Ahmad M *et al*. (2024)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 52, Issue 1, Article number 13467
DOI:10.15835/nbha52113467
Research Article

Mitigating negative impact of salinity on berseem (*Trifolium alexandrinum*) by foliar application of salicylic acid

Masood AHMAD[1], Maria NAQVE[1], Wang LIHONG[2]*, Muhammad A. ZIA[3], Athar MAHMOOD[4], Muhammad M. JAVAID[5], Muaz AMEEN[1], Afaf A. RASHED[6], Adnan RASHEED[7], Muhammad U. HASSAN[8], Sameer H. QARI[9]

[1]*University of Agriculture Faisalabad, Department of Botany, 38000, Faisalabad, Pakistan; masoodch359@gmail.com; maria.naqve@uaf.edu.pk; muazameen@outlook.com*
[2]*Baicheng Normal University, College of Tourism and Geographic Science, Baicheng 137000, Jilin, China; wlh19721108@163.com (*corresponding author)*
[3]*University of Agriculture Faisalabad, Department of Biochemistry, 38000, Faisalabad, Pakistan; anjum.zia@uaf.edu.pk*
[4]*University of Agriculture Faisalabad, Department of Agronomy, 38040, Faisalabad, Pakistan; athar.mahmood@uaf.edu.pk*
[5]*University of Sargodha, College of Agriculture, Department of Agronomy, Sargodha, Pakistan; mmansoorjavaid@gmail.com*
[6]*Umm Al-Qura University, Faculty of Applied Science, Biology Department, Makkah 21955, Saudi Arabia; aarashed@uqu.edu.sa*
[7]*Hunan Agricultural University, College of Agronomy, Changsha 410128, China; adnanrasheed@hunau.edu.cn*
[8]*Jiangxi Agricultural University, Research Centre on Ecological Sciences, Nanchang 330045, China; muhassanuaf@gmail.com*
[9]*Umm Al-Qura University, Al-Jumum University College, Department of Biology, Makkah 21955, Saudi Arabia; shqari@uqu.edu.sa*

Abstract

Salicylic acid (SA) is a plant growth regulator known to take part in defense responses against different types of stresses, including salt stress. In this study, the role of foliar applied SA in improving the growth of berseem variety 'Anmol' under salt stress was examined. Plants were sown in plastic pots in the sand. Plants were treated with different concentrations of salinity (0, 60 mM and 120 mM NaCl) and salicylic acid (0, 100 mg L^{-1} and 150 mg L^{-1}) was applied as a foliar spray. Salinity stress significantly reduced root and shoot fresh and dry weight, root and shoot length, photosynthetic pigments including Chl. *a*, *b*, *a*/*b*, total soluble proteins, total amino acids and uptake of K^+ and Ca^{2+} ions in root and shoot tissues. Exogenous application of salicylic acid improved growth traits including shoot length, shoot fresh weight, root length, root fresh and weight, shoot dry weight, pigments contents (Chl. *a*, *a*/*b* and carotenoids). Total soluble protein and amino acid contents, activities of antioxidants peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) were also enhanced by the foliar spray of SA under saline and non-saline conditions. SA played a crucial role in lowering Na^+ and Cl^- ions content in shoot and root tissues while enhancing the uptake of K^+ and Ca^{2+} ions. The study revealed that 100 mg L^{-1} SA treatment significantly influenced several plant parameters, including shoot length (8 cm), root length 6.7 cm, chlorophyll (1.2 mg/g FW), total soluble proteins (0.8 mg/g FW) and total amino acids (2.5 mg/g FW), SOD (1.22 U/mg protein), CAT (1.75 U/mg FW), potassium ions (29 mg/g DW), and calcium ions (43 mg/g DW) during salinity stress. Therefore, field use of SA (100 mg L^{-1}) is recommended to enhance the growth of berseem and other fodder crops in saline soils.

Received: 18 Oct 2023. Received in revised form: 07 Nov 2023. Accepted: 29 Feb 2024. Published online: 28 Mar 2024.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Keywords: antioxidants; growth regulator; ionic stress; SA; salinity

Introduction

Berseem (*Trifolium alexandrinum*) also called as king of fodder crops belongs to the family Fabaceae. It is the chief leguminous fodder crop for livestock feeding, being legume, it is also important as a nitrogen fixing crop (Tejveer *et al*., 2018; Salama, 2020). It is a relatively nutritive, palatable, succulent, and digestive fodder crop (Katoch, 2022). Berseem contains a great concentration of nutrients, good quality silage, primarily carotene (11-19%), minerals (15-25%) and dry matter protein, which is most effective for milk-producing animals (Singh *et al*., 2019). Though, due to low input requirements berseem clover can be considered as an important forage crop for sustainable production and livestock feed in world (Tufail *et al*., 2018). Forage deficits may result from various crucial factors mainly, environmental constraints and poor cultural practices (Philp *et al*., 2019). However, livestock production all over the globe mainly suffers from limited forage production due to salinity (Tufail *et al*., 2020). Salt stress is a crucial factor among environmental stresses. Like other legumes, berseem is ranked among the most sensitive plants toward salinization (Abbas and Awad, 2018). Salt stress induces toxicity in plants by disturbing all cellular mechanisms. It also impedes the growth and yield of plants by inducing oxidative burst, osmotic stress and nutrient imbalance (Azab *et al*., 2021).

There is a considerable break between farming and production of fodder crops, which can be managed through the cultivation of high-yielding fodder cultivars along with the adaptation of proper production technology (Ergon *et al*., 2018; Wasaya *et al*., 2021). Although, salinity tolerance is a complex mechanism. Though, plants adopt various mechanisms to resist salinity at physiological levels with the activation of various compounds such as phytohormones, compatible solutes and antioxidants. Exogenous applications of these compounds can be an effective approach to combat salinity-induced negative effects in plants (Saxena *et al*., 2019; Souri and Tohidloo, 2019).

Salicylic acid (SA) is a growth promoter, phytohormone and signalling molecule that induces salinity stress tolerance in plants (Rasheed *et al*., 2022). As a growth regulator SA also plays a vital role in enhancing the growth and yield of plants besides salinity tolerance (Hayat *et al*., 2022). It also acts as an antioxidant and a phenolic compound (Aziz *et al*., 2018). It is also involved in the production of osmolytes and antioxidants under stress conditions in plants (Shahid *et al*., 2014; Shahid *et al*., 2015; Balal *et al*., 2016). Exogenous application of SA in low concentration is reported to induce salinity tolerance in plants (Abbas *et al*., 2015) but high concentration caused oxidative stress thus, reducing the tolerance ability of plants against stress conditions (Campos *et al*., 2019). Exogenous application of SA has been reported to induce salinity tolerance in plants by increasing photosynthetic efficiency and plant biomass (Damalas and Koutroubas, 2022). In addition, SA is present in various phases of plant developmental processes including seed germination to fruit development, and also in tolerance to abiotic stressors (Koo *et al*., 2020) Although, beneficial effects of SA has been observed in terms of salinity stress tolerance in crops including peppers (Kaya *et al*., 2020), strawberry (Dedejani *et al*., 2021), common bean (Sofy *et al*., 2020) and cotton (Hamani *et al*., 2021).

