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Research Article

Effect of commercial seaweed and amino acid-derived biostimulants on germination, metabolism indices and growth of canola, green bean, barley and lettuce under heat stress

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Abstract

The current study aimed to determine the role of two commercial biostimulants, Algabon® (seaweed extract-derived) and Bonamid® (containing amino acid (85%)), on improving heat stress during the germination stage and seedling growth of 4 different crops, canola, green bean, barley and lettuce, using a factorial design within a completely randomized design. For this purpose, seeds were pretreated for 2 hours in an aqueous solution of 0.5 g/l Algabon® and 2 g/l Bonamid®. Then, the seeds were incubated in the dark for 72 hours at 28°C (control) and 35°C (heat stress). The results showed that heat stress (temperature of 35°C) significantly reduced all germination and growth indices, the activity of α-amylase and protease enzymes and radical scavenging activity (RSA), while increasing the amount of soluble carbohydrates and H_2O_2 content in the seedlings of barley and lettuce. In contrast to the results for barley and lettuce seedlings, heat stress caused an increase in all germination and growth indices, the activity of α-amylase and protease enzymes and RSA, however reduced H_2O_2 content in the seedlings of canola and green beans. In all the four species examined, under both control and stress conditions, the germination and growth indices, the activity of α-amylase and protease enzymes and RSA, and the amount of soluble carbohydrates were increased by the Algabon®. The reduction of H_2O_2 production under heat stress was observed in the seedlings of canola, green bean, barley and lettuce pre-treated with Algabon® and Bonamid®. In this study, we observed a composition-dependent effect of biostimulants in diminishing the effects of heat stress, so that Algabon® was more effective than Bonamid®. According to the results of the absolute decrease index, the lowest inhibition index and the highest relative heat tolerance index were observed in canola seeds pre-treated with Algabon®, which were identified as the most heat-resistant seedlings. In general, it seems that the pre-treatment of seeds with Algabon® by producing some compatible osmolyte compounds and stimulating the activity of enzymes involved in germination increases the germination performance and makes the seeds of canola, green bean, barley, and lettuce resistant to heat stress.

Keywords: Amylase, Biostimulant, Heat stress, Protease, Resistance indices

Introduction

Biostimulants are organic substances or microorganisms that enhance nutrient use efficiency, tolerance to abiotic stress, quality traits, and availability of confined nutrients in the soil or rhizosphere. These substances include seaweed extracts, hydrolyzed proteins, humic and fulvic acids, and all natural origin compounds that play a role beyond nutrition, leading to increased growth and reduced stress (Bulgari *et al.*, 2019). Seaweed extracts are rich in phytohormones, minerals, and polysaccharides that promote plant growth (du Jardin, 2015). Protein hydrolysates are a mixture of oligopeptides, polypeptides, and amino acids formed by the hydrolysis of plant proteins (Colla *et al.*, 2017) that promote plant growth (Amirkhani *et al.*, 2016), improve yield and quality (Colla *et al.*, 2017). Numerous

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investigations suggest that seaweed extracts improve germination (Hernandez-Herrera *et al*., 2014, 2016) and enhance crop resistance to abiotic stress, such as heat (Zhang and Ervin, 2008; Carmo *et al*., 2021).

The germination stage is one of the important phases in the growth cycle of a plant, determining its final growth and performance, however, this stage is the most vulnerable to various stress factors (Gupta *et al*., 2022). Biostimulants pre-treatment result in faster germination and greening, which have practical and experimental agronomic consequences, especially under unfavorable conditions (Yildirim *et al*., 2000). Temperature changes significantly affect seed germination by inhibiting the emergence of the radicle and post-germination growth in seedlings (Probert, 2000). The seed priming approach is an alternative tool for preparing plants to cope with stress conditions (Filippou *et al*., 2013). For this reason, the use of biostimulants to overcome heat stress has become an important method for maintaining final product yield (Bulgari *et al*., 2019; Campobenedetto *et al*., 2020). Recent studies have demonstrated the positive effects of biostimulants on increasing antioxidant enzyme activity, improving growth, yield, and health in various agricultural plants. For example, Campobenedetto *et al*. (2020) investigated the effect of KIEM as a biostimulant on cucumber seed germination at two standard temperatures (28°C) and a stress temperature (35°C) for 48 hours. Their results showed that heat stress creates oxidative stress conditions in seeds, leading to excessive ROS production. On the other hand, treated seeds exhibited a significant reduction in H_2O_2 levels, decreased oxidative stress effects, increased heat stress tolerance, and ultimately were able to enhance germination percentage and restore oxidative balance. Additionally, Masondo *et al*. (2018) examined the potential role of seed priming with Kelpak® (commercial seaweed extract) under abiotic stress during seed germination and seedling growth of *Ceratotheca triloba*. Low temperature (10°C) completely inhibited seed germination; however, changing the temperature to 15°C improved germination. Primed seeds were germinated at low temperatures, low osmotic potential, and high NaCl concentrations. However, the effectiveness of biostimulants can vary among different crop genotypes and environmental conditions (Malik *et al*., 2021). Therefore, it is necessary to determine the efficacy of different biostimulant products on specific crops before applying them on a large scale (Jimenez-Arias *et al*., 2022). The agronomic potential of different biostimulants has not been widely investigated for crops in our country. In our study, we selected four seeds from the west of Iran to test our hypotheses. Studies have indicated a correlation between seed reserve composition and germination rates (Zhao *et al*., 2018); therefore, seeds with the most prevalent storage reserve of starch (barley), protein (green bean), or lipid (canola and lettuce) were chosen in our study. The objective of this study was to investigate the effectiveness of two biostimulants, Algabon® (seaweed extract-derived) and Bonamid® (containing amino acid (85%)), on germination indices, growth, and metabolism of 4 crop types, canola, canola, green bean, lettuce and barley, and to investigate the effectiveness of these biostimulants in response to the heat tolerance of four crops.

Materials and methods

The experiment was conducted in a factorial design within a completely randomized design with three replications. The experiment was made up of three factors: a) crop types (canola, green beans, lettuce, and barley); b) biostimulant types (water, Algabon®, and Bonamid®); and c) temperature treatments (non-stress at 28°C and heat stress at 35°C). Green bean seeds were obtained from the agricultural research center of Lordegan County, while canola (*Brassica napus*) and barley seeds were sourced from the agricultural research center of Borujen County in Chaharmahal and Bakhtiari province. Lettuce seeds were purchased from Pakan Seed Company. Seeds were stored in cloth bags at room temperature until the experimental procedure began.

In this study we used two biostimulants, Algabon® and Bonamid®, available in the market (Bon Asia Cultivation Company), each with very different formulations. Algabon®, a biostimulant derived from seaweed extract, contains compounds including alginic acid (18%), NO_3 (1%), K_2O (16%), H_2PO_4 (1%), while Bonamid®, an amino acid biostimulant, contains amino acids (85%) and nitrogen (4%).

Sterilized seeds of each species were separately soaked in an aqueous solution of 0.5 g/l of Algabon® and 2 g/l of Bonamid® (the concentrations recommended by the manufacturer) for 2 hours at 25°C. After pre-treatment, the 50 seeds of each plant were dried at room temperature and then placed in 9 cm Petri dishes with two layers of filter paper moistened with distilled water. The seeds treated with the same procedure but using distilled water instead of biostimulants served as the control. The Petri dishes were then incubated separately in the dark under non-stressed (28°C) or heat-stressed (35°C) conditions for 4 days (Campobenedetto *et al*., 2020) in an incubator (Memmert). After 4 days, germination and growth indices, germination metabolism, and resistance indices were measured.

