Efficacy of Foliar Spray of Two Biostimulants in Phytoremediation and Overcoming Salinity Stress in Halophytic Grass, *Puccinellia distans*

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Abstract

Background: Plants pre-treatment with various chemicals has often been used to diminish salinity stress impact on plants. In the present study, we used foliar spray of two commercially biostimulants (Algabon® and Bomamid®) before the stress, to study interactive effects of biostimulants and salinity stress in halophytic grass, *Puccinellia distans*.

Results: Our results showed that biomass, leaf relative water content, chlorophyll content, K⁺ content, K⁺/Na⁺ ratio, and protein and N contents were also negatively affected by 300 mM NaCl. The results obtained in the present study showed the beneficial effects of the pre-treatments of two biostimulants on *P. distans* seedlings under non salinity stress conditions with respect to increasing plant biomass, photosynthetic pigments, K⁺ content, the content of proteins and nitrogen percentage. The results suggested that foliar spray of Bonamid could considerably diminish NaCl-caused stress on *P. distans* seedlings, probably due to higher accumulation of shoot biomass, photosynthetic pigments, K⁺/Na⁺ ratio, protein and N contents, phytoremediation potential, as well as upregulation of Na⁺/H⁺ antiporters located in plasma membranes and vacuoles.

Conclusions: Collectively, it could be concluded that, intensification of osmotic adjustment by the accumulation of osmotic metabolites and the compartmentalization of salts in vacuoles in pre-treated plants with amino acid-derived biostimulant (Bonamid) can diminish the NaCl-induced deleterious effects in *P. distans* seedlings.

1. Introduction

As the population grows, the demand for more food increases, and in such circumstances, the use of halophyte plants, which cover a large part of the world's land, has particular value. Therefore, today, bio-salinity research has led studies towards salinity-resistant plants. In Asia, Iran is one of the countries with the highest level of saline soils and arid and semi-arid climate of this country contributes to the establishment of saline soils in different regions. According to Food and Agriculture Organization's (FAO's), more than 81% of Iran's irrigated land is exposed to secondary soil salinization (Dewan and Famouri 1964). Considering the large area of saline, alkaline and alkaline pastures in Iran, as well as the reduction of usable forage and natural process of wind erosion, the conditions require extensive studies in the field of halophytes plants.

Halophytes are plants that are able to survive and reproduce at high concentration of salts, exceeding 200 mM NaCl (Yuan et al. 2019). According to their reaction to salinity, halophytes can be divided into three categories: euhalophytes, recretohalophytes and pseudo-halophytes. Pseudo-halophytes of the genus *Puccinellia* are able to tolerate high salinity levels due to the thickening of the root endoderm that restricts the Na⁺ entry and compartmentalizes Na⁺ into their vacuoles (Yuan et al. 2019; Dashtebani et al. 2014). Data on the basic ionic and biochemical mechanisms that cause salinity tolerance in halophytes is limited to some model species, principally dicotyledons (Dashtebani et al. 2014). In view of the fact that many commercially major crops are monocotyledonous glycophytes, understanding the salt tolerance mechanisms in monocotyledonous halophytes will help breeding programs to improve salt tolerance in cereals. *P. distans* coming from family Poaceae, is a monocotyledonous halophyte that is distributed in the north, northwest and central parts of Iran. It can typically grow in saline and alkaline soils with high underground water table or heavy soils with moderate drainage (Akhani, 2006) and is regarded as one of the most favorable grass species for saline rangelands stabilizing and for preventing erosion.

Salinity suppresses the growth and development of plants by influencing major metabolic processes and by creating ionic and osmotic stresses (Van Zelm et al. 2020). Moreover, another detrimental effect of salinity, like other abiotic stresses, is the generation of oxidative stress through increased production of reactive oxygen species (ROS). The most of review papers have shown that the mechanisms of salinity tolerance in salt tolerant plants include reduction of Na⁺

entry and their accumulation into vacuoles by Na^+/H^+ antiporters (SOS1 in plasma membrane and NHX1 in tonoplast), increment of Na^+ exclusion from shoot and intracellular compartmentations, keeping of high K^+/Na^+ ratio in cytosol in addition to biosynthesis and accumulation of osmoprotectants and enzymatic and nonenzymatic antioxidants (Bartels and Sunkar 2005; Flowers et al. 2015; Van Zelm et al. 2020).

Salt stress tolerance in plants can be improved by different approaches including genetic manipulations (Van Zelm et al. 2020), mycorrhization (Evelin et al. 2019), the application of organic matter and biofertilizers, seeds pre-treatments (Masondo et al. 2018; Abdelhamid et al. 2019), foliar application of organic and inorganic substances and the application of biostimulants (Latef et al. 2017; Zahedi et al. 2020). According to the definition provided recently by du Jardin et al. (2020) biostimulants are regulators of plant life processes that enhance growth and resources usage under stress or non-stress conditions. They categorised biostimulants into seven classes, including: seaweed and plants extracts, protein hydrolysates and N-containing compounds, humic and fulvic acids, chitosan and other biopolymers, inorganic compounds and beneficial fungi and bacteria (Du Jardin 2015).

Biostimulants contain biologically active compounds and by enhancing primary and secondary metabolisms (Bulgari et al. 2015; Yakhin et al. 2017) can improve plant growth stimulation by enhancing the efficiency of water, photosynthesis, and increasing the absorption of nutrients from soil and neutralizing abiotic stresses (Van Oosten et al. 2017; Yakhin et al. 2017). The key mechanisms induced by biostimulants are complicated and investigation is still ongoing. They are able to change some molecular processes that allow to improve plant tolerance to salinity by supporting better status of water and increasing dry biomass, chlorophyll content, and nitrate concentration, enhanced ROS scavenging enzymes, increasing endogenous osmoprotectants, reduced membrane leakage and influencing phyto-hormones (Fasciglione et al. 2015; Rady et al. 2019; Rady and Mohamed 2015; Bulgari, Franzoni, and Ferrante 2019). Biostimulants can be used as foliar spray or in soil, depending on their composition and the favourable performance (Kunicki et al. 2010).

Although earlier researches have reported an important role of biostimulants in improving stress resistance in plants (Rouphael et al. 2017; Desoky et al. 2018; Di Stasio et al. 2018; Bulgari et al. 2019), it is not clear exactly how these compounds act under stress. The efficiency of biostimulants to neutralize the stress condition in plants relies on different parameters, such as mode of application of biostimulants, timing of application and their concentrations, and variability, intensity and duration of the stresses. Hence, in this experiment, we used foliar spray of two biostimulants (Algabon® and Bonamid®) before the stress (pre-treatment) with the concentrations suggested by the manufacturer company, to study the interactive effects of biostimulants and salinity stress in halophytic grass, *P. distans*.

