



Original Scientific Paper

The effect of salicylic acid and calcium chloride on lipid peroxidation and the scavenging ability on radical of chickpeas (*Cicer arietinum*) under salt stress

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ABSTRACT:

Salinity exerts harmful morphological, physiological, and metabolic effects on plants. This research aimed to evaluate the effect of salicylic acid (SA 0, 0.75 and 1.5 mM) and calcium chloride (CaCl₂ 0, 50 and 100 mM), singly or in combination, on different morphological and physiological characteristics of chickpeas exposed to salt stress (0, 25 and 75 mM NaCl). The results showed that the addition of SA or Ca alone improved plant behaviour in the presence of NaCl. Also, the shoot and root length, dry weight, chlorophyll and carotenoids decreased under salinity, while malondialdehyde (MDA), the inhibition of DPPH radical, anthocyanine, and proline increased. However, the use of SA and Ca combined increased the shoot and root length and the dry weight, ameliorated the chlorophyll, carotenoids, and reducing sugars, and significantly reduced MDA and the inhibition of DPPH radical in the plants. These studies imply that SA and Ca caused a tolerance to NaCl which may be related to the regulation of antioxidative responses. It may also be suggested that a concentration of 1.5 mM salicylic acid and a concentration of 100 mM calcium are the most suitable concentrations to improve the physiological parameters of chickpeas under salinity conditions. Hence, by regulating the antioxidant system, SA and Ca play this role.

Keywords:

Stress, photosynthetic pigments, sodium chloride, calcium chloride, proline, reducing sugars

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INTRODUCTION

Salinity induces oxidative stress and osmotic stress in plants, resulting in marked reduction in photosynthesis activities and leaf chlorophyll concentration, where the degree of salinity tolerance depends on the type of plant species (ASHRAF *et al.* 2010; ZAFAR *et al.* 2018; TRABELSI *et al.* 2019). Accumulations of secondary metabolites such as carotenoids protect plants from salinity stress and play an essential role in osmotic regulation (DE PASCALE *et al.* 2001; WINKEL SHIRLEY 2002). The generation of anthocyanins might enhance osmotic regulation necessary for protecting plants from the overproduction of reactive oxygen species, and physiological stresses (ROUHOLAMIN *et al.* 2015). Proline is one of the foremost essential osmoprotectants in plants, and it plays a vital role in tolerating

NaCl (BARTELS & SUNKAR 2005). Hence, under saline conditions, plants show an increase in proline concentrations (LEE *et al.* 2001). Calcium (Ca) is an essential messenger in many biological systems, and plants are able to adjust to high salt environments by activating a signal transduction system involving Ca⁺². The transmission of signals related to environmental stimuli and hormones facilitates the modulation of sucrose-induced glucose uptake by increasing calcium levels and thus improving anthocyanin accumulation (KUDLA *et al.* 2010; XU *et al.* 2014). Salicylic acid is a phytohormone which plays an essential role in depletion during salinity and drought and is vital in the improvement and tolerance of salinity in several plant species (SHAKIROVA *et al.* 2003; ARFAN *et al.* 2007; SHAHMORADI & NADERI 2018). Therefore, salicylic acid may serve to modify and thus increase plant tolerance to abiotic stresses

(ERASALAN *et al.* 2007; ALINIAEIFARD *et al.* 2016).

Sodium is generally the dominant cation in saline substrates which leads to an increase in Na^+ flux which competes with Ca^{2+} at the binding sites. The addition of calcium ions prevents salinity toxicity, and high Ca levels will maintain the integrity and permeability of cell membranes from the effects of salinity (ABDEL LATEF 2011; SEFIKALHOR *et al.* 2019). Hence, the utilization of Ca^{+2} is an excellent way to promote plant growth under biological stress (MUKHTAR *et al.* 2016; ACOSTA- MOTOS *et al.* 2017; AHMAD *et al.* 2018; NAEEM *et al.* 2018). Several plants cannot be developed under these troublesome conditions because of their sensitivity to salt stress. Supplemental calcium improved the growth and photosynthetic capacity in Senna (ARSHI *et al.* 2006). A further positive effect of calcium on plant growth under salinity stress is due to the role of calcium on the electrical conductivity of roots and water uptake (NAVARRO *et al.* 2000). Therefore, at high salinity concentrations, the ratio of sodium to calcium is vital for each plant's response (MUNNS & TESTER 2008).

Since no study has been carried out on salicylic acid and calcium chloride in chickpeas under salt stress, the aim of our research was to determine the appropriate concentrations of salicylic acid and calcium to reduce the effects of salinity on chickpeas. This study was designed to determine the toxic effects of salt levels on different morphological and physiological characteristics of chickpeas and to establish the decreased phytotoxic effects of salinity by means of salicylic acid and calcium chloride.

MATERIALS AND METHODS

Culture condition and treatments. The chickpea seeds were sterilized with sodium hypochlorite (1%) for 5 min and washed in distilled water several times. A pot experiment was conducted, and for this purpose, plastic pots of 1 kg capacity were filled with sieved soil. Five chickpea seeds were sown in each pot and the pots were replicated three times per concentration. The uniform seeds germinated after 72 h at 25°C and the seedlings were transferred to pots containing sand, clay, and humus (3:1:2) under a light density of approximately $100 \mu\text{molm}^{-2} \text{s}^{-1}$, day/night temperatures of 25/20°C under 16 h photoperiod. Sodium chloride (0, 25, and 75 mM), SA (0, 0.75 and 1.5 mM) and CaCl_2 (0, 50 and 100 mM) were applied for 10 days during the vegetative growth of the plants. Irrigation was performed with saline water. In order to prevent the accumulation of salt, in addition to pot drainage following the application of salinity treatment, water leaching was also performed uniformly for all treatments. In order to control the salinity, the amount of electrical conductivity of the potting soil was controlled by means of a conductivity device. A foliar spray of SA was applied twice to the plants in the early morning, using an atomizer. The leaves were harvested 24 days after treatment. A total of 15 treatments have been considered for the present study: T₁: 0 mM NaCl

(control); T₂: 25 mM NaCl; T₃: 75 mM NaCl; T₄: 0 mM NaCl + 0.75 mM SA; T₅: 0 mM NaCl + 1.5 mM SA; T₆: 25 mM NaCl + 0.75 mM SA; T₇: 25 mM NaCl + 1.5 mM SA; T₈: 75 mM NaCl + 0.75 mM SA; T₉: 75 mM NaCl + 1.5 mM SA; T₁₀: 0 mM NaCl + 50 mM Ca; T₁₁: 0 mM NaCl + 100 mM Ca; T₁₂: 25 mM NaCl + 50 mM Ca; T₁₃: 25 mM NaCl + 100 mM Ca; T₁₄: 75 mM NaCl + 50 mM Ca; T₁₅: 75 mM NaCl + 100 mM Ca.

