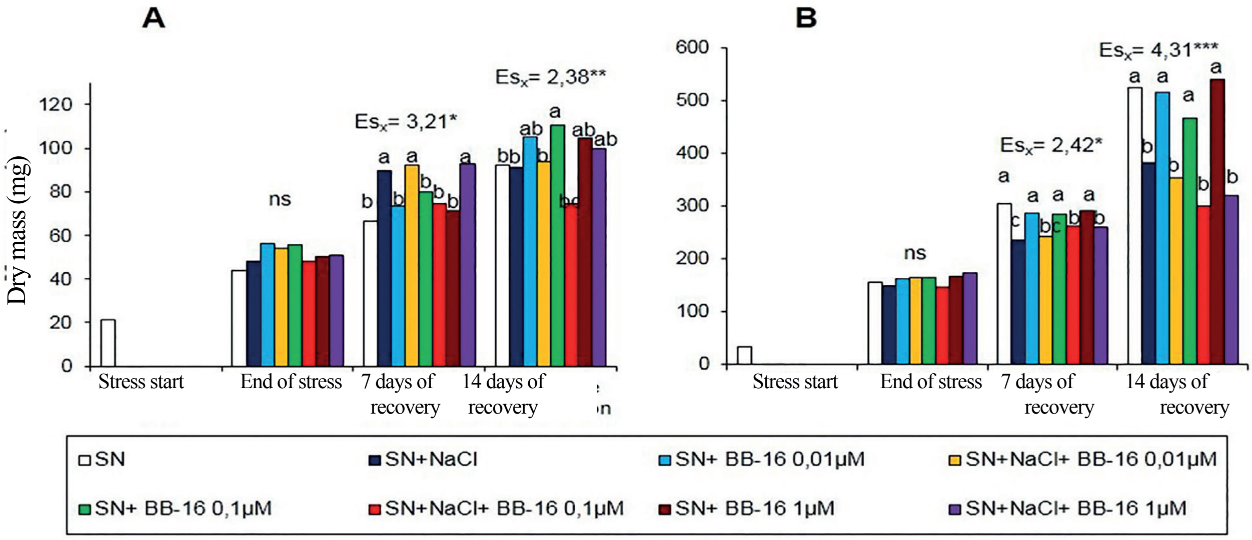

A: First Replay B: Second Replay
Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 2. Effect of foliar spraying with different concentrations of Biobras-16 (BB-16) on the length of the root of cv. J-104 subjected to NaCl 100 mmol L-1**

However, in the second repetition a significant reduction of this indicator was observed during the recovery, except for the treatments of 0,1 and 1 μmol $L^{-1}$, at seven days of recovery (Figure 3).

When evaluating the dry mass of the roots; a significant increase of this indicator was obtained in the saline stress in the first repetition (Figure 4A), which was not observed in plants sprayed with BB-16.

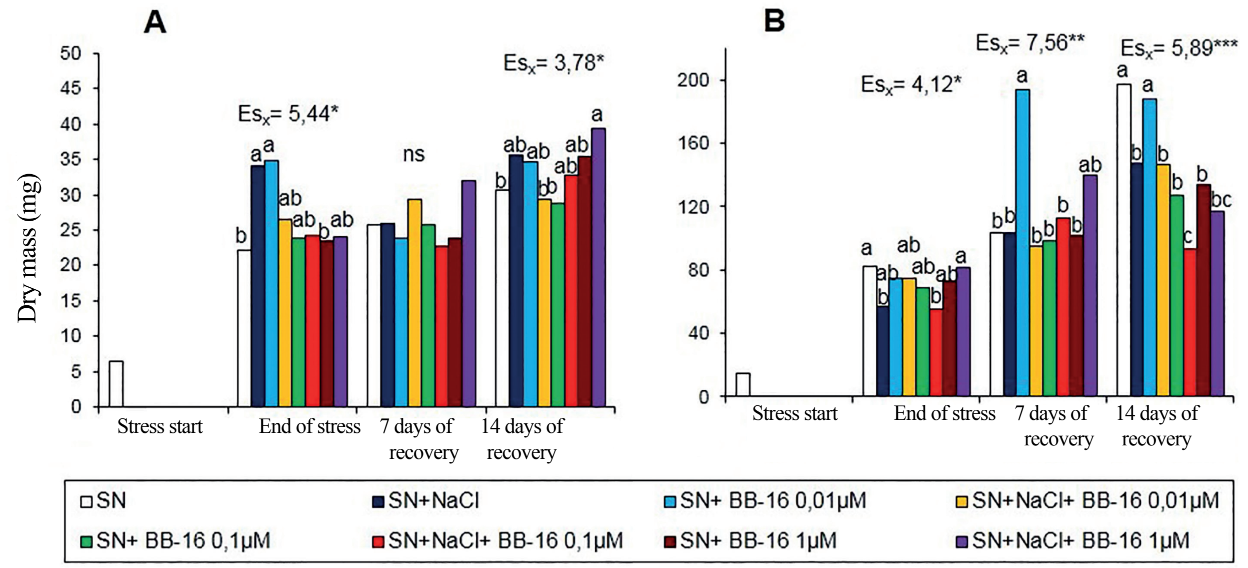
The behavior in the second repetition was different, since the salinity decreased significantly the dry mass of the roots in the control plants at the end of the treatment period with NaCl and at 14 days of the recovery; however, plants sprayed with BB-16 1 μmol $L^{-1}$ did not significantly modify dry mass levels, either after stress or during recovery (Figure 4B).