2. Material And Methods

Chemicals

In this study, we used two biostimulants (Algabon® and Bonamid®) available in the market (Bon Asia Cultivation Company) each with very distinct formulations; Algabon®, seaweed extract-derived biostimulant, containing guaranteed ingredients: alginic acid (18%), NO_3 (1%), K_2O (16%), H_2PO_4 (1%) and Bonamid® as amino acid-derived biostimulant, containing guaranteed ingredients: amino acid (85%), nitrogen (4%).

2.1. Plant culture and treatments

The seeds of *Puccinellia distans* were collected from Chargholi coastal area in Golestan Province of Iran (E 54°00', N 36°58'). The surface-sterilized seeds of *P. distans* were germinated in petri dishes for 48 hours to obtain seedlings. The seedlings were placed in plastic pots (15-cm upper diameter, 12-cm lower diameter, and 15-cm depth) that were filled with 2 kg of soil mixture (filled with soil and animal manure in a ratio of 4 to 1). All the pots were irrigated with 200 ml of

half-strength Hoagland's solution. The seedlings grew in the greenhouse condition at $25 \pm 2^{\circ}\text{C}$ with a relative humidity of 70% supplied with a 14/10 h photoperiod for 45 days to adjust the conditions and produce abundant materials for later experiments.

First experiment

In the first experiment, the 45-day old seedlings were irrigated with 200 ml of half-strength Hoagland's nutrition containing four concentrations of NaCl (0, 300, 600 and 900 mM) thrice for one a week. This experiment was performed in a completely randomized design with three replications. Plants were grown under the same condition stated in the former section. After one week, seedlings were harvested and shoot fresh and dry weights, RWC and salt tolerance index (STI) were determined by the methods described in the following sections. Based on the results, the concentration of 300 mM of NaCl (EC = 27.18 dS/m) was selected as the moderate stress in the second experiment.

Second experiment

In the second experiment, we studied the effects of foliar spray of two biostimulants (Algabon® and Bonamid®) before the stress (pre-treatment) with the concentrations 0.5 g/l and 2g/l, respectively, under normal and salinity stress conditions. To this objective, 45-day old seedlings were pre-treated with foliar spraying of 0.5 g/l Algabon and 2 g/l Bonamid thrice for two week. To improve the efficacy of foliar uptake, Tween 80 was added to the spray solutions. Control seedlings were sprayed with distilled water. Then, a half of pre-treated seedlings were irrigated with half-strength Hoagland's nutrition containing 300 mM of NaCl for 15 days. The other half of the seedlings were irrigated with half-strength Hoagland's nutrition as a control. The experiment was planned as a factorial combination of three biostimulants treatments (Water, Algabon® and Bonamid®) and two salinity solutions (control (0 mM NaCl) and salinity (300 mM NaCl)). The treatments were organized in a completely randomized design with 3 replications per treatment.

2.2. Measurement of growth characteristics

At the end of two experiments, the seedlings were harvested. The plant samples were rinsed with tap water and then separated into shoots and roots, and then weighed to determine the fresh weight. The plant parts were dried at 70°C for 48 h. Leaf relative water content (RWC) was calculated using the following formula (Barrs and Weatherley 1962): $\text{RWC (\%)} = [\text{fresh weight of leaf samples} - \text{dry weight of leaf samples} / \text{turgid weight of leaf samples} - \text{dry weight of leaf samples}] \times 100$. Salt tolerance index (STI) was calculated by the following formula (Goudarzi and Pakniyat 2008): $\text{STI (\%)} = (\text{Mean shoot dry weight in the treatment} / \text{Mean shoot dry weight in control}) \times 100$. The dried shoot and root samples were weighed, and used to determine the shoots and roots water contents ($\text{Fresh weight} - \text{Dry weight} / \text{Fresh weight} \times 100$).

2.3. Photosynthetic pigments determination

The absorbance of acetone extracts of leaves was read at 663, 645 and 470 nm for the measurement of chlorophylls and carotenoid concentrations according to the methods defined by Arnon (1949) and Lichtenthaler (1987), respectively.

2.4. Determination of proline content

Proline content was estimated using the method of Troll and Lindsley (1955). After extracting 0.1 g of leaves in 90% ethanol, the mixture was centrifuged at $14000 \times g$ for 5 min. A reaction mixture containing acetic acid, 96% (v/v) ethanol and ninhydrin was added to the supernatant. After centrifuging and boiling, proline content was measured at 520 nm and was calculated as $\mu\text{mol/g FW}$ against standard proline.

2.5. Determination of glycine betaine content

The glycine betaine content was determined according to Grieve and Grattan (1983) with minor modifications. Briefly, deionized H₂O extracts were diluted 1:1 with 2N H₂SO₄ and cooled in ice water for 1h. Then, 200 µl cold KI-I₂ reagent was added and stored at 4°C for 16h. The resulting mixture was centrifuged at 10000 rpm for 15 min at 4°C. The deposited periodide crystals in the tubes were dissolved in 9 ml of 1, 2-dichloroethane, and the mixture was shaken vigorously. After 2h, the absorbance was measured at 365 nm. The content of glycine betaine was calculated based on a standard curve of standard solutions of glycine betaine.

2.6. Determination of total phenolic content

Total phenolic content was determined by the method proposed by Singleton and Rossi (1965), which is also known as the Folin- Ciocalteu reagent technique. The basis of this method is the reduction of the reagent by phenolic compounds in alkaline medium and the formation of a blue complex that shows the maximum absorption at a wavelength of 750 nm. In a test tube, 9 ml of distilled water was added to 1 ml of the plant methanolic extract. Then, 1 ml of diluted reagent was added and the mixture was shaken vigorously. After 5 minutes, we added 10 ml of sodium carbonate (Na₂CO₃) (7%). Then, 4 ml of distilled water was added to the resulting mixture and the final volume was set to 25 ml. The reaction mixture was incubated at room temperature for 90 minutes and the absorbance was measured at a wavelength of 750 nm. Total phenolic acids were expressed in milligrams of gallic acid per gram of sample.

2.7. Determination of Protein

The protein concentration of leaves and roots of each samples was determined following the method of Bradford (1976) using BSA as a protein standard. Total protein content was expressed as milligram per gram of leaf dry weight (mg/g DW).

2.8. Determination of soluble sugar concentration

Freeze-dried leaves and roots (100 mg) were used for the determination of soluble sugar concentration. It was extracted in 5 ml 80% ethanol (v/v). After boiling the extracts and centrifuging them using anthrone reagent, absorption of samples was read at 625 nm (Porter and Villar 1997).