Biomass measurement. The length of the shoots and roots of the plants were measured after washing with deionized water. The shoots and roots were also dried in the oven for 48 hours, and the dry weight of the plants was weighed.

Pigment analysis. The fresh leaf tissues were ground with a pestle and mortar under liquid N₂. Eighty percent (v/v) acetone was added to extract the pigments and after centrifugation of the supernatant for 10 min at 10000 rpm, OD was measured at 470, 646.8, and 661.6 nm using a spectrophotometer. The extinction coefficients and the equations reported by LICHTENTHALER (1987) were used to calculate the amounts of chlorophyll *a*, *b* and carotenoids.

To determine the concentration of anthocyanins, 0.1 g fresh leaves were mixed with 10 ml of acidified methanol (methanol: HCl, 99: 1, v:v) and kept overnight in dark conditions and measured at a wavelength of 550 nm, using an extinction coefficient of $33000 \text{ mol}^{-1} \text{ cm}^{-1}$ (WANGER 1979).

Proline and reducing sugars. The method proposed by BATES *et al.* (1973) was used to measure proline. Firstly, 0.04 g of wet leaf plant tissue were weighed over ice with three replications in each concentration. Each sample was thoroughly ground with 1.7 ml of 3% (w/v) sulfosalicylic acid. The resulting extracts were transferred into 1.5 ml tubes and then centrifuged at 20000 rpm for 20 minutes. In the next step, 1 ml of the supernatant was removed from each Eppendorf and transferred to 10 ml test tubes. Then 1 ml of glacial acetic acid and 1 ml of ninhydrin reagent were added to each tube. The lid of each tube was tightly closed with aluminum foil, and the tubes were heated at 100°C in a frying pan for 1 hour. After removing the tubes from the patient and cooling them under the hood, exactly 2 ml of toluene was added to each tube. The tubes were vigorously mixed for 20 seconds and then fixed for 1 hour under laboratory conditions. For measurement, the spectrophotometer was first set to zero by toluene at a wavelength of 520 nm, and then 1 ml from each tube was removed from the upper phase and poured into a quartz cuvette. The adsorption of the colour composition of each sample at 520 nm was read.

The DNS method was used to investigate the reducing sugars by employing glucose as the standard consistent with JEFFRIES *et al.* (1988). For measuring the reducing sugars, DNS (dinitro salicylic acid) 1%, sodium 1.6%, and 25% sodium-potassium tartrate were slowly dissolved by heating. Then 2 ml of the extracted plant extract was

mixed with 1 ml of the made dye and placed in a boiling water bath at 100°C for 10 minutes. The whole solution was then mixed with 10 ml of distilled water, and the absorbance of the samples was read in 546 nm, and the amount of reducing sugars was calculated with the help of the relevant standard curve.

Lipid peroxidation. Lipid peroxidation was measured in accordance with the malondialdehyde concentration. The non-specific absorbance of the supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for the MDA measurement (HEATH & PACKER 1968), and at 455 nm for other aldehydes (MEIRS *et al.* 1992).

DPPH radical scavenging ability. The methanolic extract of the leaves was subjected to the free radical scavenging activity assay using the method described by SHIMADA *et al.* (1992). Each extract (0.2 mg ml⁻¹) in methanol (2 ml) was mixed with 2 ml of a freshly prepared methanolic solution containing 100 ppm of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The mixture was shaken vigorously and kept for 30 minutes in dark conditions. The absorbance was then measured at 517 nm.

Data analyses. The data were presented as the mean of three replicates \pm standard error (SE). The experiment was conducted in factorial format based on a completely randomized design (CRD). At 5% probability level, ANOVA was applied for data analysis by using SPSS software (Statistics Software, Version 17.0). All the data were analysed using two-way analysis of variance using SPSS software. Tukey's HSD post hoc test was applied for multiple comparisons of the means.

RESULTS

Effect of SA and Ca supplement on plant growth. The growth of the chickpea plant under salinity treatment (25 and 75 mM NaCl) was significantly reduced compared to the control plants. The shoot lengths decreased by 21% and 56%, respectively, 25 mM and 75 mM compared to the control (Fig. 1). A similar reduction was observed for the root length. Similarly, the data presented in Fig. 2 showed that NaCl significantly ($p < 0.05$) decreased the dry weight of both the shoot and root and necrosis of the leaf tips and margins was observed.

The addition of SA (0.75 and 1.5 mM) and NaCl (25 and 75 mM) alone significantly enhanced the growth parameters. The maximum increase in shoot and root length was recorded as 24% and 54% respectively, under the combined application of NaCl (25 mM) and SA (1.5 mM), as compared to the plants treated with NaCl (25 mM) alone. Similarly, the dry weight of the shoots and roots increased by 21% and 49% respectively, under NaCl (25 mM) and SA (1.5 mM) when compared with their respective controls (Figs. 1, 2).

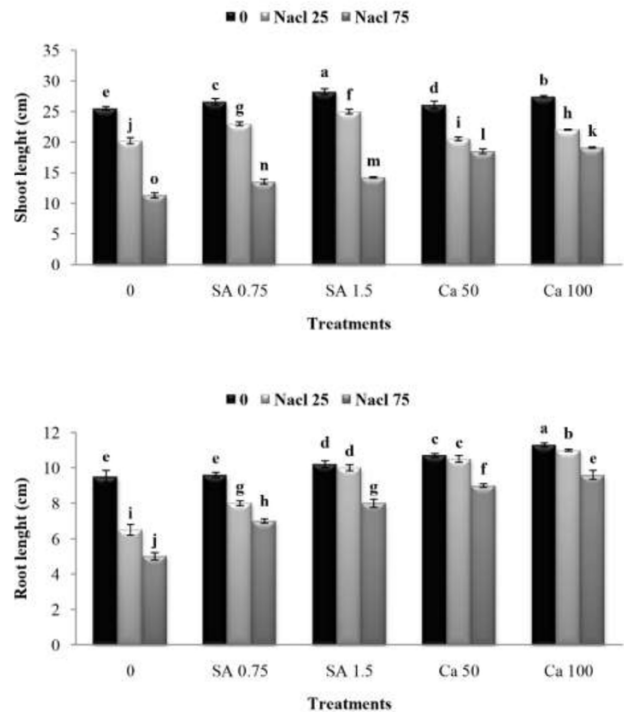


Fig. 1. The effect of SA, Ca, and salt stress on the shoot and root length of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

Similarly to SA, the application of 50 and 100 mM of CaCl₂ and 25 and 75 mM NaCl alone significantly increased all the measured parameters. The most significant effect was observed in all the measured growth parameters at 100 mM CaCl₂ alone, and the minimum was obtained with 75 mM NaCl treatment (Figs. 1, 2).

