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Bioactivity of *Ascophyllum nodosum* in Mitigating Water Stress Induced by PEG-6000 in Maize Seeds

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Abstract

Water deficit is one of the main limiting factors for crop establishment and productivity, particularly affecting seed germination and early seedling growth. This study aimed to evaluate the biostimulant effect of *Ascophyllum nodosum* extract on maize (*Zea mays* L.) seeds subjected to osmotic stress induced by PEG-6000.Three independent bioassays were conducted under controlled conditions. First, osmotic potentials ranging from 0 to −0.8 MPa were tested to determine stress levels. In the second assay, seeds were treated with increasing doses (0 to 2 mL kg^{-1}) of a commercial seaweed extract and its isolated mineral fraction. In the third assay, selected doses were evaluated under no stress, moderate stress, and severe stress conditions. Germination percentage, normal and abnormal seedlings, radicle and epicotyl length, and vigor index were assessed. Osmotic stress significantly reduced germination and seedling growth, particularly at −0.6 and −0.8 MPa. Seed treatment with *A. nodosum* did not affect final germination but improved seedling growth and vigor, showing a dose-dependent response. Maximum efficiency was observed at intermediate doses (~0.45–0.66 mL kg^{-1}), which increased the percentage of normal seedlings and promoted root and shoot development. Under water stress conditions, the complete extract outperformed the mineral fraction, indicating that the beneficial effects are mainly associated with bioactive organic compounds. These findings demonstrate that A. nodosum extract is a promising strategy to mitigate water stress effects during maize seed germination, provided that optimal doses are used.

Keywords: plant biostimulants; osmotic stress; seed germination; drought tolerance; seedling vigor

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1. Introduction

Water availability is one of the main limiting factors for agricultural growth and productivity on a global scale. The intensification of climate change has increased the frequency, duration, and severity of drought events, compromising the stability of production systems, especially in semi-arid and tropical regions [1,2]. In this context, water deficit represents one of the most impactful abiotic stresses for modern agriculture, affecting fundamental physiological processes from germination to the reproductive phase of plants.

Germination is particularly sensitive to the reduction in the water potential of the substrate, since imbibition is the first determining event for the metabolic reactivation of the seed. Limitation in water absorption compromises enzymatic activation, reserve mobilization, and cell expansion, which can result in delayed germination, reduced vigor, and an increased incidence of abnormal seedlings [3,4]. Furthermore, osmotic stress triggers hormonal and redox changes, including increased production of reactive oxygen species (ROS), which can act as signaling molecules but also cause cell damage when not properly regulated [5,6].

To simulate drought conditions in a controlled environment, polyethylene glycol 6000 (PEG-6000) has been widely used as an osmotic stress inducing agent. Due to its high molecular weight and low cell penetration, PEG reduces the water potential of the solution without exerting direct toxic effects, allowing for reproducible evaluation of the physiological responses of seeds under water restriction [4,7]. Studies demonstrate that progressively more negative osmotic potentials promote a reduction in germination and initial growth, especially in root elongation, highlighting the high sensitivity of this phase to water stress [8,9].

Given this scenario, strategies capable of increasing plant tolerance to water deficit have received increasing attention. Among them, the use of biostimulants stands out, defined as substances or microorganisms that promote greater physiological efficiency, better nutrient utilization, and greater tolerance to abiotic stresses, regardless of their nutritional content [10,11]. Unlike conventional fertilizers, biostimulants act predominantly in the modulation of metabolic, hormonal, and molecular processes, stimulating osmoregulation mechanisms, root growth, and antioxidant adjustments.

Seaweed extracts are one of the most studied classes of biostimulants, especially those derived from *Ascophyllum nodosum*. This species has a complex composition, including phytohormones (auxins, cytokinins, and gibberellins), amino acids, polysaccharides, and micronutrients, which can act synergistically in modulating plant growth and mitigating abiotic stresses [12,13,14]. Recent evidence indicates that A. nodosum extracts can stimulate the expression of genes related to stress response, increase antioxidant activity, and promote root development under adverse conditions [15].

Although several studies have demonstrated the positive effects of seaweed extracts on plants subjected to water stress, investigations that specifically evaluate their performance during the germination phase and distinguish the effects of the complete extract from those attributable exclusively to the mineral fraction present in the commercial formulation are still limited. This distinction is essential to understand whether the observed benefits result predominantly from nutritional input or from the action of bioactive organic compounds with regulatory activity.

In this context, the present study aimed to evaluate the bioactivity of Ascophyllum nodosum extract in inducing tolerance to water stress in maize seeds (Zea mays L.), during the germination phase, under different osmotic potentials induced by PEG-6000. Additionally, it sought to compare the effects of the complete extract with those of its isolated mineral fraction, in order to elucidate the role of bioactive compounds in modulating initial physiological responses under water restriction.