2.9. Determination of antioxidant capacity (DPPH scavenging activity)

Assessment of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity was performed by preparing 500 µl of sample ethanolic extract in a tube. Then, 500 µl of DPPH solution was added to the extract. The solution was thoroughly mixed and incubated at room temperature for 30 minutes. The absorbance of control ethanol sample (blank), control water sample and samples obtained from plant extracts was read at 520 nm (Kulisic et al. 2004). The capacity of scavenging free radicals of samples was calculated as follows:

$$\% \text{ Radical Scavenging Activity} = ((\text{OD control water} - \text{OD Sample}) / \text{OD control water}) * 100$$

2.10. Determination of total nitrogen

For estimation of total N, 1g of fine-ground leaves and roots dry samples was digested with sulphuric acid, and assays were carried out according to the Kjeldahl method (1883)

2.11. Determination of elements

The concentration of elements (Na⁺ and K⁺) in 0.5 g ground dried powder of leaves and roots sample was determined by ICP-OES (Genesis, Spectro Company, Germany).

2.12. Salinity phytoremediation potential

Phytoremediation potential was determined according to Fischer and Maurer (1978) by the ion uptake of the plants as follows: $PP\ (g.m^{-1}) = (Na^{+}\ \text{concentration}\ (mg.g^{-1}) \times \text{biomass}\ (g\ \text{dry weight}) / \text{pot area}\ (m^2)) / 1000$. Where, PP is the phytoremediation potential of shoots or total biomass (shoots + roots) in each treatment based on Na^{+}/m^2 of surface area.

2.13. RNA extraction, cDNA synthesis and quantitative real-time PCR

Total RNA was extracted from fresh leaf and root tissues of *P. distans* by means of the DENA zist ASIA kit (DENAzist Asia Co., # S-1010-1, Iran) according to the manufacturer's instruction. The first-strand cDNA was synthesized using PrimeScript RT Enzyme Mix I enzyme (PrimeScript™ RT reagent Kit, Takara company Inc., Otsu, Japan) according to the manufacturer's protocol. The primers for beta-actin (Actin), plasma membrane Na^{+}/H^{+} antiporter (SOS1), and tonoplast Na^{+}/H^{+} antiporter NHX1 (NHX1) genes were taken from Zhang et al. (2017). Information on primers is gave in Table 1. Quantitative real-time PCR was conducted by StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA USA) using SYBR Premix Ex Taq TaKaRa (Takara Bio, Inc., Otsu, Japan). For the reactions, the program of the thermocycler was adjusted as: 95°C/10 s, 45 (95°C/10s, 60°C/15s, 72°C/15s), 95°C/15s. To confirm the specificity of the reaction, the melting curve analysis was carried out for PCR reactions. The relative expression levels of all genes were measured using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Table 1
Sequences of primers used for quantitative real-time PCR amplification.

Gene name	Sequence (5′–3′)
<i>Actin</i>	Actin-F 5-TTGACTACGACCAGGAGATGGA-3
<i>Actin</i>	Actin-R 5-TGAAGGATGGCTGGAAGAGG-3
<i>SOS1</i>	SOS1-F 5-GACGAATAACTCAATCCACAGCAA-3
<i>SOS1</i>	SOS1-R 5-ACCGCAAACCCTTCCAATC-3
<i>NHX1</i>	NHX1-F 5-GCAATGAACTCCGCAATGATAC-3
<i>NHX1</i>	NHX1-R 5-GCTGTAATGCTTCCTTCTCTTCCT-3

Table 1

Fresh and dry weights of shoots and roots, root/shoot ratio (DW) and RWC in salinity condition (SC) and non-salinity condition (NSC) *Puccinellia distans* exposed to foliar spraying of biostimulants. Different letters indicate significant differences according to LSD tests ($p < 0.05$).

Biostimulants Treatments	Shoot fresh weight (g)		Shoot dry weight (g)		RWC (%)	
	NSC	SC	NSC	SC	NSC	SC
Control	14.60±0.49 ^c	8.20±0.05 ^e	2.75±0.04 ^c	1.38±0.07 ^f	96.38±0.59 ^b	78.50±1.80 ^d
Algabon	15.8±0.10 ^b	13.42±0.08 ^d	2.95±0.05 ^b	2.15±0.05 ^e	97.27±0.51 ^b	87.79±0.97 ^c
Bonamid	17.7±0.09 ^a	15.86±0.51 ^b	3.28±0.07 ^a	2.4±0.05 ^d	101.18±1.20 ^a	88.21±0.18 ^c
	Root fresh weight (g)		Root dry weight (g)		Root/Shoot (DW)	
	NSC	SC	NSC	SC	NSC	SC
Control	5.22±0.01 ^e	4.60±0.04 ^f	0.85±0.05 ^{cd}	0.68±0.07 ^e	0.30±0.21 ^d	0.49±0.08 ^b
Algabon	6.60±0.07 ^b	5.34±0.07 ^d	1.15±0.04 ^b	0.78±0.07 ^{de}	0.39±0.02 ^c	0.36±0.03 ^{cd}
Bonamid	9.47±0.06 ^a	6.32±0.02 ^c	2.1±0.10 ^a	0.95±0.05 ^c	0.63±0.01 ^a	0.39±0.02 ^c

3. Statistical Analysis

The first experiment was managed in a completely randomized design (CRD) with three replications. The second experiment was conducted as factorial test in a completely randomized design with three replications. Data were analysed according to the GLM procedure of SAS statistical software (version 8; SAS Institute Inc., Cary, NC, United States). A standard Fisher's test was applied to determine significant differences between treatments. Significance was determined with $\alpha \leq 0.05$.

4. Results

4.1. Plant growth measurements

Results related to the first experiment showed that shoot fresh and dry weights, RWC content and salt tolerance index (STI) were significantly affected by NaCl at all applied concentrations in comparison to the control, with the maximum reduction in seedlings treated with 900 mM of NaCl (Figure. 1). However, there was no significant difference between 300, 600 and 900 mM of NaCl concentrations in shoot dry weight and salt tolerance index (STI) in the seedlings of *Puccinellia distans*. STI values based on shoot dry weight indicated no difference in salt tolerance among the three concentration of NaCl applied in this study. Application of 300 mM NaCl decreased salt tolerance index by 50.06% in comparison to control, therefore, it was selected for the study of interaction with bio-stimulants. Dissociation of roots and the dry weight measurements were not easily feasible due to the type of bed we used for plant cultivation. Nonetheless, root density in the seedlings of *P. distans* notably decreased with increasing salinity stress level (Figure. 2).

4.2. Effects of foliar spray of biostimulants on shoot and root growth under salinity stress

Difference in growth and morphology of *P. distans* seedlings under various treatments is shown in figure 3. Evaluation of vegetative growth parameters in *P. distans* seedlings showed that two biostimulant pre-treatments considerably enhanced these parameters under both NSC and SC. Under non-salinity condition, shoot FW and DW increased by 8.2 and 7.2% and in Algabon and 21.2 and 19.4% in Bonamid-sprayed plants, respectively (Table 1). Furthermore, root FW

and DW increased by 26.3 and 35.3% in Algabon and 81% and 2.5 fold in Bonamid-sprayed plants, respectively, in comparison with water-sprayed plants in non-salinity condition. Also, in this condition, the highest RWC and root/shoot ratio (DW) was observed by the application of foliar spray of Bonamid by 5% and 2 fold, respectively. Salinity stress led to a decrease of SFW, SDW, RFW, RDW and RWC by 43, 49, 12, 20 and 18.5%, respectively, in water-sprayed plants under NaCl stress and root/shoot ratio (DW) increased by 60% compared with their NSC counterparts (Table 1). Pre-treatments of *P. distans* leaves with foliar spray of two biostimulants considerably alleviated salinity stress-induced growth reduction. Algabon enhanced SFW, SDW and RFW by 63, 55 and 16%, respectively, while Bonamid enhanced the respective parameters by 93.4, 73.5 and 37.2% as compared with salinity-stressed plants exposed to water-sprayed treatment. Although there was no significant difference between plants foliar sprayed by two biostimulants in SFW and root/shoot ratio (DW), the highest value of RDW was observed in the plants sprayed with Bonamid.

