ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 22(1) 2023, 75–85
https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 https://doi.org/10.24326/asphc.2023.4603
ORIGINAL PAPER
Accepted: 10.08.2022
Published: 24.02.2023

AMELIORATIVE ROLE OF SALICYLIC ACID IN THE GROWTH, NUTRIENT CONTENT, AND ANTIOXIDATIVE RESPONSES OF SALT-STRESSED LETTUCE

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ABSTRACT

Plant responses to biotic and abiotic stresses are regulated by salicylic acid (SA), a signaling molecule. The goal of this study was to determine the efficacy of foliar SA treatments (0.25, 0.50, or 1.00 mM) in reducing salt stress in lettuce exposed to 100 mM NaCl. Salt-stressed plants given a foliar application of SA showed alleviation of the negative effects of salinity, resulting in higher growth performance (increases of 6%–198%). The positive impacts of SA were especially noticeable as an increase in the content of photosynthetic pigments, such as total chlorophyll (31–72%) and total carotenoids (49–141%). Application of SA also helped to reduce membrane damage, as seen by significantly lower levels of MDA (31–70%) in the leaves of salt-stressed lettuce plants. Moreover, the use of SA enhanced overall flavonoid and phenolic content, as well as nutrient absorption. SA treatment also increased the activities of antioxidant enzymes, such as ascorbate peroxidase, catalase, glutathione reductase, and superoxide dismutase, resulting in a considerable reduction in salt-induced oxidative damage. The most efficient SA application concentration was 0.50 mM. Overall, the use of SA as a foliar spray could be recommended as a long-term strategy for improving the defense systems of salt-stressed lettuce.

Key words: abiotic stress, biostimulants, *Lactuca sativa* L., oxidative stress, phytohormone, salinity

INTRODUCTION

Salinity is one of the most deleterious abiotic factors affecting plant growth, development, and productivity around the world. Salt stress paves the way for undesired morphological, physiological, and biochemical responses in many crop plants by causing imbalances in nutrition, changes in metabolic processes, and disruption of cell and chloroplast membranes [Sarabi et al. 2017]. Sodium (Na) is an important cation that is soluble in many soils in arid and semiarid areas. Concentrated amounts of Na in soils result in observable harmful effects in plants, and one of the predominant effects of salinity is a nutritional imbalance. The excessive accumulation of Na and Cl in the cells causes an ionic imbalance that inhibits the uptake of other mineral nutrients including K, Ca, and Mn. Enzymes are rendered inactive and metabolic activities in plants are impacted by a high sodium to potassium ratio brought on by the buildup of large amounts of sodium ions. The uptake of K is inhibited by excess Na and Cl, which causes symptoms similar to those of K insufficiency. Under salt stress, it appears that selective K absorption as well as cellular K and Na compartmentation and distribution in the shoots are necessary to maintain appropriate K levels in plant tissue. Salt

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stress lowers the Ca/Na ratio in the root zone, which has an impact on membrane characteristics because membrane-associated Ca is replaced by Na, causing the integrity and selectivity of the membrane to dissolve [Munns and Tester 2008, Jouyban 2012]

Abiotic stressors, such as salinity, cause an excessive accumulation of reactive oxygen species (ROS), which can oxidize proteins and lipids, inactivate enzymes, and damage DNA and/or interact with other essential plant cell components. Plants have a variety of antioxidant components, both enzymatic and nonenzymatic, that can reduce the damaging effects of ROS. Superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) are enzymatic antioxidants, while reduced glutathione (GSH), ascorbate, carotenoids, and tocopherols are nonenzymatic antioxidants [Kusvuran 2021].

Plants have evolved very sophisticated crosstalk between distinct phytohormones to survive under biotic and abiotic stress conditions. The fine-tuning of the complex phytohormone network allows plants to respond to developmental and environmental stimuli in a balanced manner, thereby lowering defense-related fitness costs [Koo et al. 2020]. In addition to the use of salt tolerant plant species, several strategies, including exogenous application of SA, are being practiced by researchers and specialists in different plants to override the adverse impacts of salt stress [Rehman et al. 2022]. SA-mediated accumulation of osmolytes, such as glycine betaine, proline, soluble sugars, and amines, can also help to maintain osmotic homeostasis, regulate mineral nutrition uptake, enhance scavenging of ROS, and promote production of secondary metabolites, such as terpenes, phenolics, and nitrogen-containing compounds (alkaloids, cyanogenic glucosides) [Koo et al. 2020].