2. Materials and Methods

The study was conducted at the Mineral Nutrition Laboratory of the Federal University of Espírito Santo (UFES), São Mateus campus, under controlled germination conditions. maize seeds (*Zea mays* L.), cultivar AL Bandeirante, with a semi-early cycle, from a single commercial batch produced in 2024, were used in order to minimize physiological variations between batches. The seeds were previously treated with a Captan®-based fungicide (0.2%) and stored in kraft paper bags, in a dry environment protected from light until the experiments were set up.

The study was divided into three independent bioassays; the first bioassay aimed to determine the osmotic potential level most limiting to germination. Five osmotic potentials were evaluated: 0 (control), -0.2, -0.4, -0.6, and -0.8 MPa, obtained using polyethylene glycol 6000 (PEG 6000) solutions, according to the methodology described by Farsiani and Ghobadi (2009). The PEG-6000 concentrations were calculated using the equation proposed by Michel and Kaufmann (1973), $\Psi os = - (C) - (C2) + (CT) + (C2T)$, where: Ψos = osmotic potential (bar); C = concentration (grams of PEG

6000/Kg of distilled water); and T = temperature (°C), which was considered to be 25 °C. The quantities used to obtain the respective osmotic potentials are shown in Table 1.

Table 1. Quantities of PEG-6000 used to obtain the respective osmotic potentials.

Osmotic potential (MPa)	Concentration (g PEG 600 / L H_2O)
0	0.000
-0.2	119.570
-0.4	178.350
-0.6	223.666
-0.8	261.950

In the second bioassay, the seeds were pre-treated with five doses of the commercial seaweed extract-based product and with five doses of a solution containing exclusively the mineral fraction of the product. The doses evaluated were 0 (control), 0.5, 1.0, 1.5, and 2.0 mL kg^{-1} of seeds. The objective was to verify the effect of the pre-treatment in mitigating the damage caused by water deficit. The composition of the commercial product is shown in Table 2.

Table 2. Mineral composition of the biofertilizer.

Parameters	Unit	Quantity
Total nitrogen	%m/m	2.19
Total phosphorus	%m/m	0.3059
Total potassium	%m/m	5.71
Total calcium	%m/m	0.1305
Total magnesium	%m/m	0.0307
Sulfur	%m/m	1.925
Iron	%m/m	0.04444
Zinc	%m/m	0.0027
Copper	%m/m	0.0007
Manganese	%m/m	0.0047
Boron	%m/m	0.0042

Source: Pires et al. [16].

A solution containing the mineral fraction of the biostimulant was prepared, which was subdivided into three solutions due to the chemical incompatibility of the elements. The reagents and quantities shown in Table 3 were used to prepare this fraction. The fraction was prepared to isolate the effect of the algae extract from the effect of the mineral elements.

Table 3. Composition of the Mineral Fraction Solution.

Solution	Reagent	Quantity (g)	Makes available (%m/m)
Solution A	$ZnSO_4$	0.0118	0.0027 of Zn and 0.000132 of S
	$CuCl_2$	0.0019	0.0070 of Cu e 0.000388 of Cl
	$MnSO_4$	0.0144	0.0047 of Mn and 0.00027 of S
	$H3BO_3$	0.024	0.0042 of B
	CH_4N_2O	1.11457	0.5293 of N
	$CaSO_4$	0.56	0.1305 of Ca and 0.104 of S
	NKS	12.689	1.5227 of N, 0.1523 of S and 5.71 de K
Solution B	$FeCl_3$	0.215	0.0444 of Fe and 0.0845 of Cl

	$MgSO_4$	0.311	0.0307 of Mg and 0.0405 of S
	Na_2SO_4	7.288	1.1627 of S and 1.1792 of Na
Solution C	H_6NO_4P	1.136	0.138 of N and 0.3059 of P

Source: Pires et al. [16].

In the third bioassay, after defining the most effective doses based on the previous results, the seeds were subjected to three germination conditions: (1) moderate water deficit, (2) severe water deficit, and (3) absence of stress. The water deficit levels were established based on the analysis of the results obtained in the first experiment. Each product (complete extract and mineral fraction) was considered an independent assay, conducted in a 3 × 5 factorial scheme, consisting of three germination environments and five doses of the product or mineral fraction, considering the untreated seeds as a control.

In all bioassays, four replicates of 50 seeds per treatment were used. The seeds, previously treated with Captan® 0.2% fungicide, were distributed in rolls of Germitest® paper (three sheets per replicate), moistened with PEG-6000 solution in a proportion of 2.5 times the weight of the dry substrate. The rolls were placed in a B.O.D. type germination chamber, regulated at 25 °C, under a 12-hour photoperiod. Evaluations were carried out four and seven days after sowing, determining: percentage of normal seedlings (%PNS), abnormal seedlings (%AS), epicotyl length (EL) and primary radicle length (RL).

