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Original article

# Effect of an extract of *Sargassum fluitans* on tomato seed germination

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**ABSTRACT**

Biostimulants based on seaweed extracts can be an option to improve germination and plant growth. The objective of this work was to evaluate the effect of treating tomato seeds with different solutions of an aqueous extract of *Sargassum fluitans* on germination. Two experiments were carried out; in the first one, seeds of cv. Mariela were immersed for two hours in different concentrations (0.01, 0.05, 0.1, 0.5, 1, 1.5, 2, 2.5, 3 %) of *Sargassum fluitans* aqueous extract. Seeds were placed in Petri dishes containing distilled water and the dishes were placed in the dark in a germination chamber at 28-30 ºC for ten days. At seven days, the germination percentage was evaluated and at ten days, the dry mass of radicles was evaluated. This experiment was repeated using the three best performing concentrations and the same evaluations were made, in addition to the dynamics and speed of germination. In the second experiment, the same three concentrations of the extract were used and the seeds were placed on plates containing 50 or 75 mmol $L^{-1}$ NaCl solution and germination and growth indicators were evaluated. The results showed that seed treatment with 1.5 % solution of the extract stimulated radicle growth under both normal and saline conditions. However, the final germination percentage did not always increase significantly and there was no response when seeds were germinated in NaCl 75 mmol $L^{-1}$.

**Key words:** *Solanum lycopersicum*, algae, salinity

# INTRODUCTION

Seaweed extracts are one of the main groups of biostimulants that can improve plant growth and development and accelerate nutrient uptake [1]; because seaweeds are rich sources of secondary metabolites [2-4] such as phytohormones and other growth-promoting substances [5]. Among them, brown algae extracts have been the most widely used as biostimulants, since they not only stimulate plant growth and development, but also improve the physical and chemical properties of the soil and mitigate the harmful effect of environmental stresses on plants [6].

In the germination process, it has been found that seed pretreatment increases the speed of imbibition and the influx of water-soluble metabolites softens the seed coat, regulates the biochemical and pre-germination processes that initiate the emission and radicle growth [7]. In this sense, several studies have shown that treatment with seaweed extracts improves seed germination [8,9]) and, particularly, extracts of algae of the genus *Sargassum* [10-12].

On the other hand, salinity affects seed germination in two ways, the first due to the osmotic stress created, which hinders water absorption, and the second due to the toxic effects induced by $Na^+$ and $Cl^-$ ions. Seeds and seedlings are particularly vulnerable to increased salinity because at this stage, plants have not yet developed the physiological mechanisms to tolerate increasing salt concentrations [13].

Tomato (*Solanum lycopersicum* L) is the main vegetable grown in Cuba and it is increasingly necessary to increase the production of this crop in a sustainable manner; therefore, natural products or biological fertilizers must be used. In this context, seaweed extract and particularly, *Sargassum fluitans* seaweed extract can be an option to stimulate the germination and growth of tomato seedlings.

The aim of this work was to determine if the treatment of tomato seeds cultivar Mariela with different concentrations of *Sargassum fluitans* extract would be able to stimulate their germination when grown in both aqueous and saline medium.

# MATERIALS AND METHODS

The 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.

## Preparation of *Sargassum fluitans* aqueous extract

The sargassum aqueous extract of (*Sargassum fluitans*) was obtained from fresh material collected on Santa Fe beach coast, west of Havana province, following the methodology described below: 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. At the end of the period, the liquid was filtered to remove debris and this was considered a 100 % extract.

## Effect of different concentrations of sargassum aqueous extract on the germination of tomato seeds cv. Mariela

Seeds of tomato (*Solanum lycopersicum* L.) cv. Mariela, from the National Institute of Agricultural Sciences, which were disinfected with NaClO 5 % for 10 minutes. Subsequently, they were treated for two hours with different concentrations (0.01, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 %) of the aqueous extract of sargassum (AES). Once the treatments were completed, the seeds were placed in sterilized Petri dishes (25 seeds per dish and four dishes per treatment) to which 12 mL of distilled water were added. The plates were placed in the dark in a growth chamber at 28-30 ºC for ten days. After seven days, the final germination percentage was evaluated and after ten days, 50 radicles per treatment were selected to evaluate the dry mass of the radicles.

