Cultivos Tropicales, 2022, vol. 43, no. 2, e10
ISSN impreso: 0258-5936
ISSN digital: 1819-4087

abril-junio
Ministerio de Educación Superior. Cuba
Instituto Nacional de Ciencias Agrícolas

http://ediciones.inca.edu.cu

Original article

# Biostimulants promote rice (*Oryza sativa* L.) seed germination in saline medium

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**ABSTRACT**

Rice is one of the most important cereals and feeds more than 50 % of the world population. Its production is affected by different factors, among them, soil salinization. For this reason, it is necessary to use strategies to reduce the damage caused by salinity in the establishment, growth and development of these plants, one of them being the use of environmentally friendly bioactive products. The aim of this study was to determine if the treatment of rice seeds cultivar INCA LP-7 with Pectimorf® or with an aqueous extract of *Sargassum fluitans* is capable of stimulating their germination in a saline medium. In the first, seeds were immersed for 24 hours in solutions of different concentrations of Pectimorf® (10, 20 and 40 mg $L^{-1}$) and in the second, in different concentrations of sargassum extract (0.5, 1, 1.5, 3.5 and 5 %). In both experiments, seeds were germinated for seven days and the germination dynamics, as well as the final germination percentage, seedling dry mass and vigor index were evaluated. The results showed that the application of 10 and 40 mg $L^{-1}$ of Pectimorf® significantly stimulated the final germination percentage and vigor index in saline medium, while the sargassum extract increased both dry mass and vigor index in all the concentrations studied.

**Key words:** *Oryza sativa*, oligogalacturonide, Sargassum fluitans, seeds, salinity.

# INTRODUCTION

Rice (*Oryza sativa* L.) is a monocot belonging to the Poaceae family. It is one of the most widely produced cereals worldwide and is one of the mainstays of human nutrition (1,2). Its future yields are jeopardized by its high sensitivity to salt, which is expected to increase with global climate change (3). It is a salt-sensitive crop and under salt stress conditions, seed germination is affected (4,5).

Salinity can affect seed germination in two ways: first, it creates an osmotic stress and then the toxicity of sodium and chloride ions is evident (6,7). Seeds are particularly vulnerable to salinity because, at this stage, they have not yet developed the physiological mechanisms to tolerate increased salt concentrations in the medium (7).

One of the strategies that could be used to counteract the salinity effect, in addition to the use of tolerant cultivars, is the application of different environmentally friendly products such as biostimulants and growth regulators (8).

The Bioactive Products group of the National Institute of Agricultural Sciences has developed a mixture of oligogalacturonides commercially known as Pectimorf®. This product can activate defense mechanisms and decrease or attenuate environmental stress in plants (9,10); in addition, it also stimulates rooting, growth and cell differentiation of different plant species.

The use of seaweeds as biostimulants is a growing sector in several parts of the world. One of the reasons that make seaweeds good candidates for the production of plant biostimulants is the presence of a wide range of bioactive substances that can promote plant growth, including: phytohormones, macro and micronutrients, amino acids and vitamins. In addition to these compounds, seaweeds possess other unique metabolites such as sulfated polysaccharides and some polyphenols that, according to other research, improve stress resistance (11).

Among all marine algae, brown algae extracts and, in particular, sargassum extracts have gained special importance, being used as biofertilizers or natural stimulants for plant growth. Several previous studies have shown the stimulating effects of these extracts on seed germination (12,13).

Therefore, the aim of this work was to determine whether the treatment of rice seeds cultivar INCA LP-7 with Pectimorf® or with an aqueous extract of *Sargassum fluitans* is capable of stimulating seed germination in a saline medium.

# MATERIALS AND METHODS

In order to fulfill the aim of this work, two experiments were carried out at the Department of Plant Physiology and Biochemistry of the National Institute of Agricultural Sciences, located in San José de las Lajas municipality, Mayabeque province.