Soil salinization is a crucial issue in developing countries like Pakistan (Shahid *et al*., 2015). Thus, there is a need to introduce and adopt cost-effective techniques to grow corps on such lands. Exogenous application of compounds that are naturally produced by plants is one such approach (Zulfiqar *et al*., 2020). Such compounds are a better option than synthetic fertilizers, being organic such compounds are beneficial for animals, humans and plants as well (Naamala and Smith, 2020). Globally, soil salinization affects extensive agricultural areas, leading to toxicity due to excessive salt and chloride ions, which restrict plant growth and nutrient absorption. Plants employ various mechanisms, including ion exclusion, osmotic adjustment, and the stimulation of antioxidant enzymes, underscoring the significance of investigating potential treatments like foliar-applied salicylic acid (SA) to enhance crop tolerance (Haider *et al.,* 2022; Ali *et al.,* 2023). SA acts as plant growth regulator and phenolic compound. It interferes with plant metabolism to give tolerance to the plants under abiotic stresses, as it induce various physiological processes in plants (Kaya *et al*., 2023). Furthermore, a number of studies has been reported the significant role of SA in regulating the abiotic stress tolerance in plants. Whereas, research on the role of foliar spray of SA on berseem in tolerating the salinity stress is scarce (Saikat *et al*., 2023). The focus is on enhancing growth, photosynthesis, antioxidant properties, and nutrient management to improve plant adaptability and efficiency during saline conditions. For this purpose, growth and physiological attributes of berseem are studied. It is hypothesized that salicylic acid will mitigate the adverse impacts of salinity on barseem by improving antioxidant activities, photosynthetic pigments and maintaining the nutrient homeostasis. Therefore, current study provides novel evidence concerning a less studied plant like berseem, contributing to a useful assessment of physiological traits of berseem plant under salinity stress.

Materials and Methods

A pot experiment was conducted to elaborate the role of salicylic acid to mitigate the negative impact of salinity on berseem. The experiment was performed at the research area of the Post Graduate Agriculture Research Station (PARS) University of Agriculture, Faisalabad. Seeds of "Anmol" variety of berseem were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds were sown in plastic pots (25 cm, diameter) filled with 7 kg well-washed sand and 5 plants were maintained in each pot after thinning. Three salt treatments [0 mM (non-saline), 60 and 120 mM NaCl)] were supplied with Hoagland's nutrient solution (Hoagland and Arnon 1950). The concentration of NaCl was achieved by 5.85 g NaCl L^{-1}. NaCl level was established by adding 50 mM NaCl in aliquot parts to prevent the plants from osmotic shock. Salinity treatment was done at three leaf stage.

Foliar spray of SA at 0, 100 and 150 mg L^{-1} was applied after 6 weeks from the sowing (Vegetative growth stage), the volume of sprayed solution was 50 ml for each plant using Tween 20 (0.1%) as surfactant. Pots were arranged in a completely randomized design (CRD) with three replications of each treatment. A random sample of two plants from each treatment was uprooted carefully after three weeks of foliar spray. Uprooted plants were washed away to clear from sand/dust particles, and data were recorded for shoots and roots fresh weight and length immediately. The same plants were kept in an oven at 65 °C up to constant weight and their dry biomass was recorded. Additionally, data for the following biochemical attributes were measured.

Photosynthetic pigments

Chlorophyll *a*, *b*, *a*/*b*, total chlorophyll contents and carotenoids were determined by following Arnon (1949) method. Fresh leaf material (0.25 g) was crushed in acetone (80%). The extract was filtered and by using a spectrophotometer (IRMECO-U2020) absorbance was measured at 645 nm, 663 nm, and 480 nm. The following formulae given by Naseem *et al*. (2023) were used to calculate the chlorophyll and carotenoid content quantitively.

Total soluble proteins

Fresh plant leaves were crushed with potassium phosphate buffer in a chilled environment, the extract (5 µL) was then homogenized with 1 ml of Bradford Dye and 0.1 N HCl. Optical density was recorded at 595 nm by following the protocol of Bradford (1976).

Total amino acid

Hamilton *et al*. (1943) procedure was used for the determination of total amino acid. Fresh plant leaf sample (0.25 g) was ground in potassium phosphate buffer solution was added. The extract was homogenized with, 1 mL of 10% pyridine solution and 1 mL of ninhydrin and readings were noted at 570 nm by using a spectrophotometer (IRMECO-U2020).

Determination of antioxidants

Fresh plant material (0.25 g) was homogenized with 5 ml (50 mM) potassium phosphate buffer, supernatant was used after centrifugation for the determination of following enzymatic antioxidants activities. Phosphate buffer, H_2O_2, guaiacol and enzyme extract was homogenized as reaction solution and absorbance was recorded at 470 nm using a spectrophotometer (IRMECO-U2020) by following Chance and Maehly (1955) to record the activity of POD. The reaction mixture was prepared by adding ascribed amounts of phosphate buffer, Triton X-100, L-Methionine, NBT, enzyme extract and riboflavin as proposed by Giannopolitis and Ries (1977) to estimate SOD, absorbance was taken at 560 nm by using a spectrophotometer (IRMECO-U2020). By following Chance and Maehly (1955) enzyme extract was standardized with H_2O_2 and phosphate buffer, and absorbance was measured at 240 nm using a spectrophotometer (IRMECO-U2020) to estimate the activity of CAT.

Mineral nutrients determination

The protocol ascribed by Wolf (1982) was followed to estimate the Na^+, K^+ and Ca^{2+} ions from root and shoot by acid digestion. Shoot and root dried material (0.1 g) was digested in 2.5 ml of conc. H_2SO_4 and H_2O_2 (35%) were used as decolorising agents. The extract was used for the determination of mineral ions by using a flame photometer (Sherwood Model 410, Cambridge) and Cl^- ion content was determined by using a chloride analyser (M926 Chloride Analyser).

Statistical analysis

Snedecor and Cochran (1980) analysis of variance was used for collected data was for all the parameters and treatment means were compared using least significant difference at 5% (LSD 5%). A three-factor factorial analysis of variance was performed using COSTAT software and the graphs were created using Microsoft Excel (Version 2010).

