RESEARCH

Ameliorative effects of seed treated with phytohormones on seedling growth, soluble protein content and antioxidant enzymes of hargel (*Solenostemma argel* Hayne) seedlings under salt stress

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ABSTRACT

Salinity stress profoundly affects crop plants' morphological structures and physiological processes, decreasing plant growth and development. The application of phytohormones has been proved an efficient way to alleviate salinity stress. This study was done to evaluate the effects of exogenous application of gibberellic acid (GA_3; 0.288 mM), salicylic acid (SA; 0.362 mM), indole acetic acid (IAA; 0.285 mM) and control (0 mM) on seedling growth and physiological parameters of hargel (*Solenostemma argel* Hayne) seedlings under different salinity levels (0, 50 and 100 mM NaCl). Significant decreases due to salinity stress were observed in all the seedling growth parameters shoot and root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll *a* and *b* content, and antioxidant enzymes activities such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). In contrast, soluble protein and carotenoid content increased with increasing salinity. At high salinity level, GA_3 increased carotenoid content and APX activity by 56.4% and 53.4%, respectively, while SA increased shoot dry weight, SOD, POD and soluble protein by 1.3-fold, 1.3-fold, 1.95-fold and 93.2%, compared with the control, IAA increased root length and fresh root weight at the same salinity level by 1.58-fold and 2-fold, respectively. In conclusion, seed priming with an appropriate dosage of exogenous hormones successfully mitigates the adverse effects of NaCl by increasing photosynthetic pigment, antioxidant enzyme activity and seedling growth parameters. Furthermore, SA exhibited the best mitigating effects compared to IAA and GA_3.

Key words: Abiotic stress, antioxidant enzymes, medicinal plant, plant growth regulator, seedling growth parameters, *Solenostemma argel*.

INTRODUCTION

Crop plants are frequently subjected to environmental stresses that affect their plant growth and productivity. Salinity is an ever-increasing concern in arid, semi-arid, and irrigated environments, and it is a significant factor restricting crop plant spread in their natural habitats (Ali et al., 2021). Soil salinization is currently growing by 10% per year due to various factors such as low precipitation, high surface evaporation, native rock weathering, irrigation with saline water, and poor cultural practices (Mondal and Kaur, 2017). The detrimental effects of salts on plant growth and development could be attributed to a decrease in the osmotic potential of soil solution, which limits the amount of water available and causes water stress. Also, increased accumulation of harmful ions like Na^+ and Cl^- and nutrient imbalance inhibitory crop plant metabolism (Hmaeid et al., 2019).

Reactive oxygen species (ROS), such as superoxide radical anion (O_2^-) and hydrogen peroxide (H_2O_2), are products of cell metabolism that can cause plants to develop tolerance and adapt to environmental challenges. However, overproduction of ROS harms oxidative metabolic activities, such as the oxidation of organic molecules, including amino acids, lipids, and DNA (Khalid and Aftab, 2020). The ROS-induced oxidative stress disrupts redox equilibrium in plants, reducing photosynthetic efficiency, mineral absorption capacity, hormone concentration, and gene expression (Ma et al., 2018). On the other hand, plant cells have developed various defense systems for scavenging and detoxifying ROS to combat the adverse effects of salt stress, including enzymatic antioxidant systems and non-enzymatic antioxidants that keep ROS under control and contribute to the stress endurance mechanism (Hasanuzzaman et al., 2019). Plant hormones, also known as plant growth regulators, are frequently utilized to protect plants from the negative impacts of unfavorable environmental conditions (Sytar et al., 2019). Plant growth regulators such as salicylic acid (SA), indole acetic acid (IAA), and gibberellic acid (GA_3) are involved in the regulation of many physiological and biochemical processes and play an essential function in the growth and development of plants by regulating a variety of activities that improve salt tolerance (Rhaman et al., 2021), enhance photosynthetic activity, vascular patterning, flower development and they can coordinate different signaling pathways during exposure to abiotic stresses (Muhie, 2018; Sytar et al., 2019).