The experimental design adopted was completely randomized, with four repetitions of 50 seeds per treatment. The data were subjected to analysis of variance using the Sisvar software [17]. When significant, the means of the qualitative treatments were compared by Tukey's test, and the quantitative variables were subjected to polynomial regression analysis, selecting the models based on the significance of the coefficients and the coefficient of determination (R^2), adopting a significance level of 5% ($p \leq 0.05$).

3. Results

3.1. Bioassay 1

The results of the analysis of variance for the variables evaluated under osmotic stress induced by PEG-6000 are presented in Tables 4. Four days after sowing, a highly significant effect of the treatments was observed for all variables analyzed.

Table 4. Summary of the analysis of variance with source of variation (SV), degrees of freedom (DF) and mean square for the characteristics germination (%G), normal seedlings (%PNS), abnormal seedlings (%AS), radicle length (RL), epicotyl length (EL) and vigor of maize seeds treated with doses of *Ascophyllum nodosum* at four days under stress with PEG-6000.

SV	DF	%G	%PNS	%AS	RL	EL	Vigor
		Mean square					
Doses	4	8424.50***	6509.50***	2009.50***	10.57***	0.972***	196901.80***
Repetition	3	12.00	213.00	213.00	0.215	0.0036	2107.462
Residue	12	19.16	172.16	172.16	0.121	0.0027	1038.24
CV (%)		8.93	20.99	74.98	24.38	20.69	22.18

Legend:*** $p < 0.001$ by the F-test. CV (%) = coefficient of variation.

At seven days, no statistically significant differences were observed between the applied doses. The coefficients of variation indicated good experimental precision at four days and greater variability at seven days. At four days, the number of germinated seedlings decreased with increasing doses of PEG-6000, showing a significant linear adjustment, evidencing a proportional reduction in germination with the decrease in osmotic potential.

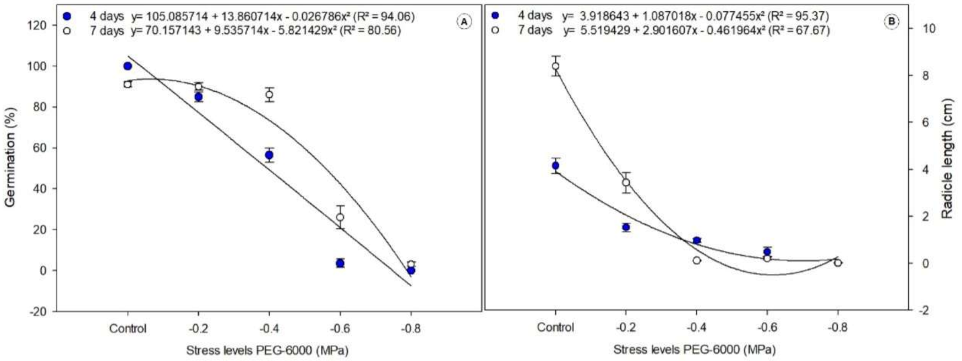

Figure 1. Effect of water stress levels induced by PEG-6000 (MPa) on germination (%) (A) and radicle length (cm) (B), evaluated at 4 and 7 days. Points represent means ± standard error. Lines correspond to quadratic regression fits for each period.

At four days, radicle length adjusted to the quadratic model as a function of PEG-6000 doses. The greatest growth was observed in the control (0 MPa), with 4.15 cm, with progressive reduction at −0.2 and −0.4 MPa, a sharp drop at −0.6 MPa, and total inhibition at −0.8 MPa (Figure 1B). At seven days, there were no significant differences between osmotic potentials.

Epicotyl length and the percentage of vigorous seedlings also showed a significant quadratic adjustment at four days, with a more intense reduction at the more negative potentials. These results, together with Table 4 and Figure 1, allowed us to define the moderate and severe stress levels used in Bioassay 3.

3.2. Bioassay 2

The results of the analysis of variance at four and seven days after sowing are presented in Tables 5 and 6. Germination (%) was not significantly influenced by the treatments at either evaluation time, both at four and seven days after sowing. In both evaluations, the treatments showed high and similar values, indicating that the doses of the seaweed extract-based product and the solution containing exclusively the mineral fraction did not compromise the germination potential of the seeds, regardless of the evaluation time.

At four days, a significant effect of the doses was observed only for epicotyl length (EL) and vigor, while the other variables did not show a statistical difference.

Table 5. Summary of the analysis of variance with source of variation, degrees of freedom and mean square for the characteristics germination (%G), normal seedlings (%PNS), abnormal seedlings (%AS), radicle length (RL), epicotyl length (EL) and vigor of maize seeds treated with doses of *Ascophyllum nodosum* at four days under stress with PEG-6000.

FV	GL	%G	%PNS	%AS	RL	EL	Vigor
		Mean square					
Doses	4	19.70ns	652.70ns	652.70ns	1.65ns	2.43**	52717.86*
Repetition	3	19.73	415.66	415.66	0.371	0.714	6448.58
Residue	12	7.56	434.16	434.16	0.973	0.339	16068.86
CV (%)		2.91	23.70	172.20	13.03	25.22	13.61

Legend: ns = not significant; * $p < 0.05$ by the F-test. CV (%) = coefficient of variation.

