ISSN 2226-3063 e-ISSN 2227-9555
Modern Phytomorphology 16: 32–40, 2022

RESEARCH ARTICLE

Salicylic acid-induced amelioration of salt stress by modulating morpho-biochemical attributes and antioxidant mechanism in black gram (*Vigna mungo* L.)

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Received: 25-Apr-2022, Manuscript No.: mp-22-61752 | **Editor Assigned:** 26-Apr-2022, Pre-QC No. mp-22-61752 (PQ) | **Reviewed:** 23-May-2022, QC No.: mp-22-61752 (Q) | **Revised:** 24-May-2022, Manuscript No.: mp-22-61752 (R) | **Accepted:** 25-May-2022 | Published: 02-Jun-2022

Abstract

As a severe and prevalent abiotic stress, salinity causes extensive crop losses by limiting plant growth and production worldwide. The present study was carried out to mitigate the salinity-induced harmful effects on plant growth, biochemical and antioxidant attributes of black gram by the foliar application of Salicylic Acid (SA). *Vigna mungo* L. Hepper (ADT-5) plants were grown in salt-treated (75 mM NaCl) and untreated (0 mM NaCl) growth medium. Three levels of SA (0.5 mM, 0.75 mM, 1.00 mM) were applied through the foliar spray. Salt stress significantly reduced the morpho-biochemical attributes in black gram. However, foliar application of 0.5 mM SA displayed ameliorated response under salt stress, as evidenced by the highest values for growth attributes and photosynthetic pigments compared to control and other experimental units. A reduced photosynthetic pigment (chlorophyll a, b, and carotenoids) under salt stress was significantly ameliorated by foliar application of 0.5 mM SA. The proline and enzymatic antioxidants like catalase, and superoxide dismutase activities were improved by salicylic acid application compared to the control group. This study indicated that SA reduced the deleterious effect of salt stress under high salt concentration by regulating morphological and biochemical indices in black gram.

Keywords: Salinity stress, salicylic acid, proline, malondialdehyde, hydrogen peroxide, antioxidant enzymes

Introduction

Agricultural productivity around the globe is under severe threat due to various biotic and abiotic stressors. Salt stress is the most prevalent abiotic threat the world faces in arid and semi-arid areas. The soluble salt concentration in these areas is high, and the rainfall is low, which allows the percolation of salts (Zhoa et al., 2007; Saddiq et al., 2021; Sharma et al. 2022). Salt stress reduces plant growth by osmotic and ionic effects that disturb ion homeostasis (Athar and Ashraf 2009; Zafar et al., 2017). Salt stress disrupts all morphological, physiological, anatomical, and biochemical parameters of a plant (Iqbal et al., 2018).The growth and development of plants, as affected by salt stress, cause a threat to global food production. It affects plant physiology by damaging cellular organelles, inhibiting enzyme activities and protein synthesis, cessation of photosynthesis and respiration, changing nutrient uptake, reducing soil osmotic potential, as well as hindering water uptake by roots (Talaat 2019; Hasanuzzaman et al., 2020). Continuous accumulation of Na^+ and Cl^- leads to overproduction of Reactive Oxygen Species (ROS) like singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radicles ($^{\cdot}OH$), and superoxide ions ($O_2^{\cdot-}$) in plant cell organelles (Hasanuzzaman et al., 2020). Higher and non-metabolized ROS leads to oxidative stress, affecting cellular metabolism, cell division, growth, membrane integrity, and photosynthetic function (Nazar et al., 2015). To restain ROS accrued upshot, plants upregulate enzymatic and non-enzymatic antioxidant defence systems to scavenge them. Salinity also hinders the uptake of certain essential elements like K^+ and negatively impacts plant water relations (Deinlein et al., 2014). The overall reduction in growth and development of plants under salt stress is the cumulative effect of disruption

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in ion homeostasis, water balance, and disruption in photosynthetic capacity (Khan et al., 2012).

Black gram [*Vigna mungo* (L.) Hepper] occupies an important place among the premier pulse crops in India. Black gram is an extensively grown grain legume and belongs to the Fabaceae family and got noticeable significance from the point of food and nutritional security in the world (Thakur et al., 2017). Black gram is a perfect combination of all nutrients, including 20% to 25% proteins, 40% to 47% starch, ash fats, carbohydrates, and essential vitamins (Manjri et al., 2018). India produces 1.45 million tons of black grams annually on about 3.25 million hectares of land (Arulbalachandran et al., 2010).

The approach and tactics to mitigate salt stress's adverse effects on plants have been an important in scientific research (Raza A. 2022). Improvement in stress tolerance in plants has significant implications for agriculture and horticulture (Rajeshwari and Bhuvaneshwari 2017). Plants growing in the saline environment have evolved diverse adaptive strategies to overcome salt stress at both cellular and whole plant levels. The osmotic stress is compensated by accumulating proline, amino acids, carbohydrates, and proteins (Ahmad et al., 2017). Several physiological and genetic manipulations have been in higher plants, which results in Na^+ exclusion at root surface and decreases root to shoot translocation of Na^+ (Schubert et al., 2009).