Medicinal plants are grown for their medicinal ingredients and utilized in various ways, including drugs. Because antibiotics are relatively expensive in developing countries, people still rely on natural treatments to treat ailments (Barakat and Fatima, 2000). Among these medicinal plants, hargel (*Solenostemma argel* Hayne; Asclepiadaceae) is a desert plant used in traditional medicine worldwide, particularly in African countries (Sudan, Libya, Chad, Egypt and Algeria), Saudi Arabia and Palestine. This plant is regarded as the richest source in Sudan and is locally called hargel; it is indigenous in the northern region and widely spread between Dongola and Barber, particularly around the Abu Hamad area. Hargel plants usually occur in dry sandy semi-desert areas with annual rainfall as low as 25-150 mm, and 8-47 °C annual temperature (Shayoub et al., 2013; Khameis and Teia, 2018). Medicinal properties of hargel shoots can be used to treat various diseases, including kidney, liver, stomach diseases, diabetes, respiratory tract infections, and some allergies (Khameis and Teia, 2018). Substantial studies indicate that hargel plants contain various compounds and provide diverse bioactivities with no toxicity. After thorough literature review, we know that little attention has been paid to the responses of seed growth of hargel under salinity stress. Although this medicinal plant is usually planted in marginal soils that face different abiotic stresses such as salinity, therefore, it is necessary to determine the salinity levels under which hargel plants could give higher yields and better quality. Researchers have used different ways to reduce the harmful effects of salinity stress. In this study, we hypothesized that treating seeds with exogenous application of GA_3, SA and IAA could improve plant establishment through increasing seedling emergence and growth characteristic and alleviate the adverse effects of salt stress on hargel seedlings. The aim of this study was to evaluate the effects of salinity stress and exogenous application of GA_3, SA, and IAA on hargel seedlings and find a suitable hormone concentration for alleviating salt stress.

MATERIALS AND METHODS

Experimental site and soil properties

A controlled pot study was conducted twice in a greenhouse on the Experimental Farm of Yangzhou University (32°30' N, 119°43' E), Yangzhou, Jiangsu Province, China, during the hargel (*Solenostemma argel* Hayne; Asclepiadaceae) growing season of 2020, to examine the effects of exogenous hormones on the seedling growth attributes and antioxidant enzymes

of hargel seedlings at different salinity levels. The soil (Typic fluvaquents, Entisols) used in this study was collected from the surface of sandy loam soil (0-20 cm) of the Experimental Farm of Yangzhou University. The soil was air-dried before being sieved at 5 mm. During the study, the average temperature was 32 °C, with a relative humidity of 76%.

Plant materials and experimental design

In this study, a hargel landrace provided by the Agricultural Research Institute in Khartoum, Sudan, was used in this research. Seeds were chosen based on their uniform color, shape, and symmetrical size. The seeds were < 18-mo-old and stored in brown paper bags under cold and dry conditions for less than 1 yr to ensure good germination. Seeds were sterilized by soaking them in a 2.5% sodium hypochlorite solution for 2 min, then washed several times with distilled water and air-dried.

The study consisted of two factorial experiments, i.e., salinity and plant growth regulator. Salinity factor included 0, 50, and 100 mM NaCl with an equivalent electrical conductivity (EC) of 0.5, 4.03, and 8.08 dS m^{-1}, respectively. Three different growth regulator solutions were chosen: control (without growth regulator, CK), gibberellic acid (GA_3) at 0.288 mM, salicylic acid (SA) at 0.362 mM, and indole acetic acid (IAA) at 0.285 mM. The different levels of growth regulator were determined by a preliminary experiment testing a wide range of concentrations (0.0, 0.144, 0.288 and 0.432 mM for GA_3; 0.362, 0.724 and 1.09 mM for SA; 0.285, 0.571 and 0.856 mM for IAA). According to preliminary experimental data, the highest germination percentage was found at the chosen concentrations. Previous publication reported the positive effects of selected concentrations of growth regulators on mitigating salinity stress effects; for example, increased seedling growth parameters, chlorophyll *a* and *b*, superoxide dismutase (SOD) and peroxidase (POD) activities were observed when sweet sorghum was exposed to salinity (Nimir et al. 2015).

The experiment run as a factorial design in a split-plot with three replicates using a randomized complete block design (RCBD). Three different salinity levels were included in the main plots, and four different growth regulator solution concentrations were included in the subplots. Seeds were soaked for 12 h at 25 ºC in 500 mL each concentration of the exogenous growth regulator solutions in the dark. After that, the solution was decanted off, and the seeds were re-dried to their original weight for 48 h under lab conditions. At the same time, the control seeds (untreated seeds) were soaked with the same amount of distilled water.

Initially, all seedlings were irrigated with tap water for 7 d. After the seed germinated, the most uniformly robust 10 seedlings were used as experimental materials for growing under control conditions in small plastic pots (n = 36), 9.5 cm in diameter, and 8.5 cm in-depth, without holes at the bottom. Each pot was filled with 400 g dry soil (20 seeds per pot).

