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Influence of a Sargassum extract on common bean seed germination

Influencia de un extracto de sargazo sobre la germinación de semillas de frijol común

Influência do extrato de sargaço na germinação de sementes de feijão comum

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ABSTRACT

In the past decade, several authors reported unusual arrivals of Sargassum to the Cuban coast; however, there is hardly any information about the possible use of this biomass in agriculture. For this reason, the objective of this work was to

determine if the treatment of seeds with an aqueous extract of Sargassum is capable of promoting the germination of common bean cv. CUL 156. For this, an experiment was carried out where the seeds, once disinfected, were soaked for 30 minutes in different concentrations of an aqueous Sargassum extract (0, 1, 2, 5 and 10 %) and subsequently placed on Petri dishes with sterile distilled water and placed in a germination chamber at a temperature of 26-28 ºC for seven days. The germination dynamics were followed and after seven days the final percentage of germination, the length of roots, hypocotyls and epicotyls, shoot and total dry weight, as well as the vigor index of the seedlings, were evaluated. The results showed that the lowest concentration used (1 %) was the best, since it was not only able to advance germination, but also increased the length of the roots, the shoot dry weight and, therefore, dry weight of the seedlings; which resulted in a significantly higher vigor index than the seedlings of the control treatment.

Key words: *Sargassum* spp.; seed imbibition; vigor index; *Phaseolus vulgaris.*

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RESUMEN

En la década pasada, varios autores informaron de las arribazones inusuales de sargazo a las costas cubanas; sin embargo, apenas existe información sobre el posible uso de esta biomasa en la agricultura del país. Por tal motivo, el objetivo del presente trabajo fue determinar si el tratamiento a las semillas con un extracto acuoso de sargazo es capaz de promover la germinación del frijol común cv. CUL 156. Para esto, se ejecutó un experimento donde las semillas, una vez desinfectadas, se sumergieron, durante 30 minutos, en diferentes concentraciones de un extracto acuoso de sargazo (0, 1, 2, 5 y 10 %), posteriormente, colocadas en placas Petri con agua destilada estéril e introducidas en una cámara de germinación a una temperatura de 26-28 ºC por siete días. Se siguió la dinámica de germinación y a los siete días se evaluó el porcentaje final de germinación, la longitud de raíces, hipocotilos y epicotilos, masa seca de vástagos y total, así como el índice de vigor de las plántulas. Los resultados demostraron que la concentración más baja utilizada (1 %) fue la mejor, ya que no sólo fue capaz de adelantar la germinación, sino que además incrementó la longitud de las raíces, la masa seca de los vástagos y por ende, de las plántulas; lo que repercutió en un índice de vigor significativamente superior a las plántulas del tratamiento control.

Palabras clave: *Sargassum* spp.; imbibición; índice de vigor; *Phaseolus vulgaris.*

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RESUMO

Na última década, vários autores relataram chegadas incomuns de sargaço à costa cubana; Contudo, quase não há informações sobre a possível utilização desta biomassa na agricultura do país. Por esse motivo, o objetivo do presente trabalho foi determinar se o tratamento de sementes com extrato aquoso de sargaço é capaz de promover a germinação de feijoeiro comum cv. CUL 156. Para isso foi realizado um experimento onde as sementes, uma vez desinfetadas, foram imersas, por 30 minutos, em diferentes concentrações de extrato aquoso de sargaço (0, 1, 2, 5 e 10 %) e posteriormente colocadas em Placas de Petri com água destilada estéril e colocadas em câmara de germinação à temperatura de 26-28 ºC por sete dias.

A dinâmica de germinação foi acompanhada e após sete dias foram avaliados a porcentagem final de germinação, o comprimento de raízes, hipocótilos e epicótilos, massa seca da parte aérea e total, bem como o índice de vigor das plântulas. Os resultados mostraram que a menor concentração utilizada (1 %) foi a melhor, pois além de promover a germinação, aumentou o comprimento das raízes, a massa seca da parte aérea e, portanto, das mudas; o que resultou em índice de vigor significativamente maior que as mudas do tratamento controle. **Palavras-chave**: *Sargassum* spp.; embebição; índice de vigor; *Phaseolus vulgaris*.

INTRODUCTION

Seaweed extracts contain different bioactive compounds and are therefore widely accepted as plant biostimulants and commonly used in agriculture (Ali et al., 2021). The mechanisms of action of algal extracts are complex, and understanding them requires a multidisciplinary approach to elucidate the various interactions that exist among the different bioactive compounds within each extract (Hernández-Herrera et al., 2023).