Results

The present study was conducted to evaluate the response of SA to alleviate the salinity-induced toxicity in berseem plants in terms of growth, biochemical attributes, antioxidative defence and ionic homeostasis. Analysed data revealed that salinity stress caused a significant reduction in in growth by reducing shoot length, shoot fresh and dry weight in tested berseem plants with a maximum reduction of 5.5 cm, 1.12 g, and 0.07 g respectively. The reduction was maximum when the concentration of salinity was high (120 mM) as compared to controlled condition. Foliar spray of SA significantly enhanced all these growth attributes, maximum growth was observed in plants supplied with 100 and 150 mg L^{-1} SA (Figure 1 A-C). Though, 100 mg L^{-1} spray of SA was more effective in increasing the overall shoot length under saline (8 cm) and non-saline (14.5 cm) regimes. A significant interaction was also observed between stress and SA application in shoot length and shoot fresh weight (Table 1).

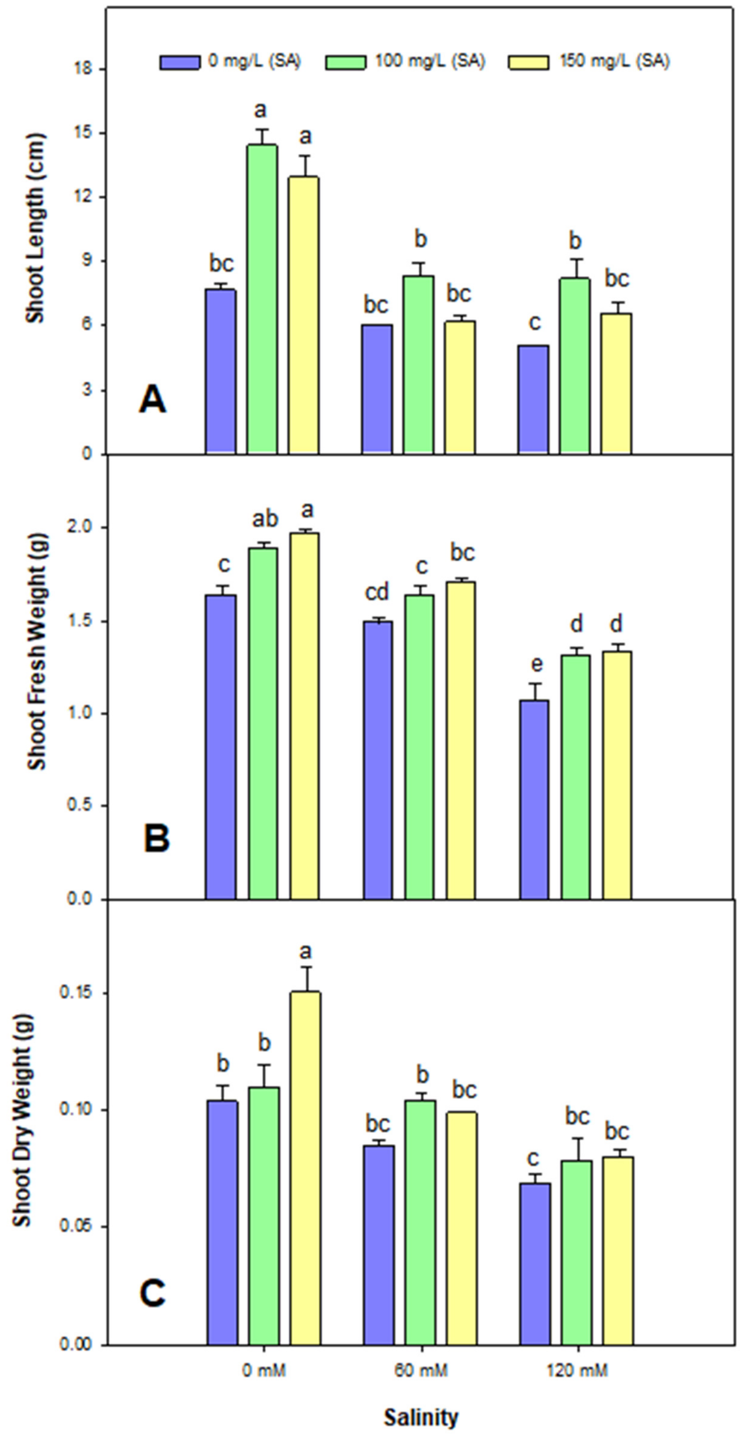

Figure 1. Effect of varying SA levels applied as foliar spray on shoot length (A), shoot fresh weight (B) and shoot dry weight (C) of berseem plants under saline and non-saline regimes

The significant reduction has also been observed in root length, and fresh and dry weight of root under salinity stress in tested berseem plants. Varying levels of NaCl affected the growth differently as maximum decrease was observed at 120 mM NaCl concentration. However, remarkable enhancement has been observed in these traits in berseem plants by the foliar application of SA (100 mg L^{-1}) under saline and non-saline regimes.

Thus, low concentration of SA was found to be more effective in enhancing root growth by 8.8 cm and 6.7 cm during 60 mM and 120 mM salt stress conditions. Significant interaction has also been observed between salinity and SA application in terms of root fresh and dry weight (Table 1; Figure 2 A-C).

Table 1. Mean square from analysis of variance of data for growth and photosynthetic pigments of berseem treated with foliar spray of SA under saline and non-saline regimes

Source	SL	SFW	SDW	RL	RFW	RDW	LA	Leaves Plant^{-1}	Chl. *a*	Chl. *b*	Chl. *a/b*	Car
NaCl	73.78***	0.81***	0.004***	36.04***	8.626***	0.003***	3981.48***	62.11***	5.03***	1.66***	0.049***	4.99ns
SA	37.26***	0.18***	0.002**	9.15***	3.902***	6.721***	3597.14***	32.33***	1.92***	1.34 ns	0.315***	0.012***
NaCl × SA	6.52**	0.003 *	5.208*	1.148*	9.916***	2.797*	163.09**	4.11*	4.87*	1.07 *	0.048***	0.001**
Error	1.34	0.007	1.264	0.71	8.176	8.775	168.96	0.96	1.104	5.63	0.002	4.98

*, **, *** Significant at 0.05, 0.01 and 0.001 levels respectively; ns = non-significant; SL: Shoot Length, RL: Root Length, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, L.A: Leaf Area; Chl. *a*: Chlorophyll *a*; Chl. *b*: Chlorophyll *b*; Chl.*a/b*: Chlorophyll *a/*

Varying levels of NaCl (60 mM and 120 mM) significantly reduced leaf area (190 m m^2 and 160 m m^2 respectively) and number of leaves plant^{-1} (10 and 8 respectively) in the tested variety of berseem plants. While foliar supplementation of SA significantly enhanced leaf area and no. of leaves plant^{-1} under saline stress and non-stress conditions. All sprayed levels of SA were found to be responsible for this enhancement while low concentration (100 mg L^{-1}) SA fertigation was more effective in maximizing the growth by increasing the leaf area and number of leaves in berseem plants under saline and non-saline conditions. Significant interactive effect has also been observed between SA application and NaCl doses in terms of no. of leaves plant^{-1} (Table 1; Figure 3 A, B).