A: First Replay B: Second Replay

Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 3. Effect of foliar spraying with different concentrations of Biobras-16 (BB-16) on the dry mass of aerial part of rice seedlings cv. J-104 treated with 100 mmol $L^{-1}$ NaCl for seven days**




A: First Replay B: Second Replay

Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 4. Effect of leaf spray with different concentrations of BB-16 on the dry mass of roots of cv. J-104 treated with 100 mmol $L^{-1}$ NaCl for seven days**

Salinity adversely affected the length of the aerial part, but this effect occurred in the recovery, where a marked reduction of the growth of the seedlings was observed. 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 saline stress on growth is widely known as both water stress and ion accumulation delay the processes of cell division and differentiation (18).

This decrease in the growth of the treated plants during recovery could be due to the toxic effects of salt accumulation taking longer to manifest but remaining in time, even after the recovery period. In order to corroborate this hypothesis, it was pointed out that once the stress period was over and the plants were submitted for seven days to the control treatment, they failed to recover, which demonstrates the irreversible nature of the damage caused (19).

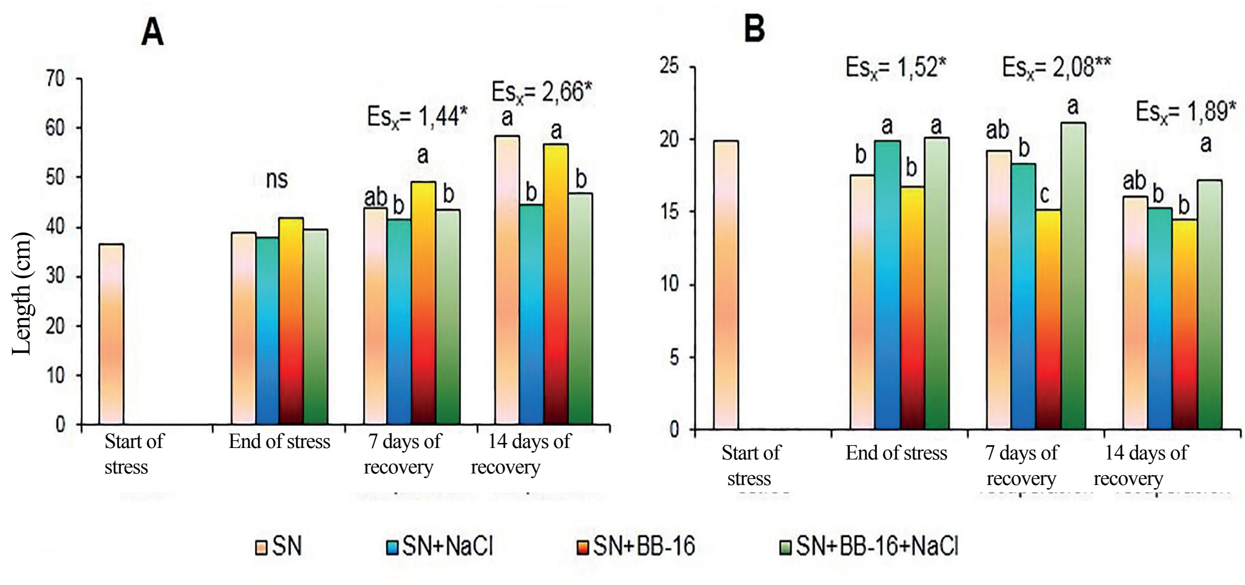
Leaf sprinkling with 0,1 mg $L^{-1}$ BB-16 stimulated shoot length 14 days after recovery, compared to the rest of the treatments studied.