4.3. Effects of foliar spray of biostimulants on chlorophyll and carotenoid content under salinity stress

As shown in figure 4A, the results demonstrated that the total chlorophyll content of *P. distans* seedlings was significantly affected by the application of biostimulants, under NSC and SC. In NSC, the highest value of the total chlorophyll (1.95 ± 0.05 mg/FW) was detected by the application of foliar spray of Bonamid. At salinity stress conditions, the total chlorophyll of Algabon and Bonamid-sprayed plants decreased by 8.8 and 22%, respectively, as compared with salinity-stressed plants exposed to water-sprayed treatment. Similarly, at the NSC, the highest value of the carotenoids content (0.05 ± 0.001 mg/FW) was also detected by the application of foliar spray of Bonamid in comparison with water-sprayed plants in NSC. We observed no significant difference between salinity-stressed plants foliar sprayed by two biostimulants in the carotenoids content compared with plants exposed to water-sprayed treatment in SC (Fig. 4B).

4.4. Effects of foliar spray of biostimulants on total phenolic, proline, glycine betaine and RSA contents under salinity stress

According to the results shown in Fig. 5, the results showed that the application of biostimulants in *P. distans* seedlings significantly influenced on total phenolic, proline and RSA content under NSC and SC. Nonetheless, there was no significant difference in the glycine betaine content between plants in NSC foliar sprayed with biostimulants and the water-sprayed plants. However the highest content of these compounds was found in water-sprayed plants in non-salinity conditions. Under salinity stress, the total phenolic, proline, and RSA content increased in *P. distans* seedlings pre-treated with two biostimulants as compared with their counterparts grown under NSC.

4.5. Effects of foliar spray of biostimulants on soluble sugar, protein and nitrogen percentage of shoots and roots under salinity stress

As shown in Table 2, the soluble sugar content of roots and shoots was influenced by foliar spray of biostimulants. Under salinity stress, the soluble sugar content of roots and shoots increased in *P. distans* seedlings pre-treated with two biostimulants as compared with their counterparts grown under NSC. Upon the application of foliar spray of biostimulants in the NSC, protein content of roots increased by 7% in Algabon and 12% in Bonamid-sprayed plants, respectively in comparison with water-sprayed plants in NSC. In salinity conditions the highest protein content of roots and shoots were observed by the application of foliar spray of Bonamid by 6.1 and 9.3, respectively, compared with plants exposed to water-sprayed treatment in SC. In consistent with protein results, the application of biostimulants in *P. distans* seedlings significantly influenced on the percentage of nitrogen content of roots and shoots under NSC and SC. In both conditions, the highest percentage of nitrogen content of roots and shoots was observed in the plants sprayed with Bonamid by 13 and 12% (in NSC) and 8.2 and 31% (in SC) compared with plants exposed to water-sprayed treatment counterparts.

Table 2

Soluble sugar, protein content and percentage of Nitrogen of roots and shoots in salinity condition (SC) and non-salinity condition (NSC) in *Puccinellia distans* exposed to foliar spraying of biostimulants. Different letters indicate significant differences according to LSD tests ($p < 0.05$).

Biostimulants Treatments	Root soluble sugar (mg/100g DW)		Shoot Soluble Sugar (mg/100g DW)		Root Protein (g/100g DW)	
	NSC	SC	NSC	SC	NSC	SC
Control	0.77±0.01 ^d	1.64±0.01 ^a	2.35±0.01 ^d	4.48±0.01 ^a	5.45±0.01 ^{cd}	5.22±0.16 ^d
Algabon	0.65±0.01 ^e	1.50±0.01 ^b	2.48±0.01 ^e	4.05±0.01 ^b	5.82±0.01 ^b	5.24±0.01 ^d
Bonamid	0.37±0.01 ^f	1.39±0.00 ^c	2.47±0.01 ^e	3.59±0.01 ^c	6.12±0.00 ^a	5.54±0.01 ^c
	Shoot Protein (g/100g DW)		Percentage of Nitrogen (Root)		Percentage of Nitrogen (Shoot)	
	NSC	SC	NSC	SC	NSC	SC
Control	14.36±0.01 ^b	11.76±0.01 ^f	0.87±0.01 ^c	0.82±0.00 ^e	2.30±0.01 ^d	1.81±0.01 ^f
Algabon	13.78±0.01 ^c	12.22±0.01 ^e	0.97±0.01 ^a	0.85±0.00 ^d	2.40±0.01 ^b	2.27±0.01 ^e
Bonamid	14.65±0.01 ^a	12.85±0.01 ^d	0.98±0.00 ^a	0.88±0.01 ^b	2.58±0.01 ^a	2.37±0.01 ^c

4.6. Effects of foliar spray of biostimulants on ions of shoots and roots under salinity stress

The effect of biostimulants and salinity stress on Na^+ , K^+ and K^+/Na^+ in roots and shoots of in *P. distans* seedlings is shown in Table 3. Salinity stress increased concentrations of Na^+ in roots and shoots in control plants, however foliar spray of two biostimulants reduced Na^+ concentration of roots and shoots by 9.5 and 11% (in Algabon), 9.6 and 13% (in Bonamid) and lessened detrimental impacts of salinity (Table. 3). Both biostimulants accumulated similar Na^+ content in roots and shoots in salinity conditions, but a lower Na^+ level was found in the Bonamid pre-treated *P. distans*. K^+ content of roots and shoots increased by the application of two biostimulants in both NSC and SC. Based on the obtained results, under salinity stress, significant reductions took place in K^+/Na^+ of both roots and shoots of control plants, however foliar spray of two biostimulants improved this ratio under both NSC and SC. In Both conditions, the highest K^+/Na^+ ratio of roots was observed in Algabon and Bonamid treatments by 2.5 and 1.6 fold, respectively, whereas there was no significant difference between plants sprayed with two biostimulants in K^+/Na^+ ratio of shoots.

Table 3

Na⁺, K⁺ and Na⁺/K⁺ ratio of shoots and roots in the shoots and total biomass in salinity condition (SC) and non-salinity condition (NSC) in *Puccinellia distans* exposed to foliar spraying of biostimulants. Different letters indicate significant differences according to LSD tests ($p < 0.05$).