In both experiments, rice seeds (*Oryza sativa* L.) cultivar INCA LP-7 were used, which were imbibed for 24 hours in different concentrations of biostimulants. Once imbibition was completed, seeds were placed in Petri dishes (20 seeds per dish and four dishes per treatment) and divided into two groups. To one group 15 mL of distilled water was added, while to the second group an equal amount of NaCl solution 100 mmol $L^{-1}$ was added. The plates were placed in the dark in a growth chamber at 28-30 ºC for seven days. The number of germinated seeds was counted on each plate at 24, 48, 72, 96 and 168 hours; seeds were considered germinated when their radicle emerged. At the end of the experiment, that is, after seven days, five samples of five seedlings per treatment were taken to determine the dry mass of the seedlings, for this purpose the seedlings were placed in an oven at 70 ºC until constant mass. With the data obtained, the following indicators were calculated: germination index and rate, final germination percentage, as well as vigor index, for which the following equations were used:

**Germination Index**

$$(GI) = \Sigma\ (Gt/Tt)$$

where:

Gt is the number of seeds germinated at day.

t and Tt is the number of days since the beginning of the germination test.

**Germination rate**

$$(GR) = \Sigma Ni\ /\ \Sigma Ti\ Ni$$

where:

Ni is the number of newly germinated seeds at time Ti.

**Final germination percentage**

$$(G\ \%) = (Gf/N)\ 100$$

where:

Gf is the total number of germinated seeds at the end of the test.

N is the total number of seeds used in the test.

**Vigor index**

$$(VI) = SDW\ G\ \%$$

where:

SDW is the seedling dry mass at the end of the test.

G % is the final germination percentage [(14)].

In the first experiment, various concentrations (10, 20 and 40 mg $L^{-1}$) of the oligogalacturonide mixture, known as Pectimorf® (Pm), obtained at INCA, were used. In the second one, different concentrations (0.5, 1.0, 1.5, 3.5 %) of an aqueous extract of sargassum were used, which was obtained from fresh sargassum collected on the coast of Santa Fe beach, west of Havana province, according to the following methodology: first washing the sargassum with sea water and then several times with running water until all the salt and sand were eliminated. Subsequently, the washed sargassum was placed in a container and completely covered with running water and left to rest for three months, with agitation twice a week [(15)]. Sargassum species used was *Sargassum fluitans*. At the end of the period, the liquid was filtered to eliminate the remains and this was considered a 100 % extract.

The data obtained, in both experiments, were processed by calculating means, standard deviation and confidence intervals at $\alpha$=0.05.

# RESULTS AND DISCUSSION

The influence of Pectimorph® on seed germination of rice cv. INCA LP-7 is shown in Table 1. As can be seen, the concentrations used, in general, did not exert significant influence on the indicators evaluated when the germination medium was distilled water; however, in saline medium (100 mmol $L^{-1}$ of NaCl) the concentration of 40 mg $L^{-1}$ of Pm (Pectimorf) was the best, since it significantly influenced the germination index and the final germination percentage, which had an impact on the vigor index of the seedlings. However, the concentration of 10 mg $L^{-1}$ also favored the final germination percentage and seedling vigor index. These preliminary results demonstrated the feasibility of using Pectimorf® to favor seed germination of this cultivar in a saline medium, an aspect that should be confirmed at a later date.

**Table 1.** Influence of Pectimorph® on the germination of rice seeds cv. INCA LP-7

| Pectimorph® concentrations | Germination medium | Germination index (GI) | Final germination percentage (G%) | Germination rate (GR) | Vigor index (VI) |
|---|---|---|---|---|---|
| Control | Distilled water | 20,8 ± 3,1 | 88,7 ± 10,9 | 1,3 ± 0,7 | 391,7 ± 57,0 |
| 10 mg $L^{-1}$ | | 20,1 ± 1,7 | 77,5 ± 6,3 | 1,8 ± 0,2 | 384,1 ± 50,5 |
| 20 mg $L^{-1}$ | | 22,2 ± 3,3 | 85,0 ± 4,0 | 1,6 ± 0,4 | 421,3 ± 19,3 |
| 40 mg $L^{-1}$ | | 23,4 ± 2,6 | 88,8 ± 7,3 | 1,6 ± 0,5 | 493,2 ± 45,7 |
| 0 (Control) | NaCl | 16,5 ± 0,9 | 73,8 ± 2,4 | 0,9 ± 0,1 | 246,6 ± 25,3 |
| 10 mg $L^{-1}$ | 100 mmol $L^{-1}$ | 19,2 ± 3,3 | 90,0 ± 8,0* | 1,0 ± 0,1 | 303,6 ± 26,4* |
| 20 mg $L^{-1}$ | | 15,6 ± 2,0 | 70,0 ± 10,6 | 1,2 ± 0,6 | 259,0 ± 39,4 |
| 40 mg $L^{-1}$ | | 19,5 ± 1,1* | 85,0 ± 4,0* | 1,1 ± 0,5 | 333,2 ± 37,0* |

*Represents treatments that differ significantly from the control treatment according to confidence interval at $\alpha$=0.05

Means ± confidence intervals

Rice plant responses to salt stress are complex and depend on the duration and type of salt stress, rice developmental stage, day length, and other factors. Generally, it is classified as a salt-sensitive crop, but the extent of its sensitivity varies during different growth stages. It is considered to be less sensitive during germination and active tillering, whereas it shows higher sensitivity during the onset of vegetative and reproductive stages [(4)].