Seedling stage

Treatments were carried out after 7 d naturally growth. The concentration of saline solutions (80 mL) at different salinity levels was gradually increased every week for 2 wk until it reached the final concentration to avoid salinity shock. The control was irrigated with the same amount of tap water (0.5 dS m^{-1}). The seedlings in the pot were irrigated with the required amount of tap water at a 2 d interval when the soil seemed dried. The seeding dates were 28 June and 10 July 2020.

Seedlings growth parameters

On the 45th day of the sowing date, three seedlings were randomly selected from every pot, harvested, cleaned and washed. The seedlings were separated into roots and shoots. Seedling growth parameters were determined, including shoot length (cm), root length (cm), shoot weight and root weight (g $plant^{-1}$). The shoots and roots were then dried at 80 °C in a forced-air oven until they reached a constant weight for dry weight determination.

Preparation of enzyme extracts

On the 45th day of the sowing date, 5 g leaves of seedlings from each replicate of each treatment were collected and immersed in liquid nitrogen for 30 min and then kept in -80 °C for determination of the enzymatic activity. Leaf protein was extracted using a phosphate buffer solution containing sodium phosphate dibasic dehydrate and sodium phosphate monobasic dehydrate. Stored leaf tissue (0.2 g) was crushed in a 2 mL phosphate buffer solution, and the slurry was centrifuged at 10 000 rpm for 20 min at 4 °C. The supernatant was used to determine the activities of SOD, catalase (CAT), POD, ascorbate peroxidase (APX) and the activity of a soluble protein.

Determination of physiological parameters

The SOD was measured following Janmohammadi et al. (2012), POD activity was measured by the method of Xu and Ye (1989), CAT activity was assayed by Janmohammadi et al. (2012), and APX activity was measured according to Nakano and Asada (1981) method. The soluble protein content was determined by Bradford (1976) method using bovine serum albumin as the protein standard.

Determination of chlorophyll *a* and *b* and carotenoid content

The photosynthetic pigments such as chlorophyll *a* and *b* and carotenoid content were determined according to the method reported by Lichtenthaler and Wellburn (1983). A spectrophotometer recorded the absorbance readings at 453, 645, and 663 nm, respectively.

Statistical analysis

Each variable's data was statistically examined for a variance for RCBD as a factorial design using MSTAT-C statistical Package (Freed et al., 1991). When F values were significant, the least significant difference (LSD) test ($P \leq 0.05$) was used to separate means.

RESULTS

According to the ANOVA table, most parameters, including seedling growth characteristics, activities of antioxidant enzymes like SOD, POD, CAT, and APX, photosynthetic pigments (chlorophyll *a*, *b*, and carotenoids content), and soluble protein content, were found to be highly significant for salinity, growth regulator, and their interactions (Table 1).

Growth parameters

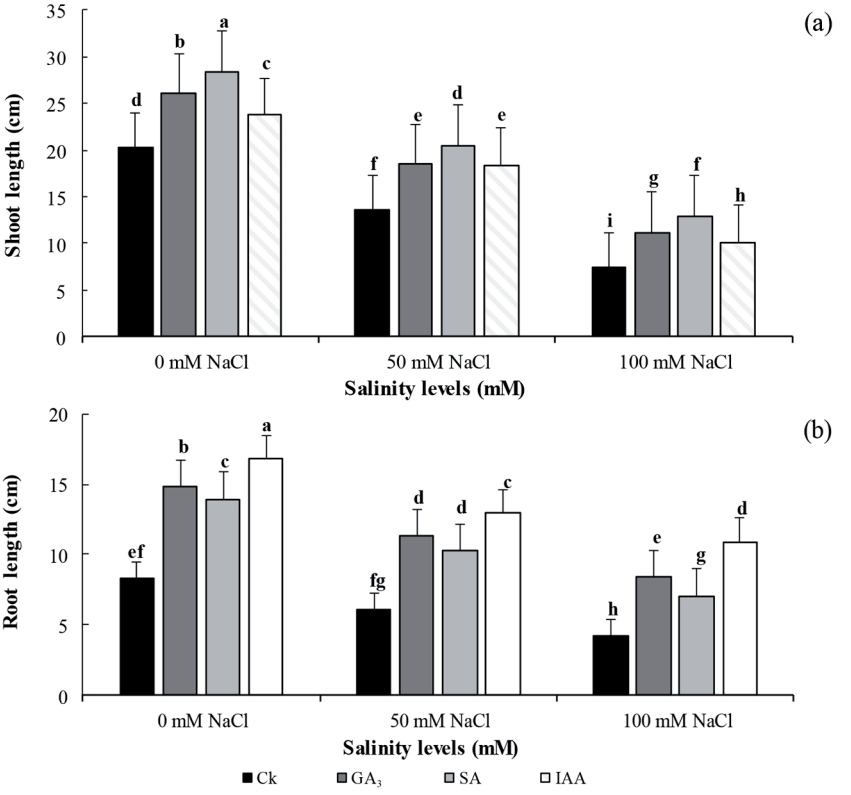
Shoot length (SL) and root length (RL) decreased with increasing salinity concentrations. The interaction between salinity and growth regulator significantly affected SL and RL. At the highest salinity level of 100 mM NaCl, SL was increased by 74.9%, 51.3%, and 36.6% with SA, GA_3 and IAA, respectively, compared with growth regulator control (Figure 1a). However, at 100 mM salinity level, RL increased by 157.5%, 98.6%, and 66.0% with IAA, GA_3 and SA, respectively, compared with growth regulator control (Figure 1b). Salinity stress significantly reduced the dry seedling weight (shoot dry weight [SDW] and root dry weight [RDW]) of hargel plants. However, the application of GA_3, SA, and IAA reduced the adverse effects of salinity. Both salinity levels of 50 and 100 mM NaCl significantly reduced RDW by 30.5% and 52.4%, respectively, compared with 0 mM NaCl (Table 2). The highest value of RDW of 0.11 g plant^{-1} was recorded with