Biostimulants Treatments	Shoot Na ⁺ (mg/g)		Root Na ⁺ (mg/g)		Shoot K ⁺ (mg/g)	
	NSC	SC	NSC	SC	NSC	SC
Control	5.35±0.10 ^e	10.62±0.10 ^a	7.22±0.10 ^c	10.23±0.09 ^a	33.46±1.08 ^d	42.30±0.91 ^b
Algabon	5.34±0.10 ^e	9.43±0.11 ^b	6.52±0.10 ^d	9.25±0.10 ^b	36.22±1.01 ^c	42.71±1.12 ^b
Bonamid	6.38±0.10 ^d	9.23±0.10 ^c	5.14±0.09 ^e	9.24±0.09 ^b	43.50±0.96 ^b	46.13±0.95 ^a
	Root K ⁺ (mg/g)		Shoot K ⁺ / Na ⁺		Root K ⁺ / Na ⁺	
	NSC	SC	NSC	SC	NSC	SC
Control	20.04±0.98 ^d	16.60±1.17 ^e	6.26±0.31 ^b	3.98±0.04 ^e	2.77±0.12 ^c	1.62±0.10 ^d
Algabon	30.37±0.75 ^b	24.12±0.95 ^c	6.77±0.06 ^a	4.52±0.17 ^d	4.65±0.04 ^b	2.60±0.07 ^c
Bonamid	35.60±0.85 ^a	24.58±1.18 ^c	6.81±0.25 ^a	4.99±0.05 ^c	6.92±0.28 ^a	2.65±0.10 ^c

4.7. Effects of foliar spray of biostimulants on phytoremediation potential (PP) under salinity stress

Estimation of phytoremediation potential (PP) in *P. distans* seedlings showed that two biostimulant pretreatments considerably enhanced this potential under both NSC and SC. Under NSC, phytoremediation potential of the shoots and the total biomass (shoots+roots) increased by 7.2 and 11.6% by Algabon and 42.6 and 52.4% by Bonamid treatments, respectively, in comparison with control treatment (Figure 6). Significant increase in phytoremediation potential of shoots and total biomass were observed in plants foliar sprayed with two biostimulants at salinity stress; the highest phytoremediation potential of shoots and total biomass was detected in the plants sprayed with Bonamid by 50.8 and 42.7% respectively, relative to that in salinity-stressed control plants.

4.8. Effects of foliar spray of Bonamid on gene expression of SOS1 and NHX1 under salinity stress

QPCR was applied to analyse the expression induction of antiporter genes, SOS1 and NHX1 in *P. distans* pre-treated with Bonamid in roots and shoots, respectively, under both conditions (Figure 7). Under the salinity stress, the expression of SOS1 gene increased, although not significantly, compared to the water sprayed plants. Although the foliar spray of Bonamid significantly increased the expression level of SOS1 (7.39 ± 1.55 , $p > 0.05$) under salinity stress, there was no significant difference between Bonamid pre-treated plants and water sprayed counterparts (Figure 7B). The expression of NHX1 in shoots was also analysed by QPCR (Figure 7B). The results indicated that salinity was able to induce NHX1 gene expression in the shoots of *P. distans* (6.04 ± 0.64 , $p > 0.05$). Furthermore, the plants pre-treated by Bonamid showed significant NHX1 gene expression in both conditions. However, the highest expression of NHX1 (8.53 ± 1.08 , $p > 0.05$) was found in Bonamid-treated plants under salinity stress.

5. Discussion

5.1 Pre-treatment of biostimulants significantly impacted growth and photosynthetic pigments in *P. distans* seedling under salinity stress

To assist to our understanding of the effects of efficiency of biostimulants pre-treatment to neutralize the stress condition, we pre-treated *P. distans* seedling with foliar spray of two biostimulants (Algabon® and Bonamid®), and then exposed them to 300 mM NaCl and compared responses with those pre-treated with foliar spray of water.

Growth of NSC plants displayed a marked response towards biostimulants pre-treatment by both Algabon and Bonamid (Figure. 3B). Generally, there was a significant increase in the shoot and root biomass and water content of NSC plants pre-treated with Bonamid. Also, the application of Bonamid resulted in higher root/shoot biomass, which is the result of increased root growth and is consistent with root density (Figure 3. C). Similarly, the increase in dry biomass, root density and RWC of the plants sprayed with Bonamid compared to the Algabon treatment and control plants was most pronounced at 300 mM NaCl (Figure 3. C), and pre-treatment with Bonamid mitigated NaCl-induced growth prevention in *P. distans* (Figure 3. B).

In this study, we observed a composition-dependent effect of biostimulants in diminishing the effects of NaCl stress, so that Bonamid was more effective than Algabon. In most plants, salinity stress prevents growth and development. One of the primitive response of plants to salinity is inhibition of shoot and root growth. The effects of salinity on alteration in growth might be attributed to changes in water and ion absorption by the roots, production of hormonal signals that exchange messages to the shoot, and changes in gene expression patterns. Typically, when glycophytes are exposed to salinity stress, shoot growth is more affected than roots, leading to the enhance of root/shoot ratio (Kravchik and Bernstein 2013). However, the salinity-induced root growth response in halophyte plants may be different (Flowers and Colmer 2008).

Several studies have reported the affirmative effect of biostimulants on plant growth in a wide range of compounds such as seaweed extract, protein hydrolysis and humic acids under stress (Lucini et al. 2015; Latef et al. 2017; Saidimoradi et al. 2019). The application of seaweed extract- and protein hydrolysates-derived biostimulants might supply defence opposed to salinity stress in plants. Latef et al. (2017) suggested that foliar applications of two seaweed extracts improved plant growth and photosynthetic pigments of *Cicer arietinum* under saline soil condition. Also, exopolysaccharide extracts from *Dunaliella salina* diminished the salinity stress and alleviated the decrease in dry weight of the plant's shoot and root systems in *Solanum lycopersicum* (Arroussi et al. 2018). Lucini et al. (2015) also demonstrated that foliar application of LISIVEG® (the plant-derived protein hydrolysate) biostimulant increased fresh yield, dry biomass and root dry weight of *Lactuca sativa* grown under salinity conditions. It is well known protein hydrolysates-derived biostimulant that play an important role in stimulation of growth, increase of yield and alleviation of the impact of salinity stress on crops, through the modulation of plant molecular and physiological processes. Protein hydrolysates-derived biostimulants directly influence on plant growth by the induction of carbon and nitrogen metabolisms, as well as regulation of N uptake and by interfering with hormonal activities, leading to the stimulation of root and shoot growth, and thus crop productivity (Colla et al. 2017).