At seven days, there was a significant effect of the doses on normal seedlings (%PNS), abnormal seedlings (%AS), radicle length (RL), epicotyl length (EL), and vigor, while germination (%G) was not influenced, although a replication effect was observed in this evaluation.

The coefficients of variation indicated good experimental precision for germination and growth variables at seven days, with greater variability for abnormal seedlings. At four days, there was no significant effect of the treatments on normal and abnormal seedlings, maintaining a pattern similar to that observed for germination at that time.

Table 6. Summary of the analysis of variance with source of variation (SV), degrees of freedom (DF) and mean square for the characteristics germination (%G), normal seedlings (%PNS), abnormal seedlings (%AS), radicle length (RL), epicotyl length (EL) and vigor of maize seeds treated with doses of *Ascophyllum nodosum* at seven days under stress with PEG-6000.

SV	DF	%G	%PNS	%AS	RL	EL	Vigor
		Mean square					
Doses	4	12.30ns	574.20***	22.70***	2.31*	1.77*	1294067.62***
Repetition	3	32.26	18.13	3.46	1.65	0.217	54274.57
Residue	12	5.76	37.80	2.30	0.571	0.500	106033.89
CV (%)		2.55	7.02	108.33	8.10	12.21	30.30

Legend: ns = not significant; * $p < 0.05$; *** $p < 0.001$ by the F-test. CV (%) = coefficient of variation.

Conversely, seven days after sowing, the number of normal and abnormal seedlings was significantly influenced by the applied doses. For the percentage of normal seedlings (PNS%), a significant fit to the quadratic regression model was observed, characterized by an increase up to intermediate doses, followed by a reduction at the highest concentrations (Figure 2), evidencing a dose-dependent response, with a point of maximum efficiency at a dose of 0.45 mL kg^{-1} of seeds.

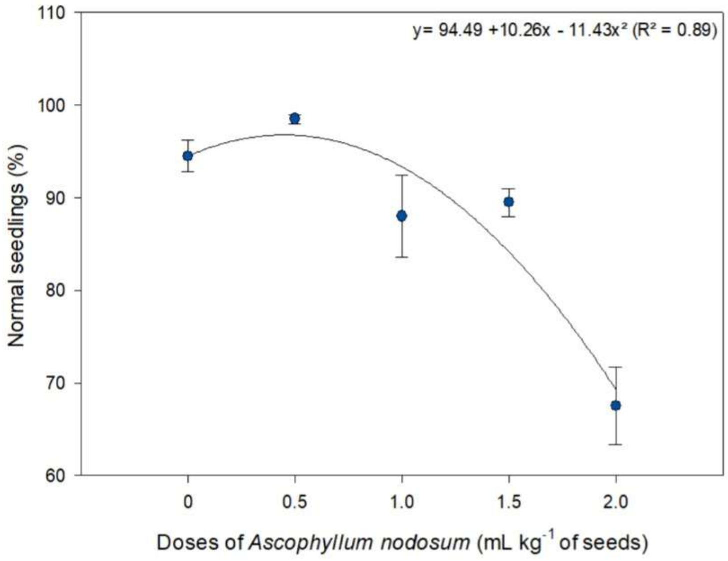

Figure 2. Effect of *Ascophyllum nodosum* doses on the percentage of normal seedlings at 7 days after sowing. Bars represent mean ± SE.

The percentage of normal seedlings showed a significant quadratic adjustment to the doses of Ascophyllum nodosum, with an increase up to the intermediate dose and a reduction at the highest concentrations. The point of maximum technical efficiency was estimated at 0.45 mL kg^{-1}, with a predicted value of 96.8%, while at the dose of 2.0 mL kg^{-1} there was a reduction to approximately 68%.

The percentage of abnormal seedlings did not fit regression models; however, the comparison of means indicated lower values at the applied doses (close to zero) compared to the control (5.5%), with a statistical difference. Epicotyl length showed a significant quadratic adjustment at four and seven days (Figure 3), with a reduction at intermediate doses and an increase at the highest concentrations, characterizing a dose-dependent response at both evaluation times.