It is important to note the difference observed in the response of the plants to the treatment with NaCl in the two replications carried out, which could be related to the prevailing meteorological conditions during the execution of the experiment. As shown in Table II, in the experiment initiated in December (first repetition), both the maximum, minimum and average temperatures during the execution of the experiment was approximately 7 to 8 ºC lower than in the initiate in May (second repetition) and is well known, the effect that temperature exerts on rice growth, since the optimum temperature for the vegetative phase of this crop is 25-30 ºC and lower temperatures cause a delay in growth.

This shows that salinity effects on seedlings also depend on other factors, since in the experiment started in December, where the temperature was lower, the control plants showed a lower growth, masking the effect caused by the saline stress. In addition, the low temperatures may also have decreased the absorption of water, and therefore of NaCl, in the rice seedlings.

Figures 5 and 6 present the results of the influence of leaf spray with Biobras-16 (0,1 µmol $L^{-1}$) on the growth indicators of rice seedlings submitted or not to treatment with 100 mmol L NaCl $^{-1}$ during seven days. It was observed that at the end of treatment with NaCl, none of the growth indicators evaluated were affected; however, plants placed in saline medium generally exhibited lower growth after fourteen days of recovery.

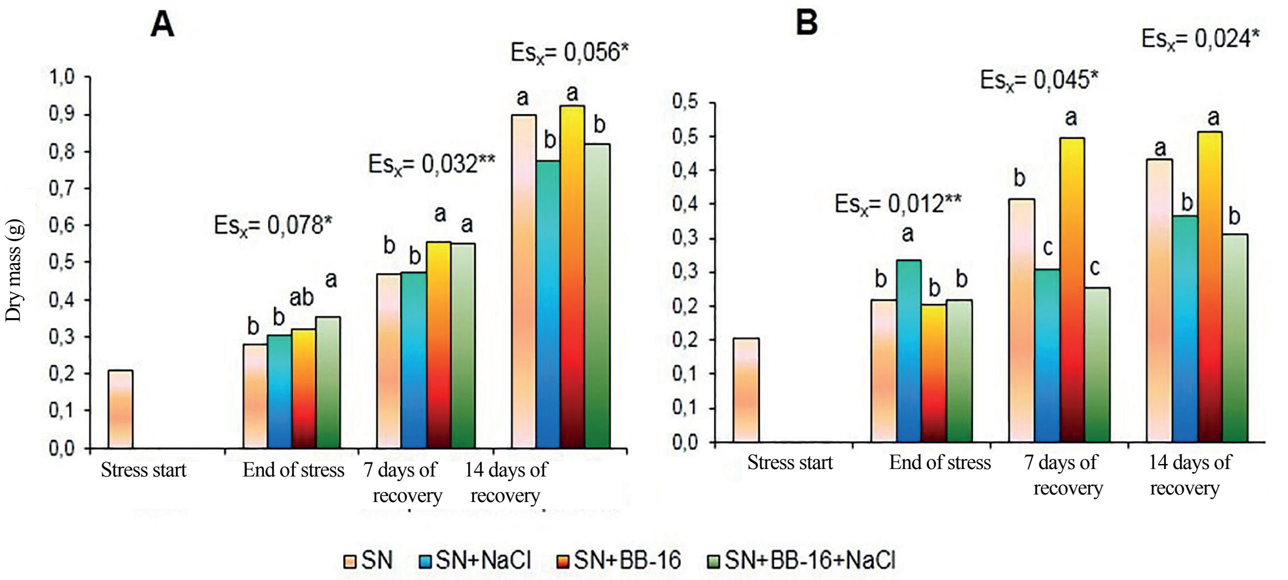
[C]Yoshida, S. *Fundamentals of rice crop science*. Ed. International Rice Research Institute, 1981, Los Baños, Philippines.