Salinization negatively impacts the photosynthetic apparatus in plants. Root medium applied NaCl significantly reduced photosynthetic pigments content including chl. *a*, *b*, *a/b* and carotenoid contents in tested variety of berseem plants. Varying levels of NaCl markedly reduced chl. *a* content with a maximum reduction of 0.05 mg/g FW during 120 mM salt stress. Foliar fertigation with 100 and 150 mg L^{-1} substantially enhanced the concentration of chl. *a* content under saline and non-saline conditions. Remarkably strong interaction within NaCl stress and SA spray exhibited that all levels of SA were responsible in enhancing the chl. *a* concentration (Table 1; Figure 4 A).

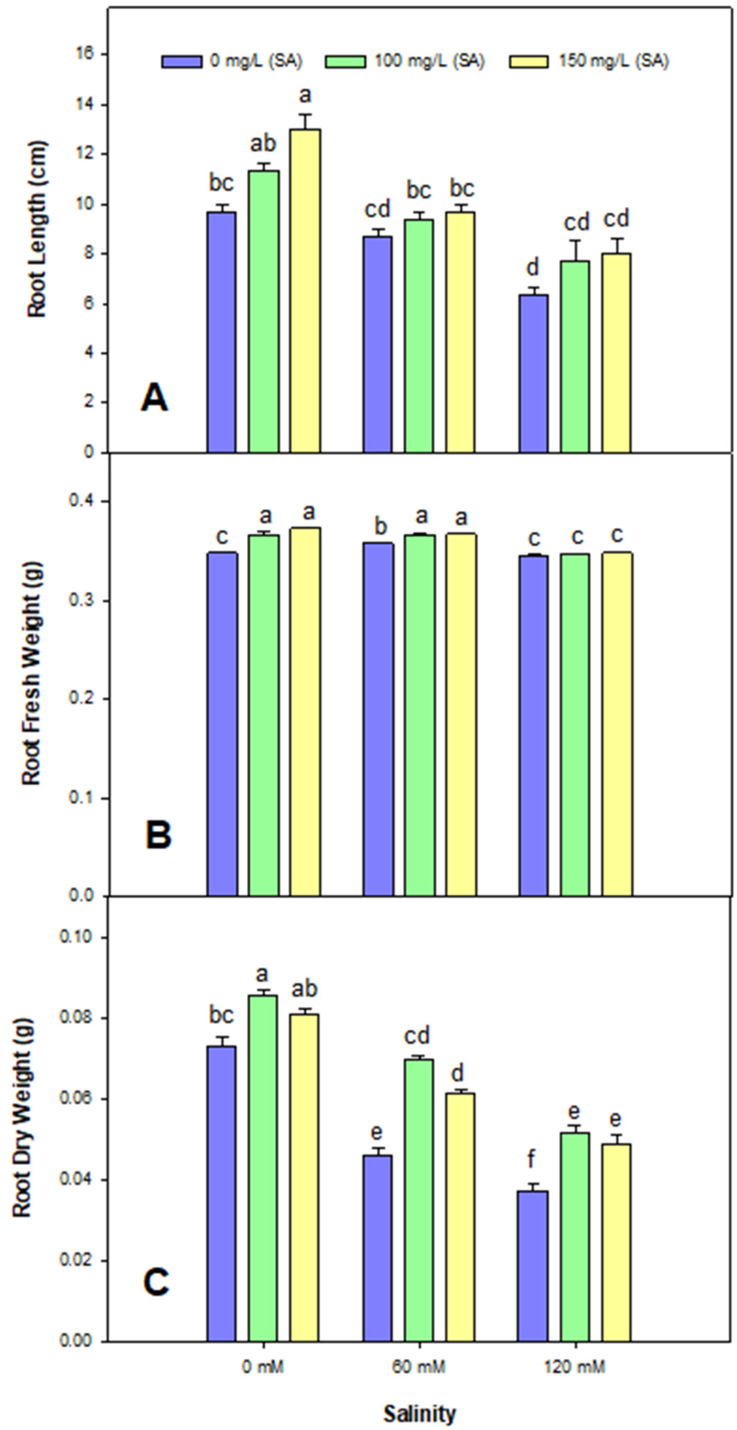

Figure 2. Effect of varying SA levels applied as foliar spray on root length (A), root fresh weight (B) and root dry weight (C) of berseem plants under saline and non-saline regimes

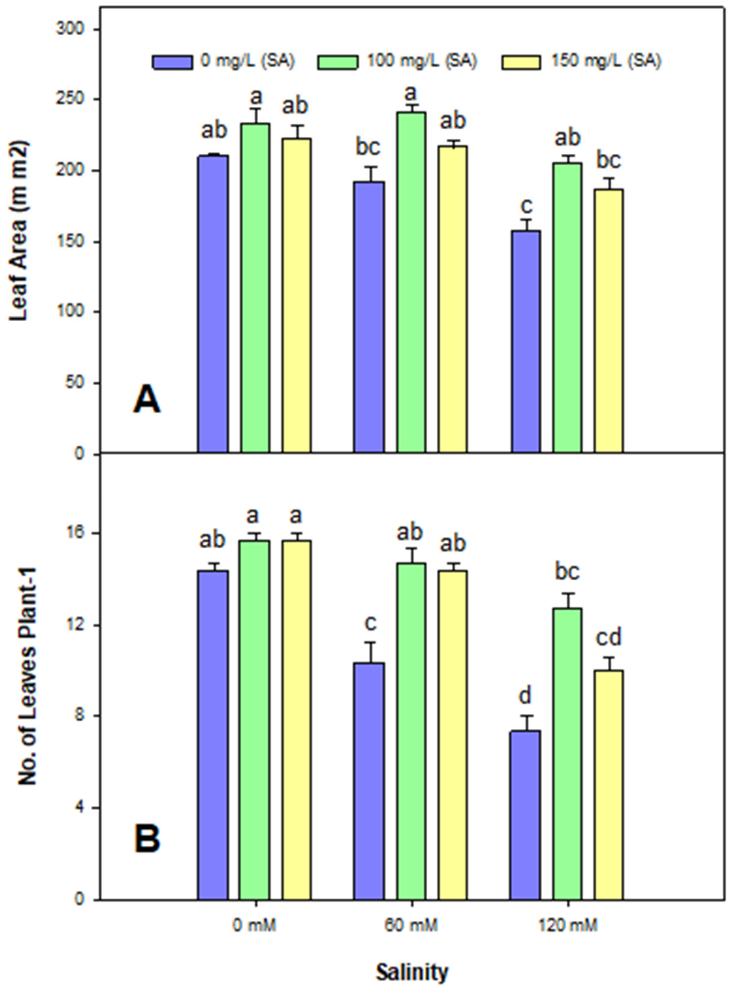

Figure 3. Effect of varying SA levels applied as foliar spray on leaf area (A) and No. of leaves Plant^{-1} of berseem plants under saline and non-saline regimes