Phytohormones are essential regulators for the growth and development of plants (Sabagh et al., 2021). They play a vital role in regulating the response of plants to different biotic and abiotic stresses. Salicylic acid (SA., 2-hydroxy benzoic acid), a phenolic endogenous plant growth regulator, acts as a signaling molecule for modifying plant response by inducing the expression of genes that code defense-related compounds like jasmonic acid and proline (Wani et al., 2017; Shaukat et al. 2022). It acts as a bio stimulator that activates the biochemical pathways associated with salt tolerance mechanisms in plants (Fariduddin et al., 2018). Salicylic acid alters plant response to abiotic stressors by signaling cross-talk with other plant hormones (Iqbal et al., 2015). Salicylic acid plays a vital role in assimilating nutrients under stressful conditions (Khan et al., 2015).

Salicylic acid is the strong candidate among stress ameliorators and has been recognized as plant growth premotor. It plays a diverse role in plants, including stomatal closure, thermogenesis, flower induction, and abiotic stress resistance. Eraslan (2007) reported that foliar spray of SA enhances plant growth, photosynthetic pigment, and antioxidant activity of carrot root and shoot. Foliar spray of SA has also been reported to maintain K^+/Na^+ ratio and boost photosynthetic activity and antioxidant system under stressful conditions (Hayat and Ali. 2010). Black gram is a very sensitive crop to high salt concentrations like many other legumes (Muas and HofSman, 1977). Several studies have shown that the application of SA improved plant salt tolerance by acting as chemical messengers in numerous metabolic processes such as mineral nutrition uptake, antioxidant activity, methylglyoxal detoxification, photosynthesis, respiration, cellular signaling, senescence, and overall cellular redox homeostasis (Bukhat et al., 2020; Hoang et al., 2020; Kaya et al., 2020; Es-sbihi et al. 2021; Shamili et al., 2021).

There is also little information available in the literature on optimal concentrations of SA required for achieving maximal productivity on salt-affected soils. In this study, the effects of SA were examined on black gram under non-saline and saline conditions. The current experiment was designed to evaluate the varied SA concentrations as a foliar application on growth, biochemical and antioxidant profile in the leaves of black gram plants and the possibility to ameliorate salt stress.

The optimal levels of SA are species-or even cultivar-dependent. Furthermore, previous studies focus on the individual effects of SA improving crop plant tolerance, while their putative integrative effects have rarely been addressed. The study was conducted to find out the (1) impact of salt stress on several morpho-biochemical, and antioxidant activities of the subject plants. (2) To analyze the response of black gram genotype towards exogenously applied SA concentration with reference to these parameters under salt stress. The information obtained from this experiment will give a future direction for expanding this crop under saline soil. We hypothesized that the foliar-applied SA alone or in combination with salt stress might reduce the detrimental effect of salinity stress by modulating growth, biochemical parameters, and increasing antioxidant activity in the black gram plant.

Materials and Methods

Plant material and stress treatment

The present study was carried out at the Department of Botany, Annamalai University, Annamalai Nagar (Lat 110 24'N and Long 790 44' East) with Black gram genotype variety ADT-5 under salt stress. Healthy seeds of black gram genotype variety ADT-5 were procured from Pulse Research Institute, Pudukottai Tamil Nadu, India. The surface sterilization was done by using Mercuric chloride (0.01%) followed by washing with double distilled water. The seeds were sown in pots containing a mixture of soil, sand, and manure in the ratio of 1:2:1. The pots were arranged in Completely Randomized Block Design (CBRD). Ten-day-old seedlings were subjected to 0 mM NaCl and 75 mM NaCl (to facilitate the seedling establishment stage) given in three successive doses on alternate days to avoid osmotic shock. Treatment was continued till the required concentration of salt was achieved. The experimental soil was sandy loam with 7.4 pH and EC values of 0.4 dSm^{-1} and 8.3 dSm^{-1} were achieved in soils, which were maintained throughout the experimental trials by monitoring at weekly intervals. After six days of emergence, thinning was done, and three plants were maintained per pot throughout the experiment.

Foliar application of salicylic acid

Salicylic Acid (SA) was obtained from Himedia chemical laboratory Chennai (RM 1476-500G). The hormonal solution was prepared by dissolving it in 5 mL ethanol, and desired concentration was achieved by the addition of distilled water. After that, surfactant teepol (0.5%) was added with SA treatment solution. SA solution of different concentrations (0.5 mM, 0.75 mM, and 1.00 mM) were sprayed 20 days after sowing with the

help of a hand sprayer. One group of plants was sprayed with distilled water and treated as a control.

