# IMPROVEMENT OF SALT TOLERANCE IN FABA BEAN (*VICIA FABA* L.) BY SALICYLIC ACID APPLICATION

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**Abstract.** In order to minimize risks associated with salinity. This study was undertaken to investigate the effect of salicylic acid (SA) application on salt stress tolerance in Faba Bean plants. So, Faba Bean plants (*Vicia faba* L.) were treated with salicylic acid (SA) of 0 and 0.5 mM, and/or NaCl, of 0.90 and 150 mM. The results showed that salt stress reduced plant growth and photosynthetic pigments contents. Salt stress also induced an accumulation of proline, a reduction in the levels of protein and relative water content. as well as an increase in enzyme activities including ascorbate peroxidase (APx) and catalase (CAT). However, SA application on Faba bean grown under salt-stressed conditions improved photosynthetic pigments (chlorophyll a and b, carotenoids), relative water content, the organic osmolytes (total soluble protein and proline) and activities of enzymes (APX and CAT) as compared to control plants. In conclusion, depending on these findings, salicylic acid can be used as a potential growth regulator to improve the salt response of Faba bean. In other words, the application of salicylic acid might propose an ecological and economical solution to deal with salt-affected soils, especially in arid and marginal regions.
**Keywords:** *salicylic acid, salt stress, photosynthetic pigments, proline, protein, ascorbate peroxidase, catalase*

## Introduction

The Faba bean or Broad bean (*Vicia faba* L). is an important legume for human nutrition. as a major source of protein, it becomes the interest of many plant scientists to increase its production especially on marginal lands of the world (Hungria and Vargas, 2000). Salinity is one of the most devastating environmental stresses that drastically curtails the productivity and quality of Faba bean which ultimately cause threat to food production globally. Excess salt in the soil reduces root growth and water uptake as well as transpiration and respiration of the plant. As a result, the hormonal balance is destroyed, photosynthesis reduction, protein synthesis decline and the plant height decrease. As this affects the fresh and dry weight of the plant, and causes a decrease in yield (Ahanger et al., 2020; Ribeiro et al., 2020).

In addition, oxidative stress generated by an excessive NaCl accumulation through reactive oxygen species (ROS) overproduction, This results in oxidative injuries to various cellular macrobiomolecules, which eventually deactivate numerous important cellular processes in plants (Halli and Angadi, 2019; Fouad et al., 2021).

Plants detoxify ROS by stimulation of ROS-scavenging enzymes such as catalase (CAT) and ascorbate peroxidase (APx) (Bernstein et al., 2010). Non-enzymatic antioxidants such as proline plays a key role in ROS scavenging (Mansour and Salama,


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2019). However, the development of methods and strategies to mitigate the negative effects of salt on plants. It is an important part of scientific research for improving stress tolerance in plants (Rajeshwari and Bhuvaneshwari, 2017). In recent years, researchers indicated the positive effect of salicylic acid on plants tolerance to various environmental stresses (Al-Taey and Al-Musawi, 2022).

Where several research have illustrated that adding SA to fruit and vegetables, including snap bean, can lower salt stress (Osman and Salim, 2016; Amirinejad et al., 2017; Kaya et al., 2020). SAactivates the ascorbate-glutathione pathway (AsA-GSH), which increase plant resistance to tolerate ion imbalance (Kaya et al., 2020). Additionally, SA modulates plant salt tolerance via maintaining redox homeostasis (Hediji et al., 2021; Lalarukh et al., 2022), up-regulating SOD and $H_2O_2$ scavenging enzymes such ascorbate peroxidase (APx) and catalase (CAT) (Hasanuzzaman et al., 2017; Rehman et al., 2022) and it encourages also the accumulation of osmoprotectants such proline (Costa et al., 2022). Therefore, the aim of this investigation was to elucidate the alleviating effect of SA on faba bean response and tolerance to salt stress conditions by studying various physiological and biochemical parameters. Thus, we anticipate that bean plants growing under salt stress suffer considerably i.e. significant increases occurred in ROS which lead to concurrent reductions in the anabolic rate of stressed plants and their growth parameters decreased considerably (Hypothesis I). SA enhances both non-enzymatic and enzymatic antioxidants, which may induce salt stress tolerance in bean to lessen ROS and consequently improve plant growth (Hypothesis II). In addition, it increases safely total soluble protein and organic osmolytes, which may lessen the impacts of sodium influx in plant roots and its translocation to shoots (Hypothesis III).

## Materials and methods

### *Plant material and experimental design*

Healthy uniform seeds of faba bean (*Vicia faba* L.) were surface sterilized using 1% sodium hypochlorite solution during 2 min followed by several washes with distilled water. After that, seeds were allowed to germinate between wet filter paper in Petri dishes in a growth room at controlled conditions in the dark 25°C for 7 days. Then the healthy germinated seedlings were transplanted to pots containing a mixture of sterilized sand and peat (3:1 ratio). The experiment was conducted in a greenhouse with 26°C/20°C day/night temperature, 50-80% relative humidity, and a 16 h photoperiod at the faculty of sciences, Souk-Ahras University, Algeria. Two weeks after transplanting, a completely randomized design with 3 replications per treatment and 10 plants per replication were adopted. Six treatments with different salt concentrations (0, 90 and 150 mM NaCl) and different SA concentrations (0 and 0.5 mM) were implemented as follows: (1): control, (2): 0.5 mM SA, (3): 90 mMNaCl, (4): 150 mMNaCl (5): 90 mMNaCl + 0. 5 mM SA, and (6): 150 mM NaCl + 0.5 mM SA. All the measurements were carried out using samples collected around 14 days after processing.