This experiment with three concentrations of best response and proceeded in a similar way to the first repetition was repeated. In this case, the germination dynamics by counting the germinated seeds 1, 2, 3, 4, 5 and 7 days after placing seeds on the plates were followed and were expressed as a percentage. In addition, the behavior of germination speed was calculated by the formula VG=ni/ti, where ni is the number of newly germinated seeds at time ti. As in the first replicate, the dry mass of radicles was determined after ten days.

## Effect of an aqueous extract of sargassum on the germination of tomato seeds cv. Mariela in a saline medium

In this experiment, the same three concentrations of the aqueous extract of sargassum used in the repetition of the previous experiment were used, the difference being that in this case the seeds were placed in Petri dishes containing NaCl 50 or 75 mmol $L^{-1}$ solution. Germinated seeds were counted 3, 4, 5, 6 and 7 days after being placed in plates and the average germination speed and time per treatment were calculated. Formulas VG= Σ(ni/di) were used, ni being the number of seeds germinated at time di and TMG= Σ(D x n)/Σn, where n is the number of newly germinated seeds on day D and D is the number of days counted from the beginning of the experiment [14]. At 7 days, the final germination percentage was calculated, and at 10 days, the dry mass of radicles was evaluated, similar to the previous experiment.

## Statistical processing

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

# RESULTS AND DISCUSSION

## Mariela Effect of different concentrations of sargassum aqueous extract on the germination of tomato seeds cv. Mariela

The influence that seed treatment with different concentrations of AES exerted on the final germination percentage and dry mass of tomato radicles cv. Mariela are shown in Table 1. The treatments of AES 0.05, 1.0 and 1.5 % significantly increased the final germination percentage compared to the control treatment, while all the concentrations tested significantly increased the dry mass of radicles.

**Table 1.** Effect of different concentrations of aqueous extract of sargassum (AES) on the final percentage of seed germination (seven days) and dry mass of tomato radicles cv. Mariela grown

| Treatments | Final germination percentage | Dry mass (mg mg rootlets$^{-1}$) |
|---|---|---|
| Control | 79 ± 6,7 | 1,1 ± 0,06 |
| AES 0,01 % | 88 ± 6,4 | 1,3 ± 0,04* |
| AES 0,05 % | 91 ± 2,0* | 1,5 ± 0,04* |
| AES 0,1 % | 84 ± 3,2 | 1,4 ± 0,07* |
| AES 0,5 % | 88 ± 5,5 | 1,5 ± 0,04* |
| AES 1,0 % | 92 ± 4,5* | 1,3 ± 0,05* |
| AES 1,5 % | 90 ± 3,9* | 1,5 ± 0,04* |
| AES 2,0 % | 85 ± 7,4 | 1,2 ± 0,02* |
| AES 2,5 % | 86 ± 2,3 | 1,4 ± 0,04* |
| AES 3,0 % | 83 ± 6, 7 | 1,3 ± 0,05* |

Means ± confidence intervals

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

Similar results in the final germination percentage and vigor index were obtained when seeds of *Solanum lycopersicum*, *Solanum melongena* and *Capsicum annum* were treated for 24-48 hours with an aqueous extract of *Sargassum johnstonii*, although in this case concentrations of 3, 4 and 5 % were used [(12)]. An increase in the germination percentage of tomato seeds up to 100 % was obtained when these were treated with an extract of *Sargassum tenerrimum* 0.8 % [(15)]. However, when *Sargassum vulgare* extracts (0.2 and 0.5 %) were added to the medium, there was no significant effect on the final seed germination percentage of Agatha and Nemadore tomato cultivars [(13)].