Research design

The experimental design consisted of one salt concentration of 75 mM NaCl combined with three SA concentrations of 0.5 mM, 0.75 mM, and 1.00 mM. One group of plants was treated as a control and was not subjected to salinity stress (T_0) or SA spray. Other treatment groups consisted of 75 mM NaCl (T_1), 0.5 mM SA (T_2), 0.75 mM SA (T_3), and 1.00 mM SA (T_4), 75 mM NaCl plus 0.5 mM SA (T_5), 75 mM NaCl plus 0.75 mM SA (T_6), and 75 mM NaCl plus 1.00 mM SA (T_7). The pots were arranged in Completely Randomized Block Design (CBRD). After 35 days, plant morphology, biochemical and antioxidant assays were carried out.

Analysis of growth and biomass

After completion of the experiment (35 days), five plants were randomly selected from each treatment. The root length and shoot length were measured by using a meter scale and were expressed as (cm plant^{-1}). After careful washing and drying, the plant parts were separated and oven-dried at 80°C. Dry weight was measured to determine the accumulation and allocation of biomass and was expressed in (grams).

Biochemical analysis

Photosynthetic pigment: To extract the photosynthetic pigment, 0.5 g of fresh leaves were grounded in a porcelain mortar with 15 ml of acetone 80% and then centrifuged for 10 minutes at 4000 rpm. Absorption of the extract was read by UV/Vis spectrophotometer at 645 mm, 663 mm, and 480 nm wavelengths for chlorophyll a, b, and carotenoid. For device adjustment, acetone 80% was used. Chlorophylls contents per gram of fresh weight were calculated (Arnon's method, 1949).

Proline assay: Proline accumulation level in the leaf samples was determined based on proline's reaction with ninhydrin following the method of Bates et al. (1973). Briefly, 0.5 g of leaf sample from each treatment was macerated with 10 mL of 3% sulphosalicylic acid, and the homogenate was filtered through filter paper. The mixture was heated at 100°C for 1 hr. in a water bath after the addition of 2 mL of 1% ninhydrin, and 2 ml of 75% glacial acetic acid. The reaction was arrested in an ice bath, and the chromophore was extracted with 4 ml toluene, and its absorbance at 520 nm was determined through spectrophotometer.

Total Protein Content: Total protein content was estimated using the Lowry et al., (1951) method. Fresh tissue weighing 0.5 g was macerated in 20 percent Trichloroacetic acid using mortar and pestle. The homogenate was centrifuged at 600 rpm for 30 minutes, and the supernatant was discarded. Five ml of 0.1 N NaOH was added to the pellet, and it was centrifuged for 30 minutes. To 0.5 ml of the extract, 5 ml of copper reagent 'C' was added (Reagent C: Mixture of reagents 'A' and 'B' in the ratio 50:1 ratio. Reagent A: 2 percent Na_2CO_3 in 0.1 N NaOH; Reagent B: equal volume of 1 percent $CuSO_4$ and two percent sodium potassium tartrate). The absorbance was read at 550 nm in a spectrophotometer against an appropriate blank, Bovine Serum Albumin (BSA) was used as the standard.

Malondialdehyde (MDA) assay: Peroxidation of membrane lipids was measured based on the formation of malondialdehyde complex with Thiobarbituric acid, using Heath and Packer (1968). Take 200 mg leaf segment was homogenized in 3 ml of 50 mM phosphate buffer (pH 7.0) and then centrifuged at 8000 g in a Remi centrifuge (R-8C) for 20 minutes. Then, 2 mL of 0.5 percent TBA in 20 percent TCA solution was added to the 0.5 ml of remaining supernatant. The mixture was warmed in the water bath at 90°C for 30 minutes, followed by rapid cooling. Then the mixture was read spectrophotometrically at 532 nm and 600 nm wavelength using spectrophotometry.

Superoxide Dismutase (SOD) activity: The SOD activity was determined using Beauchamp and Fridovich's (1971) method. The SOD activity was determined by the ability of this enzyme to retard the reduction of Nitro Blue Tetrazolium (NBT) by superoxide free radicals, which were generated in the reaction medium through photoreduction of riboflavin. The reaction mixture consists of 1 mL of buffer, I M sodium bicarbonate, 300 mM EDTA, 200 mM methionine, 100 µL of enzyme extract and 600 µM of riboflavin in test tube. The test tube was shaken and was placed 30 cm from light back consisting of six 15 W fluorescent lamps. The reaction was run for 10 min and was stopped by switching off the light. The optical density was determined at 560 nm, and the SOD activity results were calculated as µmol/mg Prot./min.

Catalase (CAT) activity: The CAT activity was determined based on the rate of H_2O_2 disappearance as measured by the decrease in absorbance at 240 nm, based on the method of Aebi (1974). The reaction mixture included phosphate buffer (50 mM, pH 7.0), H_2O_2 (3%), and 10 µL enzyme extract. The results were calculated as µmol/mg Prot./min.