The application of seaweed extracts by soil application or seed coating, either by imbibition or soaking, has been widely evaluated and both modes of application are considered suitable for improving germination rates and subsequent seedling establishment (Amirkhani et al., 2019). In this way, it has been shown that extracts of brown algae and especially extracts of different species of the genus Sargassum stimulate the seed germination of various plant species, such as: *Solanum lycopersicum* (Jumadi et al., 2023; Sasikala et al., 2016), *Oryza sativa* (Pérez-Domínguez et al., 2022), *Vigna radiata* (Makawita et al., 2021), *Capsicum annum* (Fatimah et al., 2018), *Arachis hypogea* (Koffi et al., 2018) and *Triticum aestivum* (El-Din, 2015).

The species *Sargassum fluitans* and *Sargassum natans,* within the genus Sargassum, are recognized as the only two holopelagic species, since they spend their entire life cycle floating in the sea and reproduce asexually by vegetative fragmentation (Hu et al., 2016). In recent years, large quantities of holopelagic sargassum have arrived on the coasts of the Caribbean and West Africa (Amador-Castro et al., 2021). In Cuba, arrivals have been reported on the central-southern coast (Moreira & Alfonso, 2013) and on the north-western coast (Arencibia-Carballo et al., 2020); However, there is very little information about the possible use of this biomass in Cuban agriculture.

The common bean (*Phaseolus vulgaris* L.) is a food product of great importance for the world and very traditional for the people of Latin America and the Caribbean (de Jesús Martínez et al., 2021).

In recent years, the production and yield of this crop have decreased

significantly in our country, so the consumption needs of the population are not met, despite the fact that significant quantities are imported annually. Then, it is a priority for agriculture to increase its production with strategies and technologies that are friendly to the environment (Escalona et al., 2023), for which the implementation of some actions is strengthened, such as the promotion of genetic improvement of cultivars, seed production and a correct crop management, with emphasis on achieving adequate germination to guarantee the population density established in the technical instructions (Maqueira-López et al., 2021).

For all of the above, the fundamental objective of the present research was to determine if the seed treatment with an aqueous extract of *Sargassum* is capable of promoting the seeds germination of common bean cv. CUL 156.

MATERIALS AND METHODS

The experiment was carried out in the Department of Plant Physiology and Biochemistry of the National Institute of Agricultural Sciences, located in San José de las Lajas, province of Mayabeque. For this purpose, bean seeds (*Phaseolus vulgaris* L.) cv. CUL 156, which were disinfected with 1% NaClO for 15 minutes and then washed three times with sterile distilled water. The product used was an sargassum aqueous extract (SAE) obtained from fresh material of a mixture of *Sargassum fluitans* and *Sargassum natans*, collected on the coast of Guanabo and macerated with tap water for three months, whose chemical characterization was reported by Gutiérrez et al. (2024).

The seeds, once washed, were dipped for 30 minutes in SAE solutions at concentrations of 0, 1, 2, 5 and 10 % and subsequently placed in sterilized Petri dishes (15 seeds per plate and four plates per treatment) to which 15 mL of sterile distilled water were added. The plates were placed in the dark in a growth chamber at 26-28 °C for seven days.

The count of germinated seeds per plate was carried out at 24, 48, 72 and 96 hours after beginning the experiment and after seven days (168 hours) the final percentage of germination was evaluated and at this time, at 25 seedlings per treatment, root, hypocotyl and epicotyl lengths (cm) and the root, shoot and total dry weight (mg) were, also, measured.

In addition, the seedling vigor index (Pérez et al., 2022) and some germination indices (González & Orozco, 1996) were calculated.

Seedling vigor index $VI = \%G \times DW$ [1]

Where: G is the final germination percentage and DW is the dry weight of the seedlings.

Velocity coefficient $VC = \frac{\Sigma ni}{\Sigma(niti)} x100$ [2]

Where: n_i is the number of seeds germinated on day i and t_i is the number of days since sowing.

Mean germination time $MGT = \frac{\Sigma(niti)}{\Sigma ni}$ [3]

Where: n_i is the number of seeds germinated on day i and t_i is the number of days since sowing.

Germination index $GI = \frac{\Sigma(niti)}{N}$ [4]

Where: n_i is the number of seeds germinated on day i, t_i is the number of days since sowing and N is the total number of seeds sown.

The experiment was repeated twice and all the data obtained were analyzed statistically by calculating the means, standard deviations and confidence intervals at α=0,05, for which the Excel statistical program, Windows 10 Pro was used.