Table 1. ANOVA table for seedling growth parameters, activities of antioxidant enzymes photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids content), and soluble protein content as influenced by salinity, growth regulators, and their interactions.

	F value		
Parameters	Salinity (S)	Hormone (H)	S × H
Shoot length (SL)	148.4***	70.8***	5.2**
Root length (RL)	54.0**	41.0***	6.0**
Shoot fresh weight (SFW)	1231.7***	268.7***	36.4***
Root fresh weight (RFW)	637.8***	163.8***	9.4***
Shoot dry weight (SDW)	55.3**	17.7***	3.0*
Root dry weight (RDW)	35.2**	38.5***	1.5^{ns}
Superoxide dismutase (SOD)	1.8^{ns}	8.7***	7.3***
Peroxidase (POD)	43.5**	72.5***	23.2***
Catalase (CAT)	150.0***	4.6*	2.0^{ns}
Ascorbate peroxidase (APX)	42.2**	22.3***	17.9***
Chlorophyll *a*	13.8*	36.7***	0.9^{ns}
Chlorophyll *b*	31.2**	21.6***	1.9^{ns}
Carotenoids content	68.3***	44.0***	4.1**
Soluble protein content	0.81^{ns}	12.8***	3.3*

*, **, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively; ^{ns}: nonsignificant difference.

Figure 1. Effect of interaction between different salinity and growth regulators on shoot length (a) and root length (b) of hargel seedlings.



Bars with different letters showed significant differences at the 0.05 level of probability according to LSD test.
CK: Control 0 mM; GA_3: gibberellic acid 0.288 mM; SA: salicylic acid 0.362 mM; IAA: indole acetic acid 0.285 mM.

Table 2. Effect of salinity on root dry weight, chlorophyll *a* and *b* content and catalase (CAT) activity of hargel seedlings.

Salinity	Root dry weight	Chlorophyll *a*	Chlorophyll *b*	CAT
mM NaCl	g plant^{-1}	mg g^{-1} FW	mg g^{-1} FW	U g^{-1} min^{-1}
0	0.11a	4.9a	1.7a	7.1a
50	0.07b	4.4ab	1.8a	3.4b
100	0.05c	3.9b	1.9a	1.7c

Different letters in the same column indicate significant differences at the 0.05 probability level according to LSD test.