Statistical analysis: The results presented are the means ± standard error of five replicates (n=5). The results were statistically confirmed by Analysis Of Variance (ANOVA). Tukey's HSD test was applied to find means significantly different from each other at $p \leq 0.05$ level. The means that are not sharing a letter is significantly different at $P \leq 0.05$ significance level.

Results and Discussion

Plant growth attributes

The results presented in Tab. 1 indicate that all the growth parameters of the selected plant were significantly ($p<0.005$) affected by NaCl treatment. However, SA application increased the growth characteristics compared to the control group. All the concentrations of SA increased the growth characteristics compared to T_0 under non-saline conditions. However, among the various concentrations 0.5 mM concentration of SA was most effective in alleviating the harmful effects of salt stress. The combination of salt stress with SA treatment led to a significant ($p<0.05$) growth improvement compared to the salinity treatment alone. This increase remained important for the plants treated with 0.5 mM SA, which demonstrated increases of approximately 26.57%, 14.58%, 49.85%, 28.27%, 50.00%, 23.62% for the Root Length (RL), Shoot Length (SL), Fresh Weight Root (FWR), Fresh Weight Shoot (FWS), Dry Weight Root (DWR), and Dry Weight Shoot (DWS) compared

to T_1, respectively. Under saline conditions, by increasing concentration beyond 0.5 mM, the enhancement was not profound when compared to the T_5 group.

Photosynthetic pigments

Salinity stress shows reciprocal relationship with photosynthetic pigments. Chlorophyll (a, b, total) and carotenoid contents were decreased by 63.44%, 17.07%, 87.67%, and 24.12% at 75 mM NaCl concentration over control group (Fig. 1). Under unstressed conditions salicylic acid significantly enhanced chlorophyll contents compared to water-sprayed plants. The highest significant value was obtained with SA at 0.5 mM under non-saline control conditions. There was no positive response by increasing the concentration of SA beyond 0.5 mM. The combination of salt stress with SA treatment at 0.5 mM was effective in mitigating the negative effects of salt stress led to a significant ($p<0.05$) improvement in photosynthetic parameters compared to the salinity treatment alone.

Total soluble protein content

As presented in Fig 2A, the comparison of means indicated that all treatments were significantly different. The results demonstrated that salt stress significantly decreased the protein content compared to the conditions without salinity. This protein content was reduced by 11.54% at T_1 (75 mM NaCl) when compared with T_0, respectively. However, SA application ameliorates the harmful impacts of salt treatment. The combination of salt stress and SA treatment (0.5 mM) was found to be effective in alleviating the harmful effects of salt stress and led to an increase of 10.87% compared to the T_1 treatment group.

Table 1. Effect of varying concentration of SA treatment and salt stress on the growth parameters of 567 black gram genotype. Values are means ± SE (n=5). Different letters indicate a significant difference at p<0.05..

Treatment	Root length (cm/plant)	Shoot length (cm/plant)	Fresh weight root (g/plant)	Fresh weight shoot (g/plant)	Dry weight root (g/plant)	Dry weight shoot (g/plant)
Control	12.05 ± 0.00c	24.12 ± 0.014c	5.24 ± 0.014d	8.15 ± 0.007d	2.91 ± 0.028d	3.95 ± 0.028d
T1 (75 mM NaCl)	9.22 ± 0.035f	20.85 ± 0.007g	3.41 ± 0.035h	6.26 ± 0.035g	1.86 ± 0.014f	3.09 ± 0.014f
T2 (0.5 mM SA)	15.32 ± 0.021a	28.18 ± 0.015a	7.83 ± 0.021a	11.49 ± 0.021a	4.44 ± 0.035a	5.65 ± 0.035a
T3 (0.75 mM SA)	13.41 ± 0.028b	26.04 ± 0.022b	6.43 ± 0.007b	10.67 ± 0.028b	3.87 ± 0.042b	4.89 ± 0.014b
T4 (1.00 mM SA)	12.11 ± 0.014c	23.32 ± 0.035e	5.93 ± 0.042c	9.74 ± 0.014c	3.87 ± 0.042b	4.12 ± 0.028c
T5 (75+0.5 mM SA)	11.67 ± 0.042d	23.89 ± 0.028d	5.11 ± 0.014e	8.03 ± 0.042d	2.79 ± 0.021d	3.82 ± 0.042d
T6 (75+0.75 mM SA)	11.21 ± 0.05e	22.43 ± 0.043e	4.28 ± 0.014f	7.53 ± 0.049e	2.14 ± 0.007e	3.51 ± 0.05e
T7 (75+1.00 mM SA)	9.32 ± 0.014f	21.16 ± 0.021f	3.96 ± 0.049g	6.87 ± 0.014f	1.97 ± 0.014f	3.21 ± 0.014f