Photosynthetic pigments. In this study, the chickpea plants under salinity showed a significant reduction in the concentration of chlorophylls and carotenoids compared to the control. The amounts of carotenoids, total chlorophyll and the Chla/b ratio in the leaves decreased by 41%, 13% and 9% respectively, at 75 mM NaCl, compared to the controls. However, SA and Ca ($p < 0.05$) increased the Chla, Chlb, Chla/b ratio, total Chl, and carotenoid concentrations. The maximum total chlorophyll and carotenoid concentrations were achieved in the plants treated with SA (1.5 mM) and Ca (100 mM) only, and the minimum concentrations were achieved with 75 mM NaCl treatment. The addition of 1.5 mM SA along with NaCl 25 and 75 mM considerably increased the total chlorophyll by 17% and 16% and the carotenoids by 28% and 62% respectively, in comparison with the other treatments. Also, a combination of calcium (100 mM) and NaCl (25 and 75 mM) significantly increased the total chlorophyll by 11% and 15% and the carotenoids by 30% and 62% respectively, when compared to the other treatments (Fig. 3).

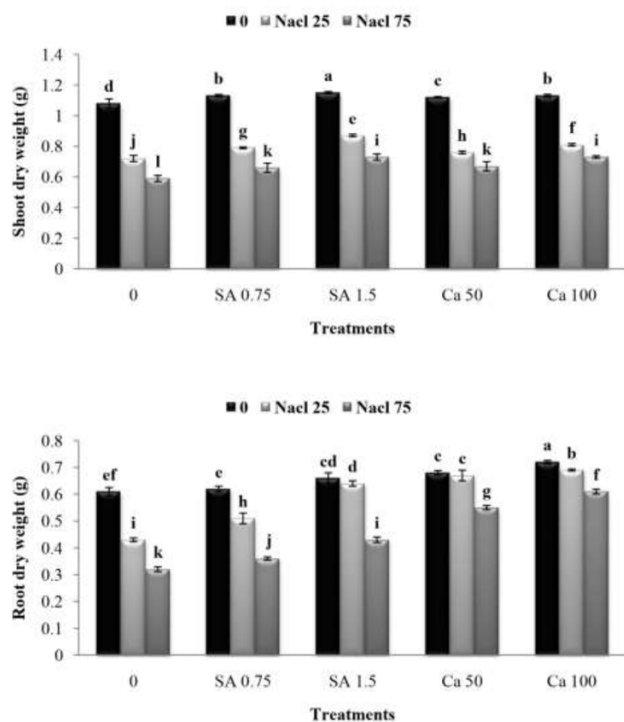


Fig. 2. The effect of SA, Ca, and salt stress on the shoot and root dry weight of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

Lipid peroxidation. Salinity induced lipid peroxidation in the chickpea leaves. Increasing the salinity concentration (25 and 75 mM) significantly increased the MDA and other aldehyde production in the chickpeas (Fig. 4). The amounts of MDA and other aldehydes in the leaves were boosted by 5% and 47% respectively, at 75 mM NaCl, compared to the controls. The addition of SA and Ca significantly decreased the production of MDA and other aldehydes in the leaves at all NaCl levels. The MDA and other aldehydes showed a 4% and 29% reduction in the leaves under NaCl 75 mM respectively, combined with 1.5 mM SA, in comparison with the controls. Also, the addition of calcium to the plants decreased the MDA and other aldehydes in the leaves at all NaCl levels. The greatest reduction in MDA and the other aldehydes was estimated as 4% and 20% respectively, under the combined use of NaCl (75 mM) and Ca (100 mM), in comparison with the plants treated with NaCl alone (Fig. 4A, B).

Reducing sugars concentration. In this study, the chickpea plants under salinity showed a significant reduction in the concentration of reducing sugars (oligosaccharides). Salinity of 25 and 75 mM reduced the concentration of reducing sugars by 24% and 36% respectively when compared to the control (Fig. 5). The use of salicylic acid and calcium reduced the harmful effect of NaCl on sugar concentration. The SA (1.5 mM) increased the concentration of the reducing sugars in the plants by 40% under NaCl 75

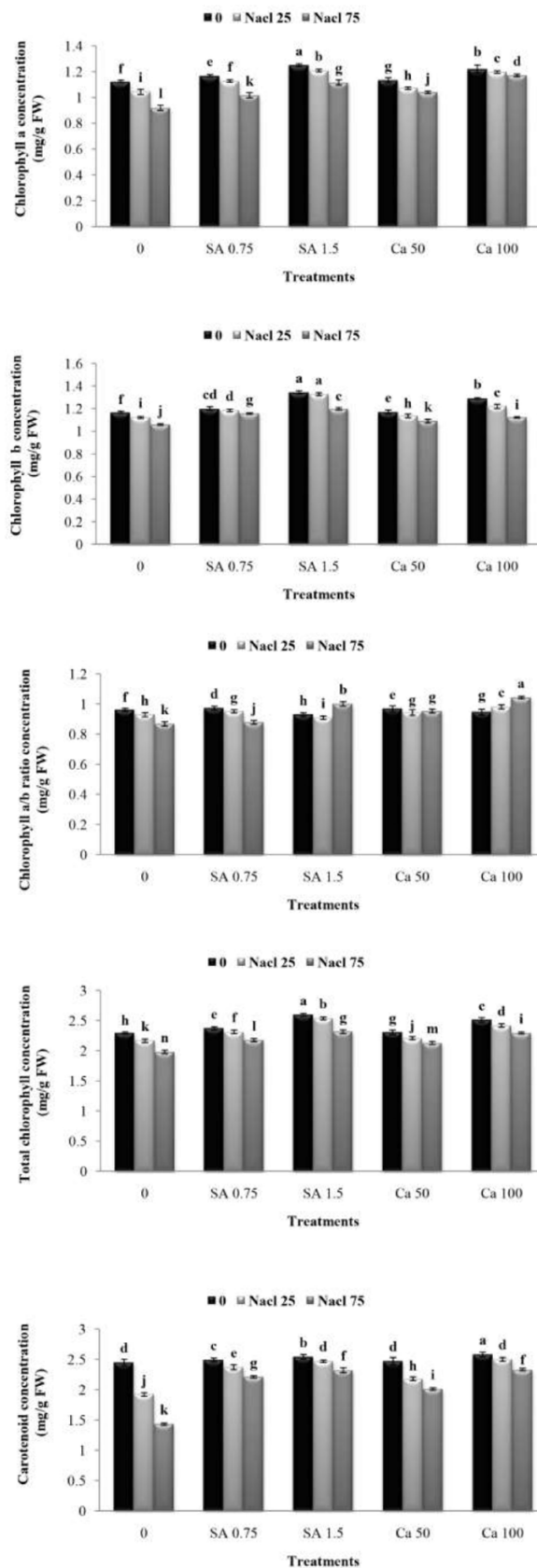


Fig. 3. The effect of SA, Ca, and salt stress on the Chla, Chlb, Chla/b ratio, total Chl, and carotenoid concentrations of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