Germination rate: The total number of seeds that germinated after 4 days for each petri dish was considered the final germination percentage (Abdelkader *et al*., 2023):

$$GP=N/N'\times100$$

Where: GP = Germination percentage, N = Number of germinated seeds, N′ = Total number of seeds.

From each Petri dish, 7 seedlings were randomly selected, and the indices of length, fresh weight, and dry weight of the seedlings were measured.

Additionally, germination metabolism was assessed by evaluating the activity of the enzyme's alpha-amylase

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and protease, as well as the content of soluble carbohydrates during the early stages of germination (after 4 days).

Measurement of alpha-amylase activity: The activity of the alpha-amylase enzyme was measured according to the method described by Roberts and Whitehouse (1976). Initially, 0.5 g of germinated seeds were ground and homogenized in 1.5 ml of cold distilled water in a mortar. The intensity of the blue color in the samples was read using a spectrophotometer at a wavelength of 620 nm. The activity of the alpha-amylase enzyme in the samples was calculated by plotting a standard curve of starch and expressed as a percentage of hydrolyzed starch.

Measurement of protease activity: The activity of the protease enzyme was measured according to the methods described by Salmia *et al.* (1978) and Haroun and Hussein (2003). Initially, 0.3 g of germinated seeds were ground and homogenized in 1.5 ml of 0.2 mM phosphate buffer (pH 7) in a mortar. The proteolytic activity of the enzyme was estimated after adding Folin's reagent in a 1:2 ratio to the samples and reading the absorbance at a wavelength of 660 nm. The enzyme activity was reported as µM of tyrosine released due to casein degradation per gram of fresh weight.

Measurement of soluble carbohydrates: The measurement of soluble carbohydrates in 0.1 g of germinated seeds was performed using the method of Poorter and Villar (1997). This method is based on spectrophotometry and the measurement of the intensity of the color produced from the reaction of sugars with the anthrone reagent.

Determination of antioxidant capacity: The evaluation of DPPH radical (1,1-diphenyl-2-picrylhydrazyl) scavenging capacity was conducted by preparing 500 µl of ethanolic extract from germinated seeds (0.5 g) in a test tube (Kulisic *et al*., 2004). The capacity to scavenge free radicals in the samples was calculated as follows:

Radical scavenging activity (%) = 100 × (Absorbance of control-absorbance of sample / absorbance of control)

Measurement of H_2O_2: The measurement of H_2O_2 was performed using the method of Alexieva *et al*. (2001).

Measurement of heat resistance indices: Absolute decrease (AD), inhibition index (II) and relative heat tolerance (RHT) were standard criteria used to determine the performance of plants subjected to heat stress, including final germination percentage under non-stress conditions ($PG_{Control}$) and final germination percentage under heat stress (PG_{Heat}) (Bolton and Simon, 2019). These measurements were calculated using the following relationships: AD $=PG_{Control}-PG_{Heat}$; II= $100\times PG_{Control}$ $(PG_{Control} - PG_{Heat})$ and RHT = $PG_{Control} / PG_{Heat}$

Statistical analysis: The experiments were conducted in a factorial within a completely randomized design with three replications. The data were analyzed using the GLM method in SAS statistical software (version 9.4; SAS Institute Inc., Cary, NC, United States). The LSD post hoc test was used to determine significant differences between treatments. The significance level was set at $\alpha \leq 0.05$. Besides, a biostimulant × heat stress × trait-biplot analysis was performed using Origin 2024 software.

Results

Effect of biostimulants on germination and seedling growth in canola, green bean, barley and lettuce under heat stress: The results obtained from the analysis of variance of germination data, dry and fresh weight, root and shoot length, hydrogen peroxide levels, free radical scavenging activity, soluble carbohydrates, and the activity of amylase and protease enzymes are shown in Table 1.

According to figure 1, the highest germination percentage was measured at 35°C (canola and green bean) and 28°C (barley and lettuce) under pre-treatment conditions with the biostimulant Algabon®. Among all pre-treatments, seeds pre-treated with water showed the lowest germination percentage. The highest fresh and dry weight in green bean and barley was measured at 35°C and 28°C, respectively, under pre-treatment with the biostimulant Algabon® (Figures 2, 3). The dry weight of green bean and barley increased significantly by 3.5 and 2.1 times compared to the control. The biostimulant Algabon® led to an increase in other seedling growth indices compared to water and Bonamid® pre-treatments. For example, pre-treatment with Algabon® in barley (at 28°C) and green bean (at 35°C) resulted in the highest root length, while in canola (at 35°C) and lettuce (at 28°C), it resulted in the highest length of aerial parts (Figures 4, 5).

Effect of biostimulants on ROS metabolism and soluble sugars in canola, green bean, barley and lettuce under heat stress: Under heat stress conditions, the hydrogen peroxide level in lettuce seedlings pre-treated with the two biostimulants Algabon® and Bonamid® decreased compared to the control condition. At 35°C, the highest hydrogen peroxide level was observed in seeds pre-treated with water. No significant difference was observed in hydrogen peroxide levels in barley seedlings under different pre-treatments at both 28°C and 35°C. In contrast, in canola and green bean plantlets, the highest hydrogen peroxide levels were observed at 28°C, and pre-treatment of canola seeds with Algabon® and Bonamid® led to a reduction in this index under both stress and control conditions. Additionally, no significant difference was observed in hydrogen peroxide levels in green bean seedlings under different pre-treatments at both 28°C and 35°C. Hydrogen peroxide levels in canola (at 28°C) and lettuce (at 35°C) seedlings both showed a significant reduction when pre-treated with Bonamid® compared to Algabon® (Figure 6).

Table 1. Three-way ANOVA test results showing levels of significance of stress, species, and biostimulants (Bios) on growth and biochemical parameters.

Parameters	Stress	Species	Bios	Stress*Species	Stress*Bios	Species*Bios	Stress*Species*Bios
GP	*	*	*	*	NS	*	*
FW	*	*	*	*	NS	*	*
DW	NS	*	*	*	NS	*	*
RL	*	*	*	*	*	*	*
SL	*	*	*	*	NS	*	*
H	NS	*	*	*	*	*	*
RSA	*	NS	*	*	NS	*	NS
S	*	*	*	*	NS	*	NS
AA	*	*	*	*	NS	*	*
PA	NS	*	*	*	NS	*	*

Level of significance: NS represents not significant; * represents P < 0.05. GP: germination percentage, FW: fresh weight, DW: dry weight, RL: root length, SL: shoot length, H: H_2O_2 content, RSA: radical scavenging activity, S: solution carbohydrate content, AA: amylase activity and PA: protease activity