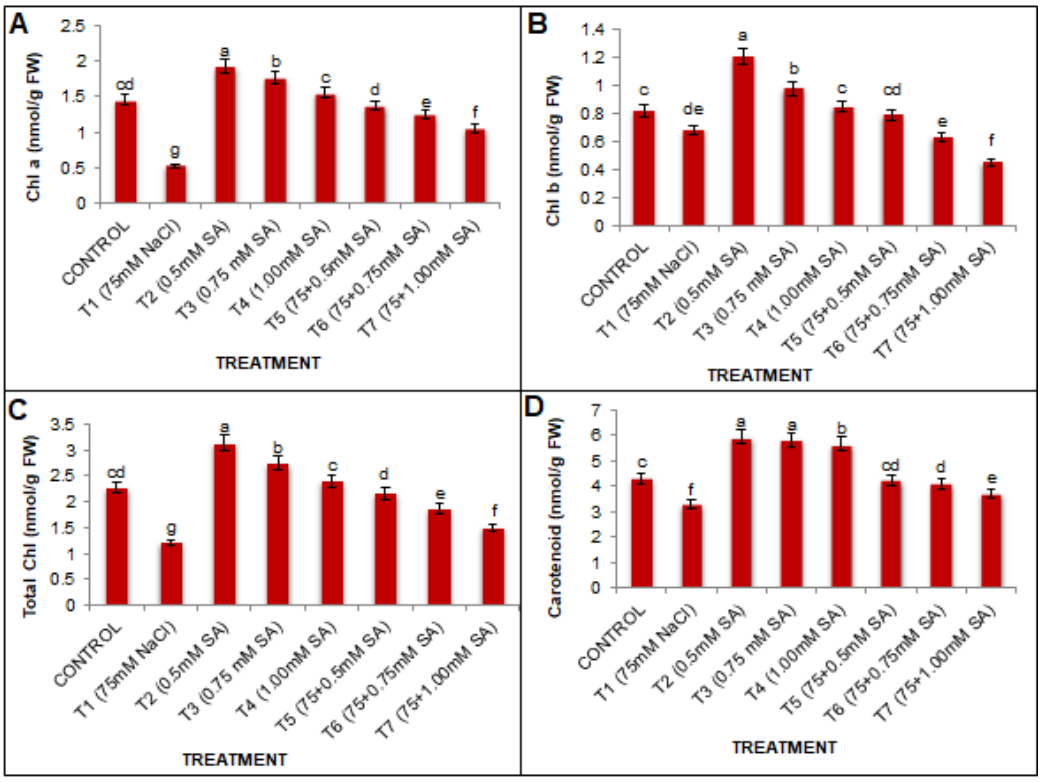

Figure 1. A): Stress-induced ethylene production by soybean plants without seed bacterization; B): inoculation with rhizobia of strain 604k; C) Tn5 mutant B1-20 in the stages of cotyledons (I), primordial leaves (II) and the first true leaf (III)); (x ± SD, n=10).

Lipid peroxidation

Plants subjected to salt stress showed an increased content of MDA in black gram leaves Fig. 2B Damage to cell membranes was studied by estimating the MDA content in leaves. The current results presented a significant increase of MDA content in the stressed plants compared to the unstressed plants. However foliar application of SA reduced the MDA content in the study plant over the control group. MDA accumulation was reduced by SA treatment in unstressed plants. However, 0.5 mM SA application was found to be most effective in T_5, which reduced the MDA content by 65.61% compared to T_1.

Proline content

The present results showed that salt stress treatment caused significant leaf proline accumulation in the leaves of stressed plants over non-saline control during plant growth. Exogenous application of SA caused maximum accumulation of proline in salt-stressed plants (Fig. 2C). However, with the increase in the concentration of SA, proline content also increased, and a 28.86% maximum increase was observed in plants treated with 1.00 mM SA under salt stress compared to T_1 treatment groups.

Catalase activity

CAT activity was significantly ($p<0.005$) affected by NaCl application (Fig. 3A). At 75 mM NaCl 16.60% increase in CAT activity was observed in the T_1 treatment over the control group. However, the exogenous application of various SA concentrations enhanced the CAT activity in the study plant