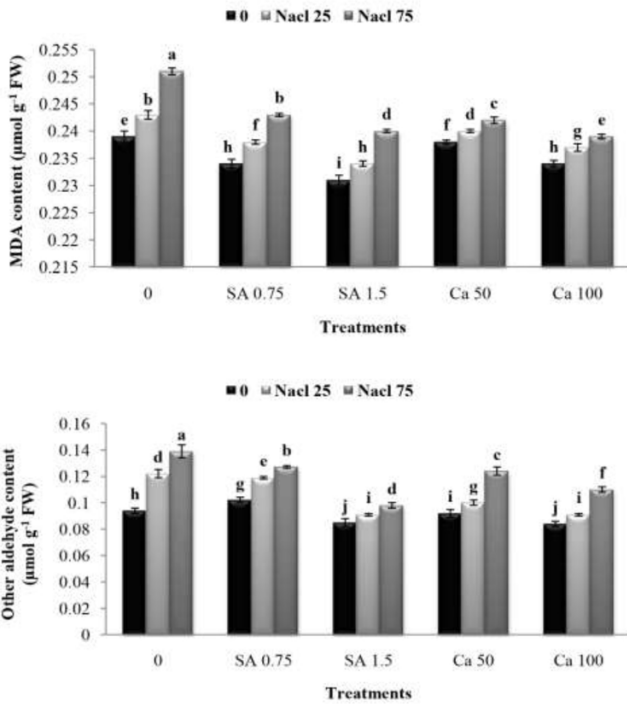


Fig. 4. The effect of SA, Ca, and salt stress on MDA and other aldehyde concentrations of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

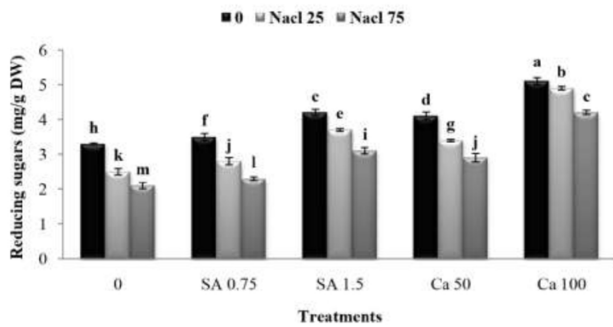


Fig. 5. The effect of SA, Ca, and salt stress on the concentration of reducing sugars of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

mM compared to the treated plants under NaCl alone. The greatest increase of 54% in the concentration of reducing sugars was obtained under Ca 100 mM compared to the respective control (Fig. 5).

Proline concentration. Salinity concentrations of 25 and 75 mM of NaCl increased leaf proline concentration by 5% and 9% respectively when compared to the control. Salicylic acid and calcium treatment alleviated the adverse effect of salinity on the proline level. The maximum increase of 16% in proline concentration occurred under NaCl (75 mM) and SA (0.75 mM) in comparison with the

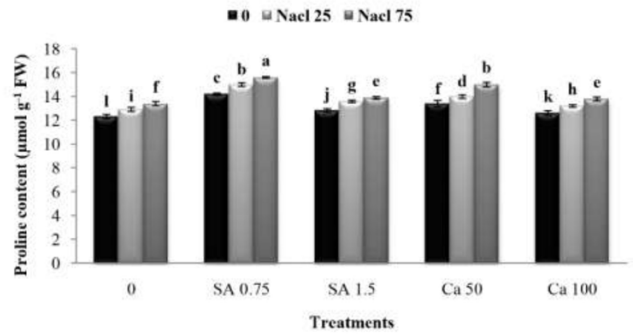


Fig. 6. The effect of SA, Ca, and salt stress on the proline concentration of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

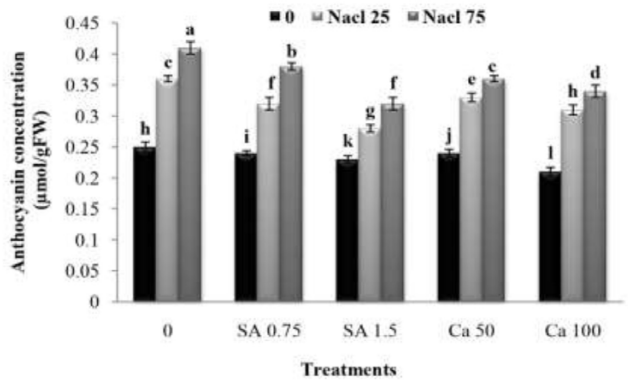


Fig. 7. The effect of SA, Ca, and salt stress on the anthocyanin concentration of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

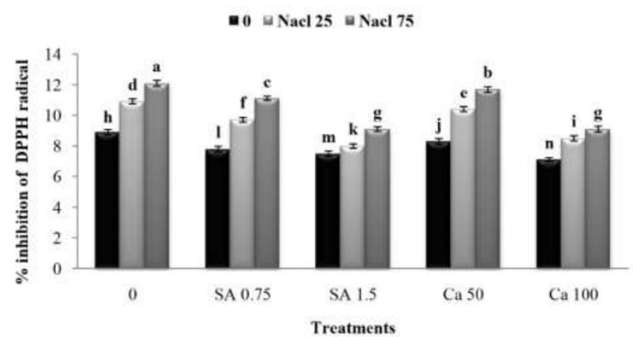


Fig. 8. The effect of SA, Ca, and salt stress on the DPPH radical scavenging activity of *Cicer arietinum*. Values with similar letters are not significantly different at $p < 0.05$.

treated plants under NaCl (75 mM) alone. Calcium (50 mM) boosted the proline concentration of the plants by 12% under NaCl 75 mM compared with NaCl treatment alone (Fig. 6).

Anthocyanin concentration. The anthocyanin concentration in the chickpea leaves significantly increased in 25 and 75 mM of salt-stressed plants by 44% and 64%, respectively when compared to the control. The addition of 0.75 and 1.5 mM SA along with NaCl 75 mM significantly reduced the anthocyanin concentration by 7% and 22% respectively as compared to NaCl used alone. The Ca (50 and 100 mM) reduced the anthocyanin concentration of the leaves by 12% and 17% under NaCl 75 mM respectively as compared with NaCl alone (Fig. 7).