A: Aerial part B: Roots

Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 5. Effect of Biobras-16 foliar spraying (0,1 µmol $L^{-1}$) on the length of rice seedlings cv. J-104 subjected to NaCl 100 mmol $L^{-1}$ for seven days**




A: Aerial part B: Roots

Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 6. Effect of Biobras-16 foliar spraying (0,1 µmol $L^{-1}$) on the dry mass of rice seedlings cv. J-104 subjected to NaCl 100 mmol $L^{-1}$ for seven days**

Thus, the lengths of the aerial part and of the roots are shown in Figure 5. A protective effect of BB-16 on root length (Figure 5B) was observed, confirming what was found in the second repetition of the previous experiment (Figure 2B).

Concerning the dry mass (Figure 6), there was a protective effect of BB-16 in the aerial part, where the spray of the compound increased the dry mass during the period of stress and seven days of recovery, compared to the plants which grew in nutrient solution (Figure 6A). It is interesting to note that salinity increased the dry mass of the roots at the end of the stress period, whereas the leaf spray with BB-16 did not modify this indicator in plants treated or not with NaCl; however, a significant reduction in the dry mass of the roots of plants undergoing treatment with NaCl was observed during the recovery regardless of whether or not they were sprayed with BB-16 (Figure 6B).

This last result, which shows the increase of the biomass of the root at the end of the saline treatment, is repeated in several experiments and several authors have been found that observe a similar behavior (20). Although the roots are in direct contact with the salt, their growth is less affected than that of the aerial part, increasing the root ratio: aerial part (18). This could be an adaptation of the plant to increase the area of water absorption and decrease the area of transpiration. However, in the recovery period, root biomass declines in seedlings that were previously stressed suggesting that the long-term effects of salinity affect the growth rate of this organ.

On the other hand, it should be noted that the effectiveness of seed treatment with BB-16 0.005 mg L-1 has been reported to reverse the adverse effects that salinity caused on the initial growth of the plants of rice cultivars J- 104 and INCA LP-7, whereas the response of another analog (BB-6) depended on the cultivar (7).

More recently, the influence of seed treatment with various concentrations of BB-16 and 24-epibrasinolide (EBL), a natural brassinosteroid, on seedling growth of two rice cultivars (J-104 and Ginés) in Half saline. Both EBL and 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; Being more effective the treatments in the tolerant cultivar Ginés (21).

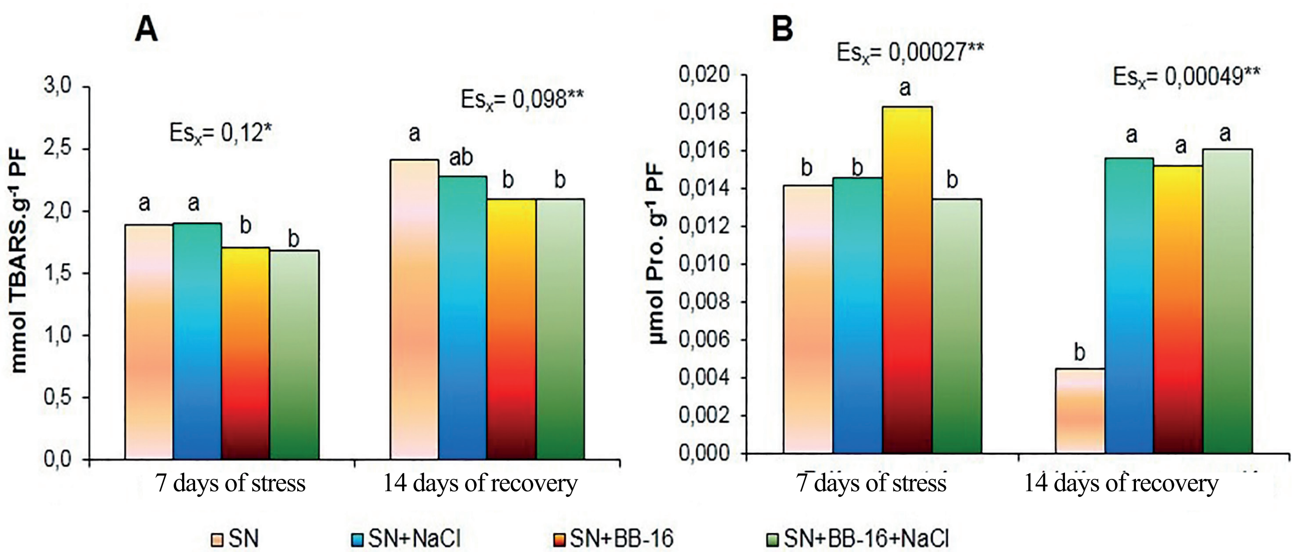
As can be seen, the results shown in the literature refer to the treatment of seed as a mode of application, so these are the first results that show that Biobras-16 leaf spray could protect young rice plants of saline stress. Saline stress did not modify the concentration of malondialdehyde (MDA) in the seedlings at any of the two evaluated moments; however, leaf spray with BB-16 decreased the concentration of this compound with respect to the control plants (Figure 7A). MDA is one of the low molecular weight end products of the decomposition of lipid hydroperoxides. This compound is the most used as a measure of lipid peroxidation in the cell membrane, as a direct consequence of the action of reactive oxygen species.