In other crops, such as peanut, it has been reported that seed treatment with an extract of *Sargassum fluitans* Borgersen (15 mg mL$^{-1}$) stimulated germination and plant growth indicators [(16)]. In Vigna mungo and *Vigna radiata* plants, it was shown that a 3 % seaweed extract stimulated growth, increased the concentration of photosynthetic pigments, proteins, reducing and total sugars and amino acids [(17)].

The responses of germination and dry mass of radicles to seed treatment with the aqueous extract of sargassum, observed in this work, may be related to the composition of the extract. It has been reported, the presence of macro (N, P, K, Mg, Ca) and microelements (Fe, Mn, Zn, Cu); as well as auxins and cytokinins in these extracts [(10)].

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In addition, it has been found that biostimulants based on brown algae, such as *Ascophyllum nodosum*, over-regulate the expression of the nitrate transporter gene NRT1.1, stimulating nitrogen sensing and auxin transport, which leads to accelerated lateral root growth and improved nitrogen assimilation [(2)].

From these results, the concentrations of 0.05, 0.5 and 1.5 % were selected for subsequent studies, taking into account that these are low, medium and high concentrations among those tested and, in addition. They were treatments that provided the highest values of dry mass of radicles and two of them (0.05 and 1.5 %) also increased the final percentage of germination.

Figure 1 shows how the two lowest concentrations used accelerated seed germination, since they showed significantly higher germination percentages two days after the experiment started (Figure 1A), as a consequence of a higher germination speed of the seeds of these treatments between the first and second day of the experiment (Figure 2A), compared to the seeds of the control treatment. However, between the second and third day, this situation was reversed, resulting in no significant differences in the final seed germination percentages seven days after the start of the experiment (Table 2).

The dry mass of the radicles increased significantly in the seeds treated with the three concentrations of sargassum extract (Table 2), confirming the results obtained in the first repetition (Table 1).

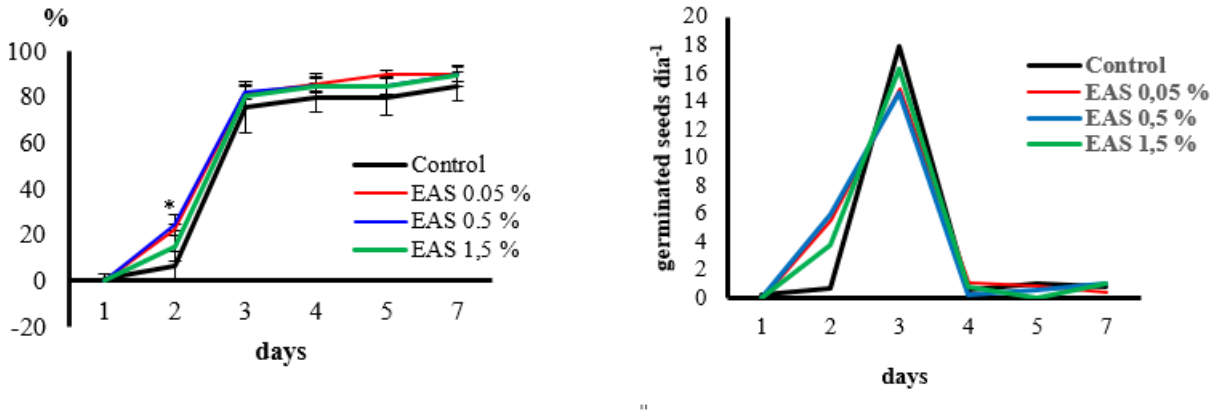


**Figura 1.** Influence of three concentrations of aqueous extract of sargassum on the dynamics of the percentage (A) and germination speed (B) of tomato seeds cv. Mariela