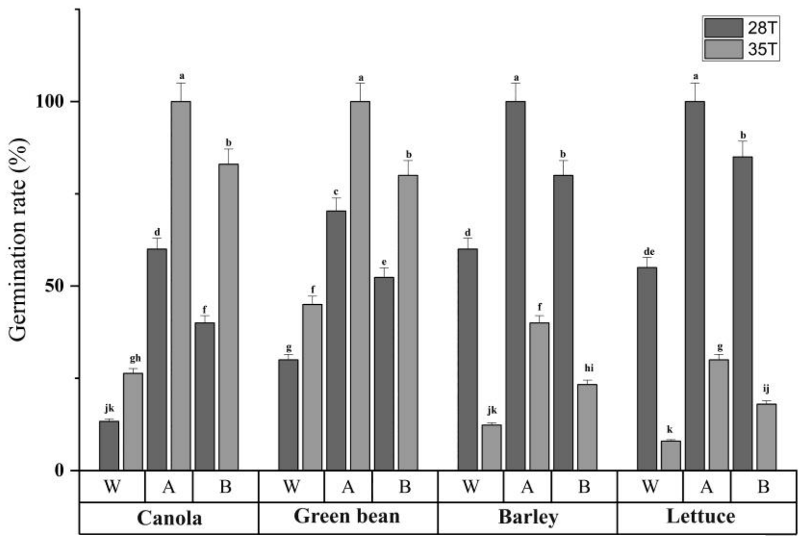

Figure 1. Germination percentage under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

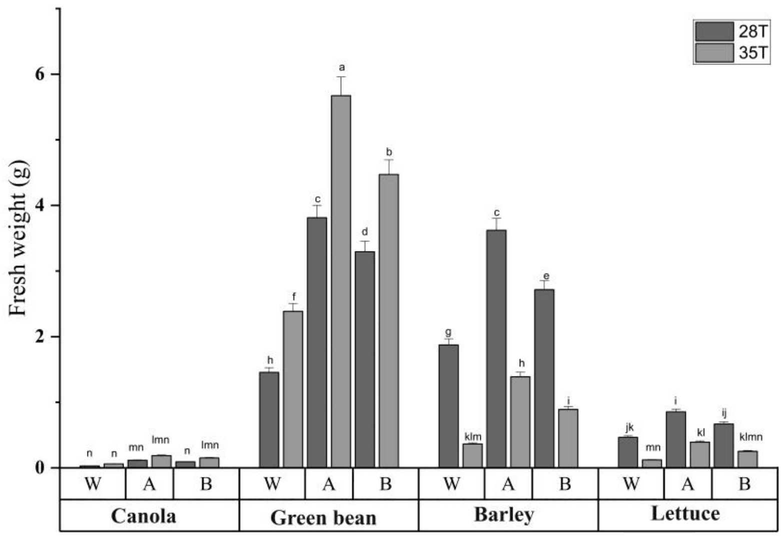

Figure 2. Fresh weight under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

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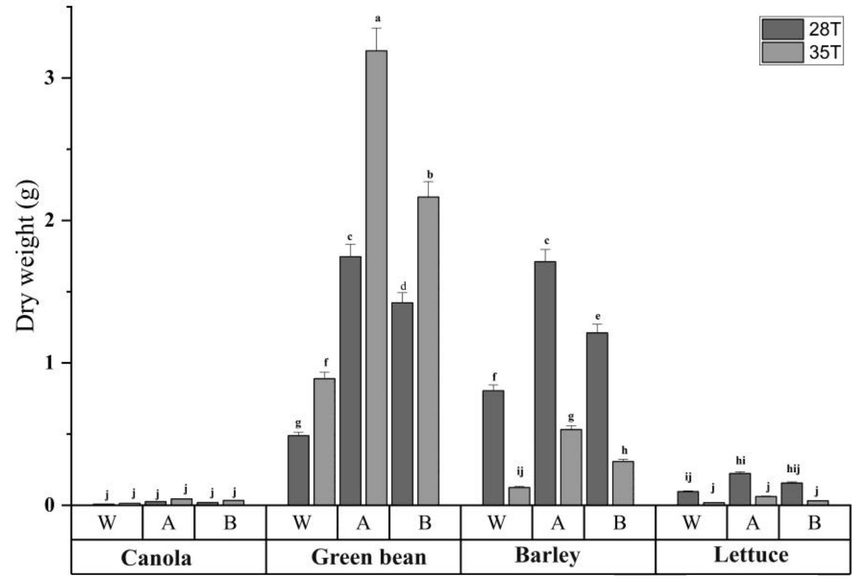

Figure 3. Dry weight under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

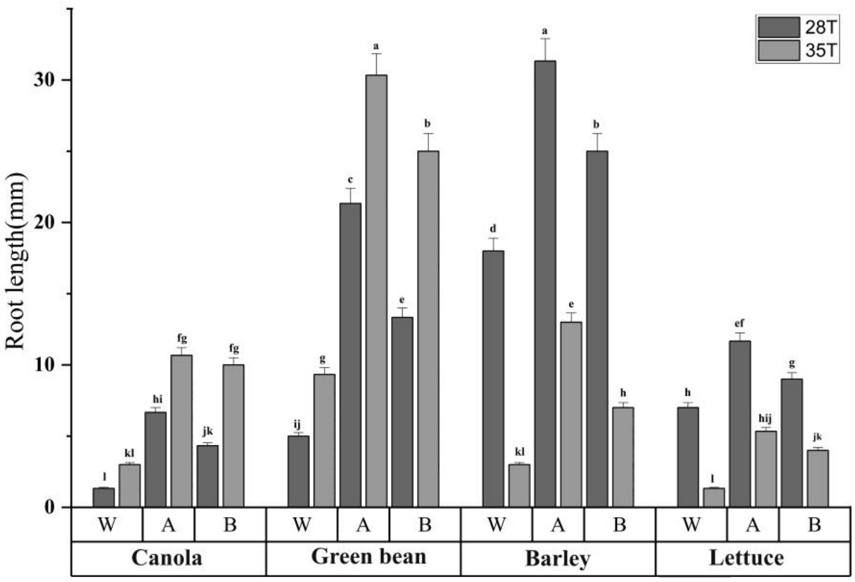

Figure 4. Root length under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

According to the results shown in figure 7, pre-treatment with biostimulants significantly increased free radical scavenging activity in seedlings of canola, green bean, barley and lettuce under control and stress conditions. In all four species at both 28°C and 35°C, the highest free radical scavenging activity was observed in seeds pre-treated with Bonamid®.

In this study, seeds of canola and green beans that had been pre-treated with the biostimulants Algabon® and Bonamid® did not show a significant impact on the amount of soluble sugar in the seedlings (Figure 8), whereas pre-treatment of barley and lettuce seeds with Algabon® and Bonamid® led to an increase in this index in both stress and non-stress conditions. In these seedlings, the greatest amount of soluble sugar was found in seeds that had been pre-treated with Algabon®.