### *Experimental details*

The experimental details are as follows: faba bean (Vicia faba L.), Variety Aguadulce.

### *Number of treatments with their different concentrations*

- *Treatment 1*: (distilled water), serving as a control.


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- *Treatment 2*: (0.5 mM SA). This concentration is used to assess the potential beneficial effects of salicylic acid, a compound known for its plant-protective properties against environmental stress.
- *Treatment 3*: 90 mM NaCl: This concentration is often used to simulate moderate salt stress, which can be found in some agricultural regions where irrigation can cause salt build-up in the soil.
- *Treatment 4*: 150 mM NaCl: This represents high salt stress, which is relevant in areas where soil salinization is a major problem, affecting crop growth.
- *Treatments 5, 6*: By combining these concentrations, we can better understand how plants respond to different levels of salt stress and how salicylic acid can mitigate these effects. This allows us to simulate scenarios that farmers might encounter in real-world conditions, providing insights into crop management in saline environments.

### *Experimental design*

Randomized block design with three replicates was applied in the experiment: 10 plants per treatment × 3 NaCl levels × 2 SA levels × 3 replicates; total of 180 plants.

### *Growth measurement*

Root and shoot length was measured using meter scale. For determination of fresh weight were weighed immediately after uprooting while as dry weight was determined after oven drying the samples at 80°C for 48 h.

### *Photosynthetic pigments measurement*

The concentration of chlorophyll and carotenoids contents were determined in fresh weight of leaves (100 mg) by sample homogenization with 80% (v/v) acetone and centrifuged at 10 000 g for 10 min at 4°C. The absorbance was recorded at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids. Pigments content was calculated using the equations of Lichtenthaler and Wellburn (1987).

### *Relative water content measurement*

The relative water content (RWC) was determined depending on the method of Sairam et al. (2002) using *Equation 1*:

$$\text{RWC (\%)} = [(\text{fresh weight} - \text{dry weight})/(\text{turgid weight} - \text{dry weight})] \times 100 \quad \text{(Eq.1)}$$

Turgid weight was determined after saturation of leaf.

For determination of turgid weight (TW), leaf disks (5 mm diameter) were submerged for 18 h in distilled water, thereafter, they were blotted to dry gently on a paper towel and weighed.

### *Proline measurement*

The proline content in leaves was determinate by the method described by Bates et al. (1973). Each sample of 100 mg fresh leaf tissue was extracted by grinding in 10 ml of 3% (v/v) sulphosalicylic acid. Then, the mixture was centrifuged at 10,000 × g for


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10 min. 2 ml of the supernatant was added to a test-tube and 2 ml of freshly prepared acid-ninhydrin solution was subsequently added. Each tube was incubated in a water bath at 90°C for 30 min. The reaction was terminated in an ice-bath. The developed color was extracted in 5 ml toluene and measured colorimetrically at 520nm. A standard curve with l-proline was used for the final calculations.

### *Total soluble protein content measurement*

The protein content in fresh leaves was determined according to the method described by Bradford (1976) using bovine serum albumin as standard. The assay is based on the stable dyee albumin complex, which can be quantified spectrophotometrically at 590 nm.

Samples of 1.0 g fresh leaves were ground in 5 ml Tris-HCl buffer (0.05 M). After that, the homogenates were centrifuged at 10,000 × g for 25 min at 4°C. The obtained extraction was used for the measurement of protein solution concentration. Also, 0.1 ml protein extraction and 5 ml biore reagent were added to the test tube, and vortexes quickly. After 25 min, their absorption was read by spectrophotometer system in 595 nm. The protein value was measured and presented by using of relevant standards curve based on mg/g fresh weight.

### *Samples preparation of antioxidants enzymes measurements*

Leaves (0.5 g, fresh weight) were homogenized in 50 mM potassium phosphate buffer pH 7.6. Then, the homogenized samples were centrifuged at 12,000 × *g* for 20 min and the supernatant crude extract was used for assays of the activities of catalase (CAT) and ascorbate peroxidase (APX) (Logginiet al., 1999).

#### *Assay of catalase activity*

Catalase (EC 1.11.1. 6) activity was assayed according to Cakmak and Marschner method (1992). The reaction mixture in a total volume of 2 ml contained potassium phosphate buffer (25 mM) and $H_2O_2$ (10 mM). The reaction was initiated by the addition of 100 µl enzyme extract and the activity was assessed by determining the initial rate of disappearance of $H_2O_2$ at 240 nm.

#### *Assay of ascorbate peroxidase activity*

The activity of ascorbate peroxidase (EC1.11.1.11) was estimated according to the method of Nakano and Asada (1981). The reaction mixture consisted of 2 ml potassium phosphate buffer (0.05 M), 0.2 ml $H_2O_2$ (3%), 0.2 ml ascorbate (0.05 mM) and 0.1 ml enzyme extract. $H_2O_2$ dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm.

### *Statistical analysis*

The results are presented as means ± standard error of three replicates (n = 3). the comparison between groups is assessed using One way ANOVA. Tukey's HSD test was used to estimate homogenous groups. Significant difference was considered at $p \leq 0.05$ level using Minitab 16 Statistical Software.


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## Results

### *Growth parameters*

The results presented in *Table 1* indicate that all the growth parameters of the (*Vicia faba* L) were affected by salt stress. Shoot and root length decreased by 52.4 and 63.8%, respectively, with high concentration 150 mM NaCl. Application of SA showed less decrease 31.2% in shoot length and 29.6% in root length with 150 mM NaCl + SA over as compared to control plants.