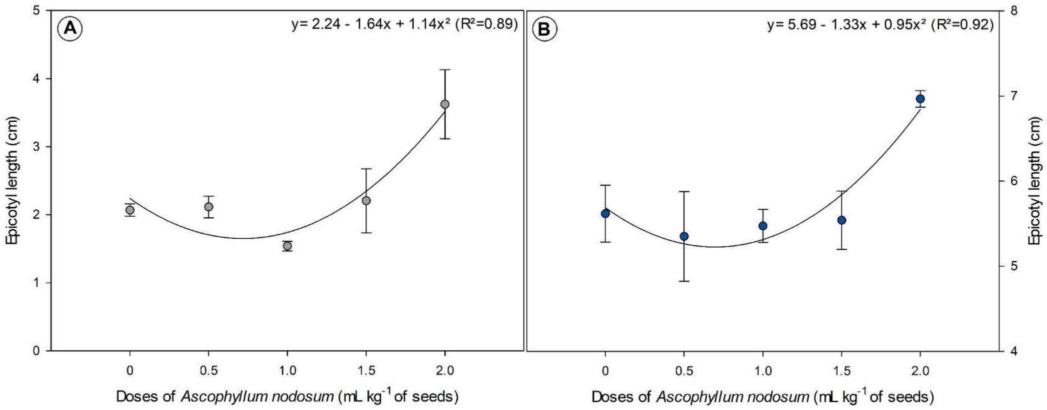

Figure 3. Epicotyl length of seedlings as affected by *Ascophyllum nodosum* doses at 4 days (A) and 7 days (B) after sowing. Data were fitted to quadratic regression models. Bars represent mean ± standard error.

Root length was not influenced by the doses at four days. At seven days, there was a significant effect, with a quadratic adjustment (Figure 4), showing an increase up to 0.66 mL kg^{-1} and a reduction at the highest concentrations. The values were higher at seven days, reflecting the advancement of development, with moderate experimental variability. Thus, the effect of the doses on root growth was dependent on the evaluation time, manifesting itself only in the final evaluation.

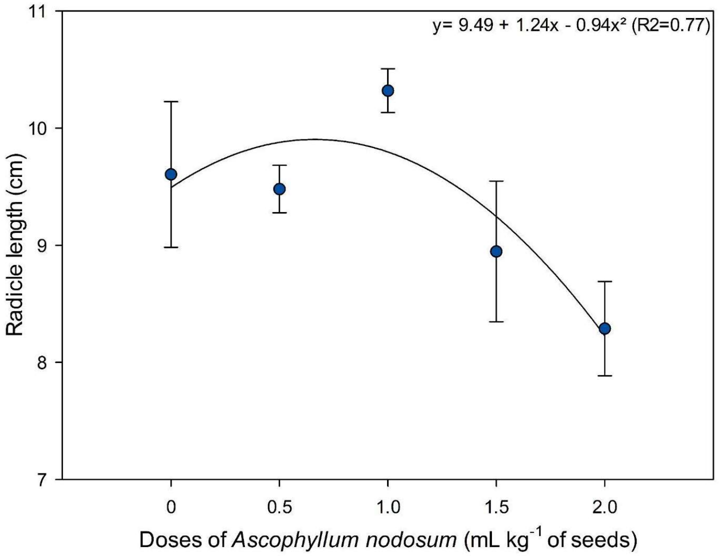

Figure 4. Radicle length as affected by *Ascophyllum nodosum* doses at 7 days after sowing. A quadratic regression model was fitted to the data. Bars represent mean ± SE.

At seven days, radicle length was influenced by *Ascophyllum nodosum* doses, with a quadratic adjustment ($R^2 = 0.77$). There was an increase up to 0.66 mL kg^{-1}, followed by a reduction at the dose of 2.0 mL kg⁻¹, indicating better performance at intermediate concentrations and a possible limiting effect at higher concentrations. The vigor index at four days also showed a significant quadratic adjustment (Figure 5), with a reduction at intermediate doses and an increase at higher concentrations, evidencing a non-linear and dose-dependent response in the initial germination phase.

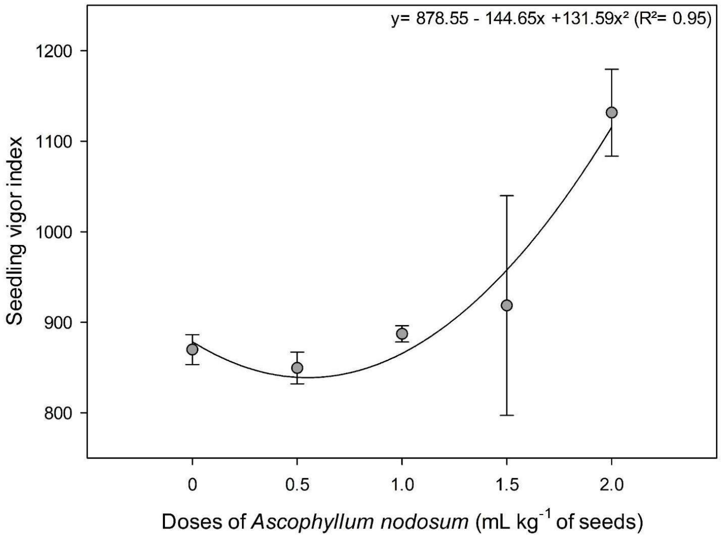

Figure 5. Seedling vigor index as affected by *Ascophyllum nodosum* doses at 4 days after sowing. Data were fitted to a quadratic regression model. Bars represent mean ± standard error.