Effect of biostimulants on reserve metabolism (amylase and protease activities) in canola, green bean, barley and lettuce under heat stress: Since the increase in germination percentage of seeds may be due to the increased activity of certain enzymes involved in germination, the activity of some germination-related

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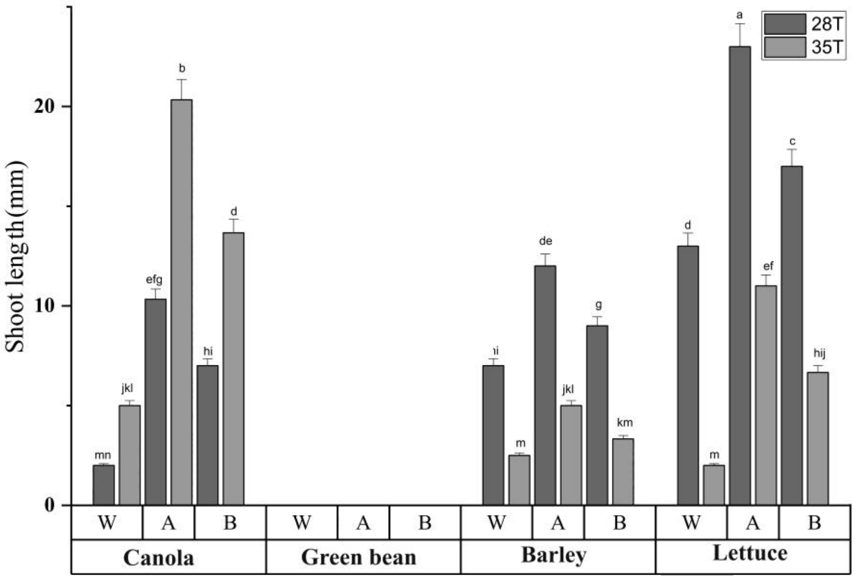

Figure 5. Shoot length under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

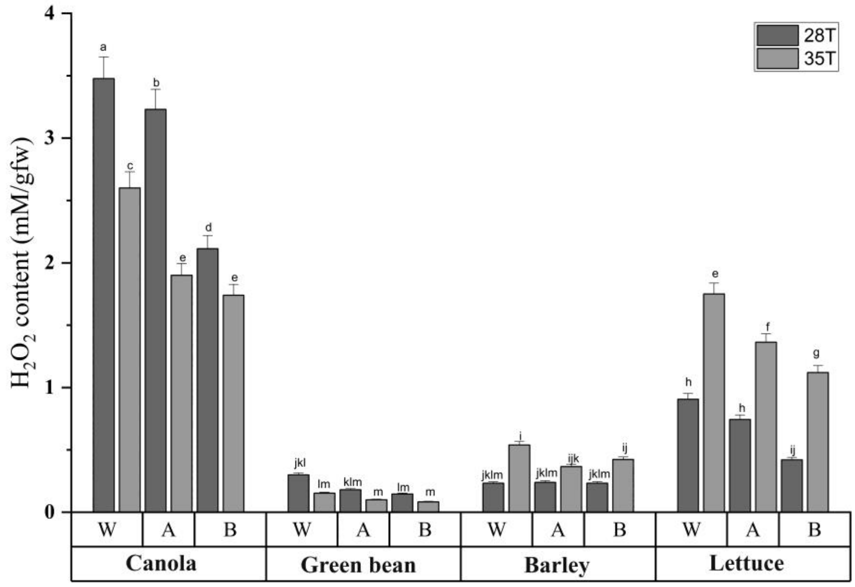

Figure 6. H_2O_2 content under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

enzymes was measured in this study. The activity of amylase and protease enzymes in canola and green bean seedlings showed that both biostimulant pre-treatments significantly increased the activity of these enzymes at both 28°C and 35°C. The highest activity of amylase and protease enzymes in seedlings was recorded with Algabon® pre-treatment at 35°C, showing increases of 3.4 and 2.3 times in canola seedlings and 2.5 and 4 times in green bean seedlings compared to water pre-treatment (Figures 9 and 10). Similar trends for both amylase and protease enzymes were observed in barley and lettuce plantlets, with the highest enzyme activity recorded with Algabon® pre-treatment at 28°C, showing increases of 1.6 and 2 times in barley seedlings and 2.4 and 3 times in lettuce seedlings compared to water pre-treatment.

Comparison of the effect of biostimulants on resistance indices under heat stress in seedlings of canola, green bean, barley and lettuce: The analysis of variance for absolute decrease index (AD), inhibition index (II), and relative heat tolerance (RHT) in seedlings of canola, green bean, barley and lettuce

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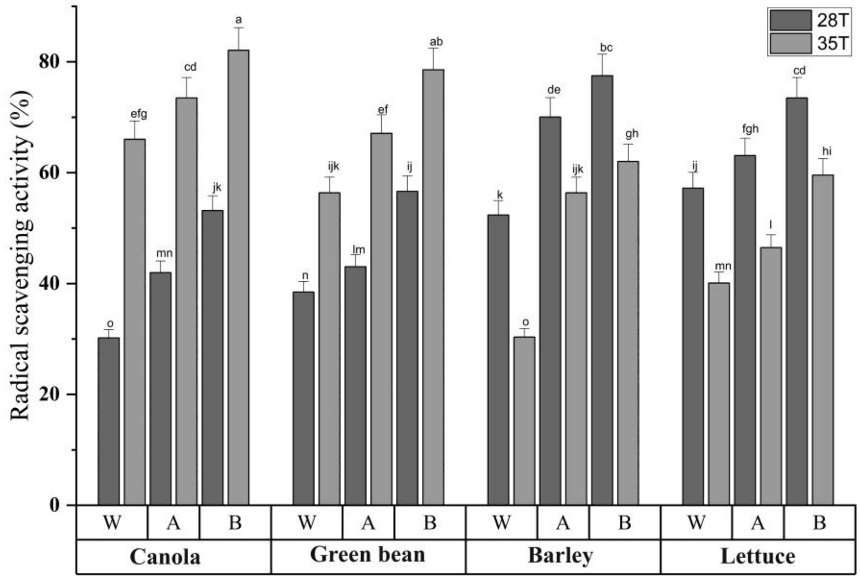

Figure 7. Radical scavenging activity under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

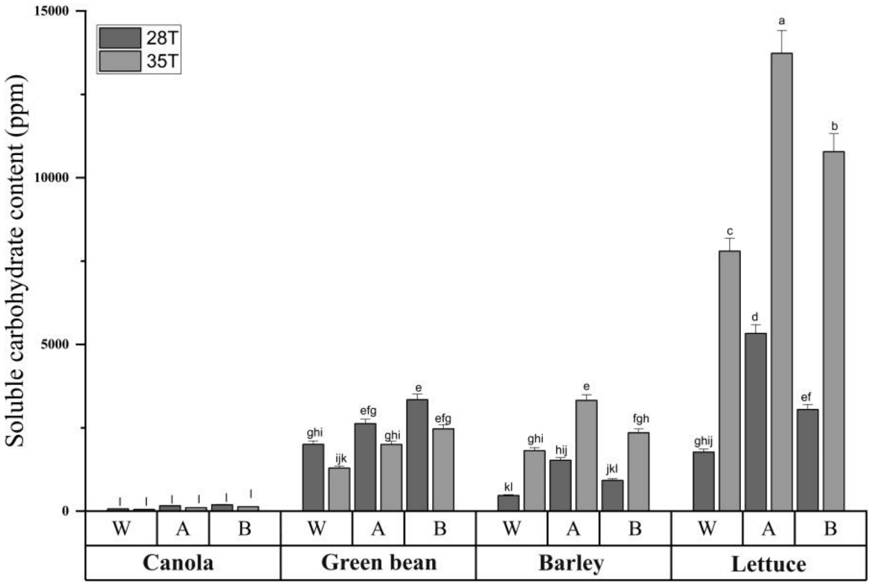

Figure 8. Soluble carbohydrate content under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on the LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

showed significant effects for species types, biostimulants and the interaction between species types and biostimulants (Table 2). The results indicated that the lowest values of the absolute decrease index belonged to the canola seeds pre-treated with Algabon® (-86), which demonstrated the highest resistance to heat stress in their seedlings (Table 3). The highest value of this index was observed in the barley and lettuce seeds pre-treated with water, indicating the sensitivity of their seedlings to heat stress. Moreover, according to the results of the absolute decrease index, the lowest value of the inhibition index was observed in the canola seeds pre-treated with Algabon®. The highest value of this index was also found in the lettuce and barley seeds pre-treated with water (Table 3). According to the results of the inhibition index, the canola seeds pre-treated with Algabon® were identified as the most heat-resistant seedlings under heat stress. The seeds of green bean species pre-treated with two biostimulants did not show differences in this index (Table 3). Furthermore, the