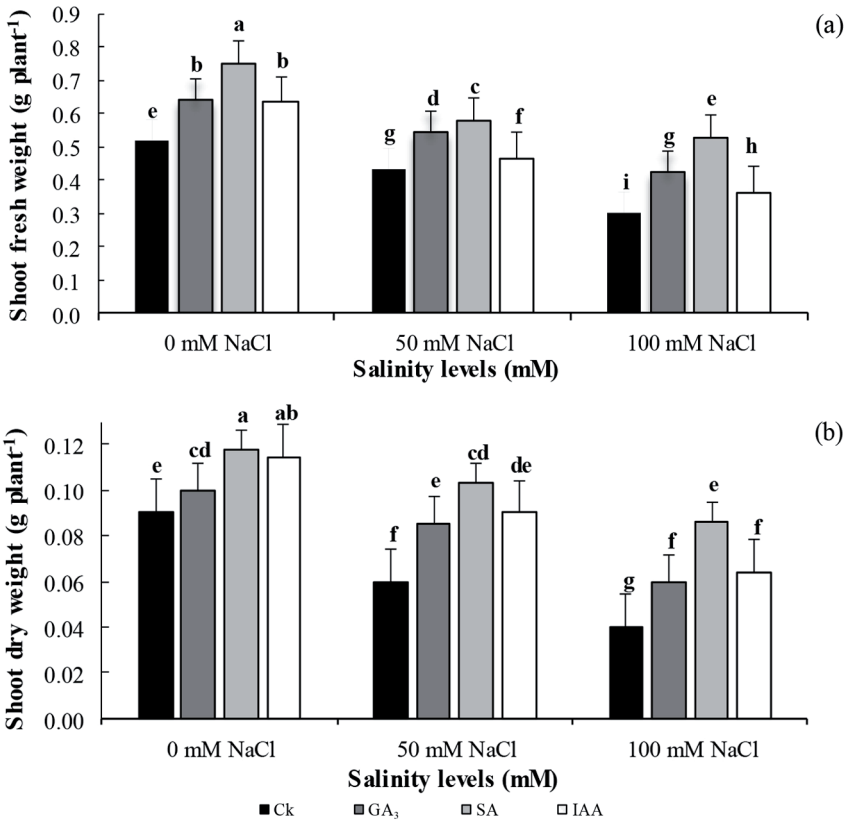
IAA application, followed by GA_3 (0.09 g plant^{-1}) and SA (0.07 g plant^{-1}) (Table 3). In the interaction between salinity and hormone application, the highest SDW value was recorded at SA, followed by IAA and GA_3 (Figure 2b). Seedlings' fresh weight (shoot fresh weight [SFW] and root fresh weight [RFW]) were increased by growth regulator application and decreased with increasing salinity levels. In the interaction between salinity and growth regulator application, at the high salinity level of 100 mM NaCl, SFW increased by 76.7%, 41.0%, and 21.0% in SA, GA_3 and IAA, respectively, as compared with the control of growth regulator (Figure 2a). For the RFW, at the 100 mM NaCl, the highest RFW value (0.15 g plant^{-1}) was recorded at IAA, followed by GA_3 (Table 4).

Table 3. Effect of growth regulators on root dry weight, chlorophyll *a* and *b* content and catalase (CAT) activity of hargel seedlings.

Hormone	Root dry weight	Chlorophyll *a*	Chlorophyll *b*	CAT
mM	g plant^{-1}	mg g^{-1} FW	mg g^{-1} FW	U g^{-1} min^{-1}
CK	0.04d	3.6c	1.2c	3.6b
GA_3	0.09b	4.7a	2.0ab	4.4ab
SA	0.07c	5.0a	2.2a	4.6a
IAA	0.11a	4.6ab	1.9ab	4.6ab

Different letters in the same column indicate significant differences at the 0.05 probability level according to LSD test.
CK: Control 0 mM; GA_3: gibberellic acid 0.288 mM; SA: salicylic acid 0.362 mM; IAA: indole acetic acid 0.285 Mm.

Figure 2. Effect of interaction between different salinity and growth regulators on shoot fresh weight (a) and shoot dry weight (b) of hargel seedlings.



Bars with different letters showed significant differences at the 0.05 level of probability according to LSD test.
CK: Control 0 mM; GA_3: gibberellic acid 0.288 mM; SA: salicylic acid 0.362 mM; IAA: indole acetic acid 0.285 mM.

Table 4. Mean values of root fresh weight and carotenoid content of hargel seedlings for the effect of interaction between salinity and growth regulators.