Foliar and root applications of protein hydrolysates-derived biostimulants have been demonstrated to increase the uptake and use efficiency of both macro and micronutrients (Colla et al. 2015). Modifications of root density in protein hydrolysate-treated plants may be involved to improve the efficiency of water and nutrient uptake, thus promoting yield production (Colla et al. 2015). Subbarao et al. (2015) also indicated a positive effect of protein hydrolysate on enhancement of root and shoot growth, and found that soil application was more effective than foliar application. Similar results were obtained by Popko et al., (2018), who recommended foliar application of amino acids-based biostimulants for an efficient agricultural production of winter wheat. In our study, the Bonamid pre-treatment mediated-

growth promotion effect on *P. distans* seedlings under salinity stress was simply explained by higher biomass and density of roots that led to the enhancement of the efficiency of water and nutrient (especially N) uptake.

Salinity stress decreased total chlorophyll content in control plants, whilst foliar spray of two biostimulants enhanced chlorophyll content and diminished detrimental impacts of salinity. Application of biostimulants plays an important role in enhancing chlorophyll content (Latef et al. 2017; Saidimoradi et al. 2019). Colla et al. (2014) also showed that a commercial plant-derived PH (Trainer) induced chlorophyll synthesis by the increasing of leaf nitrogen content. Generally, there was a significant increase in total chlorophyll in plants pre-treated with Bonamid under SC. Protein hydrolysate can also help plants to retain photosynthetic activity under stress conditions.

These results are in agreement with those of Lucini et al. (2015) and Di Mola et al., (2021) who stated that application of a plant-derived protein hydrolysate increased photochemical activity (Fv/Fm) in lettuce and hemp, respectively, grown under saline conditions. This factor ensures better photosynthetic metabolism and thus improves plant performance. Foliar spray of *P. distans* seedlings with two biostimulants enhanced carotenoid content in NSC. It has been shown that biostimulants are effective in increasing production of several classes of secondary metabolites such as carotenoids, which are involved in crop quality and stress response. Foliar application of seaweed extract from *Ecklonia maxima* increased chlorophylls and carotenoids concentrations in *Brassica oleracea* (Rengasamy et al. 2016). Application of an amino acid-derived biostimulant had positive effects on carotenoid content in two varieties of carrot (Grabowska et al. 2012). In general, under salinity conditions, the carotenoid content was unchanged in plants pre-treated with two biostimulants (Figure 4B), likely due to the great enhance in biomass produced by biostimulants, which in turn might dilute some of the compounds in the tissues.

5.2 Pre-treatment of biostimulants significantly impacted compatible solutes in *P. distans* seedling under salinity stress

The accumulation of compatible solutes, including proline, soluble sugar and glycine betaine was reported in halophytes plants likely for the modulation of osmotic signalling pathways in vacuoles, as well as stabilization of membranes and act as ROS scavengers (Sharma et al. 2019). At 300 mM of NaCl, proline, glycine betaine and soluble sugar content of roots and shoots enhanced in control plants of *P. distans*. Under salinity conditions, foliar spray of two biostimulants increased proline and soluble sugar concentrations of roots and shoots compared with their counterparts grown under NSC that diminished detrimental impacts of salinity in *P. distans* seedling. Previous studies also showed that foliar application of seaweed extract- and protein hydrolysates-derived biostimulants improved salt stress amelioration in plants by enhancing proline, soluble sugar and glycine betaine contents (Ertani et al. 2013; Arroussi et al. 2018; Latef et al. 2017). Osmotic regulation in stressed plants is highly dependent on soluble sugars, and in glycophytic plants, more than 50% of the maintenance of osmotic regulation under salinity stress is related to the production of soluble sugars. The important roles of carbohydrates in the alleviation of salinity-induced responses involves osmoprotection, scavenging of ROS and carbon storage. The enhancement of reducing sugars under salinity stress has been previously observed in various plants (Kerepesi and Galiba 2000). The upregulation genes associated with osmotic regulation has been also reported in halophytic plant of *Spartina alterniflora* under salinity stress (Baisakh et al. 2006).

Biostimulants produced based on protein hydrolysates and amino acids showed quality improvement in one cultivar of carrot by increasing soluble sugar content (Grabowska et al. 2012). Higher sugar level in plants treated with biostimulants have been detected in different species, together with higher carotenoid and chlorophyll contents, photosynthetic rates, stomatal conductance, total protein, phenols, ascorbic acid, as well as growth-promoting hormones (Abbas 2013; Abdalla 2013; Martinez Esteso et al. 2016; Arroussi et al. 2018). After the exposure of Bonamid-pre-treated plants under salinity conditions, we observed the reduction of glycine betaine in the leaves of *P. distans*. Similar to our results, some researchers revealed that by the application of biostimulants, the compatible solutes decreased in salinity-stressed plants (Aydin et al. 2012; Jarošová et al. 2016). One possible reason for this observation in plants pre-treated

with Bonamid is the more participation of glycine betaine in the **moderation** of deleterious impacts of salinity in *P. distans*. Glycine-betaine is likely the major compatible solute other than proline. Application of a seaweed-based biostimulant had the effect of glycine betaine synthesis in spinach (Fan et al. 2011). The improvement in salinity tolerance in Bonamid-pre-treated plants may be attributed to the ingredients of the Bonamid (containing amino acid (85%)), which could induced the plant's metabolism to biosynthesis of compatible solutes and these protective compounds increase the plant potential to exclude Cl^- ions.

5.3 Pre-treatment of biostimulants significantly impacted antioxidants potential in *P. distans* seedling under salinity stress

Besides the accumulation of compatible solutes, to fight against salinity-induced oxidative stress, plants increase the biosynthesis and accumulation of non-enzymatic antioxidant compounds, particularly metabolic pathways associated with polyphenolic antioxidants biosynthesis (Cheynier et al. 2013). Under salinity stress, total phenolic compounds and radical scavenging activity increased in control plants of *P. distans*, also foliar spray of two biostimulants increased the antioxidant potential and total phenolic compounds compared with their counterparts grown under NSC and moderated detrimental impacts of salinity. Induction of oxidative damage under salinity stress through forming ROS has been reported in halophytes plants (Ellouzi et al. 2011; Bose et al. 2014). Hence, halophytes plants are able to synthesize non-enzymatic antioxidants such as total phenolic compounds under salinity conditions (He et al. 2020; He et al. 2021). Hsouna et al. 2020 revealed enhancement of polyphenol compounds in the leaves of the halophyte *Lobularia maritima* when exposed to saline conditions that might be related to the up-regulation of phenylalanine ammonia-lyase activity. Many quality characteristics of plants are linked to secondary metabolites, like polyphenols. These compounds are free radical scavengers, and may preserve plants against oxidative stress. Hence, interaction of biostimulants with the flavonoid metabolism can synergistically strengthen their effects. Enhancement in polyphenols contents of plant tissues by the biostimulant application has been shown in different glycophyte plants (Gurav and Jadhav 2013; Elansary et al. 2016). However, the research on the effects of biostimulants on the levels of phenolic compounds in halophyte plants has been very limited. Kaluzewicz et al. (2017) reported that the application of amino acid-based biostimulants and *Ascomyces nodosum* filtrate increased the total phenolic content, sinapic acid content, as well as quercetin content in *Brassica oleracea* seedlings. According to the results of this study, Latef et al., (2017) and Arroussi et al. (2018), demonstrated that the foliar spray of seaweed extract-based biostimulant enhanced the levels of phenolic compounds in chickpea and tomato plants, respectively, under salinity stress, thus leading to salt stress alleviation. Amino acids-based biostimulants enhanced the activity of phenylalanine ammonia-lyase, which is the first step in phenylpropanoid pathway and starting point for secondary metabolics pathways and production of a wide range of phenolic compounds, such as flavonoids, anthocyanins, plant hormones, phytoalexins and lignins (du Jardin et al. 2020). In our study, biostimulants-mediated induction of phenylpropanoid compounds as well as the antioxidant capacity of plants under salinity stress would increase their health-related characteristics, leading to maintaining a high level of chemical defence capability and, in turn, better efficiency in respect of plant growth. Generally, a better status of the plants pre-treated with Bonamid, as well as potentially higher tolerance to salinity (Fig 3B) due to the presence of phenolic compounds, was also shown by the higher content of proline and proteins, indicative of the more magnitude of metabolic activity in plants treated with the biostimulant.