Fresh and dry weight were also declined with NaCl stress and the maximum decrease were 60.47 and 64.14% respectively. Whereas the application of SA to NaCl stressed plants restored the dry weight with both stress level (*Fig. 1*).

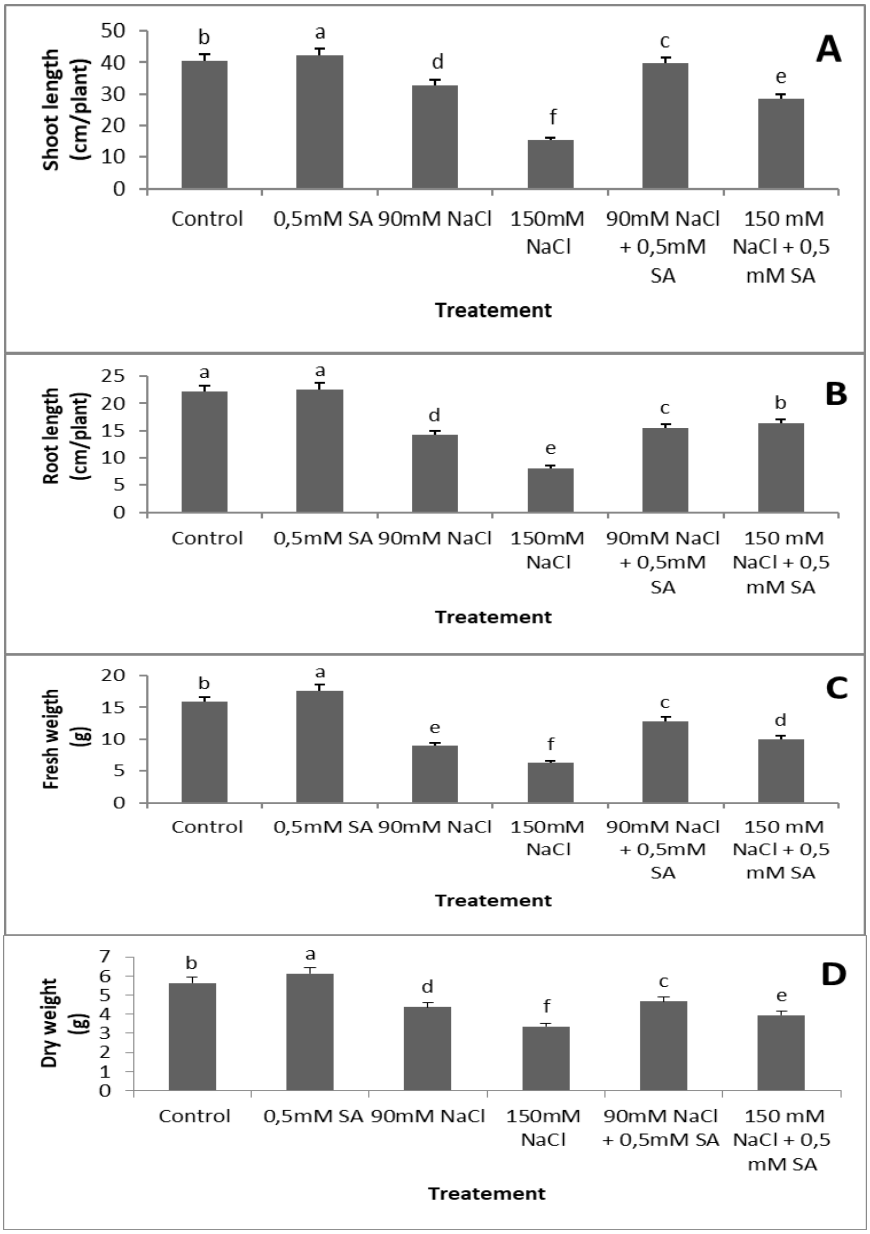


***Figure 1.*** *Effect of salicylic acid on shoot length (A), root length (B), fresh weight (C) and dry weight (D) in Vicia faba L. under salt stress condition. Error bars = standard error of means (S.E.M.); different letters (a–e) indicate a significant difference at $p \leq 0.05$*


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***Table 1.*** *Effect of salicylic acid on shoot Length, root length, fresh weight and dry weight in Vicia faba L. under salt stress condition (means ± SE)*

| Treatment | Shoot length (cm/plant) | Root length (cm/plant) | Fresh weight (g) | Dry weight (g) |
|---|---|---|---|---|
| Control | $40.52^{b} \pm 0.55$ | $22.14^{a} \pm 0.28$ | $15.87^{b} \pm 0.14$ | $5.64^{b} \pm 0.056$ |
| 0.5 mM SA | $42.26^{a} \pm 0.48$ | $22.58^{a} \pm 0.37$ | $17.66^{a} \pm 0.22$ | $6.14^{a} \pm 0.037$ |
| 90 mM NaCl | $32.76^{d} \pm 0.75$ | $14.19^{d} \pm 0.42$ | $8.94^{e} \pm 0.19$ | $4.38^{d} \pm 0.015$ |
| 150 mM NaCl | $15.25^{f} \pm 0.25$ | $8.13^{e} \pm 0.50$ | $6.25^{f} \pm 0.28$ | $3.35^{f} \pm 0.020$ |
| 90 mM NaCl + 0.5 mM SA | $39.64^{c} \pm 0.32$ | $15.43^{c} \pm 0.16$ | $12.86^{c} \pm 0.33$ | $4.67^{c} \pm 0.022$ |
| 150 mMNaCl + 0.5 mMSA | $28.32^{e} \pm 0.14$ | $16.32^{b} \pm 0.44$ | $9.98^{d} \pm 0.15$ | $3.95^{e} \pm 0.045$ |
| P Value | 0.001 | 0.009 | 0.075 | 0.098 |