Several studies in rice cultivation have shown that salinity acts mainly by delaying the germination process and does not affect the final percentage of seed germination [(16)]. However, other research revealed that salt stress (NaCl 0-300 mM) reduces germination percentage, germination rate and speed, germination energy percentage, and mean germination time [(17)]. These results could be due to the high salt concentrations used in the study.

Figure 1 shows the influence of Pm and the affectation that salinity causes on the dry mass of rice seedlings cv. INCA LP-7. It can be observed that the seed treatment with 40 mg $L^{-1}$ significantly increased the dry mass when the seeds were germinated in water. On the other hand, none of the treatments employed stimulated seedling dry mass when seedlings were germinated under saline conditions.

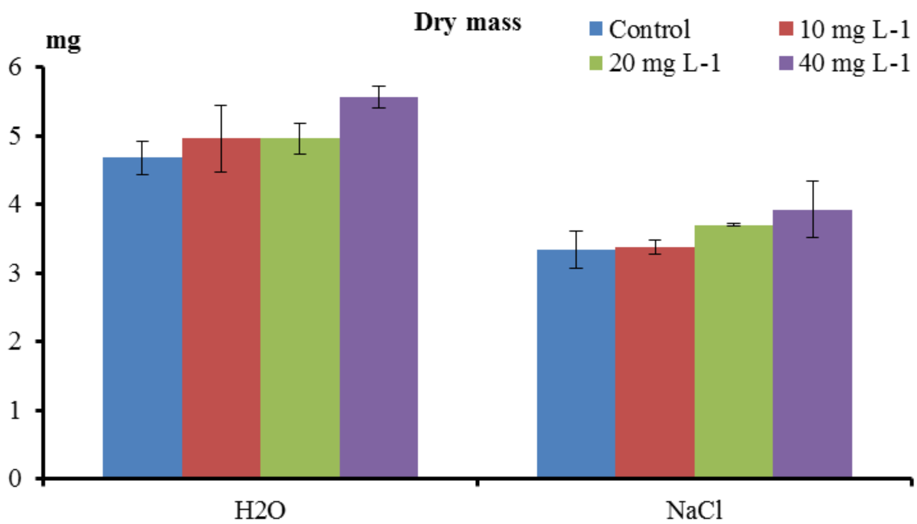


**Figure 1.** Effect of different concentrations of Pectimorf® on the dry mass of rice seedlings cv. INCA LP-7 germinated for seven days, both in water and in 100 mM NaCl

Several investigations have been carried out to determine the influence of biostimulants in the protection of plants against different types of stress. For example, seeds of rice cultivar INCA LP-5 were treated for 24 hours with different concentrations of chitosan (0, 100 and 500 mg $L^{-1}$). The germinated seeds were transferred to pots, to which diluted Hoagland nutrient solution was added, supplemented or not with NaCl 100 mmol $L^{-1}$ and placed in a growth room with controlled conditions, eleven days later the treatment to seeds with the concentration of 100 mg $L^{-1}$ of chitosan stimulated the length and dry mass of the aerial part of the seedlings grown in saline medium [(18)].

On the other hand, it has been reported that foliar spray with 24-epibrasinolide (2 μmol $L^{-1}$) stimulated the length and dry mass of rice seedlings under saline conditions [(19)].

Different concentrations (0, 10, and 20 mg $L^{-1}$) of Pm were applied both per treatment to rice seeds cv. INCA LP-7, for 24 hours, as well as by addition to the Hoagland nutrient solution supplemented with NaCl 100 mmol $L^{-1}$, once seeds had germinated. The saline treatment for thirteen days only significantly decreased the length of the roots and the stem of seedlings, being totally and partially reverted this inhibition, respectively, by the treatment to seeds with 20 mg $L^{-1}$ [(8)].