5.4 Pre-treatment of biostimulants significantly influenced protein and nitrogen percentage of shoots and roots in *P. distans* seedlings under salinity stress

Based on the results, root and shoot protein and nitrogen contents of *P. distans* can be affected by applying foliar spray of biostimulants under NSC. The highest content of proteins and nitrogen was found in roots and shoots of Bonamid pre-treated plants. Application of biostimulants have a major role in enhancing protein and nitrogen content (Ertani et al. 2013; Colla et al. 2014; Lucini et al. 2015). Abiotic stresses such as salinity increase ROS production and by upsetting

redox balance, cause oxidative damage to organic molecules such as proteins, lipids, carbohydrates, and DNA. Under salinity stress, the protein content decreased in all pre-treated plants compared with their counterparts grown under NSCs, however the maximum content of proteins in roots and shoots was detected in Bonamid pre-treated *P. distans*. Salinity stress could also alter several metabolic processes in plants, in particular, photosynthesis, respiration, phytohormone regulation, protein biosynthesis and nitrate assimilation (Colla et al. 2010). It generally leads to a decrease of production and to the lower quality of the final product, due to an inhibition of leaves and roots growth (Bulgari et al. 2019). To confirm the effects eliciting from the applications of biostimulants, Lucini et al. (2015) showed that foliar application of a hydrolysate biostimulant increased yield and dry weight in lettuce plants, leading to resistance to salinity stress through the improvement of nitrogen metabolism and an increase of the Fv/Fm-ratio efficiency. It has been also reported that application of seaweed extract-based biostimulant mitigated salinity-mediated protein reduction in tomato plants grown under different NaCl levels, through the activation of various metabolic pathways (Arroussi et al. 2018). However, abiotic stresses such as salinity increase nitrogenous compounds such as proline and soluble proteins in the plant. These proteins are essential for all plant's physiological processes including plant growth. Proteins produced under salinity stress in plants may be used as a source of nitrogen after stress. Since protein synthesis depends on the nitrate assimilation, it is obvious that the reduction of nitrogen assimilation under salinity stress can be the cause of the decrease in the protein content.

5.5 Pre-treatment of biostimulants significantly impacted Na^+ , K^+ , K^+/Na^+ and antiporters gene expression of shoots and roots in *P. distans* seedling under salinity stress

It has been shown that a disruption of Na^+ and K^+ homeostasis in cells followed by ion toxicity, adversely influences some major processes such as growth, photosynthesis, and development (Deinlein et al. 2014) and in both glycophyte and halophyte plants, sensitivity of cytosolic enzymes in front of salinity is similar. Hence, in all plants, maintaining cellular homeostasis of Na^+ and K^+ is crucial allowing plants to survive and grow under salt stress conditions (Cuin et al. 2011).

Our results showed that foliar spray of *P. distans* plants with two biostimulants reduced salinity-induced Na^+ accumulations in roots and shoots. Salinity induced a reduction of K^+ content of roots of *P. distans*; however, in the biostimulants pre-treated seedlings, it significantly compensated the negative impacts of salinity and enhanced K^+ content in roots and shoots. Similarly, Latef et al. (2017) suggested that foliar applications of two seaweed extracts decreased the extent of Na^+ accumulation and maintained higher K^+ levels of *Cicer arietinum* under salinity stress. Furthermore, Wu et al., (2021) demonstrated that Na^+ contents of roots in cucumber increased significantly under salinity stress; however, after foliar spray of 5-aminolevulinic acid (ALA), the Na^+ content in the roots decreased significantly. Osmotic adjustments play important role in plant resistance under salt stress, which include the intake of inorganic solutes from the soil to help the maintenance of leaves' turgor. Osmotic adjustments have been observed for the improvement of osmoregulation and resistance to salinity in halophyte plants (Jones and Gorham 2002; Hariadi et al. 2011). The effects of biostimulants in plant resistance against salinity can be attributed to the improvement of the osmotic adjustment by the accumulation of osmotic metabolites and the compartmentalization of salts in vacuoles. Our data indicated that application of biostimulants probably affected the mechanisms of uptake and translocation of ions in roots and shoots under both conditions. These biostimulant's beneficial effects includes the inhibition of Na^+ accumulations and improvement of K^+ uptake in the leaves, leading to an increases K^+/Na^+ ratio of roots and shoots (Table. 3).

On the basis of phytoremediation potential (shoot and total) of Bonamid pre-treated *P. distans* seedlings (Figure. 6) that is the best criterion for comparison with the other pre-treatments, we decided to investigate this criterion in Bonamid pre-treated *P. distans* seedlings under both conditions at the molecular level, through determination of genes expression

profile of SOS1: plasma membrane Na^+/H^+ and NHX1: tonoplast Na^+/H^+ antiporters. The mechanisms accepted by plants surviving in saline conditions include a balance between influx and efflux of Na^+ , either back into the apoplast across the plasma membrane or the tonoplast into the vacuole. In halophyte plants, the developed tolerance mechanisms like compartmentalization of ions in vacuoles are performed by Na^+/H^+ antiporter (NHX1) and salt extrusion through antiporter (SOS1) located in the plasma membrane (Wang et al. 2007; Hamed et al. 2013). The presence of Na^+/H^+ antiporter in the plasma membrane of plants is crucial for their growth under high salinity as it removes toxic Na^+ from the cytoplasm. Salt stress increases the gene expression of SOS genes in *Arabidopsis* (Oh et al. 2009), *Kochia scoparia* (Fahmideh and Fooladvand 2018) and sugarcane (Brindha et al. 2021). Several reports indicate that NHX overexpression confers salinity tolerance in a wide range of plant species (Brini et al. 2007), tomato (Zhang and Blumwald 2001) and cotton (He et al. 2007).