Seven days after sowing, the vigor index was significantly influenced by the treatments, with the comparison of means performed using Tukey's test, since no significant fit was observed to regression models for this variable. The treatment corresponding to the dose of 1.0 mL kg⁻¹ presented the lowest vigor index (95.0), differing statistically from the others.

In contrast, the doses of 0.5 mL kg⁻¹ (1361.2), 1.5 mL kg⁻¹ (1058.8) and 2.0 mL kg⁻¹ (1420.3), as well as the control (1438.7), presented the highest vigor index values, not differing from each other. These results indicate that, in the final evaluation, the intermediate and higher doses, as well as the control, provided greater seedling vigor when compared to the lowest dose evaluated.

3.3. Bioassay 3

Analysis of variance indicated a significant effect of the treatments on some of the variables, highlighting an interaction between germination conditions and seaweed extract doses. The effects were more pronounced on growth and vigor variables, especially at seven days, indicating an intensification of the physiological response throughout the initial development. The germination percentage was influenced by the treatments (Figure 6). At four days, the differences were mainly related to the germination speed, while at seven days there was a tendency for the averages to stabilize, reflecting the final germination potential under each condition.

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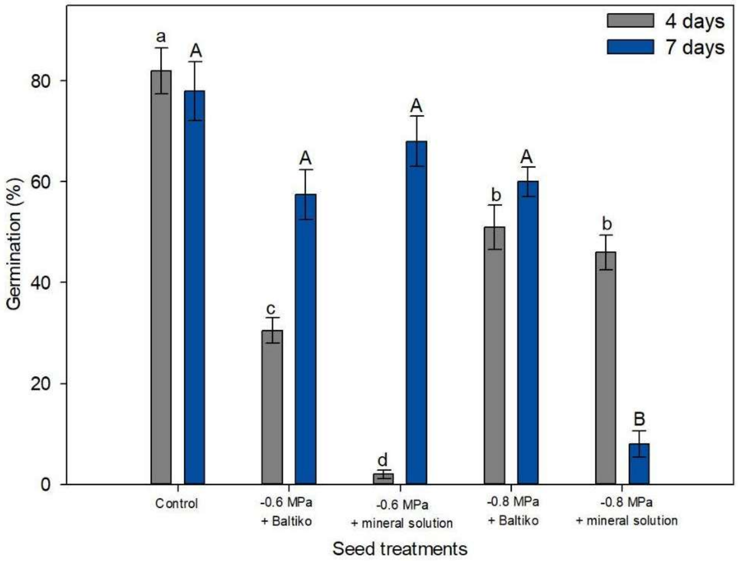

Figure 6. Germination (%) under different seed treatments at 4 and 7 days after sowing. Bars represent mean ± SE. Different lowercase (4 days) and uppercase (7 days) letters indicate significant differences among treatments according to Tukey's test (p ≤ 0.05).

The quality of the seedlings, expressed by the percentages of normal and abnormal seedlings (Figure 7), showed significant differences between treatments, with distinct responses between evaluation periods. The higher proportion of normal seedlings in certain treatments indicates a positive effect on structural development, while the reduction in abnormal seedlings suggests partial mitigation of the effects of water stress.

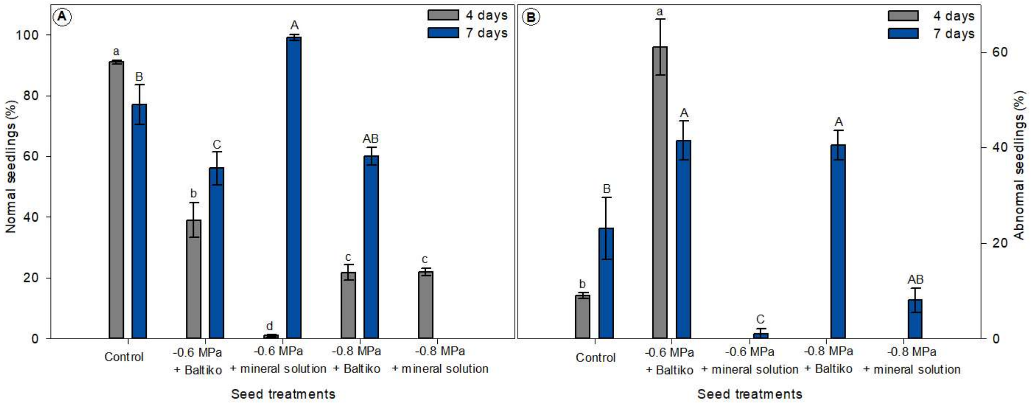

Figure 7. Percentage of normal (A) and abnormal (B) seedlings under different seed treatments at 4 and 7 days after sowing. Bars represent mean ± SE. Different lowercase letters (4 days) and uppercase letters (7 days) indicate significant differences among treatments according to Tukey's test (p ≤ 0.05). Analyses were performed separately for each evaluation time.