Different letters (a–e) indicate a significant difference at $p \leq 0.05$

### *Photosynthetic pigments*

Salt stress resulted in a decrease in the photosynthetic pigment contents “chlorophyll a and b, carotenoid contents as compared to control plants (*Table 2*). The chlorophyll a content decreased by 45.7% under 90 mM NaCl condition and 61.5% under 150 mM NaCl condition (*Fig. 2A*). However, The SA application ameliorated detrimental effect by reducing the decrease in chlorophyll a content by 25.8% under the 90 mM NaCl condition and 53.4% under 150 mM NaCl condition compared with the non-SA-treated plants (*Fig. 2A*). The chlorophyll b content decreased 59.6% under the 90 mM NaCl condition and 65.8% under 150 mM NaCl condition (*Fig. 2B*). The SA application resumed the decrease in chlorophyll b content by approximately 38.2% under 90 mM NaCl, and 59.8% under 150 mM NaCl condition. Similar responses were also observed in carotenoid. Reduction percentage of carotenoid content under 90 mM and 150 mM was recorded by 55.2 and 41.9% respectively when compared to control plants (*Fig. 2C*).

***Table 2.*** *Effect of salicylic acid on photosynthetic pigments in Vicia faba L. under salt stress condition (means ± SE)*

| Treatment | Chlorophyll a (mg/g) | Chlorophyll b (mg/g) | Carotenoids (mg/g) |
|---|---|---|---|
| Control | $0.966^{b} \pm 0.016$ | $0.816^{a} \pm 0.022$ | $4.814^{b} \pm 0.060$ |
| 0.5 mM SA | $1.023^{a} \pm 0.014$ | $0.852^{a} \pm 0.018$ | $6.017^{a} \pm 0.083$ |
| 90 mM NaCl | $0.893^{c} \pm 0.023$ | $0.393^{d} \pm 0.014$ | $4.147^{c} \pm 0.040$ |
| 150 mM NaCl | $0.726^{bc} \pm 0.02$ | $0.206^{d} \pm 0.020$ | $2.093^{d} \pm 0.048$ |
| 90 mMNaCl + 0.5 mM SA | $1^{d} \pm 0.015$ | $0.492^{d} \pm 0.014$ | $4.447^{bc} \pm 0.055$ |
| 150 mMNaCl + 0.5 mMSA | $0.793^{bc} \pm 0.011$ | $0.249^{d} \pm 0.020$ | $2.407^{d} \pm 0.045$ |
| P Value | 0.051 | 0.030 | 0.157 |

Different letters (a–e) indicate a significant difference at $p \leq 0.05$


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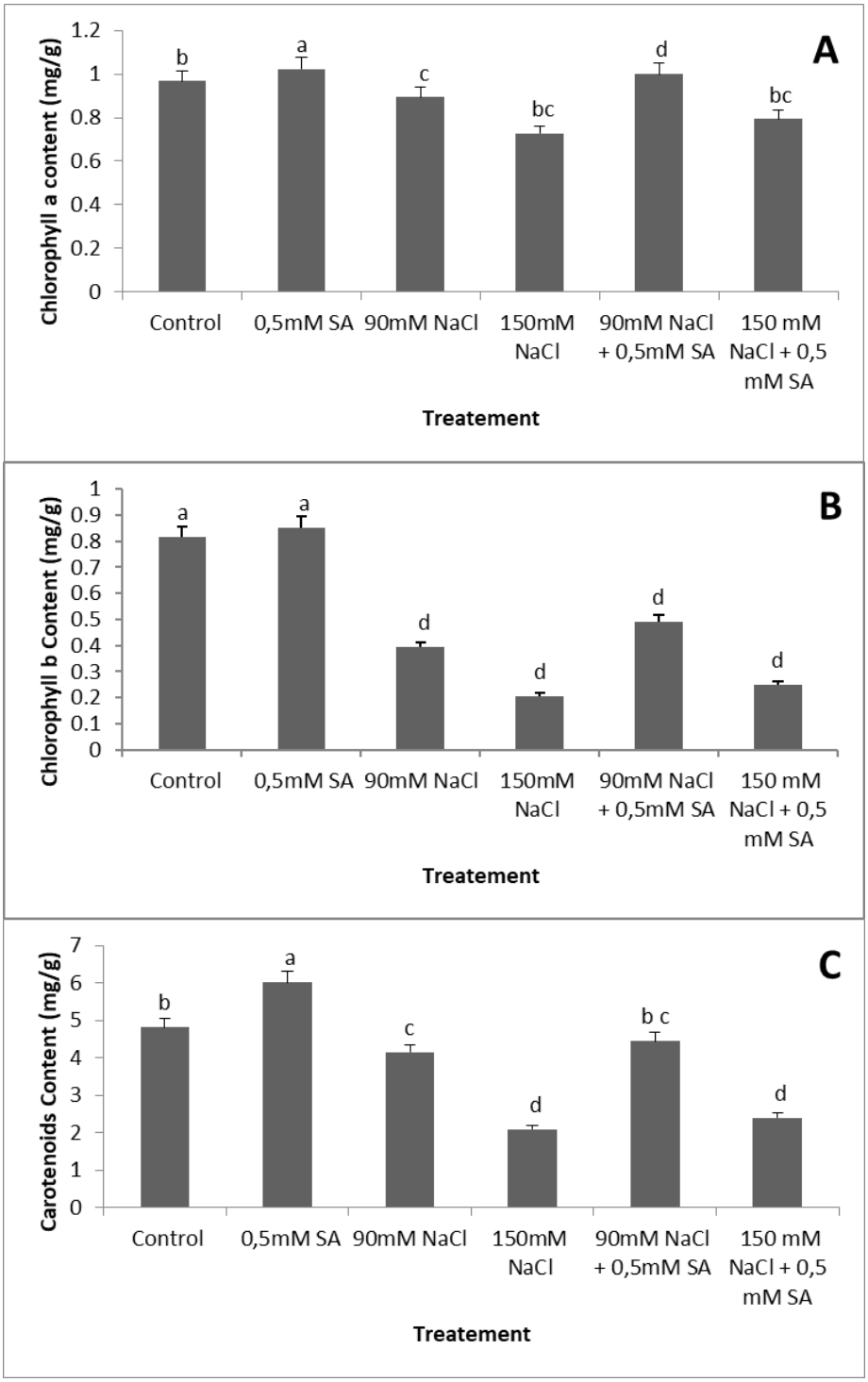