It should be taken into account that the response of the crop to biostimulants, both under normal and stress conditions, depends on several factors, among which are the intensity, duration and moment of stress implantation, as well as the sensitivity of the genotype to such stress. Thus, in this work, seeds were germinated in saline medium for seven days; however, in the previous work, seeds were germinated in water and after 48 hours were subjected to salt stress. In addition, different cultivars were used, such as cv. J-104, which is sensitive to salinity, and cv. Ginés, which is considered tolerant to salt, as well as cv. INCA LP-7, which was used in this work. The behavior of the cultivars is different according to their salt tolerance. All this could explain the non-response found in the present work to the treatment of the seeds with Pm in the dry mass, the opposite occurring in the other works where a stimulation of the seedlings in a saline medium was observed.

Pectimorf effect in stimulating the final germination percentage could be due to its hormonal effect. Previous publications have discussed the hormonal effect of oligogalacturonide mixtures, mainly their possible auxinic effect [(9)]. In particular, Pectimorf has been used as a substitute for traditional growth regulators in *in vitro* culture [(20-22)]. However, it is necessary to further investigate the mechanisms by which this biostimulant performs its anti-stress action.

Table 2 shows the results of the influence of aqueous extract of *Sargassum fluitans* on the germination of rice seeds cv. INCA LP-7. When the germination medium was distilled water the concentration of 1.5 % showed significant differences with respect to the control treatment in the final germination percentage, while the vigor index increased with the three highest concentrations of the extract. Seeds germinated in NaCl 100 mmol $L^{-1}$ increased the vigor index with all the concentrations of sargassum used and only the 1 % concentration significantly stimulated the germination index.

**Table 2.** Influence of an aqueous extract of *Sargassum fluitans* on the germination of rice seeds cv. INCA LP-7

| Concentrations of *Sargassum* extracts | Germination medium | Germination Index (GI) | Final germination percentage (G%) | Germination rate (GR) | Vigor index (VI) |
|---|---|---|---|---|---|
| 0 (Control) | Destilled water | 27,4 ± 1,8 | 91,3 ± 2,4 | 1,6 ± 0,2 | 444,0 ± 11,2 |
| 0,5 % | | 27,4 ± 3,6 | 92,5 ± 9,4 | 1,6 ± 0,2 | 483,0 ± 67,8 |
| 1 % | | 28,4 ± 2,7 | 96,3 ± 4,7 | 1,9 ± 0,2 | 434,7 ± 50,4 |
| 1,5 % | | 26,5 ± 2,0 | 95,0 ± 0,0* | 1,9 ± 0,1 | 574,8 ± 27,0* |
| 3,5 % | | 27,1 ± 2,0 | 95,0 ± 4,0 | 1,9 ± 0,1 | 523,4 ± 40,0* |
| 5 % | | 24,7 ± 1,0 | 93,8 ± 2,4 | 1,7 ± 0,2 | 523,9 ± 29,7* |
| 0 (Control) | NaCl | 18,5 ± 0,3 | 91,3 ± 4,7 | 1,1 ± 0,2 | 215,8 ± 30,8 |
| 0,5 % | 100 mmol $L^{-1}$ | 17,8 ± 2,6 | 86,3 ± 6,2 | 1,1 ± 0,1 | 291,0 ± 17,9* |
| 1 % | | 21,6 ± 0,6* | 92,5 ± 2,8 | 0,9 ± 0,1 | 320,3 ± 41,6* |
| 1,5 % | | 20,6 ± 1,8 | 93,8 ± 6,52 | 0,9 ± 0,1 | 315,3 ± 48,1* |
| 3,5 % | | 19,4 ± 2,7 | 95,0 ± 6,9 | 1,1 ± 0,2 | 309,0 ± 54,2* |
| 5 % | | 19,8 ± 2,5 | 92,5 ± 2,8 | 1,3 ± 0,3 | 295,5 ± 9,0* |

*Represents treatments that differ significantly from the control treatment according to confidence interval at α=0.05.

Means ± confidence intervals

Figure 2 shows the effect of different concentrations of an aqueous extract of *Sargassum fluitans* on the dry mass of seedlings seven days after placing the seeds in both water and 100 mM NaCl for germination. In the same, it is appreciated that the highest concentrations significantly increased the dry mass when the seedlings were grown in distilled water and in saline medium, all the concentrations of *Sargassum fluitans* used surpassed the control treatment, which demonstrated the effectiveness of this extract to stimulate the dry mass and therefore, the vigor index of seedlings under these conditions.