The results of this work indicated that the treatment with NaCl for seven days did not cause oxidative damage in the membranes of the plant cells of this rice cultivar until the 14 days of recovery and the spray with BB-16 did not modify this behavior.

As for proline, treatment with NaCl did not modify the concentration of this metabolite in the control plants at the end of the same; however, BB-16 spraying increased levels of this amino acid in non-stressed seedlings. On the other hand, the increase in the concentration of this metabolite product of the saline treatment was evidenced in the recovery and the plants sprayed with BB-16 presented similar values of proline in their leaves, independently, that had been treated or not with salt (Figure 7B). Other authors have observed that EBL was able to reduce electrolyte efflux and lipid peroxidation in bean and cucumber seedlings exposed to saline stress, which favored the membrane stability index (22, 23). Something similar has been reported in the literature in the case of wheat plants subjected to saline stress and sprayed with different concentrations of EBL (24). Similarly, in bean seedlings subjected to saline stress leaf spraying with EBL increased the proline concentration in both leaves and roots (22). However, the concentration of this amino acid decreased in leaves of stressed rice plants, whose seeds were treated with EBL (25) and in bean seeds treated with EBL (5 μmol $L^{-1}$) and subjected to saline stress for eight days, with respect to stressed seedlings (26).

Although proline accumulation has been observed in rice exposed to saline stress, it is now clear that this amino acid has multiple functions. Numerous authors have suggested that cultivars that are more susceptible to salinity have higher proline content than tolerant cultivars (27, 28). For this reason, proline accumulation correlates with the degree of stress-induced deterioration (29), and there are authors who suggest that the increase of this amino acid could be due to a need for the plant to remove excess ammonium (30).

On the other hand, the possible role of proline as a non-enzymatic antioxidant has recently been described; since it is capable of behaving as a final acceptor of free radicals such as singlet oxygen and hydroxyl radical, so proline accumulation in tissue may be important in preventing oxidative damage caused by reactive oxygen species (31). Proline also stabilizes membranes and subcellular components, including the complex II of the mitochondrial electron transport chain, and stabilizes the redox potential by NADP + replenishment (32). It has been shown that the exogenous application of proline provides a protective action against the oxidative damage induced by the saline stress. There are reports that the application of proline was able to decrease the concentration of $H_2O_2$ and increase the activity of the enzyme ascorbate peroxidase (APX) in two rice cultivars that differ in their tolerance to salinity, while also increasing the activity of the enzymes peroxidase and catalase in the sensitive cultivar, both in the stress period and in the recovery period (33).




Common letters do not differ according to Tukey's test (p≤0.05) n = 12

**Figure 7. Effect of Biobras-16 (0,1 μmol $L^{-1}$) foliar spray on malondialdehyde (A) and proline (B) levels of rice seedlings cv. J-104 subjected to NaCl 100 mmol $L^{-1}$ for seven days**

Other authors have also demonstrated that the application of exogenous proline decreases oxidative damage and increases the antioxidant defenses in different cultures under salt stress (34, 35).

When analyzing the results on the chlorophyll pigments, at the end of the treatment with NaCl, a decrease of the chlorophyll *a* and of the total chlorophylls in the stressed seedlings was observed; while leaf spray with this concentration of BB-16 was able to counteract this effect (Figure 8A). In the recovery, this behavior was maintained in the case of chlorophyll *a* and therefore, in total chlorophylls (Figure 8B). This result is in agreement with different authors who have observed a decrease in the concentration of this pigment in the presence of saline stress in many crops such as lettuce, rice, cucumber and wheat (23, 27, 28, 36).