Salt stress remarkably reduced the content of chl. *b* in tested Anmol variety of berseem plants. Whereas foliar spraying with SA increased the accumulation of chl. *b* at 100 (0.03 mg/g FW) and 150 mg L^{-1} concentrations (0.028 mg/g FW) at 120 mM salinity treatment. But SA fertigation had non-significant influence on the accumulation of chl. *b* pigment (Table 1; Figure 4 B). Significant adverse effect of salt stress was also recorded on chl. *a/b* concentration. Exogenous application of different levels of SA significantly enhanced this concentration though maximum enhancement was found in plants that were sprayed with 100 mg L^{-1}. A significant interactive effect was also recorded between SA and NaCl stress with respect to the accumulation of chl. *a/b* concentration (Table 1; Figure 4 C). Imposition of salt stress reduced the accumulation of carotenoid contents in tested berseem plants although this reduction was non-significant. Foliar spray of berseem plants with SA significantly maximized the accumulation of carotenoid contents in plants. All sprayed concentrations of SA were responsible for this enhanced accumulation, but 100 mg L^{-1} was more effective for accelerating the carotenoids accumulation (Table 1; Figure 4 D).

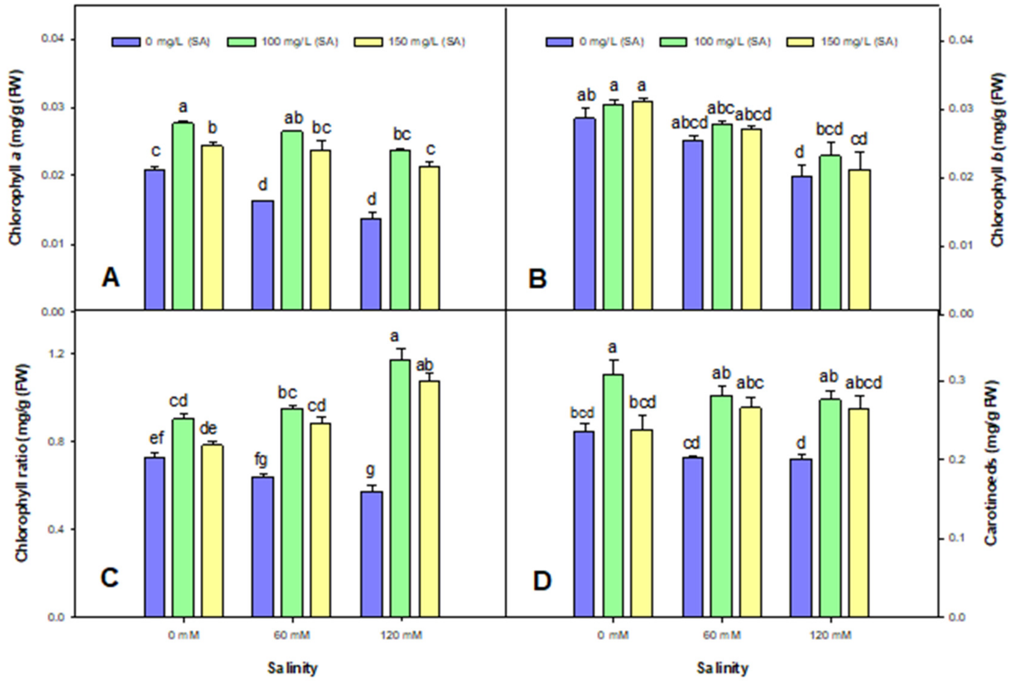

Figure 4. Effect of varying SA levels applied as foliar spray on photosynthetic pigments contents; chlorophyll *a* (A), chlorophyll *b* (B), chlorophyll *a*/*b* (C) and carotenoids (D) of berseem plants under saline and non-saline regimes

An adverse effect of salt stress has been observed in TSP (0.56 mg/g FW) and TAA (1.4 mg/g FW) content in berseem plants at 120 mM NaCl treatment. A significant decrease was recorded in these attributes due to the imposition of salinity stress. Foliar spray of different levels of SA significantly enhanced the accumulation of TSP and TAA under stressed and non-stressed conditions and the lower level (100 mg L^{-1}) of SA was more pronounced in enhancing the content of TSP (0.8 mg/g FW) and TAA (2.5 mg/g FW) in sprayed plants at 120 mM salinity stress. Significant interaction has also been observed between NaCl and SA spray for TSP content (Table 2; Figure 5 A, B).

Table 2. Mean square from analysis of variance of data for osmolytes, antioxidants and ionic contents of berseem treated with foliar spray of SA under saline and non-saline regimes

Source	TSP	TAA	POD	SOD	CAT	Shoot Na^+	Shoot K^+	Shoot Ca^{2+}	Shoot Cl^-	Root Na^+	Root Ca^{2+}	Root Cl^-
NaCl	0.07***	1.67***	9.05**	0.120***	0.28***	330.26***	94.11***	116.14***	28429.48***	6.48 ns	79.59***	26950.25***
SA	0.09***	1.84***	0.01***	0.195***	0.22***	36.04**	52.11***	188.48***	3099.70***	58.92***	221.59***	1162.70***
NaCl × SA	0.01***	0.064*	4.79**	0.005*	0.014*	3.04 **	5.55*	11.70*	350.25**	44.25***	3.759ns	153.70***
Error	2.407	0.052	1.39	0.0013	0.018	5.04	1.407	2.96	48.85	2.037	2.481	21.11

*, **, *** Significant at 0.05, 0.01 and 0.001 levels respectively; ns = non-significant; TSP: Total Soluble Proteins; TAA: Total Amino Acid; POD: Peroxidase; SOD: Superoxide Dismutase; CAT: Catalase.