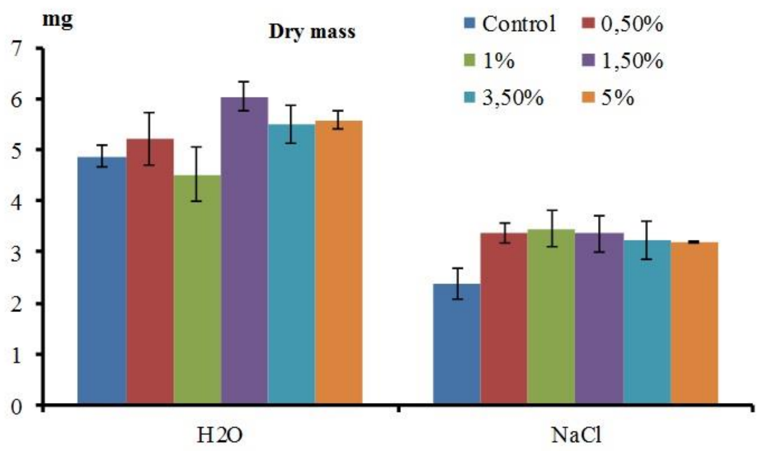


**Figura 2.** Effect of different concentrations of an aqueous extract of *Sargassum fluitans* on the dry mass of rice seedlings cv. INCA LP-7 germinated for seven days in both water and 100 mM NaCl

In previous studies, the efficiency of liquid extract of *Sargassum tenerrimum* on germination, growth, yield and quality of seeds of *Solanum lycopersicum*, applied to the plant in three different ways, as soil treatment, foliar spraying and seed treatment with different concentrations ranging from 0.2 to 1%, has been observed [(23)].

The application of liquid extracts of *Sargassum wightii* has increased the percentage of seed germination and *Triticum aestivum* growth [(13)]. Another investigation showed that 1 % aqueous extracts of *S. wightii* promoted growth and yield of *Capsicum annum* plants [(13)]. Seeds of *Vigna mungo* L. treated with 1.5 % *Turbinaria conoides* and 1.0 % *Sargassum wightii* algae extracts for three hours significantly improved seed germination, germination speed, dry matter production and vigor index [(24)].

Regarding salt stress, it has been shown that 0.2 and 0.5 % extracts of *Sargassum vulgare* increased germination and growth of durum wheat (*Triticum durum* L.) seedlings subjected to NaCl concentrations of 2 and 4 g $L^{-1}$. While higher concentrations of these extracts 25 and 50 % had inhibitory effects [(25)].

Similarly, extracts of *Sargassum vulgare* stimulated germination and plant growth of two tomato cultivars grown in saline medium [(26)].

Similarly, extracts of brown (*Sargassum muticum*) and red algae (*Jania rubens*) at a concentration of 1 % reduced the adverse effects of salt stress on the growth of chickpea (*Cicer arietinum*) plants. These results were associated with an increase in photosynthetic pigments, a decrease in Na+ ions, an increase in $K^+$ ions, and a decrease in oxidative stress [(27)].

The effects shown by sargassum extracts could be due to the large amount of active compounds that these algae possess, such as phytohormones, minerals, amino acids, and vitamins. Although it is to be assumed that at the concentrations used in this work, the predominant effect is hormonal. In brown algae such as sargassum, the presence of hormones such as auxins and cytokinins has been described, in addition to other compounds that may have hormonal action such as polyamines, betaines and sterols [(28)].

# CONCLUSIONS

- When analyzing the results obtained in both experiments, it can be concluded that the treatment of rice seeds cv. INCA LP-7 with Pectimorf® 10 and 40 mg $L^{-1}$, for 24 hours, was able to reverse the reduction that salinity caused in the final percentage of seed germination and to significantly increase seedling vigor after seven days. On the other hand, the treatment with aqueous extract of *Sargassum fluitans* did not stimulate the final germination percentage in saline medium; however, all the concentrations used significantly increased the dry mass and vigor index of seedlings.
- These results are promising and, therefore, further confirmation is necessary, as well as determining the effects of these treatments on plant growth and yield, with a view to recommending them for future use in rice-growing areas affected by salinity.

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