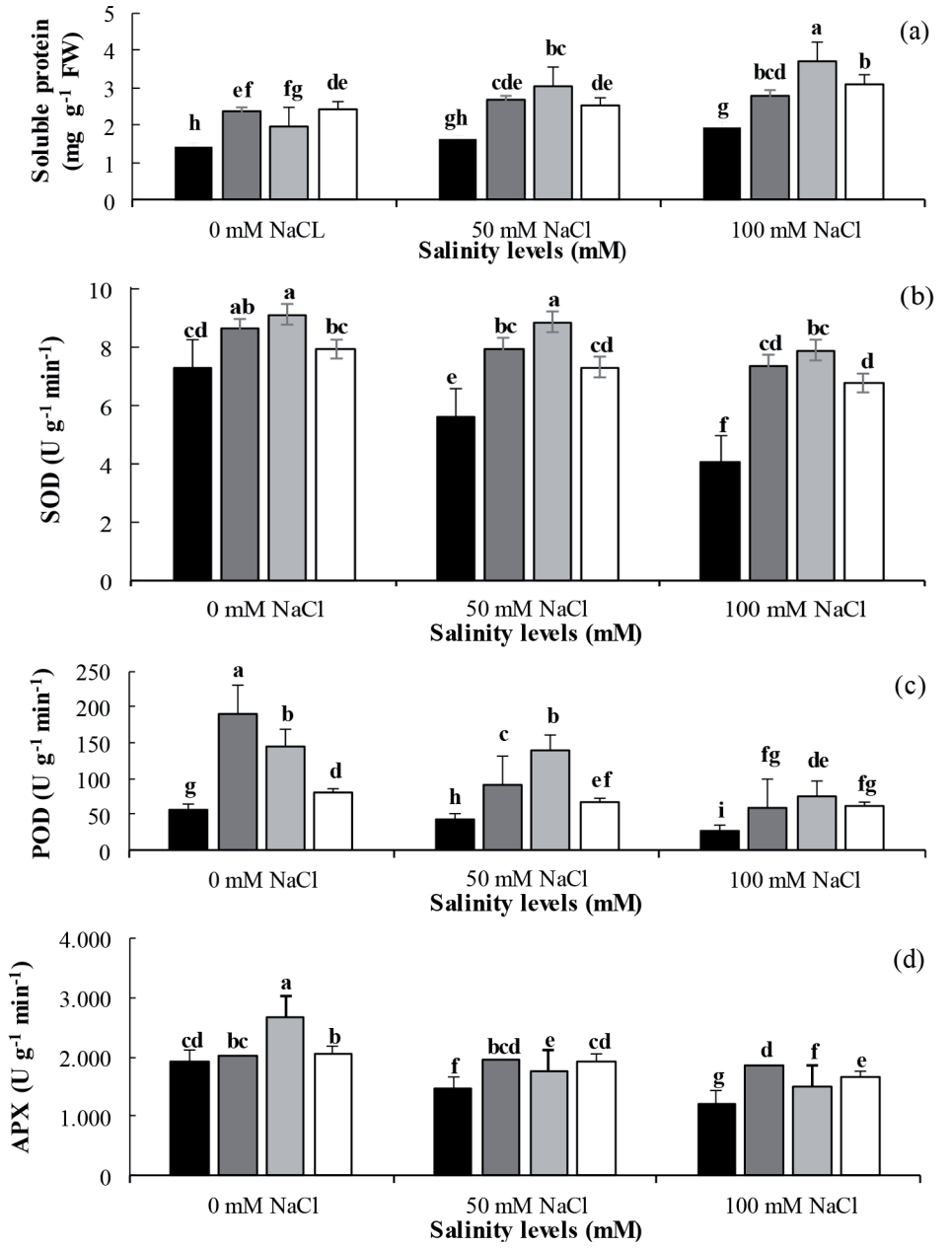
	Root fresh weight				Carotenoid content			
Salinity	Growth regulators							
mM NaCl	CK	GA_3	SA	IAA	CK	GA_3	SA	IAA
0	0.15e	0.33b	0.28c	0.39a	2.9g	4.2e	4.4e	5.5cd
50	0.11g	0.27c	0.21d	0.33b	3.2f	5.3d	5.5d	5.9cd
100	0.05h	0.13f	0.11g	0.15e	4.2e	6.5a	5.5cd	6.0bc

Different letters in the same column indicate significant differences at the 0.05 probability level according to LSD test.
CK: Control 0 mM; GA_3: gibberellic acid 0.288 mM; SA: salicylic acid 0.362 mM; IAA: indole acetic acid 0.285 mM.

Photosynthetic pigments

The photosynthetic pigments such as chlorophylls *a* and *b* decreased markedly with increasing salinity levels. In contrast, carotenoid content increased when salinity level increased. The high salinity level of 100 mM NaCl significantly reduced chlorophylls *a* and *b* by 22.1% and 13.1%, respectively, compared with 0 mM NaCl (Table 2). Chlorophyll *a*, *b*, and carotenoid content were improved by growth regulator application. The highest chlorophyll *a* and *b* (4.99 and 2.24 mg g^{-1} FW, respectively) were recorded with SA application (Table 3). In the interaction between salinity and growth regulator application, at the high salinity level of 100 mM NaCl, GA_3, IAA, and SA application increased carotenoid content by 56.4%, 43.9%, and 32.6%, respectively, compared with the control of growth regulator (Table 4).

Figure 3. Effect of interaction between different salinity levels and growth regulators on soluble protein content (a), superoxide dismutase (SOD) (b), peroxidase (POD) (c), and ascorbate peroxidase (APX) (d) activities of hargel seedlings.