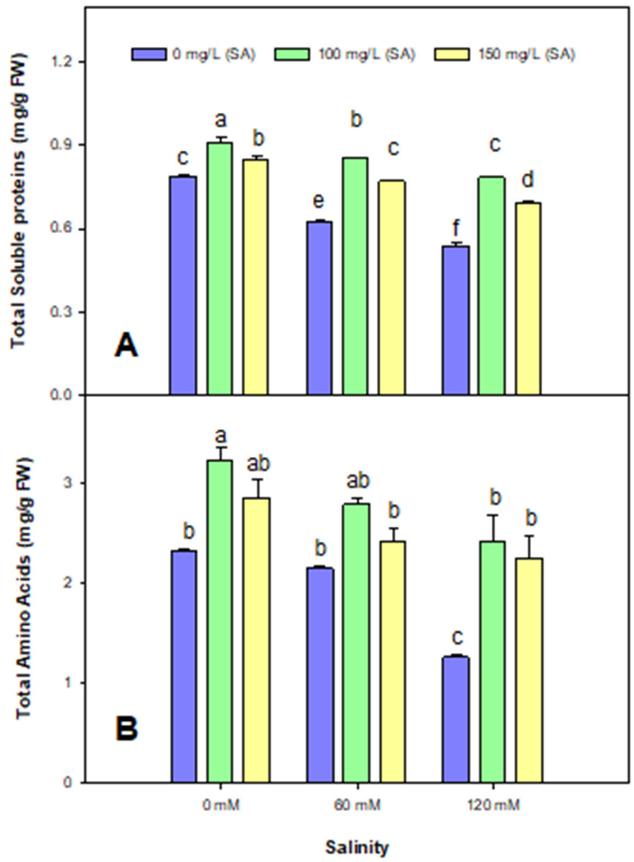

Figure 5. Effect of varying SA levels applied as foliar spray on total soluble proteins (A) and total amino acids (B) of berseem plants under saline and non-saline regimes

Activities of antioxidants (POD, SOD and CAT) in the leaf tissues of tested berseem plants were significantly enhanced under salt stress. Foliar fertigation of berseem plants with different levels of SA significantly enhanced the activity of POD enzyme under saline and non-saline regimes. A significant interaction between NaCl and SA fertigation has been recorded. In this regard, the increase in POD activity was found in plants sprayed with a low concentration of SA (100 mg L^{-1}) at highest stress level (1.15 U/mg protein) and non-stressed (1.1 U/mg protein) conditions (Table 2; Figure 6 A).

Imposition of salinity stress significant increased has been observed in the activity of in a tested variety of berseem plants. Foliar application of varying levels of SA further enhanced the activity of SOD significantly under saline and non-saline regimes. This increase was maximum in plants exogenously supplied with 100 mg L^{-1} (1.22 U/mg Protein) as compared with 150 mg L^{-1} (0.78 U/mg Protein) at 120 mM salinity stress. A significant interaction was also observed between salinity and SA spray for the enhanced activities of SOD enzyme (Table 2; Figure 6 B).

Activities of CAT were markedly enhanced in the leaf tissues of tested plants under salt stress. Exogenous application of varying SA levels further increased the activities of CAT in plants under non-saline (1.12 U/mg FW) and different saline (1.75 U/mg FW) regimes at 100 mg/L SA applications. The maximum increase in the activity of CAT was recorded at low concentration (100 mg L^{-1}) of SA was 1.68 U/mg FW at 120 mM Salinity stress (Table 2; Figure 6 C).

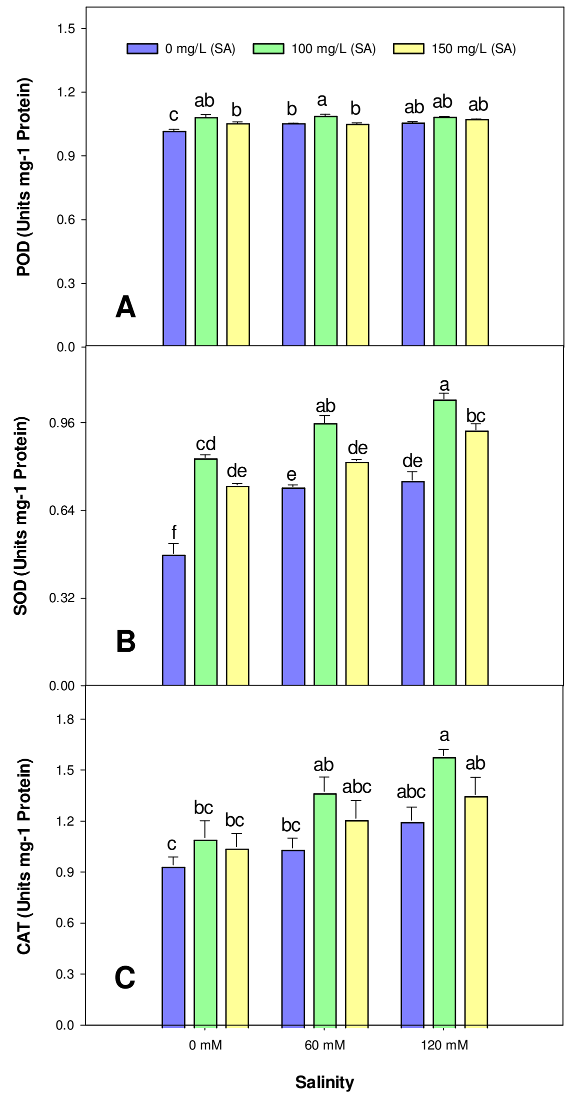

Figure 6. Effect of varying SA levels applied as foliar spray on activities of antioxidants; POD (A), SOD (B) and CAT (C) of berseem plants under saline and non-saline regimes

Salinity stress significantly increased Na^+ and Cl^- ion contents in shoot of berseem plants. Although, the significant reduction has been recorded in the accumulation of shoot K^+ (25 mg/g DW) and Ca^{2+} (30 mg/g DW) ions under varying saline regimes. Foliar application of SA (100 mg L^{-1}) significantly reduced the Na^+ (45 mg/g DW) and Cl^- ions (75 mg/g FW) concentration in shoot tissues of tested berseem plants at 120 mM salt stress as compared to other treatments. While significant increase has been recorded in the concentration of K^+ and Ca^{2+} ions in shoot tissues of berseem plants under saline (29 and 43 mg/g DW respectively) at 120 mM Salt stress and 100 mg L^{-1} SA treatment and non-saline conditions and 100 mg L^{-1} SA treatment (32, 40 mg/g DW respectively). Foliar applied SA at 100 mg L^{-1} has shown a more pronounced effect for this uptake under stressed and non-stressed conditions. A significant interactive effect was recorded between SA and NaCl stress for shoot K^+ and Ca^{2+} ions concentration. (Table 2; Figure 7 A-D).