Lettuce (*Lactuca sativa* L.) is a leafy vegetable that is commonly eaten raw or in salad mixes. Because of its marketability, sensory characteristics, and health-promoting properties, lettuce has experienced great increases in production and cultivation. Vitamins, carotenoids, polyphenols, antioxidants, and fiber are among the key lettuce phytochemicals. Negative effects of salt stress on lettuce biomass production have been documented due to ionic imbalances, salt toxicity, and osmotic effects [Khalifa et al. 2016]. The goals of the present study were: (1) to determine the effects of different salt levels on nutrient uptake, photosynthetic pigment content, plant growth, and lettuce production, and (2) to determine whether exogenous administration of SA can offset the negative effects of salinity in lettuce plants.

MATERIAL AND METHOD

Lettuce (*Lactuca sativa* L.) cv. 'Velvet' seeds (Enza Zaden Seed Company) were germinated in a peat-perlite (2 : 1) substrate under greenhouse conditions with 22/16 ±2°C, relative humidity of 65 ±5%, and photoperiod of 16 h. The uniformly developed seedlings were transplanted into plastic pots (57 × 16 × 12 cm) with 11 L of vermiculite 20 days after sowing. Four plants were planted in each pot, with five pots in each replicate (three replications).

Plants in the control group were cultivated and developed for the same amount of time under stress-free settings. A total of 5 groups were formed in each experiment, as follows: (1) control group (C): irrigation with nutrient solution – following that of Ergun et al. [2018], (2) salt-stressed group (S-100 mM NaCl), (3) S + salicylic acid (SA1) at 0.25 mM, (4) S + salicylic acid (SA2) at 0.50 mM, (5) S + salicylic acid (SA3) at 1.00 mM. Upon conclusion of the experiment, evaluation of the plants was performed via the following morphological, physiological, and biochemical parameters.

Leaf water relations (RWC). Estimation of the RWC was performed using the method of Türkan et al. [2005].

Ion concentration. For the determination of ion contents, the leaves were dried for 48 h at 65°C. After which, a mill with a 20-mesh sieve was used to grind the samples. The leaf powder was turned into ash for about 6 h at 550°C, which was then dissolved in 3.3% HCl. Atomic absorption spectrometry was used to determine the Na, K, Ca, Mg, Fe, Mn, Zn, Cu concentrations in the leaves. The Cl concentration in the tissue samples was determined used in the titrimetric analysis with silver nitrate ($AgNO_3$) via the Mohr method [Dasgan et al. 2018].

Total flavonoid and total phenolic contents. A colorimetric assay was used to determine the flavonoid concentration. The leaf total phenolic (TP) contents were measured using the Folin-Ciocalteu reagent and expressed in milligrams [Sabir et al. 2019].

MDA content. The MDA level determined by the thiobarbituric acid (TBA) reaction was used to calculate lipid peroxidation level [Heath and Packer 1968]. The absorbance was assessed at 532 nm after centrifugation of the supernatant at 10,000 g for 10 minutes at 4°C, and values were subtracted if they related to non-specific absorption at 600 nm.

Photosynthetic pigments. The total chlorophyll and carotenoid contents were determined using Arnon's [1949] technique. The leaf pigment was extracted with 80% (v/v) acetone, and the absorbance of the extraction was measured at 652 and 470 nm using a Shimadzu UV mini-1240 spectrophotometer (Kyoto, Japan).

Antioxidant enzyme activities: The enzymes were extracted from 0.5 g of leaf tissue using a mortar and pestle, as well as 5 mL of extraction buffer containing 50 mM potassium-phosphate buffer, pH 7.6 and 0.1 mM disodium ethylenediaminetetraacetate. The SOD assay was involved measuring the reduction of O_2^- induced nitro blue tetrazolium at 560 nm. The activity of the CAT was determined by monitoring the disappearance of H_2O_2. The APX activity was determined by measuring ascorbate intake using its absorbance at 290 nm. The GR activity was calculated using the absorbance of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm and the enzyme-dependent oxidation of NADPH [Cakmak and Marschner 1992].