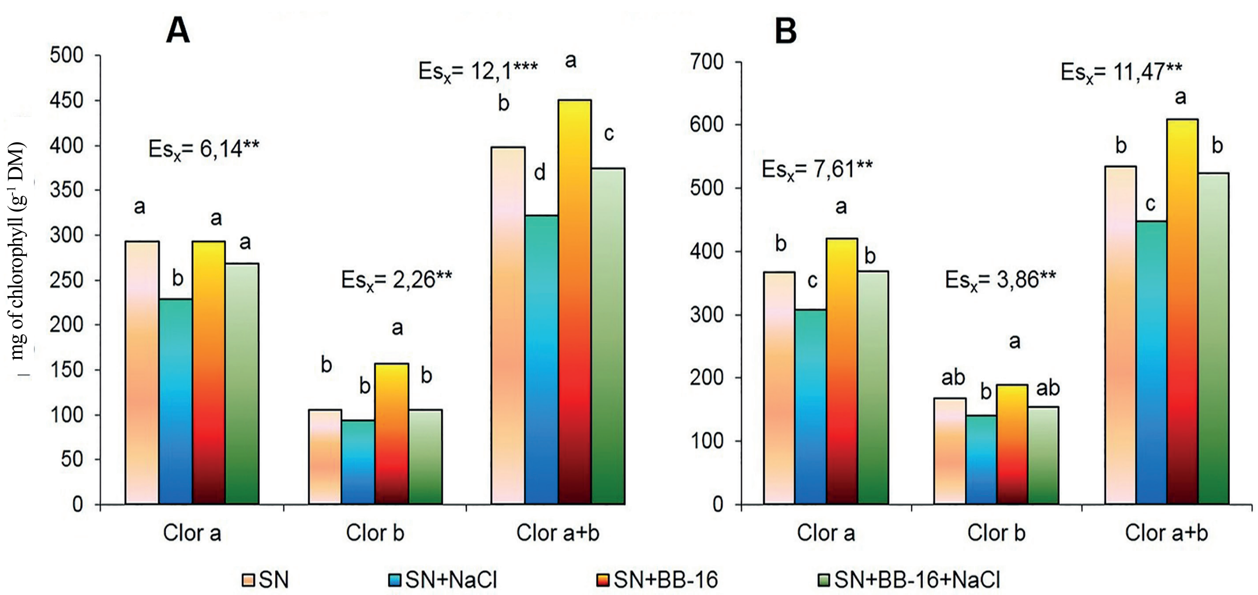
The reduction in chlorophyll content in leaves under conditions of saline stress has been attributed to several causes: the destruction of chlorophyll pigments by the increase of chlorophyllase activity and the suppression of enzymes of biosynthesis as porphobilinogen desaminase. It may also be caused by the interference of saline ions with de novo synthesis of proteins and structural components of chlorophyll (37).

The stimulation of the chlorophyll *a* concentration in the leaves of the plants sprayed with BB-16 and submitted to the treatment with NaCl, confirms the results obtained by other authors who have reported that the treatment to the seeds with Brasinolid, 24-epibrasinolid and homobrasinolid (3 µmol $L^{-1}$) decreased the loss of photosynthetic pigments caused by saline stress (NaCl 150 mmol $L^{-1}$) in rice seedlings (3).

In other cultures, the effect of BRs on the restitution of chlorophyll molecules to saline stress was also demonstrated. For example, in *Phaseolus vulgaris* and *Hordeum vulgare*, pre-treatment of the seeds with brasinosteroid (5 µmol $L^{-1}$) reversed the decrease in chlorophyll content caused by saline stress (150 mmol $L^{-1}$) reaching levels similar to the control plants (26). Similarly, EBL leaf spray in bean plants subjected to saline stress reversed the decrease in chlorophylls caused by this stress (22).