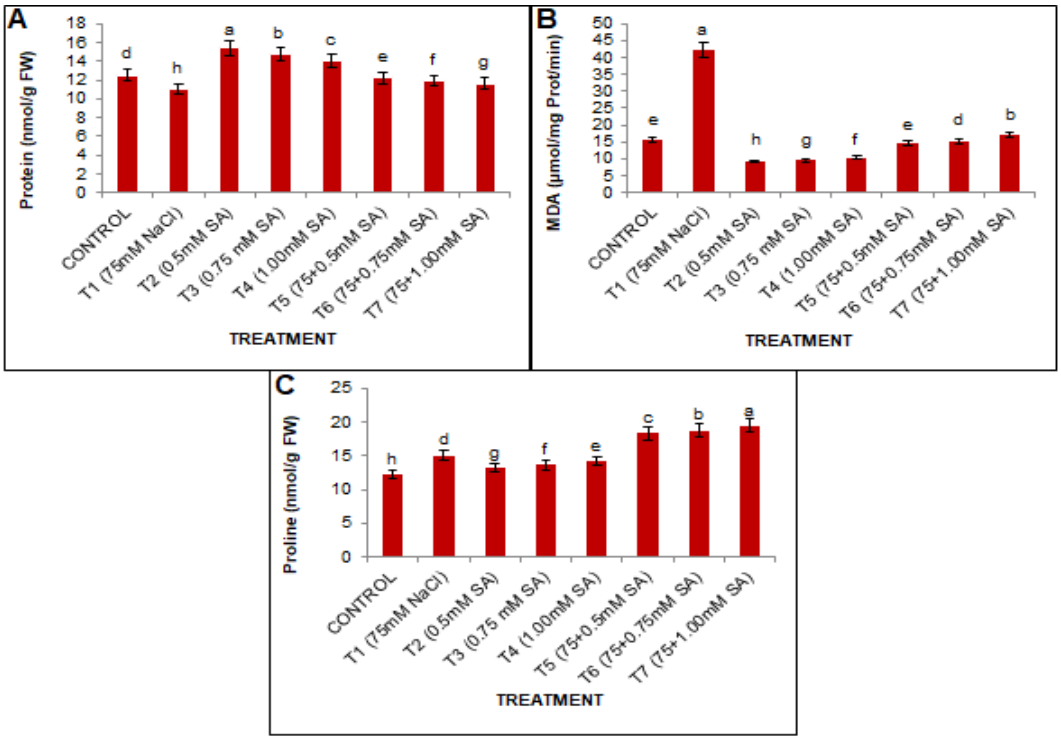

Figure 2. Effects of SA and salt stress on (A) total protein contents, (B) MDA contents, and (C) proline contents in black gram leaves. The obtained values were analyzed by using one-way analysis of variance (ANOVA)-using SPSS 16.0 software. Different letters indicate a significant difference between the mean (±SE) of treatments (n=5) at P<0.05 significant level.

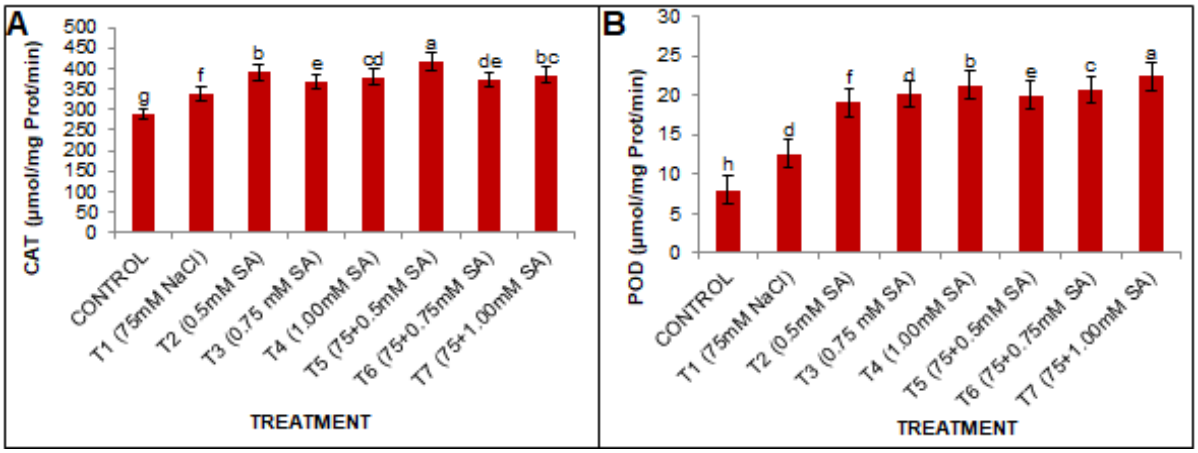

Figure 3. Effects of SA and salt stress on (A) CAT and (B) POD activities in black gram leaves. The obtained values were analyzed by using one-way Analysis of Variance (ANOVA)-using SPSS 16.0 software. Different letters indicate a significant difference between the mean (± SE) of treatments (n=5) at P<0.05 significant level.

under non-saline conditions. Among various concentrations of SA, 0.5 mM was found to be the most effective and to 24.03% high CAT activity compared to the T1 treatment group.

Peroxidase activity

POD activity was significantly affected by NaCl application. The data presented in (Fig. 3b) indicate that the POD activity was increased by 56.66% at 75 mM NaCl significantly ($p<0.05$) as compared to the control group. Under various SA treatments for the unstressed plants (approximately 138.24%, 151.30%, and 165.62% for the T_2, T_3, and T_4 treatments, respectively) compared to the control group. Furthermore, in response to the salt stress, the POD activity significantly increased compared to conditions without stress. However, the application of SA, i.e. 1.00 mM, was found to be most under salt stress and enhanced the POD activity by 77.90% for T_7, compared to T_1.