The experiment employed a completely randomized plot design with three replicates. The Tukey's multiple range test was used to compare the mean values. Using the SPSS v.13.0 software for Windows, statistical significance was defined as $P < 0.05$. (SPSS Inc., Chicago, IL, USA). In all of the figures, data were presented as means ± standard deviation (SD), with error bars representing standard deviation of the means. All of the independent variables were submitted to principal component analysis (PCA), multiple variable analysis using the Pearson correlation matrix, and ClustVis software was used to create a heat map utilizing correlation distance and average linkage.

RESULTS

Plant growth assessment. Growth components, including the shoot fresh and dry weight, leaf number, and area per plant of the lettuce plants, were significantly reduced by salt stress at rates of 73%, 72%, 66%, and 76%, respectively (Tab. 1). When compared to the S group, the S + SA group showed enhanced growth amelioration of 49%–187%. In addition, application of 0.5 mM SA was more effective in improving these growth attributes of lettuce under salt stress, at 100 mM NaCl. Of the different doses of SA, the application of 0.5 mM SA resulted in the maximum improvement in growth components under salt stress (increases of 86%–304% when compared to the S group that received no SA application).

Relative water content (RWC) and photosynthetic pigments. The water status of lettuce plants, measured as leaf RWC, showed that salt stress lowered the leaf RWC by 40% when compared to the non-stressed control. Spraying SA on lettuce leaves enhanced leaf RWC in salt-exposed plants by 23–54% compared to lettuce plants subjected to salt stress alone. When compared to the S + SA (0.5 mM) and S + SA (1 mM) groups, the 0.25 mM SA group showed smaller effects and had values similar to those in the S group not treated with SA. Plants exposed to 0.5 mM SA, by contrast, showed the highest RWC values (increases of 54%). The total chlorophyll and carotenoids in lettuce plants exposed to salinity were reduced by 45% and 59%, respectively ($P < 0.001$). Under saline conditions, foliar application of SA, particularly at 0.5 mM, greatly increased the levels of photosynthetic pigments in the leaves (Tab. 1).

MDA content. The MDA levels in the leaves of salt-stressed lettuce plants increased by 335% when compared to non-stressed control plants (Tab. 1). Spraying lettuce plants with SA protected membranes from salt-mediated oxidative stress, as evidenced by a 31–70% reduction in leaf MDA in the S + SA plants compared to plants subjected to salt stress alone. The MDA levels were lowest and statistically significant in plants treated with 0.5 mM SA (decrease of 70%).

Total phenolic and flavonoid contents. Under salt stress, when compared to the C group, the TF content of lettuce increased by 7%. The exogenous application of 0.5 mM SA resulted in a more considerable increase in TF ($P < 0.05$) (increase of 71%) when compared to the other SA applications under salt stress. For leaf TP, the response of lettuce was significantly different between the SA treatments under saline conditions; however, under salinity, the interactive term was not significant (Tab. 1).

Table 1. Effects of salicylic acid (SA) applications on growth parameters of lettuce under saline conditions