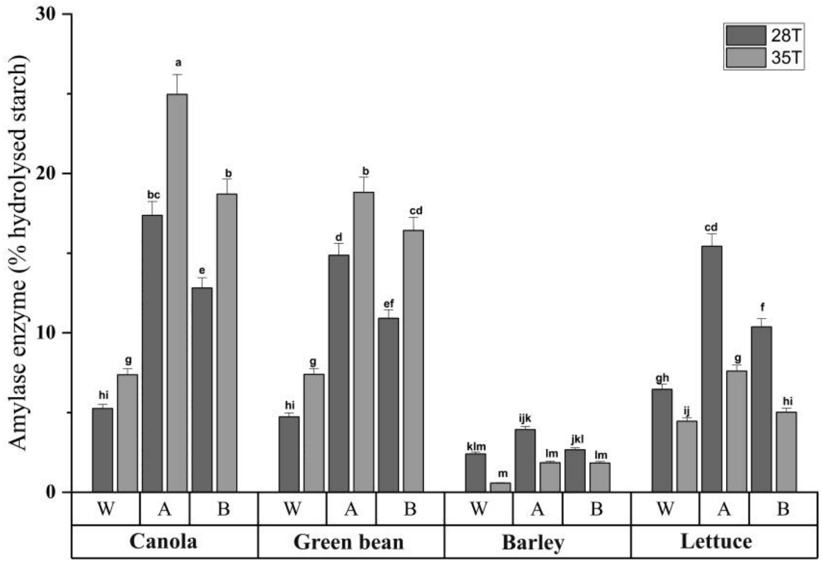

Figure 9. Amylase activity activity under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

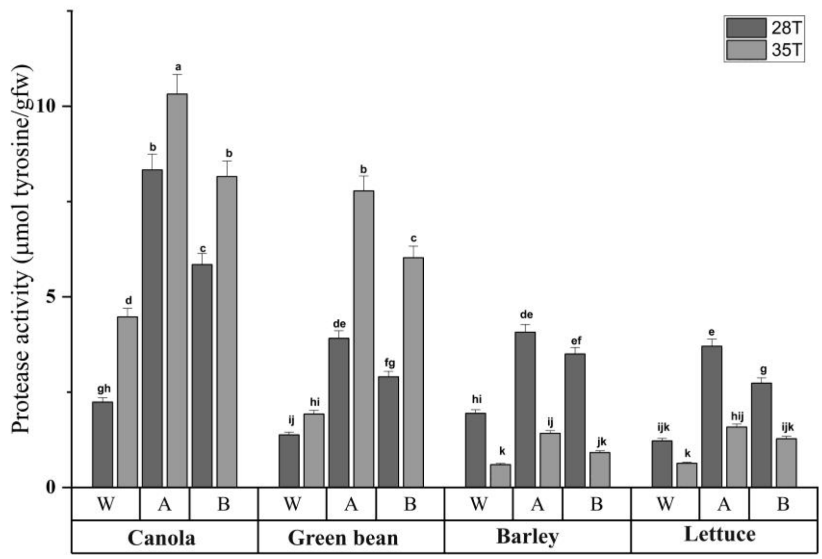

Figure 10. Protease activity under heat stress (35°C) and non-stress (28°C) conditions in canola, green bean, barley and lettuce seeds pretreated with biostimulants. Different letters indicate significant differences based on LSD test (P≥0.05). W: Water; A: Algabon®; and B: Bonamid®.

Table 2. Two-way ANOVA test results showing levels of significance of species and biostimulants on resistance indices under heat stress at 35°C.

Parameters	Species	Biostimulant	Species* Biostimulant
AD	*	*	*
II	*	*	*
RHT	*	*	*

Level of significance: * represents P < 0.05. Absolute decrease index: (AD), inhibition index: (II), and relative heat tolerance: (RHT).

relative heat tolerance index results indicated that the highest value of this index was found in the canola seeds pre-treated with Algabon®.

Principal component analysis: The PCA biplot (Figure 11B) shows that the two axes jointly explained more than 89% of the variation among the variables in

Table 3. Effect of biostimulants and species types on resistance indices under heat stress at 35°C. Different letters indicate significant differences based on LSD test ($P \geq 0.05$).

Biostimulants Treatments	Canola		RHT	Green bean		RHT
	AD	II		AD	II	
Water	-13±4.35^{d}	-106.7±63.59^{cd}	2.06±0.63^{de}	-15±5^{d}	-51.7±20.34^{bc}	1.51±0.2^{e}
Algabon	-86.7±2.9^{g}	-677.8±192.71^{g}	7.2±0.9^{a}	-70±5^{f}	-239.7±57^{e}	3.4±0.6^{c}
Bonamid	-69.6±5^{f}	-446.5±13.3^{f}	5.9±0.8^{b}	-50±4.3^{e}	-171.4±44de	2.7±0.43^{cd}
	Barley		RHT	Lettuce		RHT
	AD	II		AD	II	
Water	47.66±4.6^{a}	79.4±3.85^{a}	0.2±0.03^{f}	47±6.24^{a}	85.2±4.7^{a}	0.14±0.04^{f}
Algabon	20±13.2^{c}	32.6±19^{ab}	0.67±0.19^{f}	25±8.6^{c}	44.9±11^{ab}	0.55±0.11^{f}
Bonamid	36.7±2.9^{b}	61.6±9.8^{a}	0.23±0.07^{f}	37±4.3^{b}	67.2±3.5^{a}	0.18±0.02^{f}

Absolute decrease index: (AD), inhibition index: (II), and relative heat tolerance: (RHT).