***Figure 2.*** *Effect of salicylic acid on chlorophyll a (A), chlorophyll b (B) and carotenoids (C) in Vicia faba L. under salt stress condition. Error bars = standard error of means (S.E.M.). Different letters (a–e) indicate a significant difference at $p \leq 0.05$*

### *Relative water content*

The impact of salt stress and SA application on the relative water content (RWC) in *Vicia faba* L. leaves was shown in *Figure 3*. Salt stress reduced the RWC by 25.7%


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under 90 mM NaCl condition and 53.4 under 150 NaCl condition. Whereas, SA treated plants revealed less decrease of 14.2% and 38.8% under 90 and 150 mM NaCl + SA treatments, respectively, as compared to controls.

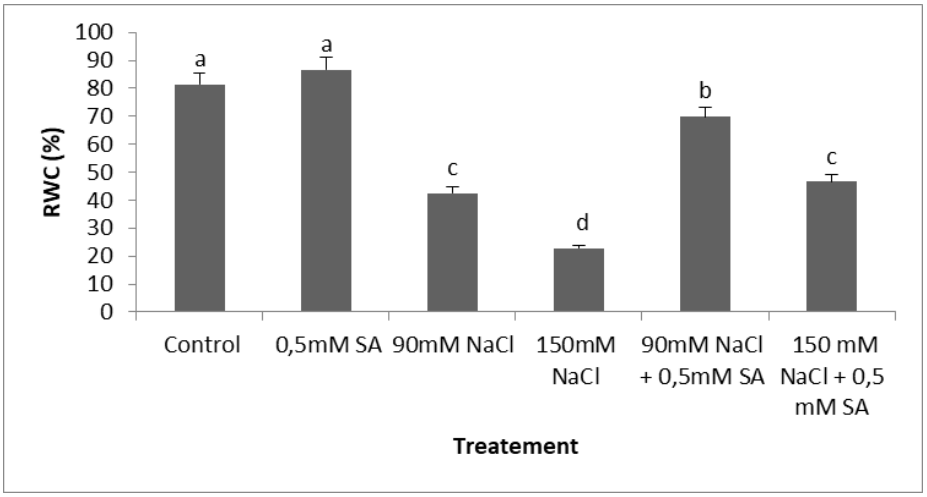


***Figure 3.*** *Effects of salicylic acid on the relative water content (RWC) in Vicia faba L. under salt stress condition. Error bars = standard error of means (S.E.M.). Different letters (a–e) indicate a significant difference at $p \leq 0.05$*

***Table 3.*** *Effect of salicylic acid on the RWC, proline and protein content in Vicia faba L. under salt stress condition (means ± SE)*

| Treatment | RWC (%) | Proline content (µg/mg FW) | Protein content (mg/g FW) |
|---|---|---|---|
| Control | $81.273^{a} \pm 1.55$ | $1.5 \pm 0.002$ | $0.650^{a} \pm 0.06$ |
| 0.5 mM SA | $86.610^{a} \pm 2.25$ | $1.8 \pm 0.037$ | $0.700^{a} \pm 0.08$ |
| 90 mM NaCl | $42.520^{c} \pm 2.20$ | $2.6 \pm 0.016$ | $0.296^{d} \pm 0.03$ |
| 150 mM NaCl | $22.747^{d} \pm 1.16$ | $3.1 \pm 0.05$ | $0.189^{e} \pm 0.05$ |
| 90 mM NaCl + 0.5 mM SA | $69.867^{b} \pm 2.28$ | $5.5 \pm 0.15$ | $0.379^{c} \pm 0.02$ |
| 150 mM NaCl + 0.5 mM SA | $46.610^{c} \pm 2.56$ | $5.7 \pm 0.12$ | $0.235^{e} \pm 0.01$ |
| P Value | 0.053 | 0.02 | 0.069 |

Different letters (a–e) indicate a significant difference at $p \leq 0.05$

### *Proline content*

The proline content in *Vicia faba* L. was increased in response to salt stress compared to their control plants (*Table 3*). In other words, the proline content was increased by 27.4% and 36.9% under 90 and 150 mM NaCl stress respectively. SA application led to further increase in proline content by 68.5% (90 mM NaCl + SA) and 70.1% (150 mM NaCl + SA) as compared to control plants (*Fig. 4*).