Application	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Leaf number (num. plant^{-1})	Leaf area (cm^2 plant^{-1})	RWC (%)
C	238.69 ±5.74^{a}	18.03 ±3.84^{a}	29.00 ±2.94^{a}	3083.17 ±51.84^{a}	91.00 ±2.00^{a}
S	62.24 ±6.95^{c}	5.02 ±1.33^{c}	10.67 ±1.15^{b}	758.72 ±25.10^{d}	54.00 ±5.01^{d}
S + SA1	83.69 ±0.70^{c}	6.96 ±0.67^{c}	11.33 ±1.13^{b}	970.92 ±23.67^{d}	66.33 ±4.16^{c}
S + SA2	143.97 ±3.11^{b}	12.55 ±0.39^{b}	18.00 ±1.00^{b}	2261.07 ±87.33^{b}	83.33 ±4.51^{ab}
S + SA3	120.37 ±8.26^{b}	10.04 ±0.76^{bc}	16.33 ±0.58^{b}	1784.92 ±22.50^{c}	76.00 ±3.61^{bc}
Application	Total Chl (mg g^{-1} FW)	Total carotenoids (mg g^{-1} FW)	MDA (µmol g^{-1} FW)	Total phenolic (µg GAE ml^{-1})	Total flavonoid (mg QE 100 g^{-1})
C	2.572 ±0.21^{a}	0.769 ±0.08^{a}	1.98 ±0.22^{d}	52.32 ±6.44 ^{ns}	88.07 ±9.30^{b}
S	1.409 ±0.09^{d}	0.311 ±0.05^{c}	8.63 ±0.51^{a}	59.38 ±3.57	94.25 ±3.25^{b}
S + SA1	1.853 ±0.10^{cd}	0.466 ±0.06^{bc}	5.88 ±0.43^{b}	76.07 ±5.52	108.59 ±9.12^{ab}
S + SA2	2.436 ±0.28^{ab}	0.750 ±0.10^{a}	2.51 ±0.25^{cd}	87.83 ±3.53	150.62 ±3.73^{a}
S + SA3	2.095 ±0.09^{bc}	0.541 ±0.04^{b}	3.61 ±0.93^{c}	79.84 ±3.05	130.90 ±5.20^{ab}

* Each value represents the mean of three replicates. For each parameter of each different letters are significantly different at $P < 0.05$ according to Tukey's test. ns: not significant
C: control; S: salt stress (100 mM NaCl); S + SA1: 100 mM NaCl + 0.25 mM salicylic acid; S + SA2: 100 mM NaCl + 0.50 mM salicylic acid; S+ SA3: 100 mM NaCl + 1.00 mM salicylic acid

Table 2. Effects of salicylic acid (SA) applications on ion concentration in leaves of lettuce grown under saline condition

Application	Na (%)	Cl (%)	K (%)	Ca (%)	Mg (%)
C	0.28 ±0.07^{c}	0.38 ±0.05^{d}	4.56 ±0.26^{a}	0.83 ±0.04^{a}	1.36 ±0.08^{a}
S	1.87 ±0.12^{a}	1.84 ±0.13^{a}	2.67 ±0.17^{d}	0.57 ±0.03^{b}	0.76 ±0.06^{c}
S + SA1	1.32 ±0.10^{ab}	1.57 ±0.06^{b}	2.93 ±0.24^{cd}	0.63 ±0.07^{ab}	0.92 ±0.17^{bc}
S + SA2	1.19 ±0.06^{b}	1.16 ±0.03^{c}	3.75 ±0.11^{b}	0.75 ±0.04^{ab}	1.29 ±0.11^{a}
S + SA3	1.24 ±0.04^{b}	1.41 ±0.02^{b}	3.24 ±0.10^{c}	0.71 ±0.06^{ab}	1.12 ±0.15^{ab}
Application	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	
C	251.25 ±1.25^{a}	21.50 ±1.95^{a}	58.10 ±1.23^{a}	16.53 ±0.95^{a}	
S	85.33 ±2.06^{e}	13.17 ±1.53^{b}	40.13 ±0.55^{c}	7.20 ±1.39^{c}	
S + SA1	107.60 ±1.60^{d}	13.83 ±2.67^{b}	41.40 ±1.47^{bc}	8.60 ±0.60^{c}	
S + SA2	204.87 ±2.41^{b}	18.17 ±1.84^{ab}	46.67 ±1.39^{b}	14.53 ±0.95^{ab}	
S + SA3	155.63 ±3.75^{c}	15.25 ±1.25^{b}	45.73 ±1.05^{bc}	12.53 ±1.40^{b}	

* Each value represents the mean of three replicates. For each parameter of each different letters are significantly different at $P < 0.05$ according to Tukey's test
C: control; S: salt stress (100 mM NaCl); S + SA1: 100 mM NaCl + 0.25 mM salicylic acid; S + SA2: 100 mM NaCl + 0.50 mM salicylic acid; S + SA3: 100 mM NaCl + 1.00 mM salicylic acid