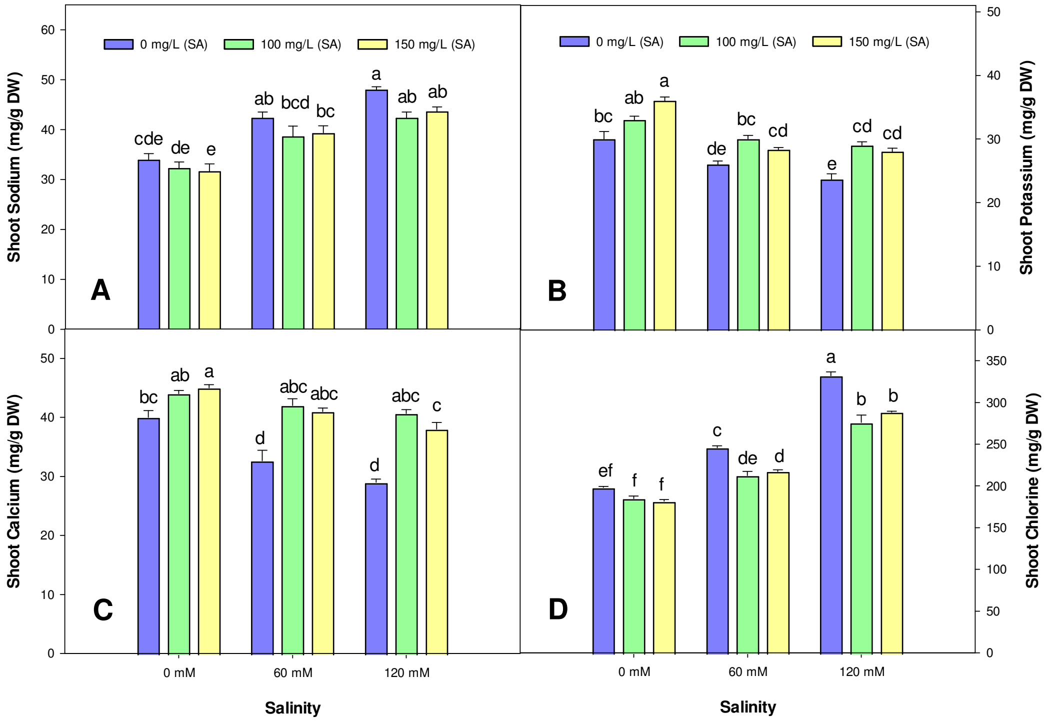

Figure 7. Effect of varying SA levels applied as foliar spray on ionic status of shoot; Sodium (A), Potassium (B), Calcium (C) and Chlorine (D) of berseem plants under saline and non-saline regimes

Salt stress considerably increased the accumulation of Cl^- ions (190 mg/g FW) in root tissues at 120 mM salinity stress of tested berseem (Table 2). Applied salinity stress significantly decreased the uptake of K^+ and Ca^{2+} ions in root tissues of tested plants at 120 mM level. While SA applied at 150 mg L^{-1} as foliar spray significantly reduced the uptake of Na^+ (38 mg/g FW) and Cl^- (165 mg/g DW) ions at 120 mM salinity stress. A remarkable increase has been observed in the uptake of K^+ (43 mg/g DW) and Ca^{2+} (41 mg/g DW) ions in the root tissues of berseem plants sprayed with SA at 100 mg L^{-1} and 60 mM Salt stress. Foliar applied SA (100 mg L^{-1}) proved more effective in reducing the Na^+ and Cl^- ions concentration and increasing the concentration of K^+ and Ca^{2+} ions under saline and non-saline regimes (Table 2; Figure 8 A-D).

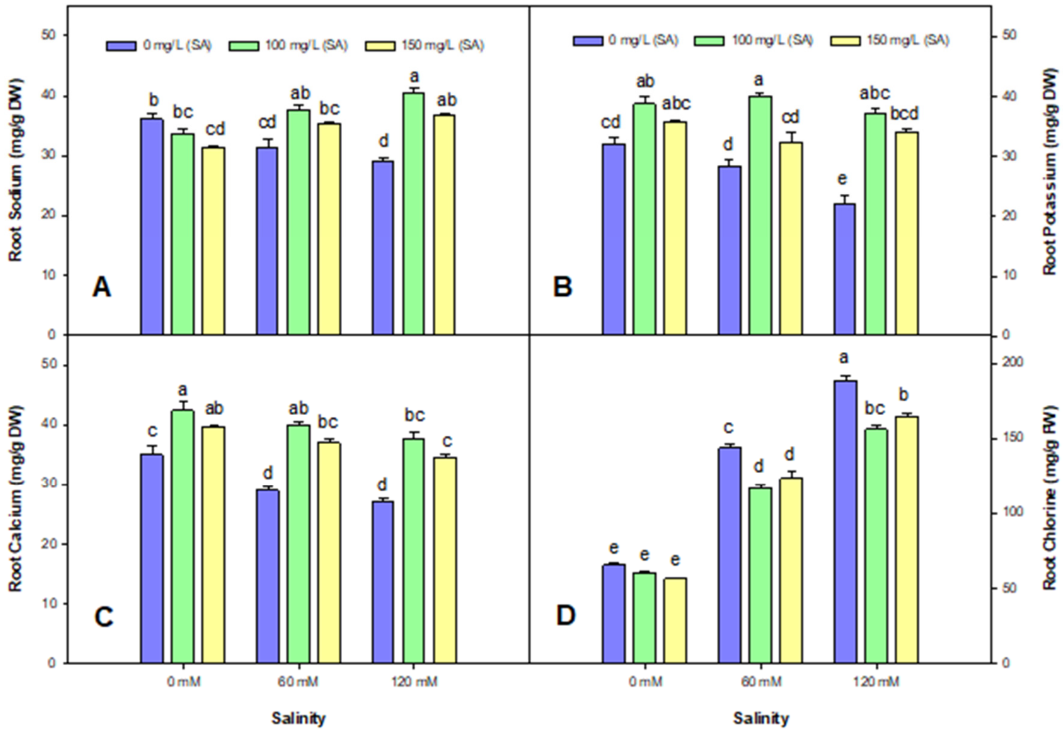

Figure 8. Effect of varying SA levels applied as foliar spray on ionic status of root; Sodium (A), Potassium (B), Calcium (C) and Chloine (D) of berseem plants under saline and non-saline regimes

Discussion

Salinization of agricultural lands is a major problem worldwide, causing adverse effects on the growth and productivity of crops by affecting physiological mechanisms (Etesami and Noori, 2019; Srivastava *et al.*, 2019). Salicylic acid acts as a growth regulator and regulates various physiological mechanisms in plants under abiotic stresses (El-Sharkawy *et al.*, 2022). Thus, the exogenous application of SA can be considered an effective strategy to combat salinity-induced toxicity in plants (Alsahli *et al.*, 2019). Therefore, the present study was conducted to check the effect of foliar spray of SA on berseem plants to mitigate the brutal impacts of salinity stress.

The results revealed that salt stress significantly reduced the growth of tested berseem plants. Decreased growth is linked with reduced cell division, elongation, nutrient imbalance and impaired photosynthesis (El-Sharkawy *et al.*, 2022). Hormonal imbalance is also linked with salt stress (Yu *et al.*, 2020). However, SA applied as a foliar spray on berseem plants significantly improved root and shoot length, and fresh and dry weight under salinity stress. Sustained growth results from SA application as it is expected to control growth by sustained turgor, water balance, and stomatal regulations (Mimouni *et al.*, 2016). Reduced levels of growth under varying levels of salinity in the current study was likely due to disruption in the process of photosynthesis due to reduction in the chlorophyll and accessory pigments (carotenoids) (Omar *et al.*, 2023). The role of SA to increase the growth of plants under salinity may be useful for increasing the plant development and yield (Nassar *et al.*, 2023).