**Table 2.** Influence of different concentrations of an aqueous extract of sargassum on final germination percentage (seven days) and dry mass of radicles (10 days) of tomato cv. Mariela

| Treatments | Final germination percentage | Dry mass of radicles (mg) |
|---|---|---|
| Control | 85,0 ± 6,3 | 1,3 ± 0,07 |
| AES 0,05 % | 89,5 ± 3,1 | 1,6 ± 0,04* |
| AES 0,5 % | 90,0 ± 3,5 | 1,5 ± 0,04* |
| AES 1,5 % | 89,5 ± 4,5 | 1,6 ± 0,04* |

Means ± confidence intervals

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

Several authors using various modes of application have reported the positive influence of Sargassum extracts on growth indicators of tomato plants previously. Thus, the application of an extract of *Sargassum vulgare* to the germination and growth medium increased the fresh and dry mass of seedlings, as well as the length of radicles 14 days after initiation of treatments [13].

Favorable results were also reported by other authors [15], who used the same mode of application but with an extract of *Sargassum tenerrimum*. In addition, these authors used other modes of application such as soil application, seed treatment and foliar spraying and found an increase in several growth indicators 40 days after sowing.

In sugar beet, it was found that microalgae extracts over-regulated the expression of genes related to primary and secondary metabolism associated with nutrient consumption, which stimulated root growth [7]. On the other hand, it has been reported that the stimulatory effect of aqueous extracts of algae is related to all the substances present in them such as: carbohydrates, proteins, vitamins, amino acids, lipids, macro and micronutrients, pigments, as well as natural phytohormones such as auxins, gibberellins and cytokinins [18-20]. These increase cell metabolism in treated seeds, stimulate the processes of cell division and elongation and therefore, seedling growth. These findings could explain the increase in the dry mass of radicles found in this work.

The results obtained in the two replicates of this experiment indicated that the treatment of tomato seeds cv. Mariela for two hours with a 0.05 % aqueous extract of sargassum did not always stimulate the final germination percentage; however, it did increase the dry mass of radicles ten days after the start of the experiment. This can be very useful for the production of quality tomato seedlings; therefore, it is necessary to continue research on this subject.

Given the favorable response of the dry mass of radicles to the treatment of seeds with the aqueous extract of sargassum, it was decided to evaluate the effectiveness of the extract when seeds germinate in a saline medium, for which the same concentrations were used.

## Effect of an aqueous extract of sargassum on the germination of tomato seeds cv. Mariela in saline medium

The effect that the three concentrations of the aqueous extract of sargassum exerted on some germination indicators and the dry mass of the radicles is shown in Table 3. As can be seen, the concentration of 1.5 % significantly increased the germination speed, achieving the final germination percentage (87.2 %) after three days in the NaCl 50 mmol $L^{-1}$ solution, in agreement with the TMG obtained for that treatment.

The increase of the NaCl concentration up to 75 mmol $L^{-1}$ did not produce variations in the germination indicators in the control treatment; however, these did not respond to the treatment of the seeds with any of the three concentrations of the extract tested.

**Table 3.** Influence of different concentrations of an aqueous extract of sargassum on germination and dry mass of tomato radicles cv. Mariela in saline medium

| Treatments | Concentrations of NaCl | GR (seed days$^{-1}$) | MGT (days) | % G | DMR (mg) |
|---|---|---|---|---|---|
| Control | | 6,2 ± 0,2 | 3,3 ± 0,3 | 80,0 ± 4,3 | 1,70 ± 0,04 |
| AES 0,05 % | | 7,1 ± 0,7 | 3,1 ± 0,2 | 87,2 ± 5,2 | 1,22 ± 0,03 |
| AES 0,5 % | 50 mmol L$^{-1}$ | 6,8 ± 1,2 | 3,1 ± 0,1 | 83,2 ± 12,7 | 1,74 ± 0,01 |
| AES 1,5 % | | 7,3 ± 0,5* | 3,0 ± 0,0 | 87,2 ± 5,8 | 1,90 ± 0,02* |
| Control | | 6,2 ± 0,96 | 3,4 ± 0,1 | 81,3 ± 11,4 | 1,38 ± 0,04 |
| AES 0,05 % | 75 mmol L$^{-1}$ | 6,1 ± 0,3 | 3,2 ± 0,1 | 76,0 ± 4,3 | 1,15 ± 0,02 |
| AES 0,5 % | | 6,4 ± 0,7 | 3,2 ± 0,2 | 80,8 ± 4,6 | 1,23 ± 0,04 |
| AES 1,5 % | | 6,1 ± 0,9 | 3,2 ± 0,1 | 75,2 ± 8,7 | 1,64 ± 0,01* |