RESULTS AND DISCUSSION

The dynamics of seed germination, during the first 96 hours, are presented in Figure 1. As can be seen, the seed dipping in the different concentrations of sargassum did not modify the dynamics of germination; although the lowest concentration used (1 %) increased the germination percentage by 16 and 8 % at 24 and 48 hours, respectively, compared to the control treatment. This meant that the seeds from this treatment were the only ones that achieved 100 % germination after 72 hours.

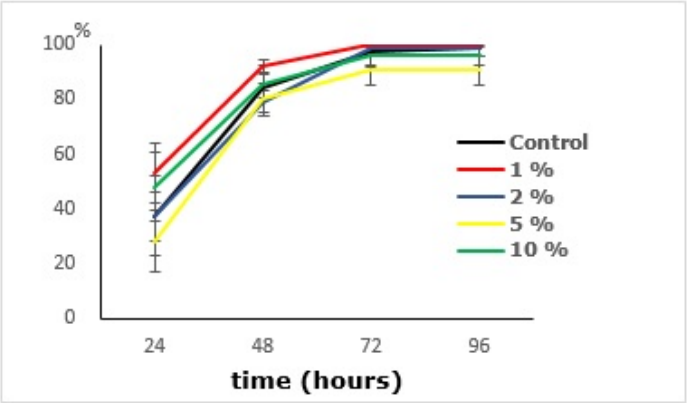

Figure 1. Effect of different concentrations of an aqueous *Sargassum* extract on the germination dynamics of bean seeds cv. CUL-156. **Legend**: The bars represent the confidence intervals at α=0,05.

Seed treatment with 1 % SAE did not significantly influence the final germination percentage evaluated at 168 hours; however, it was the only treatment that significantly increased and decreased the velocity coefficient (VC) and the mean germination time (MGT), respectively; which is associated with a better distribution of germination and an advance in it compared to the control treatment (Table 1). It should also be noted that the highest concentrations of the extract (5 and 10%) significantly decreased the final germination percentage. The treatments did not

influence the germination index (GI), which is more related to the germination capacity of the seeds.

Similar results regarding the non-influence of treatments on the final germination percentage have been reported by Machado et al. (2018) in beans treated with *S. vulgare*, Pérez-Domínguez et al. (2022) by imbibition of rice seeds, for 24 hours, with an aqueous extract of *S. fluitans* and Mohamed et al. (2023) by soaking seeds of *Vicia faba* and *Helianthus annus*, prior to sowing, with *S. polycystum*.

Table 1. Influence of different concentrations of a *Sargassum* extract on some germination indices of bean seeds cv. CUL 156.

Treatments	G %	VC	MGT	GI
Control	100,0 ± 0,0	54,0 ± 2,3	1,9 ± 0,1	1,8 ± 0,2
1 %	100,0 ± 0,0	65,0 ± 4,3*	1,5 ± 0,1*	1,5 ± 0,1
2 %	98,7 ± 2,6	57,9 ± 5,5	1,8 ± 0,1	1,8 ± 0,2
5 %	90,7 ± 5,2*	54,1 ± 2,6	1,8 ± 0,2	1,6 ± 0,2
10 %	96,0 ± 3,2*	62,6 ± 5,8*	1,6 ± 0,2	1,5 ± 0,1

Legend: G %: Final germination percentage, VC: Velocity coefficient, MGT: Mean germination time, GI: Germination index. *Represents treatments that differ significantly from the control treatment according to confidence interval at α=0,05. **Source**: Own elaboration

On the other hand, an increase in germination has been found when seeds of *Vigna radiata*, *Pachyrhizus erosus* and *Vigna mungo* were dipped for 24 hours in *Sargassum* spp., *Sargassum liebmannii* and *S. myriocystum* extracts, respectively (Makawita et al., 2021; Nicolás-Álvarez et al., 2014 and Kalaivanan & Venkatesalu, 2012). Similarly, *Trigonella foenum-graecum* L. seed dipping in an extract of *S. vulgare* for 12 hours also increased the germination percentage (El-Sheekh et al., 2016).

The use of *Sargassum* extracts as a germination medium, in certain concentrations, can also increase (Sasikala et al., 2016), inhibit (Hernández-Herrera et al., 2023) or have no influence (Salma et al., 2014) in the final percentage of seed germination.