Ion contents. Salt stress generally increased the levels of Na and Cl in the tissue samples by 1235 and 384%, respectively. The SA-treated plants showed lower Na and Cl concentrations in the leaves (an average decrease of 33% and 25%, respectively) when compared with the untreated salt-stressed plants. The foliar spray of 0.5 mM SA resulted in a greater reduction in leaf Na and Cl contents at 100 mM NaCl (decreases of 36 and 37%, respectively). As shown in Tab. 2, the SA was tested for its capacity to promote the accumulation of K, Ca, Mg, Zn, Mn, Cu, and Fe in lettuce plants under salt stress. Salt stress caused a considerable reduction in macro- and micronutrient accumulation in the plants. Overall, the SA treatments contributed significantly to the mineral acquisition by lettuce plants exposed to salinity. Compared to the C group, these reductions ranged from 38% to 82% under salt stress. The application of SA had a beneficial effect on nutrient content, with significant increases in macro and micro ion contents (19–213%) in all the S + SA groups compared to the S group. When compared to the S group, lettuce plants that received 0.5 mM SA exhibited better K, Ca, Mg, Zn, Mn, Cu, and Fe accumulations in the leaves (increases of 98%, 54%, 200%, 97%, 38%, 333%, and 338%, respectively).

Antioxidant enzyme activities. Antioxidant enzyme activities, such as SOD, CAT, APX, and GR, were upregulated and elevated by 22%, 29%, 13%, and 30%, respectively, in the S group compared to the C group, as shown in Fig. 1. Compared to the C and S groups, foliar application of SA, in general, had a significant and noticeably favorable effect on lettuce plants. When compared to the S group, enzyme activities of SOD, CAT, APX, and GR increased by 36%, 56%, 29%, and 77%, respectively, in the S + SA groups. Plants treated with 0.5 mM SA had higher SOD, CAT, GR, and APX activity than those that received 0.25 and 1 mM SA, with increases of 43%, 101%, 53%, and 126%, respectively.

Principal component analysis (PCA) of the data. When the principal component analyzes are examined in terms of the investigated parameters, principal component 1 and principal component 2 constitute 95.5% and 3.2% of the total variance under salt stress conditions. Salt application had the lowest values and separated from other applications, had the highest Na, Cl and MDA contents. In addition, among these parameters, Na, MDA and Cl were separated from other variables. However, SA0.50 (SA2) application came to the fore with the highest RWC, total Chl, and total TP content (Fig. 2).

DISCUSSION

High levels of salinity have two distinct effects on plants: 1) high salt concentrations in the soil interfere with root water absorption, and 2) high salt concentrations within the plant can disturb a variety of biochemical and physiological processes, including nutrient absorption and assimilation. When these disruptions occur together, the result is reduced development and growth and often failure of the very survival of the plant [Acosta-Motos et al. 2019]. Salinity has a detrimental impact and imposes restrictions on plant growth that have important effects in terms of agricultural production, largely due to the decrease in crop yield. Studies carried out in different species consistently show decreases in key plant growth parameters as a result of NaCl exposure. These salinity responses typically include decreases in plant biomass and disturbance of the osmotic balance due to the uptake of Na and Cl ions into the plant [İbrahimova et al. 2021].

In the present study, lettuce plants exposed to saline water irrigation (100 mM NaCl) showed reductions of 66–76% in all the growth parameters measured (FW, DW, NL, and LA). Exogenous administration of salicylic acid influences plant salt tolerance by influencing a number of physiological processes and biochemical pathways [Ghassemi-Golezani and Farhadi 2021]. The application of SA successfully reduced the harmful effects of salt stress on lettuce seedling development and growth by 49–187% in the present study (Tab. 1). The favorable effects of SA on growth parameters could be ascribed to a restriction of ethylene synthesis, decreased Na and Cl uptake, and/or enhanced uptake of important minerals like K and Ca. These mechanisms could all help plants thrive by reducing ionic and osmotic stressors [Lamnai et al. 2021]. Exogenous application of SA can modulate stomatal opening to improve CO_2 absorption and minimize transpirational water loss under saline circumstances [Da Silva Ribeiro et al. 2020]. The protective action of SA on cell membranes, which may boost plant tolerance to salt stress, could explain the improvement

