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OLIGOGALACTURONIDES ENHANCE RICE SEEDLING GROWTH CULTIVATED IN SALINE MEDIUM

Oligogalacturónidos estimulan el crecimiento de plántulas de arroz cultivadas en medio salino

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ABSTRACT. Oligogalacturonides compounds are able to enhance defense responses and regulate plant growth and development; however, there is scarce information about the effects of these compounds on plants grown under abiotic conditions, particularly, under salt stress. For this reason, an experiment was performed where different concentrations (0, 10 and 20 mg L^{-1}) of an oligogalacturonides mixture was applied by INCA LP-7 rice seed treatment during 24 hours and by addition to Hoagland nutritive solution supplemented with NaCl 100 mmol L^{-1} after seed germination. The pots containing the germinated seeds were placed in a light room with a 16 hours photoperiod and a temperature of 25±2 °C for 13 days. At the end of this period, growth indicators such as srems and root length, dry weight of both organs and some leaf biochemical indicators were evaluated. Salt treatment during 13 days only decreased significantly the length of both seedling organs of this cultivar and the inhibition on root and shoot length was total and partially reverted by the seed treatment with MOGs 20 mg L^{-1}. This response was corresponded to a significant decrease of leaf proline concentration and an increase of peroxidases enzyme activity.

Key words: abiotic stress, oligosaccharines, *Oryza sativa* L.

RESUMEN. Los oligogalacturónidos son compuestos capaces de estimular respuestas de defensa y de regular el crecimiento y desarrollo de las plantas; sin embargo, no existe apenas información acerca de los efectos que estos compuestos ejercen cuando las plantas crecen en condiciones de estrés abióticos y particularmente, en condiciones de estrés salino. Por tal motivo, se ejecutó un experimento donde se aplicaron diferentes concentraciones (0, 10 y 20 mg L^{-1}) de una mezcla de oligogalacturónidos (MOGs) tanto por tratamiento a las semillas de arroz cv. INCA LP-7 durante 24 horas como por adición a la solución nutritiva Hoagland suplementada con NaCl 100 mmol L^{-1}, una vez germinadas las semillas. Los potes que contenían las semillas germinadas se colocaron en un cuarto de luces con un fotoperíodo de 16 horas y una temperatura 25±2 °C durante 13 días. Al final de este período, se evaluaron los indicadores de crecimiento longitud de vástago y de raíces, así como la masa seca de ambos órganos y algunos indicadores bioquímicos de las hojas de las plántulas. El tratamiento salino por 13 días solo disminuyó, significativamente, la longitud de ambos órganos de las plántulas de este cultivar, siendo revertida total y parcialmente la inhibición que se produjo en la longitud de las raíces y del vástago, respectivamente por el tratamiento a las semillas con MOGs 20 mg L^{-1}. Esta respuesta se correspondió con una disminución significativa en la concentración de la prolina y un incremento en la actividad de las peroxidasas en las hojas.

Palabras clave: estrés abiótico, oligosacarinas, *Oryza sativa* L.

INTRODUCTION

Saline stress affects vegetative growth, flowering and fruit formation causing a marked reduction in the growth and development of plants, in addition to causing an accelerated production of reactive oxygen species that must be strictly controlled to avoid oxidative stress and therefore, a possible cell death. Among the recommended strategies to mitigate the effects of this type of stress, in addition to the use of tolerant cultivars, the improvement of irrigation water management, is the use of various agricultural practices (1).

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One of the agricultural practices currently used is the application of biostimulants. In this way, brassinosteroids alone or in combination with polyamines have been shown to be able to increase the tolerance of plants to salt stress (2-4). Particularly, in rice plants, not only the brassinosteroids but also the application of products based on their spirostanic analogs and oligosaccharins such as chitosan have stimulated the growth of plants subjected to stress by NaCl (5,6).

Among the biostimulants, there are also oligogalacturonides, compounds capable of stimulating defense responses in plants, including the accumulation of reactive oxygen species and proteins related to pathogenesis, which protect plants against pathogen infections. Currently, they are considered as true molecular patterns associated with damage that activates innate immunity in plants. In addition, they may be involved in the activation of responses to mechanical wounds and may act as regulators of the growth and development of plants, mainly through their antagonism with auxins (7).

In Cuba, several investigations have been carried out to evaluate the effectiveness of the application of a mixture of oligogalacturonides not only in different biotechnological processes (8,9), but also in the growth, yield and quality of the fruits of plants of different plant species (10-12). However, there is very little information about the effects that the application of this mixture exerts on plants when they are subjected to abiotic stress and especially to salt stress. For this reason, the main objective of this work was to determine the effects that the application of a mixture of oligogalacturonides exerts in rice seedlings subjected to stress by NaCl.