The inhibition of germination caused by the seed treatment, with the highest concentrations of the extract (5 and 10 %) used in the present research, confirmed previous results reported by Kalaivanan and Venkatesalu (2012) when treating *Vigna mungo* seeds, for 24 hours, with different concentrations of an *S. myriocystum* extract.

The acceleration of germination evidenced by the decrease in the seed mean germination time treated with 1 % SAE, which was observed in this investigation, has been previously reported by other authors, in this species (Salma et al., 2014), in *Sesamum indicum* (Jupri et al., 2021) and in *Plumbago zeylanica* (Kakade et al., 2022), when extracts of *S. vulgare*, *S. polycystum* and *S. ilicifolium*, respectively, are used as germination medium.

The influence that the seed treatment with different concentrations of *Sargassum* extract exerted on some indicators of the initial growth of the seedlings is presented in Table 2. The treatment with *Sargassum* significantly

increased the shoot dry weight, regardless of concentration; however, only the 1% concentration was able to promote root length. The treatments did not influence the rest of the evaluated indicators.

Table 2. Effect of seed treatment with different concentrations of a *Sargassum* extract on the initial growth of bean seedlings cv. CUL 156.

Tratamientos	R.L. (cm)	H.L. (cm)	E.L. (cm)	Root dry weight (mg seedling^{-1})	Shoot dry weight (mg seedling^{-1})
Control	1,85 ± 0,29	5,77 ± 0,51	0,45 ± 0,07	3,83 ± 0,52	18,2 ± 1,1
1 %	2,72 ± 0,37*	6,60 ± 0,45	0,49 ± 0,10	4,52 ± 0,67	23,9 ± 1,7*
2 %	2,09 ± 0,28	6,26 ± 0,61	0,56 ± 0,14	3,77 ± 0,82	23,5 ± 2,2*
5 %	2,28 ± 0,42	6,09 ± 0,67	0,41 ± 0,08	3,78 ± 0,81	24,8 ± 3,4*
10 %	2,11 ± 0,29	6,57 ± 0,49	0,51 ± 0,05	4,04 ± 0,42	23,3 ± 2,0*

Legend: R.L. - Root length; H.L. - Hypocotyl length; E.L. - Epicotyl length
*They represent the means that differ significantly from the control treatment according to confidence intervals at α=0,05. **Source**: Own elaboration

The increase in root length confirmed what was recently obtained by Hamouda et al. (2023), by treating *Pisum sativum* seeds, for two hours, with an aqueous extract of *Sargassum vulgare.*

On the other hand, several authors have reported an increase in the seedling length with the use of different *Sargassum* extracts. In this way, the treatment of *Vigna radiata* seeds with a *Sargassum* sp. extract increased the root and shoot lengths of the seedlings (Makawita et al., 2021); while the use of an aqueous extract of *Sargassum plagiophyllum*, as a germination medium, increased the root and shoot lengths of rice seedlings (Ramu & Nallamuthu, 2012). The addition to the MS medium of an extract of *Sargassum* sp. and *S. ilicifolium* stimulated root and shoot lengths of tomato seedlings (Fatimah *et al*., 2018) and shoot length of *Plumbago zeylanica* seedlings (Kakade et al., 2022), grown under *in vitro* conditions. However, the addition of *S. polycystum* to the agar medium did not influence the root length or the seedling height of bean (Jupri et al., 2021).

The increase observed in the shoot dry weight of the seedlings, whose seeds were treated with the extract confirmed what was previously reported by Koffi et al. (2018) when applying different concentrations of an extract of *S. fluitans* to the peanut crop.

The response of this indicator influenced the behavior of the seedling dry weight, so Figure 2 shows the behavior of this variable (Figure 2 A) and their vigor index (Figure 2 B).