Bars with different letters showed significant differences at the 0.05 level of probability according to LSD test.
CK: Control 0 mM; GA_3: gibberellic acid 0.288 mM; SA: salicylic acid 0.362 mM; IAA: indole acetic acid 0.285 mM.

Soluble protein content and antioxidant enzyme activity

Growth regulator treatment and salinity levels increased soluble protein content. However, in the interaction between salinity and growth regulator application, the 100 mM NaCl+CK treatment increased soluble protein content by 36.4% compared with the 0 mM NaCl+CK treatment (Figure 3a). At the same salinity level, SA growth regulator application increased protein content by 93.2%, followed by IAA (62.3%) and GA_3 (46.6%), over the control of growth regulator (Figure 3a).

The activities of antioxidant enzymes were significantly increased by seed treated with growth regulators under salinity stress. In the interaction between salinity and growth regulators, salinity stress decreased SOD by 23.2% and 44.5% (Figure 3b), POD by 23.5% and 54.7% (Figure 3c) and APX by 23.2% and 36.1% (Figure 3d) in 50 mM NaCl+CK treatment and 100 mM NaCl+CK treatment respectively, as compared with the 0 mM NaCl+CK treatment. At the high salinity level (100 mM NaCl), the highest value of SOD (7.91 U g^{-1} min^{-1}) was recorded at the SA application (Figure 3b). At the same salinity level, growth regulator treatment increased POD activity by 194.9% in SA, then 141.7% in IAA and 133.9% in GA_3 compared with CK (Figure 3c). However, in the interaction between salinity and growth regulator, at the high salinity level of 100 mM NaCl, APX activity increased by 53.4%, 35.7%, and 23.5% in GA_3, IAA and SA, respectively, compared with CK (Figure 3d). Moreover, salinity stress decreased CAT activity by 52.7% and 76.1% at 50 and 100 mM NaCl, respectively, compared with 0 mM NaCl (Table 2). All the exogenous growth regulators had beneficial effects on CAT activity. SA application increased CAT activity by 27.20%, followed by IAA and GA_3 by 25.3% and 21.2%, respectively, compared with the control of growth regulator (Table 3).

DISCUSSION

Growth parameters

The seedling stage is the most vulnerable in the plant life cycle. In the present study, salt stress harmfully affected the seedling growth of the hargel plant by decreased growth parameters (SL, RL, fresh and dry weight). The results showed that hargel is sensitive to salt stress during seedling emergence and early seedling growth. Other researchers have reported similar finding in sorghum (Ali et al., 2019). The reductions in seedling growth affected by salinity stress might be due to the decreased capacity of the plant to absorb water, lowered mobility of reserved minerals, delayed cell division, injured hypocotyls, and ion toxicity (Nimir et al., 2015). However, our results differed from Qados (2011), who found that salinity stress increased both fresh and dry shoots in faba bean (*Vicia faba* L.) The discrepancy between prior and current studies could be due to differences in the salt tolerance of the studied crops and the salinity levels used.

In the present study, GA_3, SA and IAA application increased seedling growth and alleviated adverse impact of salinity. The positive effects of GA_3, SA and IAA are in agreement with Chauhan et al. (2019) in oat. This increase may result from the enhanced rate of photosynthesis and physiological parameters of crop plants (Shaddad et al., 2013). However, our findings disagreed with Shaddad et al. (2013), who found that GA_3 harmed SL and RL in soybean (*Glycine max*) plants. The disparity between these investigations is most likely due to crop species diversity and GA_3 levels. Our study showed that SA increased the seedling's growth parameters (SL and RL, fresh and dry weight) of hargel plant. This result agreed with Alsahli et al. (2019), who stated that SA mitigated the negative effects of salinity stress by enhancing seedling's growth, which could be due to an increased cell division within the apical meristem of the seedling shoot and roots. Rhaman et al. (2021) reported that IAA priming of wheat seed mitigated salinity stress by regulating ionic homeostasis and auxin-induced biosynthesis of SA in leaves, which improved CO_2 assimilation rate and ultimately increased grain yield.