MATERIALS AND METHODS

Based on the results of previous trials where several concentrations of the oligogalacturonide mixture were tested and different ways of applying it (unpublished data), an experiment was carried out where rice seeds were treated cv. INCA LP-7, for 24 hours, with different concentrations (0, 10 and 20 mg L^{-1}) of a mixture of oligogalacturonides, MOGs, (product obtained by the Department of Physiology and Plant Biochemistry of the National Institute of Agricultural Sciences, a from the enzymatic hydrolysis of pectic acid from citrus pectin). Once the treatments were finished, they were placed in Petri dishes with distilled water for germination in the dark at a temperature of 25 ± 2 ºC. At 48 hours, the germinated seeds were transferred to pots to which 50 x 10^{-3} L of diluted Hoagland nutrient solution (1:1) supplemented with 100 mmol L^{-1} NaCl was added, except for a part of the seeds imbibed in distilled water, which were placed in pots containing the previous solution with the addition of the MGOs in two concentrations (10 and 20 mg L^{-1}).

The pots were placed in a room of lights with a photoperiod of 16 hours and a temperature of 25±2 ºC.

At 13 days, 25 seedlings were selected per treatment to measure the lengths of the stem and roots and five samples of five plants each were formed to determine the dry mass of aerial part and roots. In addition, three leaf samples were taken per treatment to perform the determinations of proline concentrations and total soluble proteins and the activities of antioxidant enzymes peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6). The techniques used to carry out said determinations are those referred to above (5).

The experiment was repeated twice and the average values of all the indicators were statistically processed using analysis of variance of simple classification according to Statgraphics plus 5.1. To discriminate the differences between the means, the Tukey multiple range test was used for $p \leq 0.05$.

RESULTS AND DISCUSSION

The data showed that the presence of 100 mmol L^{-1} NaCl in the nutrient solution for 13 days only significantly affected the length of the stem and the roots of rice seedlings cv. INCA LP-7, while the dry masses of these organs did not show significant differences. The treatment of rice seeds with MOGs at a concentration of 20 mg L^{-1}, for 24 hours, totally and partially reversed the inhibition induced by the presence of NaCl in the length of the roots and the stem, respectively. This treatment significantly stimulated the dry mass of the seedlings, in comparison with seedlings from seeds treated with water and grown in the presence of salt (Table 1). The inclusion of the MGOs in the growth medium of the seedlings, in any of the two concentrations tested, only favored the growth of the aerial part of the seedlings.

Previous research has shown the stimulating effect of oligogalacturonides in the elongation of the roots of cucumber plants (13) and alfalfa (14). This effect depended on the concentrations used, which is confirmed by these results (Table 1). It should be noted that in these cases oligogalacturonides with a degree of polymerization between 10 and 15 were applied; however, the use of trimers resulted in active elicitors of plant defenses, but inhibited the growth of *Arabidopsis* postures (15).

Recently, it was reported that the addition to the culture medium of a mixture of oligosaccharins (1 mg L^{-1}) derived from the cell wall of the plants did not stimulate the length of the roots of *Arabidopsis* seedlings after 15 days of culture with the presence of NaCl (16).

Additionally, to the degree of polymerization of the oligogalacturonides and the concentration that is used, in the present work the importance of the way of application of these compounds was revealed, since the treatment to the seed with 20 mg L^{-1} was more effective in the stimulation of the growth of rice seedlings, that the addition of this same concentration in the growth medium.

On the other hand, it has been reported that the application of the oligogalacturonides mixture known as Pectimorf® to bean plants modified patterns of distribution and stomatal morphogenesis, which could favor the adequate growth of plants in unfavorable environments (17). This response of the stomata to the application of the oligogalacturonides could favor a greater assimilation of CO_2 under stress conditions and, therefore, be associated with the greater dry mass of the aerial part that was found in all the rice seedlings treated with the MOGs, regardless of the mode of application and the concentrations used.

Knowing that proline is an amino acid that accumulates in plants subjected to abiotic stress and that salt stress can generate an oxidative stress and therefore, it can stimulate the antioxidant defense in plants, in Table 2 the behavior results are shown of some biochemical indicators related to the response of plants to this stress condition.

The presence of NaCl 100 mmol L^{-1} in the nutrient solution significantly stimulated the concentration of

Table 1. Influence of different concentrations and application modes of a mixture of oligogalacturonides (MOGs) in some indicators of the growth of rice seedlings cv. INCA LP-7 subjected to treatment of NaCl 100 mmol L^{-1} for 13 days

Treatments	Length (cm)		Dry mass (mg $plant^{-1}$)	
	Stem	Rootss	Aerial part	Roots
Imb. water (Control)	19,4 a	8,1 a	9,9 ab	6,1 ab
Imb. water + NaCl	12,4 d	6,8 cd	9,2 b	5,5 b
Imb. MOGs 10 mg L^{-1}+ NaCl	14,6 bc	7,1 bc	11,2 a	5,9 ab
Imb. MOGs 20 mg L^{-1}+ NaCl	14,9 bc	8,0 ab	11,0 a	6,3 a
Imb. water + MOGs 10 mg L^{-1} + NaCl	14,2 c	6,4 cd	10,9 a	6,2 ab
Imb. water + MOGs 20 mg L^{-1} + NaCl	15,8 b	6,1 d	11,1 a	5,9 ab
E.S.$\bar{x}$	0,37*	0,25*	0,30*	0,18*