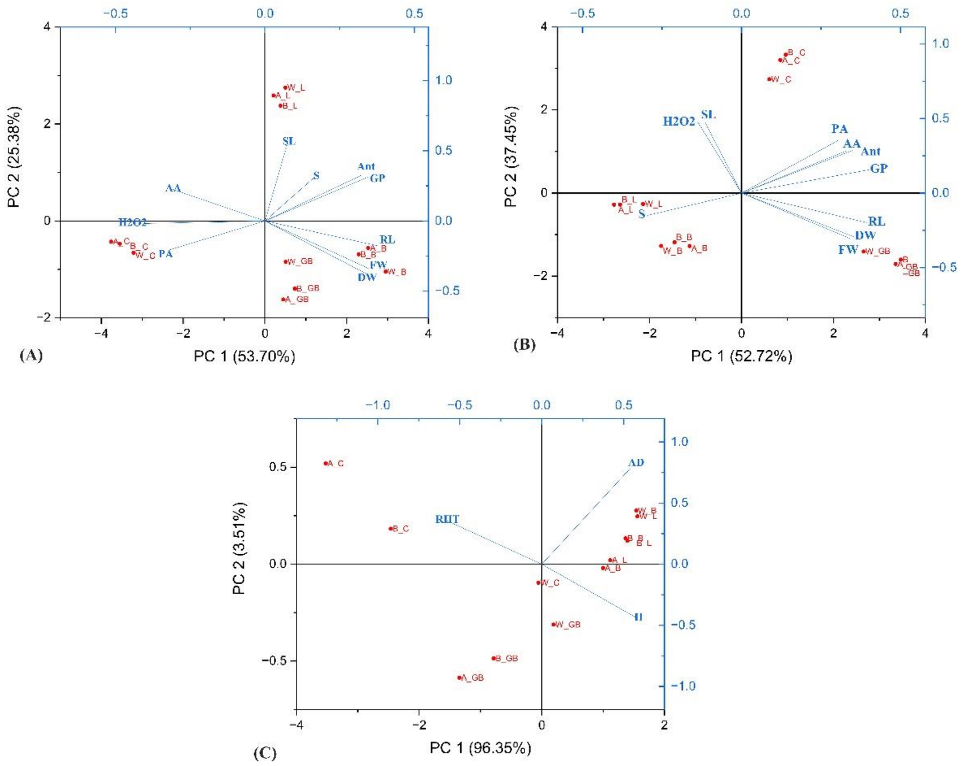

Figure 11. Biplot showing grouping of canola (C), green bean (GB), barley (B), and lettuce (L) species pre-treated with seeds with Algabon® (A), Bonamid®, and water (W) under non-stressed (A), heat-stressed (B), and heat tolerance indices (absolute decrease index (AD), inhibition index (II), and relative heat tolerance (RHT)) conditions. The traits are shoot length (SL), root length (RL), fresh weight (FW), dry weight (DW), germination percentage (GP), H_2O_2 content (H_2O_2), protease activity (PA), amylase activity (AA), soluble carbohydrate content (S), and antioxidant content (Ant).

all biostimulant pre-treatments under heat stress. The PCA biplot represents clear segregation between cool-season species (lettuce and barley) and warm-season species (canola and green bean). All studied variables were positively correlated with PC1 except shoot length and H_2O_2 concentration. Meanwhile, the main contributors to the variation in PC1 under the control condition (Figure 11A) among the measured traits were Ant, GP, FW, DW, and SY, which were correlated under the NS environment. Figure 11C shows the relationships between heat tolerance indices and the species in biostimulant pre-treatments. Seeds pre-treated with biostimulants Algabon® [A_C] were aligned with the vector for RHT, indicating high germination performance under heat stress. Both cool-season species pre-treated with water [W_B and W_L] and Bonamid® [B_B and B_L] were moderately aligned with the vectors for [AD and II], which were negatively correlated with [RHT], indicating low germination performance under drought stress.

Discussion

The effect of biostimulant pre-treatment on growth indices and germination metabolism in seedlings of

canola, green bean, barley and lettuce under heat stress: Plants can be pre-treated at various growth stages; however, for decades, there has been a focus on seed fortification to mitigate environmental stresses on germination and early seedling growth (Bulgari *et al*., 2019). The results showed that heat stress (temperature of 35°C) significantly reduced all germination and growth indices in the seedlings of barley and lettuce. It is well known that high temperatures can inhibit seed germination, a process often triggered by the production of abscisic acid (Toh *et al*., 2008; Essemine *et al*., 2020). In lettuce and barley, under both control and stress conditions, the germination rate, root length, shoot length, and fresh and dry weights were increased by the Algabon®. In contrast to the results for barley and lettuce seedlings, heat stress caused an increase in all germination and growth indices in the seedlings of canola and green beans. In these two species, the germination rate, root length, shoot length, and fresh and dry weights were also increased by the Algabon® under both control and stress conditions. Several studies have reported the positive effects of biostimulants on plant growth in a wide range of compounds, such as seaweed extract, hydrolyzed protein, and humic acids under stress (Latef *et al*., 2017; Lucini *et al*., 2015). Anjos Neto *et al*. (2020) showed that priming of spinach seeds with seaweed extract improved germination percentage, germination speed, and seedling vigor under conditions of heat stress (30°C) compared to nonprimed controls. The use of seaweed extract has been reported to enhance seed germination, improve plant growth, increase yield (Fakhrabad and Abedi, 2019), and it results in elevated levels of cytokinins, auxins, and gibberellins, while abscisic acid and ethylene levels decrease, which in turn contributes to increased antioxidant production and enhanced stress tolerance (Zhang *et al*., 2003).

Pre-treatment of seeds increases the activity of amylase and protease enzymes, as well as the amount of water absorbed by the seeds, ultimately leading to an increased germination percentage and potentially reducing the number of days until sprouting. Our results indicated that under heat stress conditions, the activity of amylase and protease enzymes was significantly increased by the Algabon® compared to the control plants of barley, lettuce, canola, and green bean. A significant increase in protease enzyme activity under heat stress supports the hypothesis of oxidative damage in these conditions. The activation of protease enzymes in plant cells is essential during the production of H_2O_2. Therefore, it can be assumed that the increase in protease activity may be at least partially related to disruptions in redox homeostasis. They also participate in the normal growth and development processes of plants. The promoter regions of cysteine protease-encoding genes in corn are enriched with regulatory elements responsive to stress, indicating the active involvement of these proteases in processes that counteract abiotic stress (Li *et al*., 2021). Amylase is the predominant enzyme synthesized during germination, aiding in the mobilization of starch reserves in the endosperm. The favorable nutritional changes that occur during germination are mainly due to the breakdown of complex compounds into simpler forms, the conversion into essential components, and the degradation of undesirable components (Hasanuzzaman *et al*., 2013). Similar to the results of this study, some researchers have shown that biostimulants increase the activity of amylase and protease enzymes in plants under heat stress. Reis *et al*. (2021) reported an increase in the activity of α and β-amylase and protease enzymes under heat stress in seeds of *Melanoxylon brauna*. The growth-promoting effect of Algabon® pre-treatment on the four studied species under heat stress can be simply explained by the increase in the activity of amylase and protease enzymes, which leads to improved water absorption efficiency by the seeds and ultimately results in increased germination percentage and growth.

Studies indicate that using biostimulants as a pre-treatment under heat stress conditions reduces H_2O_2 production and prevents the accumulation of reactive oxygen species, thereby mitigating the detrimental effects of heat stress (Campobenedetto *et al*., 2020). The reduction of H_2O_2 production under heat stress was observed in the seedlings of canola, green bean, barley and lettuce pre-treated with Algabon® and Bonamid®. Similarly, Anjos Neto *et al*. (2020) showed that priming spinach seeds with seaweed extract resulted in lower contents of H_2O_2 and malondialdehyde (MDA) under heat stress than the control. Seaweed extracts target several pathways to increase stress tolerance and generally help plants in scavenging ROS, membrane stability, and osmotic protection. In the aerial part, they regulate stomata and xylem water conductivity, while in the root zone, they enhance water availability and regulate auxin and ethylene levels (Sudozai *et al*., 2013).

An increase in the ability to scavenge DPPH radicals under heat stress was observed in the seedlings of canola, green bean, barley and lettuce pre-treated with Algabon® and Bonamid®. Goyal *et al*. (2023) reported that application of SWE on *Brassica juncea* significantly alleviated the impact of heat stress by reducing the membrane injury percentage and levels of MDA and increasing the radical scavenging activity. Seed germination performance is associated with antioxidant activity, according to a study conducted by Bailly *et al*. (2000). According to our results, canola, green bean, barley and lettuce seeds treated with pre-treated Algabon® before heat stress exhibited better physiological performance compared to untreated seeds. Therefore, the metabolites present in Algabon® may have enhanced the membrane and DNA repair process, as well as nucleic acid synthesis, protein functions, ATP generation, and the overall efficiency of the antioxidant system (Chen and Arora, 2013, Paparella *et al*., 2015).