Initial growth was significantly affected by the treatments, as observed for epicotyl and radicle length (Figure 8). The differences were more pronounced at seven days, indicating that the physiological modulation promoted by the seaweed extract is more evident as development progresses. The behavior of the variables suggests a dose-dependent response, a typical characteristic of biostimulants.

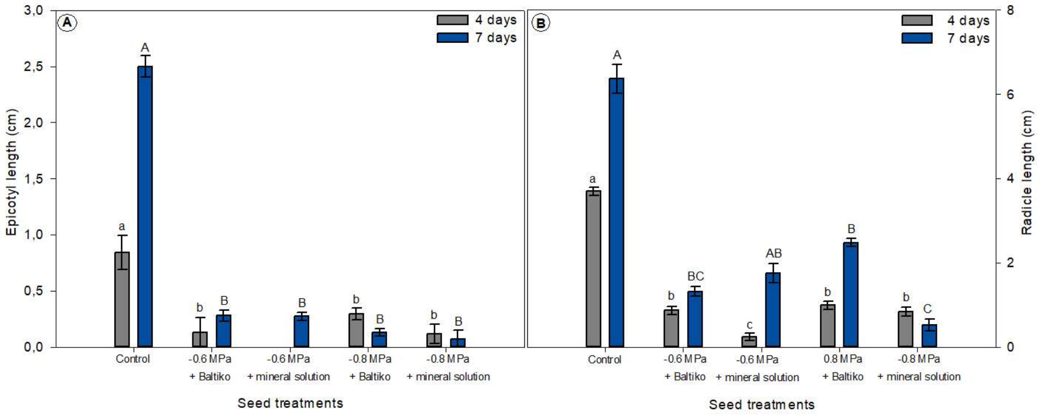

Figure 8. Epicotyl length (A) and radicle length (B) of seedlings as affected by seed treatments at 4 and 7 days after sowing. Bars represent mean ± standard error. Different lowercase letters indicate significant differences among seed treatments at 4 days, and different uppercase letters indicate significant differences among seed treatments at 7 days (Tukey’s test, $p \leq 0.05$).

The seedling vigor index (Figure 9), by integrating germination and growth, synthesizes the effects observed in the other variables. The significant differences between treatments confirm that the use of seaweed extract influenced the physiological performance of the seedlings in an integrated manner, especially under water restriction conditions.

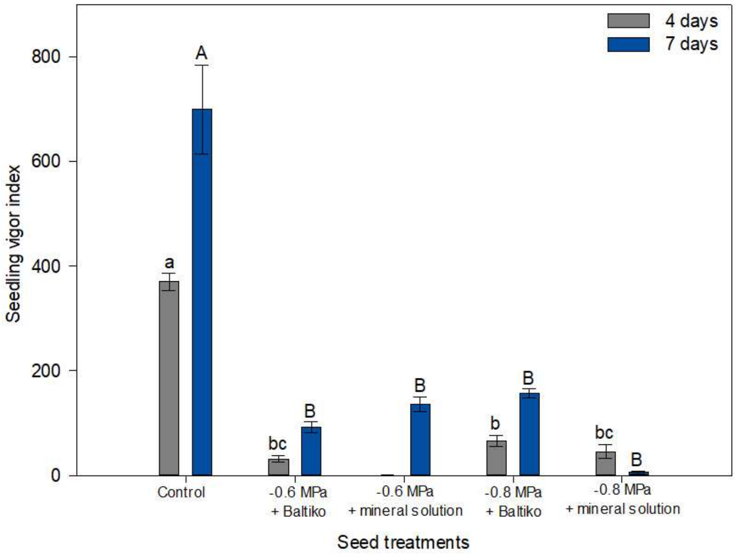

Figure 9. Seedling vigor index as affected by seed treatments at 4 and 7 days after sowing. Bars represent mean ± standard error. Different lowercase letters indicate significant differences among seed treatments at 4 days, and different uppercase letters indicate significant differences among seed treatments at 7 days (Tukey’s test, $p \leq 0.05$).

Taken together, Figures 6 to 9 show that seed treatment with Ascophyllum nodosum extract promotes relevant physiological changes in the initial phase of maize development, with responses varying depending on the dose applied and the evaluation time.

4. Discussion

The results of Bioassay 1 demonstrate that osmotic stress induced by PEG-6000 significantly reduced germination and initial seedling growth, especially four days after sowing. This response confirms that the reduction in water potential restricts imbibition and delays metabolic activation, compromising the initial developmental processes [7]. The progressive decline in germination with increasing PEG concentrations is consistent with previous reports in agricultural crops subjected to water restriction [3, 5, 6].