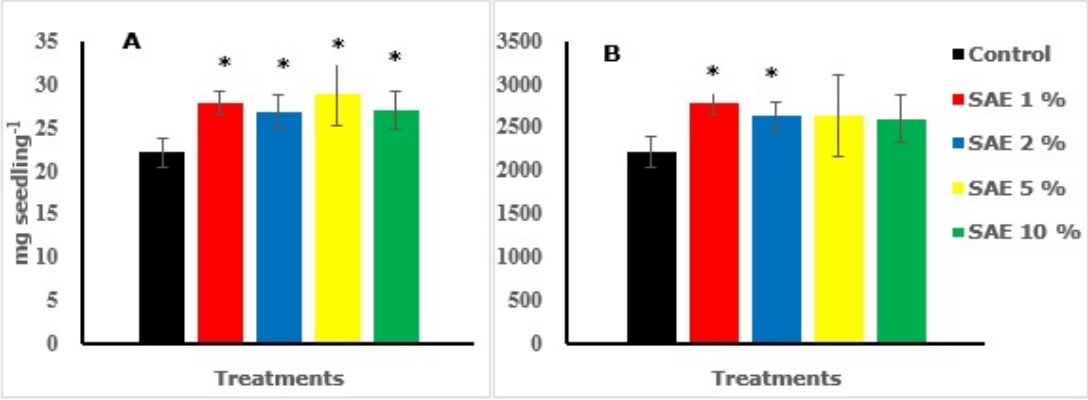

Figure 2. Influence of seed treatment with different concentrations of a *Sargassum* extract on the dry weight (A) and the vigor index (B) of bean seedlings cv. CUL 156.
Legend: The bars represent the confidence intervals at α=0,05 and the asterisks represent the treatments that differ significantly from the control treatment. **Source**: Own elaboration.

It should be noted that, although all the studied concentrations of the extract significantly increased the seedling dry weight, only the seedlings from the seeds that were treated with the two lowest concentrations of the extract presented values of vigor indexes significantly higher than the control treatment. This is because the highest concentrations evaluated significantly decreased the final germination percentage of the seeds as shown in Table 1.

Several authors have reported the increase in the seedling dry weight treated with different species of *Sargassum*, such as, for example, in *Vigna mungo* whose seeds were treated with *S. myriocystum* (Kalaivanan & Venkatesalu, 2012); in *Triticum aestivum* treated with *S. vulgare* (El-Din, 2015) and in *Vicia faba* and *Helianthus annus* treated with aqueous extract of *S. polycystum* (Mohamed et al., 2023). These last authors also reported that the seedling vigor index exhibited increases of 24,09 and 22,73 %, respectively. Previously, Hernández-Herrera et al. (2014) had reported that the use of an aqueous extract of *Sargassum liebmannii* J. Agardh 0.2 % as a germination medium increased the vigor index of tomato seedlings by 52 %.

The increases in the seed germination speed coefficient, the root length and the shoot and seedling dry weights, which were obtained in this research, with the seed treatment with a low concentration (1 %) of a aqueous extract of *Sargassum* (*Sargassum fluitans* and *Sargassum natans*), may be related to its chemical composition. In this sense, it is known that aqueous extracts of *Sargassum* sp. contain various nutrients and bioactive metabolites, including plant growth regulators; which may be responsible for such effects.

For example, the presence of plant growth-promoting hormones such as auxins, gibberellins and cytokinins has been reported in the extracts of different *Sargassum* species (Fitriyah et al., 2022; Uthirapandi et al., 2018); indole acetic acid

(IAA) being the most common found in all *Sargassum* species (Sunarpi et al., 2021).

The presence of IAA in the extract of *S. polycystum* was associated with the increase in the germination speed of sesame seeds, obtained by Jupri et al. (2021), who propose that IAA induces the activity of catalytic enzymes such as amylases, nucleases and proteases, which degrade polymers in the endosperm of seeds, generating the metabolites necessary to initiate germination. Then, this could explain the increase in the germination speed coefficient and root length found in the present research.

On the other hand, in the *Sargassum* extract used, the presence of metabolites such as carbohydrates, proteins and pigments was detected, among others (Gutiérrez et al., 2024), which could explain, in part, the stimulation of seedling growth found in this experiment, reflected in the increase in the shoot dry weight; although the reported concentrations are lower than those obtained when using other extract preparation methods and those found in other species of the genus Sargassum by other authors (El-Sheekh et al., 2016; Sasikala et al., 2016). .The results of this research revealed the potential for using the biomass of holopelagic *Sargassum* species that arrive on the coast in Cuban agriculture; therefore, currently, the effects on the germination of other plant species are confirmed and research is being carried out to determine the effects that these extracts can exert on the growth and yield of some crops of economic importance, among which is the common bean.

CONCLUSIONS

Dipping seeds, for 30 minutes, in a 1 % aqueous extract of holopelagic *Sargassum* (*Sargassum fluitans* and *Sargassum natans*) not only advances germination but also stimulates the growth of bean seedlings cv. CUL 156.

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AUTHORS CONTRIBUTION

Núñez Vázquez, M. de la C.: conceptualization, statistical analysis of data, preparation of figures and tables, writing of the manuscript.

Martínez González, L.: setup and execution of the experiment, primary data collection and processing, manuscript review.

Reyes Guerrero, Y.: conceptualization of the project and revision of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest regarding the publication of this article.