Under both conditions, pre-treatment of barley and lettuce seeds with Algabon® biostimulant significantly increased the level of soluble carbohydrates in comparison with that of controls. Numerous reports indicate that carbohydrate content increases under abiotic stress conditions (Roitsch, 1999; Kaur *et al*., 2021; Ozturk *et al*., 2021). The increase in soluble carbohydrate content in this study is in accordance with the results of research by Masondo *et al*. (2018) on seedlings of *Ceratotheca triloba* and by Fakhrabad and Abedi (2019) on tomato seedlings. Higher carbohydrates levels in biostimulant-treated plants of various species were also observed alongside increased total protein, phenols, ascorbic acid, and growth-promoting hormones (Martinez Esteso *et al*., 2016; Arroussi *et al*., 2018). Carbohydrate accumulation plays a crucial role in stress responses due to soluble carbohydrat acting as compatible solutes that accumulate under stress conditions, thereby helping to regulate osmotic pressure, decrease cellular water loss, and maintain turgor pressure. They are also associated with stabilizing membranes and proteins and buffering cellular redox potential against stresses (Bohnert *et al*., 1995; Reis *et al*., 2021).

Effect of species type and biostimulants on resistance indices under heat stress in seedlings of canola, green bean, barley and lettuce: The findings revealed that the lowest absolute decrease index belonged to biostimulant-treated canola seeds with Algabon®, indicating the highest resistance of canola compared to other species under heat stress. The low value of this index at 35°C suggests that heat stress increases germination compared to non-stressed conditions. On the other hand, the highest values of this index were observed in lettuce and barley seeds pre-treated with water, indicating their sensitivity to heat stress. According to the results of the absolute decrease index, the lowest inhibition index and the highest relative heat tolerance index were observed in canola seeds pre-treated with Algabon®, which were identified as the most heat-resistant seedlings. Biostimulants derived from hydrolyzed proteins directly impact plant growth by inducing carbon and nitrogen metabolism, regulating nitrogen uptake, and interfering with hormonal activities, leading to enhanced root and shoot growth and, consequently, increased crop yield (Colla *et al*., 2017). The results of the standard criteria for determining plant performance under heat stress were fully consistent with the highest increase in germination rate and amylase and protease activities in canola seeds pre-treated with Algabon®. Principal component (PC) analysis identified two PCs accounting for 100% of total species variation based on heat tolerance indices. Biplot analysis using assessed traits allowed selection of heat tolerant speices designated as A_C (canola seeds pre-treated with Algabon®) with higher percentage germination.

Conclusion

The reason for the better readiness of pre-treated seeds for abiotic stresses may be the activation of antioxidant system responses through priming or may result from the activity of hormones such as salicylic acid, abscisic acid, and ethylene. The positive effects of biostimulant pre-treatment can be attributed to some compatible osmolyte compounds produced in all four pre-treated seed species, including soluble carbohydrates and proteins. These compounds participate in the physiological and biochemical activities of the plant, leading to increased growth potential under heat stress conditions. This study's findings revealed that seed pretreatment, as an effective method, enhanced the germination performance of canola, green bean, barley and lettuce seeds and rendered them more resilient to heat stress. Consequently, the study's findings suggest that the use of superior fertilizers such as seaweed extract is advisable to prevent environmental deterioration, lower the expense of acquiring chemical fertilizers, and ultimately enhance growth productivity.

References

Abdelkader, M., Voronina, L., Puchkov, M., Shcherbakova, N., Pakina, E., Zargar, M., & Lyashko, M. (2023). Seed priming with exogenous amino acids improves germination rates and enhances photosynthetic pigments of onion seedlings (*Allium cepa* L.). *Horticulturae*, *9*, 80.

Abdel Latef, A. A., Saber, A. K., Alwaleed, E. A., & Tran, L. (2017). *Sargassum muticum* and *Jania rubens* regulate amino acid metabolism to improve growth and alleviate salinity in chickpea. *Scientific Reports*, *7*, 1-12.

Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environment*, *24*, 1337-1344.

Amirkhani, M., Netravali, A. N., Huang, W., & Taylor, A. G. (2016). Investigation of soy protein-based biostimulant seed coating for broccoli seedling and plant growth enhancement. *Hortscience*, *51*, 1121-1126.

Anjos Neto, A. P., Oliveira, C. R. F., Mello, S. D. C., Silva, M. S. D., Gomes-Junior, F. G., Novembre, A. D., & Azevedo, R. A. (2020). Seed priming with seaweed extract mitigate heat stress in spinach: Effect on germination, seedling growth and antioxidant capacity. *Bragantia*, *79*, 502-511.

Arroussi, H. E., Benhima, R., Elbaouchi., A., Sijilmassi, B., Mernissi, N. E., Aafsar, A., & Smouni, A. (2018). *Dunaliella salina* exopolysaccharides: A promising biostimulant for salt stress tolerance in tomato (*Solanum lycopersicum*). *Journal of Applied Phycology*, *30*(5), 2929-2941.

Bailly, C., Benamar, A., Corbineau, F., & Come, D. (2000). Antioxidant systems in sunflower (*Helianthus annuus* L.) seeds as affected by priming. *Seed Science Research*, *10*, 35-42. https://doi.org/10.1017/S0960258500000040

[DOI: DOI: 10.22034/14.70.43]