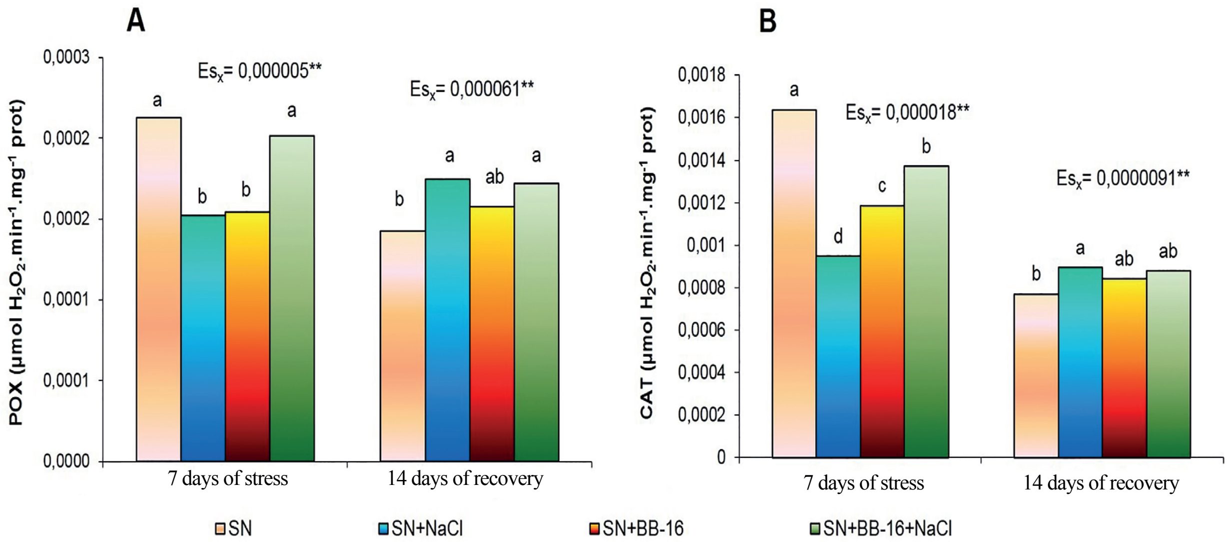
Figure 9 shows the responses of the peroxidase and catalase enzymes of J-104 cultivated rice seedlings subjected to saline stress. The activity of both enzymes decreased significantly at the end of treatment with NaCl. Foliar spraying with BB-16 reversed this behavior, increasing the specific activity of both enzymes (Figure 9A).

Fourteen days after the recovery, it was found that the plants treated with NaCl showed a significant increase of the activities of both enzymes and the foliar spray of BB-16 did not modify this behavior (Figure 9B).




A: Final period of stress B: Final period of recovery
Common letters do not differ from each other according to Tukey's test (p≤0,05) n = 5

**Figure 8. Effect of Biobras-16 (0.1 µmol $L^{-1}$) foliar spray on the content of chlorophyll a, b and total leaflets of cv. J-104 subjected to NaCl 100 mmol $L^{-1}$ for seven days**




A: Peroxidases (POX) B: Catalase (CAT)
Common letters do not differ according to Tukey test (p≤0.05) n = 6

**Figure 9. Effect of foliar spraying of Biobras-16 (0.1 µmol L$^{-1}$) on the specific activity of two antioxidant enzymes of rice seedlings cv. J-104 subjected to NaCl 100 mmol L$^{-1}$ for seven days**

The influence of the BB-16 analogue on the behavior of some antioxidant enzymes from rice seedlings grown *in vitro* in the presence of 75 mM NaCl showed that the exposure for 16 days to BB-16 0,01 mg L$^{-1}$, the activity of the enzymes catalase (CAT), superoxide dismutase (SOD), glutathione reductase and slightly the activity of ascorbate peroxidase (3). On the other hand, the application of 1 µmol L$^{-1}$ of DI-31, BB-16 active ingredient, to lettuce plants treated with 100 m mol L$^{-1}$ NaCl for five days was able to reverse the adverse effects that salinity caused in these plants (38).

In bean subjected to saline stress, EBL foliar spraying caused an increase in SOD activity and did not produce modifications in POX activity in comparison with the control plants (22). An increase in SOD and CAT was also obtained by The exogenous application of brassinosteroids in cucumber positions subjected to saline stress (23). On the other hand, in pea, treatment with EBL seeds caused an increase in the activity of CAT, POX and SOD enzymes compared to control plants (39). Recently, it has been demonstrated that in rice, the effectiveness of the application of EBL in protecting seedlings from heavy metal stress (Cr) is associated with over-regulation of antioxidant enzymes (40).

## CONCLUSIONS

In general, it can be concluded that foliar spraying with Biobras-16 stimulates the growth of rice plants subjected to saline stress which may be associated with an increase in the activities of the antioxidant enzymes catalase and peroxidase at the end of the stress, could also be associated with the increase in chlorophyll concentration, mainly of chlorophyll *a*.

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