Exposure of *P. distans* seedlings under salinity increased the expression of SOS1 and NHX1 in roots and leaves. In halophytic plants as opposed to glycophytes, accumulation of Na^+ in vacuoles and the regulation of activity of Na^+/H^+ exchanger (NHX1) have been seen under salinity conditions. For example, Wang et al. (2009) showed that the significant mechanism of halophytic species of *P. tenuiflora* to succeed in dealing with NaCl was excreting Na^+ . Also, Zhang et al., (2017) showed that SOS1, HKT1;5, and NHX1 synergistically regulate Na^+ homeostasis by controlling Na^+ transport systems at *P. tenuiflora* plant under both lower and higher salt conditions. In accordance with the results of the present study, the upregulation of NHX of *Suaeda salsa* (Qiu et al. 2007) and two antiporters of SOS1 and NHX in *Kochia scoparia* (Fahmideh and Fooladvand 2018) under salinity stress have also been reported.

It has been shown that biostimulants can elicit plant response to environmental stress, thus activating genes associated with signalling pathways related to stress response (Trevisan et al. 2017; Jithesh et al. 2019). In this regard, it is believed that the genes expression of Na^+/H^+ transporters generally increases by exogenously application of biostimulants. The foliar spray of 5-aminolevulinic acid (ALA) significantly upregulated the transcriptional level of SOS1 and NHX1 under normal and salinity conditions (Wu et al. 2021). This indicated that ALA application can improve the compartmentation of Na^+ into vacuoles and enhance the salt resistance of cucumber seedlings. Similar to our results, stimulation of the expression of SOS1 in *Arabidopsis* with a commercially biostimulant (BC204) treatment (Loubser and Hills 2020) and the expression of vacuolar Na^+/H^+ exchanger (NHX1 gene) with silicon treatment have been reported in cucumber (Gou, 2020). On the contrary, Jithesh et al. (2018) found that the treatment of *sos1 Arabidopsis* mutant with *Ascophyllum nodosum* extract in media containing 75 mM NaCl did not reverse the lethality of salt. The results of our study showed that foliar application of Bonamid increased gene expression of SOS1 in roots, which coincided with a reduction of Na^+ concentration in roots of Bonamid-pre-treated plants probably due to SOS1 proteins function that acts in loading of Na^+ into the xylem of roots for regulating Na^+ release to the leaves and contribution in osmotic adjustment. Furthermore, foliar application of Bonamid enhanced the gene expression of NHX1 under 300 mM NaCl, which suggested that the sequestration of Na^+ to the vacuole increased to reduce Na^+ toxicity in the cytoplasm.

6. Conclusion

One of the important approaches for the improvement of salinity stress tolerance in plants is the application of biostimulants. However, the effectiveness of biostimulants depends on their penetration into the plant tissue; hence, the selection of a suitable biostimulant is crucial as the efficiency can vary considerably between species. This research studied the effects of the pre-treatment of two biostimulants (seaweed extract-derived and amino acid-derived biostimulants) on *P. distans* seedling under salinity tolerance. The results obtained in the present study show the beneficial effects of the pre-treatments of two biostimulants on *P. distans* seedlings under non salinity stress conditions with respect to increasing plant biomass, photosynthetic pigments, K^+ content, the content of proteins and nitrogen

percentage. The results suggested that foliar spray of Bonamid could considerably diminish NaCl-caused stress on *P. distans* seedlings, probably due to higher accumulation of shoot biomass, photosynthetic pigments, K⁺/Na⁺ ratio, protein and N content and phytoremediation potential. Also, foliar spray with Bonamid amplified the expression of SOS1 and NHX1 in the roots and leaves, respectively, under salinity stress, that likely led to the loading of Na⁺ into the xylem and reduction of Na⁺ content in the cytoplasm and contribution in osmotic adjustment. Considering the failure of pre-treatment of *P. distans* seedlings as foliar spray with Algabon in overcoming salinity stress, it seems that the application method or proposed concentration for this biostimulant is not appropriate and should probably be used in soil. Collectively, it could be concluded that, intensification of osmotic adjustment by the accumulation of osmotic metabolites and the compartmentalization of salts in vacuoles in pre-treated plants with amino acid-derived biostimulant (Bonamid) can diminish the NaCl-induced deleterious effects in *P. distans* seedlings.

Abbreviations

NSC, Non- salinity condition; RSA, Radical Scavenging Activity; ROS, Reactive oxygen species; RWC, relative water content; SC, Salinity condition; STI, Salt tolerance index; PP, Phytoremediation potential.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Agree.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceived and designed the experiments: Leila Shabani, Mohammad R. Sabzalian. Performed the experiments: Saeed Hosseini. Analyzed the data: Saeed Hosseini, Leila Shabani, Shima Gharibi. Wrote the paper: Leila Shabani. Edited the manuscript: Leila Shabani, Mohammad R. Sabzalian.

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Figures

Figure 1

The effects of various concentrations of NaCl (0, 300, 600, and 900 mM) on shoot fresh weight (A), shoot dry weight (B) RWC (C) and salt tolerance index (STI) (D) in the seedlings of *Puccinellia distans*. Different letters indicate significant differences according to Duncan's tests ($p < 0.05$).

Figure 2

Image of 52-day old seedlings of *Puccinellia distans* under the stress of different concentrations of NaCl (A) and (B) Left to right: roots of *P. distans* seedlings grown under 0, 300, 600 and 900 mM NaCl.

Figure 3

Representative visual image of aerial organs (A) and root density (B) of *Puccinellia distans* seedlings treated with water (control), Algabon, Bonamid, NaCl, Algabon+NaCl and Bonamid +NaCl.

Figure 4

Chlorophyll content (A) and the amounts of carotenoid (B) in salinity condition (SC) and non-salinity condition (NSC) in *Puccinellia distans* exposed to foliar spraying of biostimulants. Different letters indicate significant differences according to LSD tests (p < 0.05).

Figure 5

Total phenolic (A), proline (B), glycine betaine (C) and RSA (D) content in salinity condition (SC) and non-salinity condition (NSC) in *Puccinellia distans* exposed to foliar spraying of biostimulants. Different letters indicate significant differences according to LSD tests ($p < 0.05$).

Figure 6

Phytoremediation potential of the shoots (A) and the total biomass (shoots+roots) (B) in salinity condition (SC) and non-salinity condition (NSC) in *Puccinellia distans* exposed to foliar spraying of biostimulants. Different letters indicate significant differences according to LSD tests ($p < 0.05$).

Figure 7

The expression induction of antiporter genes, SOS1 (A) and NHX1 in salinity condition (SC) and non-salinity condition (NSC) in *Puccinellia distans* exposed to foliar spraying of Bonamid. Different letters indicate significant differences according to LSD tests ($p < 0.05$).