Antioxidant activity by using the DPPH scavenging assay. The DPPH radical scavenging activity in the chickpea leaves significantly increased in 25 and 75 mM of salt-stressed plants by 22% and 35% respectively when compared to the control. The use of salicylic acid and calcium reduced the harmful effect of NaCl on the DPPH radical scavenging activity. The SA (1.5 mM) reduced the DPPH radical scavenging activity of the leaves by 26% and 24% respectively under NaCl 25 and 75 mM when compared with the NaCl treatment alone. Also, the Ca (50 and 100 mM) reduced the DPPH radical scavenging activity of the leaves by 3% and 24% under NaCl 75 mM when compared with NaCl treatment alone (Fig. 8).

DISCUSSION

Decreased plant growth under salinity conditions has been shown to be a common phenomenon in mesophytes (HERNÁNDEZ 2019). The findings of this research showed that NaCl reduced the plant growth of chickpea plants. The use of Ca and SA considerably boosted the growth in chickpea plants by changing the biochemical and physiological processes. It may be assumed that NaCl reduces growth and biomass production, which can result in impaired photosynthetic activity (ASHRAF & ALI 2008) in absentia (AKHTAR *et al.* 2013), olives (TRABELSI *et al.* 2019; LARBI *et al.* 2020), and wheat (ZAFAR *et al.* 2016).

The role of calcium in plant salinity tolerance has been previously shown (TUNA *et al.* 2007; TANVEER *et al.* 2020). In this study, calcium chloride application to chickpea plants grown under salinity conditions increased the measured growth parameters. The most significant effect was observed in all the measured growth parameters at 100 mM CaCl_2 alone. Similar results were gained in olive trees (LARBI *et al.* 2020), tomatoes (TUNA *et al.* 2007), strawberries (KAYA *et al.* 2002), and amaranth (HOANG *et al.* 2020).

The application of CaCl_2 alone and with NaCl significantly improved the dry weight of the shoots and roots. Similar results were reported by MOHAMMAD *et al.* (1998) and TUNA *et al.* (2007). The application of 10 mM of calcium to olive plants grown at 200 mM sodium chloride enhanced all the parameters measured with the exception of the shoot dry weight (LARBI *et al.* 2020).

In this study, the effect of salicylic acid on the growth parameters of the chickpea plants was considerably enhanced compared with the control. The main positive effect of SA is its role as a plant growth regulator because of its ability to increase nutrient uptake, protein synthesis, photosynthetic activity and enzymatic activities, and to protect against biotic and abiotic stresses, and thus boost the antioxidant capacity of the plant (BLOKHINA *et al.* 2003; SAHU 2013). Elevated SA increases carbon dioxide uptake, the photosynthesis rate, mineral uptake, and nutrient mobility which in turn increases the number of leaves per plant (SZEPESI *et al.* 2005; MAGDA *et al.* 2013). Our results are consistent with previous studies (EL-KHALLAL *et al.* 2009; DELAVARI *et al.* 2010; ABD EL-HAMEID ASMAA *et al.* 2017).

In this research, NaCl considerably reduced chlorophyll concentrations, resulting in a blockage in their synthesis (ASHRAF & ALI 2008). In this study, salinity reduced photosynthetic pigments in the chickpeas. These results are consistent with the findings of previous studies in plants under the influence of salinity (AKHTAR *et al.* 2013; RADI *et al.* 2013; LARBI *et al.* 2020). This means that the reduction in chlorophyll concentrations in plants under salinity conditions results from the decomposition of leaf chlorophyll (TSUCHIYA *et al.* 1999). However, some studies suggest that the reduction in chlorophyll concentrations in plants is due to a decline in the synthesis of 5-aminolevulinic acid, which is converted to chlorophyll when exposed to light (RADI *et al.* 2013). Moreover, due to the reduction in the chlorophyll concentration of the leaves, chloride absorption is high (TAVAKKOLI *et al.* 2010). In this study, the application of calcium and salicylic acid mitigated the negative effects of NaCl on chlorophyll and carotenoids in the chickpea plants. This confirms previous findings related to calcium application (LARBI *et al.* 2020). The application of calcium helps maintain the chloroplast membrane and prevent chlorophyll decomposition (NAEEM *et al.* 2018). This study showed that treatment with salicylic acid increased the chlorophyll concentrations, but was not as effective as calcium, indicating that in pigment maintenance, the role of calcium was more prominent than salicylic acid. Studies have shown that the application of SA ameliorated the photosynthetic pigments of plants under NaCl, which can improve plant tolerance to environmental stresses by osmotic stress, and chlorophyll (ANANIEVA *et al.* 2004; ABD EL-HAMEID ASMAA *et al.* 2017).

Salinity will induce changes in the levels of pigments such as chlorophyll and carotenoids. Carotenoids are necessary for the photosystem integrity and will scavenge reactive oxygen species generated under salinity conditions (DE PASCALE *et al.* 2001; AHMAD *et al.* 2005).

The quantification of malondialdehyde is an indicator of membrane damage under salinity stress conditions (KATSUHARA *et al.* 2005). Malondialdehyde is usually used to indicate oxidative damage in fatty acids (AZEVEDO

NETO *et al.* 2008), and its accumulation has been determined in plants under salinity stress such as cotton (MELONI *et al.* 2003), sugar beet (BOR *et al.* 2003), cowpeas (CAVALCANTI *et al.* 2004), maize (AZEVEDO NETO *et al.* 2006) and rice (DEMIRAL & TURKAN 2006). In this study, increasing the concentration of NaCl increased the MDA and other aldehyde production in chickpeas. Although we did not measure reactive oxygen species, the improved level of lipid peroxidation, measured by the increased MDA concentration, could have resulted from the generation of ROS under salinity conditions (BOR *et al.* 2003). Indeed, the addition of SA and Ca considerably reduced the MDA content in the leaves at all NaCl levels. It could be hypothesized that the SA and Ca include the induction of antioxidant enzyme activity and lipid peroxidation (GUNES *et al.* 2007). This may be explained on the basis of their roles, as SA and Ca act as signal endogenous molecules to induce the antioxidant response to protect the membrane from oxidative damage (METHENNI *et al.* 2018).

Proline accumulation was a typical response to salt stress and was associated with enhancing sodium chloride concentrations (AGHAEI *et al.* 2009). It is known that proline can enhance sodium chloride tolerance in potato plants (MARTINEZ *et al.* 1996). Most studies have shown an increase in proline levels by salicylic acid (JANDA *et al.* 2007). The supplementation of salicylic acid increases the amount of proline in plants under salinity (ABD EL-HAMEID ASMAA *et al.* 2017). It has also been reported that salicylic acid plays an important protective role against plant salinity stress by synthesizing proline (MISRA & SAXENA 2009; AL-WHAIBI *et al.* 2012; HEIDARIAN & ROSHANDEL 2021).