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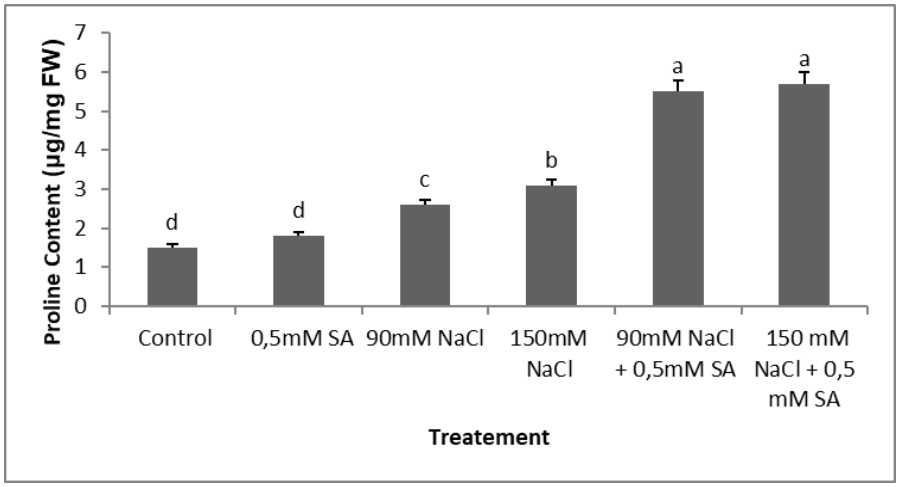


***Figure 4.*** *Effects of salicylic acid on the proline content in Vicia faba L. under salt stress condition. Error bars = standard error of means (S.E.M.). Different letters (a–e) indicate a significant difference at $p \leq 0.05$*

### *Protein content*

The protein content in *Vicia faba* L. was affected by salt stress (*Table 3*). The content of protein decreased by 43.2% under 90 Mm NaCl and 68.7% under 150 mM level of salinity. However, SA application improved the protein content by 37.4% and 53.9% with 90 and 150 mM NaCl levels respectively as compared to controls (*Fig. 5*).

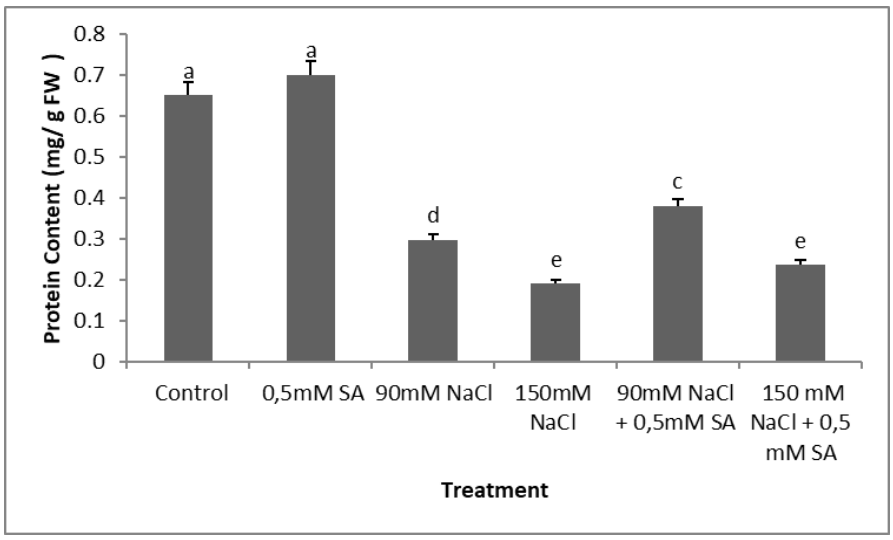


***Figure 5.*** *Effects of salicylic acid on the protein content in Vicia faba L. under salt stress condition. Error bars = standard error of means (S.E.M.). Different letters (a–e) indicate a significant difference at $p \leq 0.05$*

### *Antioxidant enzymes activity*

The role of SA against oxidative stress was assessed by determining the enzymes activities of APX and CAT, in the Vicia faba L. plants under NaCl conditions (*Fig. 6*). APX increased by 44.6% and 46.7% under 90 mM and 150 mM NaCl conditions,


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respectively (*Fig. 6A*). Whereas, CAT increased by 40.5% under the 150 mM NaCl condition (*Fig. 6B*). The SA application enhanced the APX and CAT activities as compared to the non-SA-treated plants under salt stress. So, APX and CAT activities raised by 49.2%, 47.4% and 52.7%, 46.3% under the respective 90 and 150 mM NaCl salinity when compared to non SA treated plant (*Table 4*).