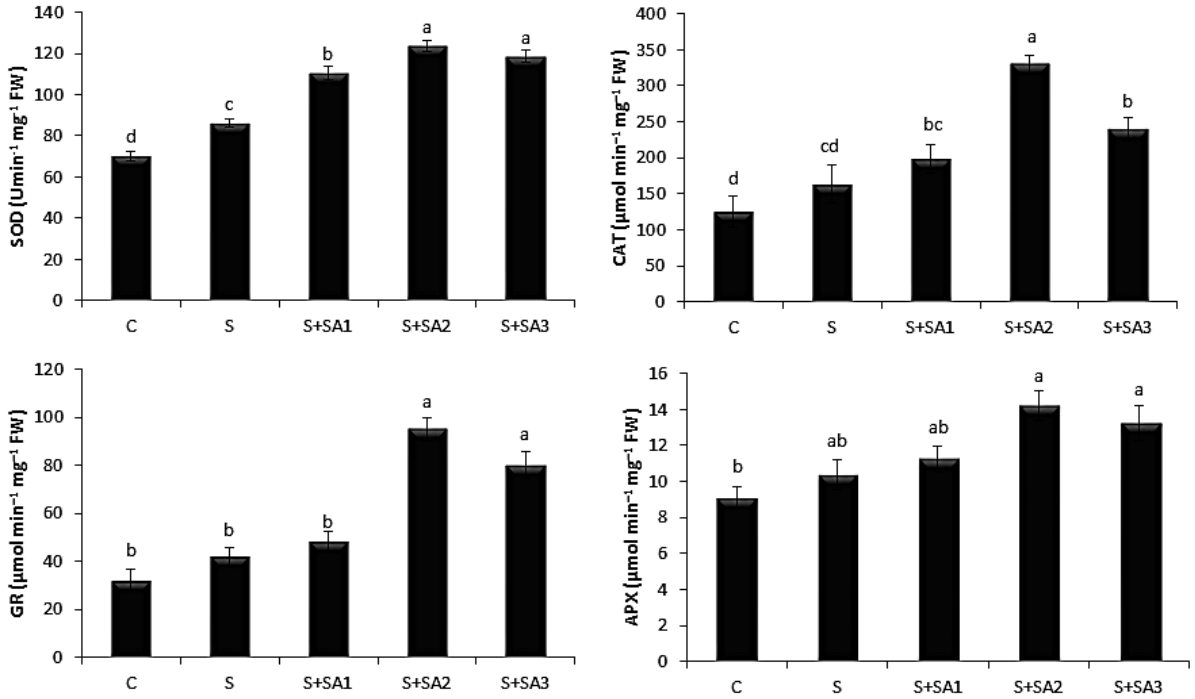

Fig. 1. Effects of salicylic acid (SA) applications on antioxidant enzyme activity in leaves of lettuce grown under saline condition. C: control; S: salt stress (100 mM NaCl); S + SA1: 100 mM NaCl + 0.25 mM salicylic acid; S + SA2: 100 mM NaCl + 0.50 mM salicylic acid; S + SA3: 100 mM NaCl + 1.00 mM salicylic acid. Each value represents the mean of three replicates. For each parameter, different letters represent statistically significant differences at $P < 0.05$ according to the results of the Tukey's test. Data are shown as the mean ±SD and, in all of the figures, error bars represent the standard error of the means