Discussion

Salinity is one of the challenging environmental factor affecting plant growth and development aspects. Excessive uptake and accumulation of ions, particularly sodium (Na^+) and chloride (Cl^-), causes osmotic stress and cytotoxicity, restricting plant growth and productivity. Several Physiological and molecular processes govern osmotic and ionic stress tolerance by enhancing antioxidant activity, osmolyte buildup, and ion equilibrium (Roussos et al., 2013; Isayenkov and Maathuis 2019; Saddiq et al., 2021). Our present study shows that salt stress retards the plant growth in terms of root length, shoot length, fresh weight, dry weight, and biomass exclusively by excessive uptake of Na^+ and Cl^- ions and antioxidant activity of *Vigna mungo* L. plants under salt stress. The results indicate that the exposure of black gram plants to salt stress negatively affected their growth. The plants, under saline water irrigation (75 mM NaCl), demonstrated a decrease in all studied growth parameters. These results are similar to those documented in several plant species, such as wheat (Abdel-Lattif et al., 2019), soybeans (Ghassemi-Golezani et al., 2018), and mung beans (Salingpa et al. 2018). According to Acosta-Motas et al., (2017), decline in growth is a key aspect for depicting the extent of salt stress-led devastation independent of type of species. Ahmad et al. (2016) suggested that salt stress-induced suppression of cell division and cell elongation is the key cause of reduced growth and biomass and our results also showed higher sensitivity of *Vigna mungo* to salinity. SA-induced advantageous effect on growth characteristics could be endorsed to reduced Na^+ and Cl^- uptake, ethylene synthesis inhibition, or increased uptake of essential nutrients, such as K^+, Ca^{2+}, Mg^{2+}, Fe, Mn, N, and Cu^{2+} (Ghassemi-Golezani et al., 2018). These measures can help plants thrive well by reducing ionic and osmotic stress. Exogenous application of SA can modulate stomatal opening, promote CO^2 assimilation, and reduce transpirational water loss under saline circumstances (Ribeiro et al., 2020). As a result, the plants could maintain their turgor, boost their photosynthetic capacity and increase their yield (Fghire et al., 2015; Ribeiro et al. 2020).

Photosynthesis, the prime photosynthetic machinery, gets easily targeted by salt stress. Chlorophyll (a, b, total) and carotenoid contents decreased with increasing NaCl concentration (Fig. 1). Under unstressed conditions, salicylic acid significantly enhanced chlorophyll contents compared to water-sprayed plants. The highest significant values were obtained with salicylic acid at 0.5 mM under non-saline conditions. The combination of salt stress with SA treatment led to a significant ($p<0.05$) improvement in photosynthetic parameters compared to the salinity treatment alone. The present study results agree with the findings of earlier researchers where salt-induced reduction in the chlorophyll contents is alleviated by the foliar application of salicylic acid in crops such as tomatoes (Shahba et al., 2010). Exogenous SA application protects chlorophyll from degradation by regulating antioxidant molecules synthesis, suppressing genes involved in the senescence process, up-regulating chlorophyll biosynthesis genes, and down-regulating chlorophyll degradation genes (Altaf et al., 2021). SA plays a pivotal role in chloroplast biosynthesis, maintaining chlorophyll stability, regulates photosynthesis by guarding this vital organelle against toxic effects of ROS to survive under salt stress. The oxidation of chlorophyll and other chloroplast pigments, as well as the instability of the pigment-protein complex under salt stress, could explain the decrease in chlorophyll concentration in salt-affected black gram genotypes Stępień and Kłbus (2006).

Surprisingly, foliar applications of SA significantly neutralized the salt injuries and maintained a high concentration of carotenoids in salt-stressed plants (Fig. 4). The decomposition of beta carotene and the zeaxanthin production in the xanthophyll cycle results decrease in carotenoid content under stress conditions (Sultana et al., 1999). A similar observation was also reported by Arnao and Hernandez-Ruiz (2019); Bukhat et al., (2020); Faraz et al., (2020) that SA could regulate steps in carotenoid biosynthesis and influence cellular signaling, and trigger redox-sensitive regulatory pathways. Salinity stress increases the concentrations of growth regulators such as abscisic acid and ethylene that activate Chlorophylase, causing break down of chlorophyll.