The quadratic adjustment observed for radicle length suggests the existence of a physiological threshold beyond which water limitation exceeds the seedling's adaptive capacity. The reduction in root elongation at more negative osmotic potentials may be associated with the restriction of cell expansion in the elongation zone, a process dependent on the maintenance of turgor and regulated by hormonal interactions involving auxins, abscisic acid (ABA), and redox signaling [6,18]. Recent evidence indicates that water deficit modulates gene expression related to cell division and expansion, promoting structural and metabolic adjustments in the root system [18,19].

Impaired epicotyl growth and vigor under osmotic stress may also reflect alterations in hormonal balance and increased production of reactive oxygen species (ROS), which act as signaling molecules but can cause cellular damage when accumulated in excess [6]. The smaller difference between treatments at seven days suggests that only seedlings with greater osmotic adjustment capacity maintained active growth, possibly through compensatory mechanisms such as accumulation of compatible solutes and hormonal reprogramming.

In Bioassay 2, the doses of seaweed extract did not affect the germination percentage, indicating an absence of deleterious effects on seed viability, in agreement with the literature [20,21]. However, the variables related to growth showed dose-dependent responses. The vigor index fit a quadratic model, with a slight reduction near 0.5 mL kg^{-1}, followed by a progressive increase from 1.0 mL kg^{-1} and maximum values at the dose of 2.0 mL kg^{-1}. This pattern is characteristic of biostimulants, in which suboptimal concentrations may not fully activate physiological pathways, while adequate doses favor the mobilization of reserves and hormonal signaling. Although higher doses promoted an increase in vigor, reductions observed in some structural variables indicate the existence of an optimal application range.

Epicotyl length showed a significant quadratic response at both evaluated times, with a greater increase at higher doses, especially at seven days. The radicle responded significantly only at seven days, also with quadratic behavior and an optimum point at an intermediate dose. These results indicate concentration-dependent physiological modulation, consistent with the action of bioactive compounds present in the extract, such as substances with phytohormone-like activity and secondary metabolites [13,14]. Promoting effects on initial growth have been reported in different cultivated species [22,23].

The quadratic response observed for normal seedlings, with a reduction at higher doses, reinforces the existence of a window of physiological efficiency, typical of biostimulants with action related to hormonal mechanisms [11, 24]. The greater sensitivity of growth variables at seven days indicates that the expression of biostimulant effects intensifies with the advancement of initial establishment and metabolic activity [21,25].

Comparatively, Bioassay 1 showed that isolated osmotic stress compromised germination and growth, while the seaweed extract maintained germination and positively modulated growth variables, reinforcing evidence of the physiological potential of biostimulants under adverse conditions [12,15].

In Bioassay 3, seed treatment with *Ascophyllum nodosum* extract under water deficit primarily influenced the physiological quality and vigor of seedlings, more than the final germination percentage. The maintenance of initial performance under osmotic restriction suggests partial mitigation of stress effects, as reported for algae extracts [3,12,14]. The increase in the proportion of normal seedlings and the increase in root growth at intermediate doses indicate positive modulation of structural development, possibly associated with hormonal regulation and attenuation of oxidative stress [25,14]. Similar results were described by Jannin et al. [15] in plants treated with *A. nodosum*.

In general, the recurring quadratic patterns observed for epicotyl, radicle, and normal seedlings confirm a dose-dependent response, highlighting the importance of defining an optimal application range [11,12]. Additionally, the superior performance of the complete extract compared to the mineral fraction suggests that the observed effects are predominantly due to bioactive organic compounds, and not solely to nutritional input. Thus, seed treatment with *Ascophyllum nodosum* represents a promising strategy to improve the initial performance of maize under water deficit, provided that the doses are adequately optimized.

5. Conclusions

The results demonstrate that water stress induced by PEG-6000 significantly compromises germination and, above all, the initial growth of maize seedlings, highlighting the high sensitivity of this phase to water deficit. The reduction in osmotic potential mainly affected root elongation, epicotyl development, and vigor index, indicating that water limitation directly interferes with cell expansion processes and initial physiological establishment.

Seed treatment with *Ascophyllum nodosum* extract promoted dose-dependent physiological responses, especially in variables related to growth and vigor. The recurring quadratic pattern shows the existence of an optimal application range, in which the biostimulant effects are maximized. In general, intermediate doses provided better physiological performance, with increased root growth and a higher proportion of normal seedlings.

Under water stress, the complete extract showed superior performance to the mineral fraction, suggesting that the observed effects are predominantly associated with the presence of bioactive organic compounds, and not only with nutritional contribution. Thus, seed treatment with *Ascophyllum nodosum* appears to be a promising strategy to mitigate the effects of water deficit in the initial phase of maize. However, the precise definition of the optimal dose is crucial to maximize physiological benefits and ensure agronomic efficiency.

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Abbreviations

The following abbreviations are used in this manuscript:

%G	Germination
%PNS	Percentage of normal seedlings
%AS	Abnormal seedlings
EL	Epicotyl length
RL	Radicle length

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