Bohnert, K., Nelson, D., & Jensen, R. (1995). Adaptation to environment stresses. *The Plant Cell*, *7*, 1099-1111.
Bolton, A, & Simon, P. (2019). Variation for salinity tolerance during seed germination in diverse carrot [*Daucus carota* (L.)] germplasm. *Hort Science*, *54*, 38-44.
Bulgari, R., Franzoni, G., & Ferrante, A. (2019). Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy*, *9*, 306.
Campobenedetto, C., Grange, E., Mannino, G., van Arkel, J., Beekwilder, J., Karlova, R., Garabello, C., Contartese, V., & Bertea, C. M. (2020). A biostimulant seed treatment improved heat stress tolerance during cucumber seed germination by acting on the antioxidant system and glyoxylate cycle. *Frontiers in Plant Science*, *11*, 836.
Carmo, L. P., Moura, C. W. d. N., & Lima-Brito, A. (2021). Effects of heat stress and seaweed derived biostimulants on the germination of *Comanthera mucugensis*, an endemic plant of fire-prone *Campos rupestres* of Chapada Diamantina (Brazil). *South African Journal of Botany*, *141*, 49-53. https://doi.org/10.1016/j.sajb.2021.04.017
Chen, K, & Arora, R. (2013). Priming memory involves seed stress-tolerance. *Environmental Experimental Botany*, *94*, 33-45. https://doi.org/10.1016/j.envexpbot. 2012.03.005
Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., & Rouphael, Y. (2017). Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Frontiers in Plant Science*, *8*, 2202.
Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae*, *196*, 3-14.
Essemine, J., Ammar, S., & Bouzid, S. (2020). Impact of heat stress on germination and growth in higher plants: Physiological, biochemical and molecular repercussions and mechanisms of defence. *Journal of Biological Sciences*, *10*, 565-572.
Fakhrabad, M. S, & Abedi, B. (2019). Investigation of the effect of foliar application of seaweed extract as growth biostimulants (*Ascophyllum nodosum*) on quantitative and qualitative characteristics of three tomato cultivars (*Solanum lycopersicon* Mill). *World Journal of Environmental Biosciences*, *8*, 19-22.
Filippou, P., Tanou, G., Molassiotis, A., & Fotopoulos, V. (2013). Plant acclimation to environmental stress using priming agents. In: Plant Acclimation to Environmental Stress. (eds. Tuteja, N., and Singh Gill, S.) Pp. 1-27. Springer, New York, NY. https://doi.org/10.1007/978-1-4614-5001-6_1
Goyal, V., Kumari, A., Avtar, R., et al. (2023). Orthosilicic acid and seaweed extract alleviate the deteriorative effects of high temperature stress in *Brassica juncea* (L.) Czern and Coss. *Silicon*, *15*, 4909-4919. https://doi.org/10.1007/s12633-023-02376-9
Gupta, S., Dolezal, K., Kulkarni, M. G., Balazs, E., & Van Staden, J. (2022). Role of non-microbial biostimulants in regulation of seed germination and seedling establishment. *Plant Growth Regulation*, *97*, 271-313.
Haroun, S, & Hussein, M. (2003). The promotive effect of algal biofertilizers on growth, protein pattern and some metabolic activities of *Lupinus termis* plants grown in siliceous soil. *Asian Journal of Plant Sciences*, *2*, 944-951.
Hasanuzzaman, M., Nahar, K., Alam, M. M., Roychowdhury, R., & Fujita, M. (2013). Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *International Journal of Molecular Sciences*, *14*, 9643-9684.
Hernandez-Herrera, R. M., Santacruz-Ruvalcaba, F., Ruiz-Lopez, M. A., Norrie, J., & Hernandez-Carmona, G. (2014). Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.). *Journal of Applied Phycology*, *26*, 619-628, 10.1007/s10811-013-0078-4
Hernandez-Herrera, R. M, & Santacruz-Ruvalcaba, F. (2016). Activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants. *Journal of Applied Phycology*, *28*, 2549-2560. 10.1007/s10811-015-0781-4
Jimenez-Arias, D., Morales-Sierra, S., Borges, A. A., Herrera, A. J., & Luis, J. C. (2022). New biostimulants screening method for crop seedlings under water deficit stress. *Agronomy*, *12*, 728.
Kaur, H., Manna, M., Thakur, T., Gautam, V., & Salvi, P. (2021). Imperative role of sugar signaling and transport during drought stress responses in plants. *Physiologia Plantarum*, *171*, 833-848.
Kulisic, T., Radonic, A., Katalinic, V., & Milos, M. (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, *85*(4), 633-640.
Li, Y., Niu, L., Wu, X., Faleri, C., Tai, F., Zhang, M., Liu, H., Wang, W., & Cai, G. (2021). Genome-wide identification and comparison of cysteine proteases in the pollen coat and other tissues in maize. *Frontiers in Plant Science*, *12*, 709534. doi: 10.3389/fpls.2021.709534
Lucini, L., Rouphael, Y., Cardarelli, M., Canaguier, R., Kumar, P., & Colla, G. (2015). The effect of a plant-derived biostimulant on metabolic profiling and crop performance of lettuce grown under saline conditions. *Scientia Horticulturae*, *182*, 124-133.
Malik, A., Mor, V. S., Tokas, J., Punia, H., Malik, S., Malik, K., Sangwan, S., Tomar, S., Singh, P., Singh, N., et al. (2021). Biostimulant-treated seedlings under sustainable agriculture: A global perspective facing climate change. *Agronomy*, *11*, 14.
Martinez Esteso, M. J., Vilella Anton, M. T., Selles Marchart, S., Martinez Marquez, A., Botta-Catala, A., Pinol-Dastis,

R., & Bru-Martinez, R. (2016). A DIGE proteomic analysis of wheat flag leaf treated with TERRA-SORB® foliar, a free amino acid high content biostimulant. *Journal of Integrated OMICS*, *6*(1), 1-9.

Masondo, N., Kulkarni, M., Finnie, J., & Van Staden, J. (2018). Influence of biostimulants-seed-priming on *Ceratotheca triloba* germination and seedling growth under low temperatures, low osmotic potential and salinity stress. *Ecotoxicology and Environmental Safety*, *1*, 43-48.

Ozturk, M., Turkyilmaz Unal, B., Garcia-Caparros, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum*, *172*, 1321-1335.

Paparella, S., Araujo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, D. (2015). Seed priming: State of the art and new perspectives. *Plant Cell Reports*, *34*, 1281-1293. https://doi.org/10.1007/s00299-015-1784-y

Poorter, H, & Villar, R. (1997). The fate acquire carbon in plants: Chemical composition and constructions costs. In: Plant Resource Allocation. (eds. Bazzaz, F. A., and Grace, J.) Pp. 39-72. Harvard University The Biological Laboratories: Cambridge, Massachusetts.

Probert, R. (2000). The role of temperature in the regulation of seed dormancy and germination. *Seeds: The Ecology of Regeneration in Plant Communities*, *2*, 261-292.

Reis, L. P., de Lima e Borges, E., Brito, D., Bernardes, C., & dos Santos Araujo, R. (2021). Heat stress-mediated effects on the morphophysiological, biochemical, and ultrastructural parameters of germinating *Melanoxylon brauna* Schott. seeds. *Plant Cell Reports*, *40*, 1773-1787.

Roberts, J, & White house, D. G. (1976). Practical Plant Physiology. Sciences, New York.

Roitsch, T. (1999). Source-sink regulation by sugar and stress. *Current Opinion in Plant Biology, 1999*, 198-206.

Salmia, M. A., Nyman, S. A., & Mikola, J. J. (1978). Characterization of the proteinases present in germinating seeds of Scots pine, *Pinus sylvestris*. *Physiologia Plantarum*, *42*, 252-256.

Sudozai, M. I., Tunio, S., Chachar, Q., & Rajpar, I. (2013). Seedling establishment and yield of maize under different seed priming periods and available soil moisture. *Sarhad Journal of Agriculture*, *29*, 515-5.

Toh, S., Imamura, A., Watanabe, A., Nakabayashi, K., Okamoto, M., Jikumaru, Y., Hanada, A., Aso, Y., Ishiyama, K., Tamura, N., et al. (2008). High temperature-induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. *Plant Physiology*, *146*, 1368-1385.

Yildirim, E., Dursun, A., Guvenc, I., & Kumlay, A. M. (2000). The effects of different salt, biostimulant and temperature levels on seed germination of some vegetable species. *II Balkan Symposium on Vegetables and Potatoes*, *579*, 249-253.

Zhang, X, & Ervin, E. H. (2008). Impact of seaweed extract-based cytokinins and zeatin riboside on creeping bentgrass heat tolerance. *Crop Science*, *48*, 364-370. 10.2135/cropsci2007.05.0262

Zhang, X., Ervin, E. H., & Schmidt, R. E. (2003). Seaweed extract humic acid, and propiconazole improve tall fescue sod heat tolerance and post-transplant quality. *HortScience*, *38*, 440-443.

Zhao, M., Zhang, H., Yan, H., Qiu, L., & Baskin, C. C. (2018). Mobilization and role of starch, protein, and fat reserves during seed germination of six wild grassland species. *Fronteirs in Plant Science*, *9*, 234. doi: 10.3389/fpls.2018.00234