The stressed plants showed decreased protein content compared to the control group. However, there was an increase in the protein content in SA-treated plants under saline conditions. Similar results have been found with wheat (Alsahli et al., 2019), tomatoes (Arbaoui and Belkhodja, 2018), and faba beans (Anaya et al., 2017). Salt stress negatively affects protein biosynthesis by assisting protein degradation. A decrease in soluble protein level of salt-treated *Vigna mungo* may be possibly due to catabolism of proteins under salt stress. The salt stress condition could affect different stages of nitrogen metabolism, such as absorption, ionic reduction, and protein synthesis. Under stress conditions, plant produce several proteins that can regulate intracellular signaling and gene expression related to critical metabolic processes (Alsahli et al., 2019). Arbaoui and Belkhodja (2018) and Rajeshwari and Bhuvaneshwari (2017) have explained that the application of SA under abiotic and biotic stresses can improve plant tolerance by protecting proteins ultrastructure and increasing metabolic enzyme activity.

The oxidative reactions induce a sequence of reactions that lead to membrane peroxidation. In this work, the salt stress resulted in a significant increase in MDA content in black gram leaves due to oxidative stress (Tab. 1). This is supported by a

positive correlation between H_2O_2 and MDA (Moustafa-Farag et al., 2017). Similar reports have been provided for *Arabidopsis thaliana* L. by Yu et al., (2020). However, under saline stress conditions, the treatment with SA remarkably reduced the MDA accumulation in the treated plants compared to those under salinity treatment alone. Similar results have been found by Torun (2019) in barley plants and Wang et al., (2018) in wheat plants; those studies indicated that exogenous SA treatment could reduce the higher MDA levels caused by salt and cold stress, respectively. The findings suggest that SA effectively limits cell membrane damage through the antioxidant defence system. Indeed, SA application ameliorated the plants' stress tolerance by reducing membrane oxidation (Wang et al., 2018), protecting cellular components, and strengthening cell walls (Ahanger et al., 2020).

SA treatment and salt stress in T_5 increased proline accumulation by 17.69% compared to T_1. A similar increase in proline by SA treatment has earlier been observed in Panax (Ali et al., 2007), Tomato (Mimouni et al., 2016), and Cucumber (Youssef et al., 2018). To protect thylakoidal protein complexes, salt stress stimulates the production of reactive oxygen species in the chloroplast as well as the accumulation of several osmoprotectants and antioxidants. Proline is one of the potential osmoprotectant with roles in osmotic regulation, protection of PSII activity, antioxidant activity, and cell membrane stabilization (Szabados and Savouré, 2010).

In the present study, salt stress enhances the antioxidant activity in the studied plant. The increase in antioxidant enzyme activity was highly significant in SA-treated plants under saline conditions. The foliar spray with SA significantly increased POD and CAT activity which is inversely related to MDA and H_2O_2, respectively. SA foliar spray ameliorated the salt-induced oxidative damage to PSII by modulating the activities of antioxidant enzymes. These findings are explained by Bose et al. (2017), who claim that SA triggers numerous pathways in the chloroplast to scavenge ROS and protect the photosynthetic apparatus, including a battery of antioxidant enzymes. The enhancement of salt tolerance against oxidative stress is usually related to the improving antioxidant enzyme activities in plants (Torun 2019). This increase in antioxidant enzyme activity was highly significant in SA-treated plants under saline conditions. Several recent studies have proven SA's significance in activating antioxidant enzymes under various stress conditions both directly and indirectly. Cai et al. (2015) investigated the effects of SA treatment on the antioxidative system. They found that the jasmine plant shows greater resistance to cold stress, which was likely linked to higher antioxidant enzyme activity, which resulted in decreased ROS generation, and altered gene expression. Other similar studies have found that SA improved antioxidant enzyme activity such as POD and SOD, when applied exogenously in black gram (Solanki Mital et al. 2018). According to Faried et al. (2017), SA ability to bind with antioxidant enzymes (POD and CAT) involved in ROS metabolism and redox homeostasis explains its beneficial effect on the antioxidant system of salt-stressed potato plants.

Conclusions

To summarize, our results clearly show that saline circumstances have a detrimental effect on the morphological, biochemical, and antioxidant characteristics of black gram by retarding growth, disrupting photosynthetic machinery, accumulating ROS, and modifying nutrient absorption and translocation. On the other hand, SA application alleviates the above-mentioned detrimental effects of salt stress by increasing osmolyte buildup, antioxidant machinery, and its effect on Na^+ and K^+ uptake, which is a prerequisite for metabolic processes. SA application not only nullified the impacts of salt stress but also enhanced the photosynthetic function and growth. However, the mode of action of SA in remedying the harmful impacts depends on the effective concentration. Notably, 0.5 mM SA was found to be most effective in mitigating the harmful impacts of stress, as higher concentrations were inhibitory in most cases. As a result, it is fair to conclude that foliar SA exerted stimulatory effects on the black gram genotype when exposed to salt. Our study opens a new research window that might bring revolution in the global agriculture sector and provide new insights into the application of salicylic acid to enhance tolerance to salt stress and minimize crop production yield losses under field environments.

Declaration of Competing Interest

The authors declare that they have competing interests.

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