The exogenous Ca induces anthocyanin synthesis by regulating related structural and regulatory genes (MICHAELIDIS *et al.* 2017; ZHU *et al.* 2019). Reducing sugars play a role in regulating salinity stress and plant survival whereby reducing sugars accumulate in the plant cytoplasm during salinity stress (FALLON & PHILLIPS 1989; REJSKOVA *et al.* 2007). Increasing the availability of sugars also promotes the synthesis of anthocyanins (HIRATSUKA *et al.* 2001) which is consistent with our results from this study.

The results of the present research showed that the DPPH radical scavenging activity in the chickpea leaves significantly increased in the plants under salinity in comparison with the control. In terms of the activity of inhibiting DPPH-free radicals in response to salinity, the plants treated with salicylic acid had a greater ability to control ROS production than the control groups. Also, salicylic acid treatment increased DPPH radical inhibition activity in safflower plants under sodium chloride-stressed compared to the control group (SHAKI *et al.* 2017).

This study shows that the use of salicylic acid and calcium improves chickpea NaCl tolerance by protecting photosynthetic pigments and osmolality accumulation, which has a positive impact on plant growth.

CONCLUSION

The current study shows that the application of salicylic acid and calcium to the chickpeas counteracted the harmful effects of salinity stress, facilitated the protection of the pigment system, and improved lipid peroxidation and osmolality accumulation, which in turn enhanced the plants' antioxidant defense system and promoted growth. Finally, it could be stated that the treatment with 1.5 mM salicylic acid and 100 mM calcium was considered the most effective among all these treatments and could significantly improve the physiological parameters of chickpea plants affected by salinity.

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REFERENCES

- ABD EL-HAMEID ASMAA R, AHMED MA, GAMAL EL-DIN KARIMA M, SHALABY MAGDA AF & ELNAGGAR HODA MH. 2017. Role of salicylic acid to improve physiological characters and bio-chemical markers of soybean (*Glycine max* L.) under sea salt stress. *International Journal of Environmental Research* **11**: 547–556.
- ABDEL LATEF AAH. 2011. Ameliorative effect of calcium chloride on growth, antioxidant enzymes, protein patterns and some metabolic activities of canola (*Brassica napus* L.) under sea water stress. *Journal of Plant Nutrition* **34**(9): 1303–1320.
- ACOSTA-MOTOS JR, ORTUÑO MFA, BERNAL-VICENTE P, DIAZ-VIVANCOS MJ & HERNANDEZ JA. 2017. Plant responses to salt stress: Adaptive mechanisms. *Agronomy* **7**(1): 18–38.
- AGHAEI H, EHSANPOUR AA, SHAH AHS & KOMATSU S. 2009. Proteome analysis of soybean hypocotyl and root under salt stress. *Amino Acids* **36**: 91–98.
- AHMAD P, ABD-ALLAH EF, ALYEMENI MN, WIJAYA L, ALAM P, BHARDWAJ R & SIDDIQUE KHM. 2018. Exogenous application of calcium to 24-epibrassinosteroid pre-treated tomato seedlings mitigates NaCl toxicity by modifying ascorbate–glutathione cycle and secondary metabolites. *Scientific Reports* **8**(1): 13515.
- AHMAD S, WAHID A, RASUL E & WAHID A. 2005. Comparative morphological and physiological responses of green gram genotypes to salinity applied at different growth stages. *Botanical Bulletin Academia Sinica* **6**: 35–42.
- AKHTAR J, AHMAD R, ASHRAF MY, TANVEER A, WARRICH EA & ORABY H. 2013. Influence of exogenous application of salicylic acid on salt-stressed mungbean (*Vigna radiata*): Growth and nitrogen metabolism. *Pakistan Journal of Botany* **45**: 119–125.
- AL-WHAIBI M, SIDDIQUI M & BASALAH M. 2012. Salicylic acid and calcium-induced protection of wheat against salinity. *Protoplasma* **249**: 769–778.
- ALINIAEIFARD S, HAJILOU J & TABATABAEI SJ. 2016. Photosynthetic and growth responses of olive to proline and salicylic acid under salinity condition. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **44**(2): 579–585.
- ANANIEVA K, MALBECK J, KAMÍNEK J & VAN STADEN J. 2004. Methyl jasmonate down-regulates endogenous cytokinin levels in cotyledons of *Cucurbita pepo* (zucchini) seedlings. *Physiologia Plantarum* **122**(4): 496–503.