Photosynthesis pigments

In the present study, increasing salinity levels significantly decreased chlorophyll *a* and *b*. The decreased photosynthetic pigments under salinity stress might be due to chloroplast destruction, change in thylakoid membrane structure, and loss of membrane proteins in the chloroplasts (Shams et al., 2019). Similar results were reported by Hessini et al. (2019) in maize plants. In this study, induced salinity stress increased carotenoid content in hargel plants. Salinity stress, unlike other biotic and abiotic stresses, causes the production of abscisic acid (ABA) from carotenoids via the mevalonic acid pathway, which regulates plant development and tolerance to salinity. Thus, the activation of the mevalonic acid pathway may result in the buildup of carotenoid content due to NaCl salt treatment (Sarker and Oba, 2018). The results showed that exogenous growth regulators have a protective role in the mitigating the adverse effect of salinity stress by increasing the photosynthetic pigments of the hargel plant.

Soluble protein content and antioxidant enzymes activity

In the present study, the soluble protein content was substantially higher than in control in hargel seedlings under salinity stress. Increased protein content in response to IAA treatment could be due to auxin's stimulating influence on K^+ uptake channel activation, allowing the plants to tolerate the damaging effects of salt stress via osmotic adjustments or ionic homeostasis (Claussen et al., 1997). Higher content of soluble proteins has been associated with salt- tolerant than in salt -sensitive plant species; however, Ashraf and Harris (2004) reported that the proteins produced under salt stress are not always associated with salt -tolerance, thus using proteins as salt tolerant indicators depending on the nature of the plant species or cultivar. The same result has been reported by Ali et al. (2019), while Hussain et al. (2017) observed a contrary result, stating that protein content was decreased in the NaCl-stressed seedlings.

In this investigation, salt-sensitive hargel plants showed a decrease in antioxidant enzyme activity (POD, CAT, APX) at different levels of NaCl. This reduction could be due to damage to the antioxidant system. Reduced CAT activity in stressed plants may have facilitated H_2O_2 accumulation, resulting in a Haber-Weiss reaction that produces hydroxyl radicals, which are known to cause harm to biological systems (Gill and Tuteja, 2010). The decrease in SOD activity might be due to decreases in Mn-SOD and Fe-SOD; this reduces seedlings' ability to scavenge O_2 radicals. Furthermore, these reduction which might be due to ineffective enzyme synthesis or change in the enzyme subunits; on the contrary, in salt-tolerant plants (Ali et al., 2019). Similar results were reported by Janmohammadi et al. (2012) and Shahzad et al. (2021). The salt tolerance in different species or cultivars might involve an overproduction of SOD, CAT, and APX when the seedlings are exposed to salinity. This pattern of antioxidant enzymatic activity was not observed in salt sensitivity, where salinity led to a reduction in the activity of these enzymes (Plumb et al., 2018). Also, Cunha et al. (2016) reported that differential antioxidant enzyme activity varies according to crop species, salinity extent, exposure time, plant developmental stages, and crop responses to salt stress.

In this investigation, exogenous growth regulators significantly increased the activity of antioxidant enzymes (SOD, POD, CAT, and APX). Exogenous growth regulators application can regulate the synthesis of antioxidant enzymes in plants and thereby ameliorate salt stress in plants. Similar results were reported by Alsahli et al. (2019), who reported that seed treated with growth regulators (SA, GA_3 and IAA) significantly increased antioxidant enzymes activity like SOD, CAT, POD, and APX when the seed was grown under salinity stress; which improves the removal of ROS and protects oxidative stress (Shahzad et al., 2021). This result was contrary to Zhu et al. (2019), who reported that exogenous growth regulator treatment reduced SOD activity in salt-stressed plants. The discrepancy in antioxidant enzyme activity between these investigations is most likely due to crop species and their response to exogenous hormone levels.

CONCLUSIONS

We studied the effects of exogenous growth regulator application of gibberellic acid (GA_3), salicylic acid (SA), and indole acetic acid (IAA) on seedling growth parameters and physiological traits of hargel seedlings under salinity stress. Our results showed that salinity reduced all the attributes tested except soluble protein content and carotenoid content. The exogenous application of those growth regulators successfully improved seedlings' growth parameters, antioxidant enzyme activity, soluble protein content, chlorophyll *a*, *b*, and carotenoid content in hargel seedlings under salinity stress, resulting in better stress tolerance. Salicylic acid was the most effective plant growth regulator in seedling growth parameters and physiological traits; it induced the highest shoot length, fresh and dry weight, chlorophyll *a* and *b*, soluble protein content, and superoxide dismutase, peroxidase, and catalase activities. The present study indicated that the hargel plant at the early seedlings stage is sensitive to salinity (under-examined levels). It also provides evidence for a beneficial effect of growth regulators application in enhancing salinity tolerance of plants by altering the antioxidant activity. However, further research is needed to study the effects of salt and seed treatments with different plant growth regulators on different growth stages and secondary metabolites of hargel plants under different stages.

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