Equal letters represent means that do not differ significantly according to Tukey's multiple range test for p≤ 0.05. Imb, imbibition

Table 2. Effect of the exogenous application of a mixture of oligogalacturonides (MOGs) on the behavior of some biochemical indicators of the leaves of rice seedlings cv. INCA LP-7 subjected to NaCl 100 mmol L^{-1} for 13 days

Treatments	Proline (µmol g^{-1} M.F.)	Total soluble protein (mg g^{-1} M.F.)	Peroxidase (µmolH_2O_2 min^{-1} mg^{-1} protein)	Catalase (µmolH_2O_2 min^{-1} mg^{-1} protein)
Imb. Water (Control)	0,016 c	1,10 a	0,202 a	0,598 c
Imb. Water + NaCl	0,027 a	0,90 bc	0,123 c	0,883 a
Imb. MOGs 10 mg L^{-1} + NaCl	0,023 b	0,80 c	0,166 ab	0,871 a
Imb. MOGs 20 mg L^{-1} + NaCl	0,015 c	0,9 bc	0,191 a	0,572 c
Imb. Agua+ MOGs 10 mg L^{-1}+NaCl	0,018 c	1,0 ab	0,121 c	0,590 c
Imb. Water + MOGs 20 mg L^{-1}+NaCl	0,025 ab	1,0 ab	0,140 bc	0,750 b
E.S.$\bar{x}$	0,0064*	0,03*	0,008*	0,02*

Equal letters represent means that do not differ significantly by Tukey multiple test at p≤0.05 (n = 6). Imb, imbibition proline and the activity of the catalase enzyme in the leaves of rice seedlings cv. INCA LP-7, while there was a significant reduction in the concentration of total soluble proteins and the activity of the enzyme peroxidase. A similar response of proline, total soluble proteins and the specific activity of the catalase enzyme were obtained in rice plants var. Pusa Basmati-1 of twelve-day-old subjected to stress by NaCl (1).

The exogenous application of the mixture of oligogalacturonides was able to modify this behavior, to such an extent that the plants coming from the treatment to the seeds with 20 mg L^{-1} during 24 hours, they showed values of all the indicators, except for the concentration of total soluble proteins, which did not differ significantly from those shown by the seedlings of the control treatment. This behavior, together with the fact that this treatment was the one with the best behavior in the growth indicators, suggests that it may induce the protection of the seedlings of this cultivar under conditions of NaCl stress.

Currently, it is clear that proline has multifunctional roles in plants, that in addition to being an osmoprotective, proline can act as a powerful non-enzymatic antioxidant, can stabilize DNA, membranes and protein complexes, and provides a source of carbon and nitrogen for post-stress growth (18). This explains the increase of this indicator in rice seedlings subjected to stress by NaCl and the decrease in the concentration of proline in the leaves of the seedlings whose seeds were treated with the oligogalacturonide test confirms the result reported previously by other authors who found, during the acclimatization phase, a decrease in free proline foliar in banana plants treated with oligogalacturonide solutions suggesting that they suffered less the stress caused by the transfer from an *in vitro* condition to an *ex vitro* (19).

The significant reduction that occurred in the concentration of total soluble proteins in the leaves of plants subjected to stress was reversed partially, although not significantly, by the presence of the mixture of oligogalacturonides in the growth medium (Table 2). Similar results were reported in basil plants whose roots were submerged in a solution of a mixture of oligogalacturonides prior to transplantation and later, subjected to water deficit (20).

Regarding the influence of the mixture of oligogalacturonides in the activities of antioxidant enzymes in plants subjected to salt stress, no background has been found, since it is generally pointed out that oligogalacturonides stimulate proteins related to pathogenesis, both alone (21) as in combination with chitosan oligomers (22). However, in this work it was observed that the treatment of seeds with the MOGs significantly stimulated the activity of the enzyme peroxidase in saline medium in comparison with the plants whose seeds were imbibed in water; however, the inclusion of this in the medium, in the two concentrations tested, did not influence this indicator.

These results reflect once again the importance of the mode of application in the response of plants to oligogalacturonides. However, these are the first reported results related to the influence that a mixture of oligogalacturonides exerts on rice plants subjected to stress by NaCl. For this reason, it is necessary to continue researching and deepening in this sense, in such a way that, if confirmed, these results will not only clarify the mode of action of these compounds in plants subjected to abiotic stresses, but also, to reach propose a methodology for the practical application of this type of compound to mitigate the adverse effects that salt stress causes in rice cultivation.

CONCLUSIONS

The exogenous application of a mixture of oligogalacturonides and especially, the treatment to the seeds during 24 hours with a solution of 20 mg L^{-1} significantly stimulates the growth of rice seedlings cv. INCA LP-7 in saline medium. This response corresponded with a significant decrease in the concentration of proline in the leaves and the activity of the catalase enzyme and with an increase in the activity of the enzyme peroxidase, reaching values similar to the plants of the control treatment without NaCl and without oligogalacturonides.

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