- ARFAN M, ATHAR HR & ASHRAF M. 2007. Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *Journal of Plant Physiology* **6**: 685–694.
- ARSHI A, ABDIN MZ & IQBAL M. 2006. Sennoside content and yield attributes of *Cassia angustifolia* Vahl. as affected by NaCl and CaCl₂. *Scientia Horticulturae* **111**: 84–90.
- ASHRAF M & ALI Q. 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.). *Environmental and Experimental Botany* **63**(1–3): 266–273.
- ASHRAF MY, ASHRAF M, MEHMOOD K, AKHTAR J, HUSSAIN F & ARSHAD M. 2010. Phytoremediation of saline soils for sustainable agricultural productivity. In: ASHRAF M, OZTURK M & AHMAD MSA (eds.), *Plant adaptation and phytoremediation*, pp. 335–355, Springer Dordrecht.
- AZEVEDO NETO AD, GOMES-FILHO E & PRISCO JT. 2008. Salinity and oxidative stress. In: KHAN NA & SINGH S (eds.), *Abiotic stress and plant responses*, pp. 58–82, IK International Group, Aligarh.
- AZEVEDO NETO AD, PRISCO JT, ENEAS-FILHO J, ABREU CEB & GOMES-FILHO E. 2006. Effect of salt stress on antioxidative enzymes and lipids peroxidation in leaves and roots of salt-tolerant and salt sensitive maize genotypes. *Environmental and Experimental Botany* **56**: 87–94.
- BARTELS D & SUNKAR R. 2005. Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences* **24**: 23–58.
- BATES LS, WALDREN RP & TEARE ID. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205–207.
- BLOKHINA O, VIROLAINEN E & FAGERSTEDT KV. 2003. Antioxidants, oxidative damage and oxygen deprivations stress. *Annals of Botany* **91**: 179–194.
- BOR MO, ZDEMIR F & TURKAN I. 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritima* L. *Plant Science* **164**: 77–84.
- CAVALCANTI FR, OLIVEIRA JTA, MIRANDA ASM, VIÉGAS RA & SILVEIRA JAG. 2004. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. *New Phytologist* **163**: 563–571.
- DE PASCALE S, MAGGIO A, FOGLIANO V, AMBROSINO P & RITIENI A. 2001. Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. *The Journal of Horticultural Science and Biotechnology* **76**: 447–453.
- DELAVARI PM, BAGHIZADEH A, ENTESHARI SH, KALANTARI KHM, YAZDANPANAH A & MOUSAVI EA. 2010. The effect of salicylic acid on some of biochemical and morphological characteristic of *Ocimum basilicum* under salinity stress. *Australian Journal of Basic Applied Sciences* **4**(10): 4832–4845.
- DEMIRAL T & TURKAN I. 2006. Exogenous glycinebetaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. *Environmental and Experimental Botany* **56**: 72–79.
- EL-KHALLAL SM, HATHOUT TM, ABDEL RAHEIM A, ASHOUR A & KERRIT AA. 2009. Brassinolide and salicylic acid induced growth, biochemical activities and productivity of maize plant growth under salt stress. *Research Journal of Agricultural and Biological Sciences* **5**(4): 380–390.
- ERASLAN F, INAL A, GUNES A & ALPASLAN M. 2007. Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. *Scientia Horticulturae* **113**: 120–128.
- FALLON KM & PHILLIPS R. 1989. Responses to water stress in adapted and unadapted carrot cell suspension cultures. *Journal of Experimental Botany* **40**: 681–687.
- GUNES A, INAL A, ALPASLAN M, ERASLAN F, BAGCI EG & CICEK N. 2007. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *Journal of Plant Physiology* **164**: 728–736.
- HEATH RL & PACKER L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **125**: 189–198.
- HEIDARIAN F & ROSHANDEL P. 2021. Salicylic acid improves tolerance against salt stress through boosting antioxidant defense system in Black Bean. *International Journal of Horticultural Science and Technology* **8**(2): 175–189.
- HERNÁNDEZ JA. 2019. Salinity tolerance in plants: trends and perspectives int. *Journal of Molecular Sciences* **20**: 2408.
- HIRATSUKA S, ONODERA H, KAWAI Y, KUBO T, ITOH H & WADA R. 2001. ABA and sugar effects on anthocyanin formation in grape berry cultured in vitro. *Scientia Horticulturae* **90**: 121–130.
- HOANG H, GUZMAN CD, CADIZ NM, HOANG T, TRAN DH & REHMAN H. 2020. Salicylic acid and calcium signaling induce physiological and phytochemical changes to improve salinity tolerance in red amaranth (*Amaranthus tricolor* L.). *Journal of Soil Science and Plant Nutrition* **20**: 1759–1769.
- JANDA T, HORVATH E, SZALAI C & PALDI E. 2007. Role of salicylic acid in the induction of abiotic stress tolerance. In: HAYAT S & AHMAD A (eds.), *Salicylic acid: a plant hormone*, pp. 91–154, Springer, Dordrecht.
- JEFFRIES TW, YANG VW & DAVIS MW. 1988. Comparative study of xylanase kinetics using dinitrosalicylic, arsenomolybdate, and ion chromatographic assays. *Applied Biochemistry and Biotechnology* **70–72**: 257–265.
- KATSUHARA M, OTSUKA T & EZAKI B. 2005. Salt stress-induced lipid peroxidation is reduced by glutathione S-transferase, but this reduction of lipid peroxides is not enough for a recovery of root growth in *Arabidopsis*. *Plant Science* **169**: 369–373.
- KAYA C, AK BE, HIGGS D & MURILLO-AMADOR B. 2002. Influence of foliar-applied calcium nitrate on strawberry plants grown under salt stress conditions. *Australian Journal of Experimental Agriculture* **42**: 631–636.
- KUDLA J, BATISTIC O & HASHIMOTO K. 2010. Calcium signals: the lead currency of plant information processing. *Plant Cell* **22**: 541–563.
- LARBI A, KCHAOU H, GAALICHE B, GARGOURI K, BOULAL H & MORALES F. 2020. Supplementary potassium and calcium improves salt tolerance in olive plants. *Scientia Horticulturae* **260**: 108912.
- LEE DH, KIM TS & LEE CB. 2001. The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Journal of Plant Physiology* **158**: 737–745.
- LICHTENTHALER HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**: 350–382.
- MAGDA AFS, AHMED MA, ABDALLAH MSA & EBTESAM A. 2013. Physiological role of salicylic acid in improving growth and productivity of barley (*Hordeum vulgare* L.) under sandy soil conditions. *Middle East Journal of Agriculture Research* **2**(2): 68–75.
- MARTINEZ CA, MAESTRI M & LANI EG. 1996. In vitro salt tolerance and proline accumulation in Andean potato (*Solanum* spp.) differing in frost resistance. *Plant Science* **116**: 177–184.
- MEIRS S, PHILOSOPH HADAS S & AHARONI N. 1992. Ethylene in-