Process of photosynthesis is greatly affected by salt stress in plants. High salinity levels affected photosynthetic pigments by affecting chloroplast, photosystems and biosynthetic processes of chlorophyll (Etesami and Noori, 2019). Results of the current study revealed that salt stress significantly reduced Chl. *a*, *b*, *a/b* and carotenoid contents in berseem plants. Photosynthetic pigments are a good indicator of plants vigour and reduction in the plants pigments content is directly linked with reduced activity of enzymes and chlorophyll breakdown under salinity stress (Aazami *et al*., 2023). Foliar applied SA significantly enhanced pigments content in tested plants under saline and non-saline conditions. This enhancement shows impairment of chloroplast and oxidase enzyme activation involved in chlorophyll biosynthesis (Kang and Cao, 2014). SA is also reported to scavenge reactive oxygen species (ROS) by a higher build-up of antioxidants (Agarwal *et al*., 2014). Enzymatic antioxidants quench reactive oxygen species and prevent cellular membranes from oxidation (Gill and Tuteja, 2010). Carotenoids also act as antioxidants and exogenous application of SA has been reported to increase carotenoid contents in plants (Shaki *et al*., 2018). In the current study, foliar spray of SA might have increased chlorophyll content in berseem plants by quenching ROS mainly H_2O_2 accumulation thus, inducing antioxidants activity to shield plant pigments and membranes under salinity (Alam *et al*., 2023).

In the current study, salt stress markedly reduced the content of TSP and TAA whereas, SA application significantly enhanced these osmolytes under salt stress. Improved production of osmolytes or compatible solutes is a preferential mechanism adopted by plants to combat salinity stress. Higher build-up proteins and amino acids help plants to adjust osmotically under saline environments. Salt stressed plants increase free amino acid content for osmotic adjustment and plants accumulate stress-responsive proteins, which contribute in salt tolerance mechanisms and ameliorate deleterious effects of salinity by exogenous application of SA (Bastam *et al*., 2013). A higher concentration of total soluble proteins also enhanced the activities of antioxidants (SOD) in plants which also helps in the mitigation of salinity-induced damages (Sairam *et al*., 2002).

Induction of oxidation by the production of reactive oxygen species is linked with salinity stress in plants. Plants adopt protection from NaCl toxicity by up-regulation of antioxidants enzymes like SOD, POD and CAT to quench salinity-induced ROS (Chakraborty *et al*., 2016). It has been observed that activities of POD, SOD and CAT has been significantly increased in tested berseem plants under saline and non-saline conditions. Foliar spray of SA significantly boosted the activities of these enzymes in tested plants under non-saline and varying saline regimes. Exogenous application of SA alleviates salinity-induced damages in plants by boosting the activities of antioxidants (Ahanger *et al*., 2020). Increased activities of enzymatic antioxidants has been reported due to foliar application of SA in various crops including rice (Rizwan *et al*., 2019), sunflower (Sibgha *et al*., 2009) and cotton (Hamani *et al*., 2020).

Nutrient imbalance is a major effect of salt stress that hampers various cellular processes in plants. In the current study, varying levels of NaCl significantly enhanced Na^+ and Cl^- ions concentration while significantly reduced Ca^{2+} and K^+ ions content in root and shoot tissues of berseem plants. Enhanced concentration of Na^+ and Cl^- ions in plant tissues reduces the uptake of Ca^{2+} and K^+ ions. Plant metabolism is disturbed by high levels of NaCl in plants by disturbing enzyme activities and photosynthetic process (Alsahli *et al*., 2019). Foliar Spray of SA proved to be significantly effective in lowering the toxic levels of Na^+ and Cl^- ions and enhanced the concentration of Ca^{2+} and K^+ ions in root and shoot tissues of berseem plants under saline and non-saline regimes suggesting the role of SA as osmotolerant in plants, SA also plays vital role in increasing the uptake of Ca^{2+} and K^+ ions by decreasing uptake of Na^+ and Cl^- ions. It also improves nutrients accumulation process and lowers Na^+ and Cl^- an accumulation (El-Sharkawy *et al*., 2022; Hayat *et al*., 2022; Mimouni *et al*., 2016) . Ca^{2+} and K^+ play vital role to mitigate salinity stress in plants by giving structural stability and nutrients balance (Dedejani *et al*., 2021). Based on available literature it is concluded that exogenous application of SA at low rates helps the plants to withstand abiotic stresses (Ur Rahman *et al*., 2023). To cope with abiotic stressors, beneficial effect of SA has been reported in various plants including strawberry (Singh *et al*., 2023), barley (Islam *et al*., 2023), lettuce (Alavilli *et al*., 2023), okra (Godara and Ramakrishna, 2023), cotton (Swain *et al*., 2023), rosemary (Afrouz *et al*., 2023), Indian mustard (Mahajan *et al*., 2023) and sweet basil (Avasiloaiei *et al*., 2023).

It is concluded that effective concentration of SA and mode of application (seed priming, foliar spray, hydroponic solutions etc) can play vital role in managing the salinity stress in plants (Abdelsattar *et al*., 2023). While, concerning the foliar application plant growth stage and concentration is also important. Therefore, extension of the current research can be further useful in identifying the best range of SA levels of applications for improving tolerance of plants under salinity stress and, it is also important to further explore the role of SA on other forage crops as the data is scarce In this regard, the current study adds to a better understanding of SA roles in plants to cope with salinity stress (Horchani *et al*., 2023).

Conclusions

In conclusion, the present study demonstrated that soil salinity severely affected the growth, photosynthetic pigments and nutrients status of berseem plants. While SA improved the growth by minimizing the deleterious effects of salinity stress through boosting the antioxidants defence system, ionic homeostasis, and accumulation of total soluble proteins and total amino acids. Lower concentration (100 mg L^{-1}) of SA proved more effective in minimizing the toxic effects of salinity in berseem plants. The results of the current study can be a progressive contribution toward the cultivation and promotion of other fodder crops under saline soils. While researchers should explore the most effective methods for applying SA, including different concentrations, application timings, and frequency, to maximize crop growth and salt stress tolerance.

Authors' Contributions

Conceptualization, AM and MMJ. Writing–original draft preparation, AM, and MA. Writing – review and editing, MN, MAZ, WL, MAJ, MA, AAR, AR, MUH and SHQ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

Social Research Science Institute of Jilin Provincial Department of Education during the 13th Five-Year Plan Period: Research on the Ecological Environment Protection Mechanism of Momoge Wetland, project number: JJKH20200004SK.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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