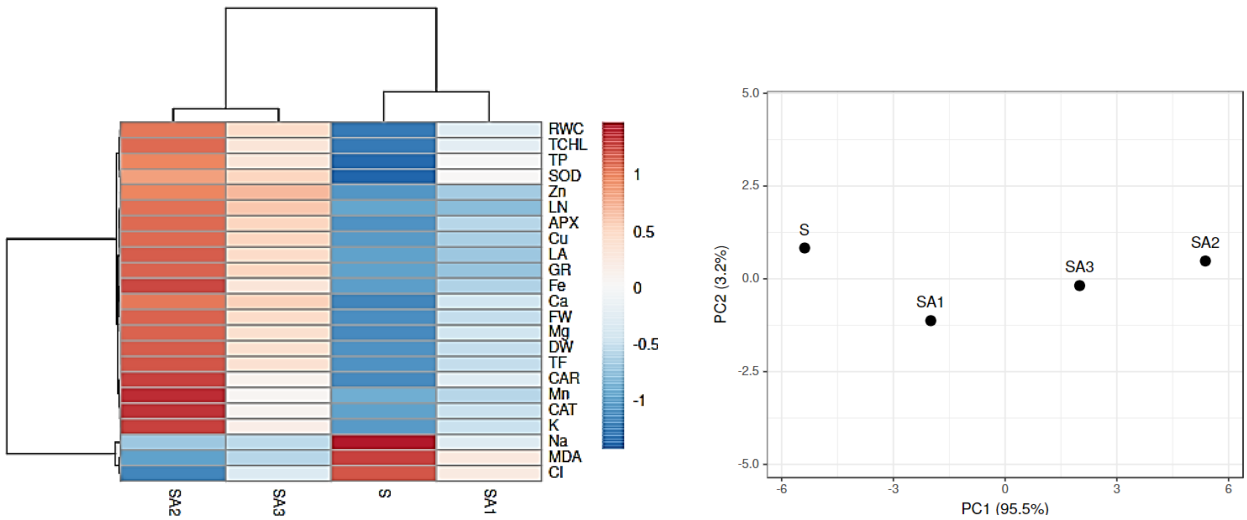

Fig. 2. Heat map cluster (left) and principal component analysis (PCA) plot (right) of quality parameters profiles of salt stress. S: salt stress (100 mM NaCl); SA1: 100 mM NaCl + 0.25 mM salicylic acid; SA2: 100 mM NaCl + 0.50 mM salicylic acid; SA3: 100 mM NaCl + 1.00 mM salicylic acid

in growth characteristics in salt-stressed plants treated with SA [Faghih et al. 2017].

The RWC of lettuce tissues was reduced dramatically (a 40% decrease) under saline stress, indicating that plants were losing substantial amounts of water. Both ionic imbalances and osmotic stress could be linked to this decrease in water content [Behdad et al. 2021, Lamnai et al. 2021]. However, comparison to plants exposed to saline conditions alone confirmed that the SA treatment improved water conservation in salt-stressed plants. This effect could be explained by an increase in SA-induced proteins that serve as osmoregulators to reduce the degree of tissue water loss [Lamnai et al. 2021].

Maintenance of photosynthetic activity in response to SA would also aid in turgor maintenance by the production of osmolytes, and photosynthetic efficiency depends greatly on the levels of photosynthetic pigments in the plant [Jannesar et al. 2021]. The total chlorophyll and total carotenoid contents decreased by 45% and 59% in lettuce plants exposed to salt stress compared to unstressed control plants (Tab. 1). Only in salt stressed plants resulted in pigment decreases of 2–39% compared to the control plants, whereas pigment levels improved with SA applications by 31–141% compared to the non-treated plants grown under salt-stress conditions. Comparisons of 0.25 and 1.00 mM SA applications in lettuce plants under salt stress revealed a significant improvement in the photosynthetic pigment content in the plants treated with 0.5 mM SA. SA mitigates the detrimental effect of salinity on chlorophyll by reducing ROS levels and increasing the activity of the plant antioxidant systems. This boosts plant development by encouraging cell division and elongation, and perhaps by blocking chlorophyll oxidase enzymes to prevent chlorophyll breakdown and maintain photosynthesis [El-Taher et al. 2021].

The oxidative reactions triggered by salt stress initiate a chain of events that culminate in membrane peroxidation. DNA can also be damaged by oxidative reactions, which can also denature enzymes and other proteins and induce carbohydrate oxidation and pigment destruction [Vázquez et al. 2021]. Salt stress results in the formation of free radicals, which cause irreversible damage in lipids and proteins. The destruction of cell membrane integrity results from lipid peroxidation, which causes cell death over time. Often, MDA is used as an indicator for the assessment of damage resulting from abiotic stress. The lipid peroxidation increase is due to compounds like OH, H_2O_2, and O_2^- in chloroplasts. MDA, which plays the role of a cellular toxicity bioindicator, is a well-known oxidation that emerges from lipid peroxidation during oxidative stress [Singh et al. 2018]. In lettuce leaves, salt stress significantly increased the MDA content (336%; $P < 0.0001$) as a result of oxidative stress. However, in plants exposed to saline stress, SA treatment significantly reduced the MDA levels (32–70% decrease) compared to salinity treatment alone, in agreement with previous studies on black bean [Heidarian and Roshandel 2021] and pistachio [Jannesar et al. 2021]. SA effectively protects cell membranes from damage via the antioxidant defense system, and SA supplementation can improve plant stress tolerance by reducing membrane oxidation, protecting cellular components, and fortifying cell walls [Lamnai et al. 2021].