- creased accumulation of fluorescent lipid peroxidation products detected during senescence of parsley by a newly developed method. *Journal of the American Society for Horticultural Science* **117**: 128–132.
- MELONI DA, OLIVA MA, MARTINEZ CA & CAMBRAIA J. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany* **49**: 69–76.
- METHENNI K, ABDALLAH MB, NOUAIRI I, SMAOUI A, AMMAR WB, ZARROUK M & YOUSSEF NB. 2018. Salicylic acid and calcium pretreatments alleviate the toxic effect of salinity in the Oueslati olive variety. *Scientia Horticulturae* **233**: 349–358.
- MICHAILIDIS M, KARAGIANNIS E, TANOU G, KARAMANOLI K, LAZARIDOU A, MATSI T & MOLASSIOTIS A. 2017. Metabolomic and physico-chemical approach unravel dynamic regulation of calcium in sweet cherry fruit physiology. *Plant Physiology and Biochemistry* **116**: 68–79.
- MISRA N & SAXENA P. 2009. Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Science* **177**: 181–189.
- MOHAMMAD M, SHIBLI R, AJLOUNI M & NIMRI L. 1998. Tomato root and shoot responses to salt stress under different levels of phosphorus nutrition. *Journal of Plant Nutrition* **21**(8): 1667–1680.
- MUKHTAR I, SHAHID MA, KHAN MW, BALAL RM, IQBAL MM, NAZ T, ZUBAIR M & ALI HH. 2016. Improving salinity tolerance in chili by exogenous application of calcium and sulphur. *Soil and Environment* **35**(1): 56–64.
- MUNNS R & TESTER M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**(1): 651–681.
- NAEEM M, NAEEM MS, AHMAD R, IHSAN MZ, ASHRAF MY, HUSSAIN Y & FAHAD S. 2018. Foliar calcium spray confers drought stress tolerance in maize via modulation of plant growth, water relations, proline content and hydrogen peroxide activity. *Archives of Agronomy and Soil Science* **64**(1): 116–131.
- NAVARRO JM, MARTINEZ V & CARVAJAL M. 2000. Ammonium bicarbonate and calcium effects on tomato plants grown under saline conditions. *Plant Science* **157**: 89–96.
- RADI AA, FARGHALY FA & HAMADA AM. 2013. Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *Journal of Biology and Earth Sciences* **3**: 72–88.
- REJŠKOVÁ AB, LENKA P, EVA S & HELENA L. 2007. The effect of abiotic stresses on carbohydrate status of olive shoots (*Olea europaea* L.) under in vitro conditions. *Journal of Plant Physiology* **164**: 174–184.
- ROUHOLAMIN S, ZAHEDI B, NAZARIAN-FIROUZABADI F & SAEI A. 2015. Expression analysis of anthocyanin biosynthesis key regulatory genes involved in pomegranate (*Punica granatum* L.). *Scientia Horticulturae* **186**: 84–88.
- SAHU GK. 2013. Salicylic acid: Role in plant physiology and stress tolerance. In: ROUT GR & DAS AB (eds.), *Molecular stress physiology of plants*, pp. 217–239, Springer, India.
- SEIFIKALHOR M, ALINIAEIFARD S, SHOMALI A, AZAD N, HASSANI B, LASTOCHKINA O & LI T. 2019. Calcium signaling and salt tolerance are diversely entwined in plants. *Plant Signaling and Behavior* **14**(11): 1665455.
- SHAHMORADI H & NADERI D. 2018. Improving effects of salicylic acid on morphological, physiological and biochemical responses of salt-imposed winter Jasmine. *International Journal of Horticultural Science and Technology* **5**(2): 219–230.
- SHAKI F, EBRAHIMZADEH H & NIKNAM MV. 2017. Central role of salicylic acid in resistance of safflower (*Carthamus tinctorius* L.) against salinity. *Journal of Plant Interactions* **12**(1): 414–420.
- SHAKIROVA FM, SAKHABUTDINOVA AR, BEZRUKOVA MV, FATKHUTDINOVA RA & FATKHUTDINOVA DR. 2003. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Science* **164**: 317–322.
- SHIMADA K, FUJIKAWA K, YAHARA K & NAKAMURA T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry* **40**: 945–948.
- SZEPESI A, CSISZAR J, BAJKAN S, GEMES K, HORVATH F, ERDEL L, DEER AK, SIMON ML & TARI I. 2005. Role of salicylic acid pretreatment on the acclimation of tomato plants to salt and osmotic stress. *Acta Biologica Szegediensis* **49**: 123–125.
- TANVEER K, GILANI S, HUSSAIN Z, ISHAQ R, MUHAMMAD ADEEL M & ILYAS N. 2020. Effect of salt stress on tomato plant and the role of calcium. *Journal of Plant Nutrition* **43**: 28–35.
- TAVAKKOLI E, RENGASAMY P & McDONALD GK. 2010. High concentrations of Na and Cl ions in soil solution have simultaneous detrimental effects on growth of *Faba bean* under salinity stress. *Journal of Experimental Botany* **61**(15): 4449–4459.
- TRABELSI L, GARGOURI K, BEN HASSENA A, MBADRA C, GHRAB M, NCUBE B, VAN STADEN J & GARGOURI R. 2019. Impact of drought and salinity on olive water status and physiological performance in an arid climate. *Agricultural Water Management* **213**: 749–759.
- TSUCHIYA T, OHTA H, OKAWA K, IWAMATSU A, SHIMADA H, MASUDA T & TAKAMIYA K. 1999. Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: Finding of a lipase motif and the induction by methyl jasmonate. *Proceedings of the National Academy Sciences* **96**(26): 15362–15367.
- TUNA AL, KAYA C, ASHRAF M, ALTUNLU H, YOKAS I & YAGMUR B. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. *Environmental and Experimental Botany* **59**(2): 173–178.
- WANGER GJ. 1979. Content and vacuole/extra vacuole distribution of neutral sugars, free amino acids, and anthocyanins in protoplasts. *Plant Physiology* **64**: 88–93.
- WINKEL SHIRLEY B. 2002. Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology* **5**: 218–223.
- XU WP, PENG H, YANG TB, WHITAKER B, HUANG LH, SUN JH & CHEN P. 2014. Effect of calcium on strawberry fruit flavonoid pathway gene expression and anthocyanin accumulation. *Plant Physiology and Biochemistry* **82**: 289–298.
- ZAFAR S, ASHRAF MY, ANWAR S, ALI Q & NOMAN A. 2016. Yield enhancement in wheat by soil and foliar fertilization of K and Zn under saline environment. *Soil and Environment* **35**(1): 46–55.
- ZAFAR S, ASHRAF MY & SALEEM M. 2018. Shift in physiological and biochemical processes in wheat supplied with zinc and potassium under saline condition. *Journal of Plant Nutrition* **41**(1): 19–28.
- ZHU M, YU J, TANG W, FAN S, BAI M, CHEN M & YANG G. 2019. Role of calcium in regulating anthocyanin accumulation in 'Manicure Finger' grape berries. *Scientia Horticulturae* **256**: 108585.



REZIME

Efekat salicilne kiseline i kalcijum hlorida na lipidnu peroksidaciju i sposobnost uklanjanja radikala leblebije (*Cicer arietinum*) pod stresom soli

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Slanost izaziva štetne morfološke, fiziološke i metaboličke efekte kod biljaka. Ovo istraživanje je imalo za cilj da proceni uticaj salicilne kiseline (SA 0, 0.75 and 1.5 mM) i kalcijum hlorida (CaCl_2 0, 50 and 100 mM), pojedinačno ili u kombinaciji, na različite morfološke i fiziološke karakteristike leblebije izložene stresu soli (0, 25 and 75 mM NaCl). Rezultati su pokazali da dodavanje samo SA ili Ca poboljšava ponašanje biljaka u prisustvu NaCl. Takođe, dužina izdanaka i korena, suva masa, hlorofili i karotenoidi opadaju pod uticajem soli, dok se malohdialdehid (MDA), inhibicija DPPH radikala, antocijanini i prolin povećavaju. Međutim, upotreba SA i Ca povećala je dužinu izdanaka i korena, suhu masu, poboljšala hlorofil, karotenoide i reducirajuće šećere i značajno smanjila MDA i inhibiciju DPPH radikala u biljkama. Ove studije impliciraju da su SA i Ca uzrokovali toleranciju na NaCl koja može biti povezana s regulacijom antioksidativnih odgovora. Takođe, sugeriše se da su koncentracija od 1,5 mM salicilne kiseline i koncentracija od 100 mM kalcijuma najprikladnije koncentracije za poboljšanje fizioloških parametara leblebije u uslovima slanosti. Dakle, SA i Ca ostvaruju ovu svoju ulogu, regulišući antioksidativni sistem.

Ključne reči: stres, fotosintetski pigmenti, natrijum hlorid, kalcijum hlorid, prolin, reducirajući šećeri