Means ± confidence intervals

*Represents means that differ significantly from the control treatment according to confidence intervals at α=0.05

GR- Germination rate MGT - Mean germination time % G - Final germination percentage DMR - Dry mass of radicles

A different behavior was shown by radicle dry mass, which decreased significantly in the control treatment when NaCl concentration increased. This showed that radicle dry mass was a more sensitive indicator of salt stress than seed germination. Similar results were reported when studying the behavior of germination and dry mass of the cultivar Poncho Negro and the wild species *Solanum peruvianum* in 100 mM NaCl, they found that salt stress significantly affected the dry mass of plants; while the germination percentage was not affected when compared to the control treatment without salt [21].

Treatment of seeds with the aqueous extract of sargassum at 1.5 % significantly increased the dry mass of the radicles, independently of the concentration of NaCl present in the medium.

Several authors have reported the beneficial effects of Sargassum extracts on the germination behavior of seeds of various plant species under saline conditions. For example, in the case of tomato [13], it has been found that aqueous extracts (0.2 and 0.5 %) of *Sargassum vulgare* increased germination of two cultivars by 2 and 5 %, respectively in 2 and 4 g L$^{-1}$ NaCl solutions; however, these increases were not statistically significant. Favorable effects on germination of durum wheat [22] and bean [23] seeds were found with the addition of *Sargassum vulgare* extracts to solutions of the same NaCl concentrations. In the present work, the Sargassum extract concentration of 1.5 % increased germination in NaCl 50 mmol L$^{-1}$ by 9 % and this increase was not statistically significant. However, it should be noted that the mode of application of the extracts was different, since in this work the seeds were treated for two hours, while in the information reviewed the extracts were added to the germination medium, i.e., to the NaCl solutions.

The increase in the dry mass of the radicles, found in the present work, confirms the results obtained by other authors [13], who reported that the application of an extract of *Sargassum vulgare* significantly improved the growth of the radicle of two tomato cultivars subjected to salt stress. According to these authors, this increase in radicle length is due to the presence in the extracts of some growth-promoting substances such as IAA,

AIB, gibberellins, cytokinins, micronutrients and amino acids. In relation to this, it has also been reported that canola plants treated with macroalgae extracts and subjected to salt stress exhibited significantly higher contents of plant hormones compared to untreated plants, which explains the growth stimulation induced by them under these conditions [24].

These results reveal the potential of the aqueous extract of sargassum that arrives to Cuban coasts to be used as biostimulant in agriculture. It is necessary to continue investigating this subject and to evaluate the influence that the application of this extract can exert on the growth and development of tomato plants cultivated under normal conditions as well as under stress conditions; as well as to test other doses, modes and moments of application.

## CONCLUSIONS

- The treatment of tomato seeds cv. Mariela with an aqueous extract of *Sargassum fluitqans* significantly increased the dry mass of radicles regardless of the concentration used; however, the final percentage of germination did not always increase significantly, although some concentrations (0.05 and 0.5%) accelerated germination during the first two days.
- When seeds germinated in saline medium, the response was different and only the treatment with the 1.5 % extract promoted radicle growth in both concentrations of NaCl (50 and 75 mmol $L^{-1}$); while, of the germination indicators evaluated, only an increase in germination speed was found in the concentration of 50 mmol $L^{-1}$.

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