***Table 4.*** *Effect of salicylic acid on ascorbate peroxidase (APX) and catalase (CAT) activities in Vicia faba L. under salt stress condition (means ± SE)*

| Treatment | APX (UI/mg protein) | CAT (UI/mg protein) |
|---|---|---|
| Control | $0.493^{e} \pm 0.03$ | $1.49^{c} \pm 0.15$ |
| 0.5 mM SA | $0.583^{e} \pm 0.02$ | $1.55^{c} \pm 0.01$ |
| 90 mM NaCl | $0.690^{d} \pm 0.04$ | $1.6^{c} \pm 0.02$ |
| 150 mM NaCl | $0.870^{c} \pm 0.05$ | $1.9^{b} \pm 0.014$ |
| 90 mM NaCl + 0,5 mM SA | $1.033^{a} \pm 0.15$ | $2.1^{a} \pm 0.007$ |
| 150 mM NaCl + 0.5 mM SA | $0.953^{b} \pm 0.05$ | $1.97^{b} \pm 0.014$ |
| P Value | 0.004 | 0.0002 |

Different letters (a–e) indicate a significant difference at $p \leq 0.05$

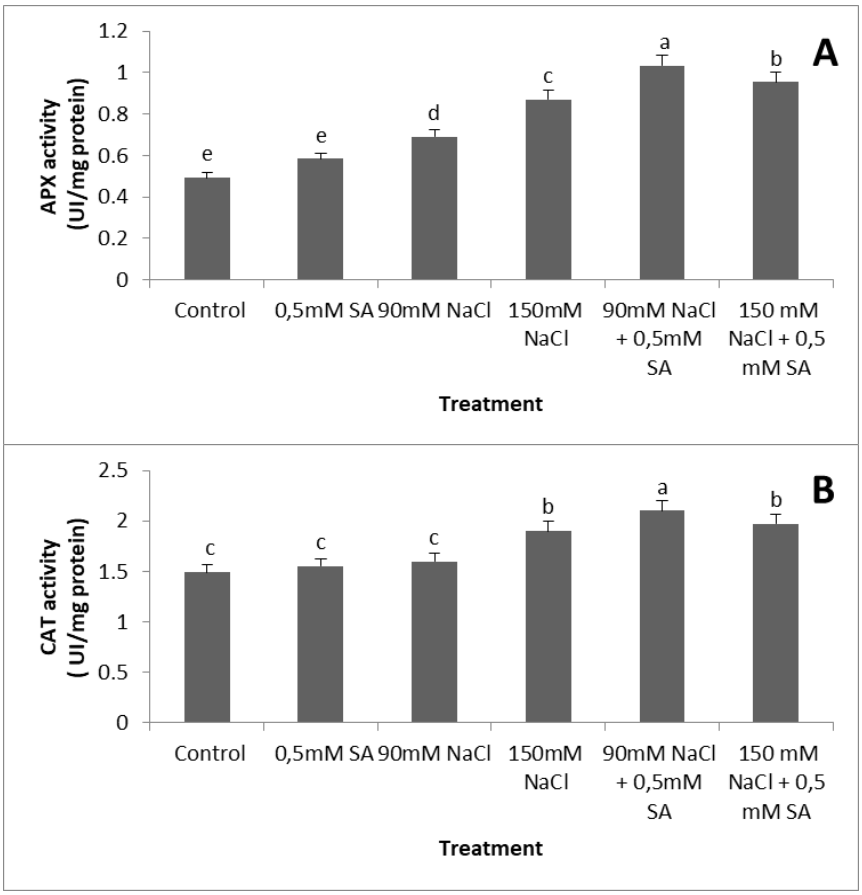


***Figure 6.*** *Effects of salicylic acid on ascorbate peroxidase (A) and catalase (B) activities in Vicia faba L. under salt stress condition. Error bars = standard error of means (S.E.M.). Different letters (a–e) indicate a significant difference at $p \leq 0.05$*


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## Discussion

Salt stress is among the major challenges in the agricultural world by inducing several limitations the growth and productivity of many crops (Shahid et al., 2018). Therefore, concerted efforts are needed to develop new technologies for ameliorating plants salinity tolerance. In this context, the objective of this study was to evaluate the potential effect of salicylic acid on the salinity tolerance of the Faba bean plant (*Vicia faba* L.).

In this study, salt stress resulted in a significant growth limitations for Faba bean plant expressed by the decrease in the length and both fresh and dry weights of plants. Similar findings reporting growth reduction have been previously reported in several legumes including common bean under salt stress (Delgado et al., 1994; Taïbi et al., 2013, 2016, 2021). This decrease is due to the combined effects of osmotic and ionic stress produced as a result of restricted water, nutrient uptake and reduced turgor pressure (Cheeseman,2013; Abdallah et al., 2016), minimized photosynthetic activity, excessive $Na^+$ and $Cl^-$ accumulation (Alvarez-Aragon et al., 2016; Bose et al., 2017), and cell membrane damages under salt stress (Alamri et al., 2020). However, the results indicated that SA application significantly improved the growth of saline-stressed plants. These results are consistent with previous studies (Mehboob et al., 2015; Tahjib-Ul-Arif et al., 2018), which have shown that SA induced beneficial effect on growth characteristics could be attributed to ethylene synthesis inhibition, reduced $Na^+$ and $Cl^-$ uptake, or increased uptake of essential nutrients, such as $K^+$, $Ca^{2+}$, $Cu^{2+}$, Fe, Mn, N, and $Mg^{2+}$ (Ghassemi-Golezani et al., 2018). These measures can help plants thrive well by reducing osmotic and ionic stress. Application of SA can regulate stomatal opening, promote $CO_2$ assimilation, and reduce transpirational water loss under saline conditions. (Ribeiro et al., 2020). Consequently, the plants could maintain their turgor, boost their photosynthetic capacity and increase their production (Fghire et al., 2015; Ribeiro et al., 2020).

In the present investigation, it was found also that elevated salt levels in the soil reduced the photosynthetic pigments of faba bean plants. The reduction in the photosynthetic pigments can be attributed to the breakdown of the ultrastructure of chloroplast, increase of chlorophyllase enzyme and inhibition of chlorophyll synthesis (Shams et al., 2019). The reductions of photosynthetic pigments are in an agreement with the reductions of plant growth parameters. Application of SA to salt-stressed faba bean increased the chlorophyll contents. SA plays a crucial role in chloroplast biosynthesis, maintaining chlorophyll stability, regulates photosynthesis by guarding this vital organelle against toxic effects of ROS. These findings are consistent with studies on French Bean (Youssef et al., 2023), Wheat (Loutfy et al., 2020), and Mustard (Husen et al., 2018). The SA induced enhancement of chlorophyll contents in stressed plants can be attributed to the stimulation of chlorophyll biosynthesis and/or reduction of their degradation (Fathi et al., 2019).

Relative water content (RWC) is an important physiological parameter of plant water status, reflecting the water holding capacity and metabolic activity in plant tissues (Anjum et al., 2011). In the current investigation, RWC decreased in faba bean plants under salt stress conditions. This decrease could be associated with an ionic imbalance and osmotic stress (Amirinejad et al., 2017; Ghassemi-Golezani et al., 2018). SA application attenuated the salt damage and maintained high RWC levels in treated plants. This effect could be explained by the accumulation of SA-induced proteins that it can act as osmoregulators, leading to the decrease of tissue water loss (Salingpa et al., 2018).


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Furthermore, several studies pointed that the SA-mediated improvement in leaf water content is mediated by the accumulation of osmolytes, particularly proline (Nasir, 2009).

Proline accumulation is a common response to salt stress in many plants; in addition to its role in osmotic adjustment, proline may participate in stress tolerance mechanisms as an osmoprotectant, by the direct stabilization of proteins and macromolecular structures, as a ROS-scavenger and/or a signaling molecule (Akram et al., 2018). The results indicated that proline contents increased substantially by increasing the salt concentration of the soil compared to control plants. These results agree with those of Wu et al. (2015). It was suggested that the accumulation of Pro might be caused by decreased protein synthesis or by increased proteolysis (Abdelhamid et al., 2013). Furthermore, it has been shown that proline also protects enzymes and increases membrane stability under salt stress (Wutipraditkul et al., 2015). Further proline accumulation has been observed in plants treated with SA. The augmented accumulation of proline could be attributed to the enhanced enzymatic activity of γ-glutamyl kinase and pyrroline-5-carboxylate reductase (P-5- CR) under the effect of salt stress and SA (Misra and Saxena, 2009; Nasir Khan, 2009). In this regard, Ahmad et al. (2018) elucidated that the SA application enhanced the antioxidant enzyme activity as well as the proline levels in salt-stressed Vicia faba L. Furthermore, it was argued that enhanced proline accumulation by SA application promoted the assimilation of nitrogen and improved photosynthesis (Sharma et al., 2019).

The total protein content was negatively affected in salt-stressed V. faba plant. This result is in agreement with those Mahboobe and Akbar (2013) who found that in many plant species grow under salt stress, the protein content deceased as well as protein pattern changes recorded. Decreasing of protein under saline stress may be attributed to salinity that causes physiological disorders due to the elimination of $K^+$ ions by roots of plant where $K^+$ is the key element in the synthesis of protein. Also, the reduction of protein under salinity conditions is due to the prevention of nitrate reductase activity (Chen et al., 2007). However, increasing nitrate reductase activity by salicylic acid depends on the material action with special inhibitor of nitrate (Ahmed et al., 2003). Arbaoui and Belkhodja (2018) with Rajeshwari and Bhuvaneshwari (2017) have explained that the application of SA under salt stresses can improve plant tolerance by protecting proteins ultrastructure and increasing metabolic enzyme activity.

Plant cells possess a variety of defense strategies against the oxidative injury caused by salt stress. Such strategies involve enzymes such as APX and CAT. So, the activity of the antioxidant enzymes APX and CAT increased in response to salt stress. The increased activities of these enzymes could scavenge effect against the toxic ROS. ROS reduction prevents the plants from oxidative injury and osmotic stress by maintaining the photosynthesis mechanism (Iqbal et al., 2021). The application of salicylic acid increased APX and CAT activities under salt conditions to improve the survival of plants by decreasing ROS production. Salicylic acid has an affinity to bind with the enzymes like APX and CAT, which are involved in ROS metabolism and redox homeostasis (Ruffer et al., 1999; Slaymaker et al., 2002). The current data are concordant with Nazar et al. (2011), who suggested that the salicylic acid treatments could be an effective strategy for reduction and detoxification of ROS.

On the bases of present results, SA application in saline soils can be recommended to alleviate the adverse effects of salinity and minimize crop production yield losses.


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## Conclusion

In conclusion, application of SA could alleviate the negative effect of salt on faba bean through the enhancement of the protective parameters such as soluble osmolytes and antioxidant enzyme. This could suggest that the protection mechanism had helped the plants to increase their tolerance against salt stress. The application of salicylic acid would provide a practical basis for wide cultivation, and might constitute a sustainable approach and effective alternative to restore the adverse effects of salinity. and might propose an ecological and economical solution to deal with salt-affected soils, especially in arid and marginal regions. However, more studies are direly needed to optimize the SA concentration under different environmental stressors, as well as in other crop plants, before making its use on a large scale. Future molecular studies with different levels of SA application could provide a deeper understanding of their role in modulating salinity tolerance mechanisms in bean so that they can determine what concentration is beneficial.

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