Flavonoids are a wide subset of secondary plant metabolites and are classified as phenolic chemicals [Shaki et al. 2018, Kusvuran 2021]. They accumulate in stressed plant tissues and can operate as free radical scavengers due to the hydroxyl groups in their structures. In the present study, lettuce plants irrigated with non-saline water alone responded to an application of SA with a considerable increase in TP (13–67%) and TF (7–71%), indicating that SA stimulated the accumulation of phenolics (Tab. 1). Shaki et al. [2018] reported an increase in flavonoid levels in SA-treated plants, which they believed was attributable to an increase in phenolic compounds. Flavonoids appear to ameliorate the oxidative damage imposed by salinity and other stresses, but mechanisms other than flavonoid accumulation are required to correct the ion imbalances caused by excess ion uptake under saline conditions.

Among the reasons for the negative effects of high salt concentrations in the cell is ion toxicity caused by high concentrations of Na and Cl ions. High Na ion levels cause oxidative damage by disrupting the osmotic potential balance, causing damage at the cellular and plant levels, as well as molecular damage [Yang and Guo 2018]. In the present study, application of 100 mM NaCl caused an increase of 568% in Na ions and 384% in Cl ions in the leaves, but SA treatment

significantly reduced this accumulation (14–37% reduction). Under saline conditions, 0.5 mM SA induced the greatest decreases in Na and Cl concentrations. As documented in several other studies, the administration of SA has been shown to change Na and Cl contents in salt-stressed strawberry [Faghih et al. 2017], sorghum [Rajabi Dehnavi et al. 2019], pennyroyal [Ghassemi-Golezani and Farhadi 2021], and lemongrass [Rehman et al. 2022]. The reduction in the levels of Na and Cl in salt-stressed plants following SA treatment could be attributable either to a reduction in cell membrane damage due to enhanced antioxidant activity or to a dilution effect caused by increased dry matter synthesis in response to SA application [Rajabi Dehnavi et al. 2019]. Plants can increase their cellular osmotic potential and promote water entry by the active uptake and accumulation of potassium [İbrahimova et al. 2021]. In the present study, the K content in lettuce leaves decreased by 41% under salt stress conditions, whereas SA applications increased K uptake by 9–40%, apparently by limiting Na uptake. K uptake helps to limit Na uptake, thanks to Na/K antiport mechanisms, which rely on the K-dependent expulsion of Na ions [Hauser and Horie 2010]. Plants also require calcium to regulate cell and tissue growth, and this ion serves as an osmoticum in vacuoles, as a membrane stabilizing element, as a cell wall strengthening agent, and as a secondary messenger to several signaling pathways, and all these responses can aid in adjustment to diverse stress situations [Gafur and Putra 2019]. Similarly, magnesium catalyzes many enzymatic reactions by creating chelate connections between enzymes and substrates. Magnesium also has a role in glucose, protein, and lipid synthesis and in metabolite transport and storage. In lettuce plants under salt stress, the concentrations of Ca and Mg were dramatically reduced (31% and 43% decreases, respectively). In pennyroyal plants, increases in tissue Na concentrations have also been shown to reduce the levels of these two cations and this reduction in Mg and Ca levels occurs due to substitution of Mg and Ca with Na, as well as Ca and Mg precipitation and low mobility in roots during salt stress [Ghassemi-Golezani and Farhadi 2021]. SA treatment apparently lowered the uptake and accumulation of Na in lettuce plants, thereby minimizing the detrimental effects of Na on Ca and Mg. As with potassium, application of SA improved Ca and Mg uptake by 10–69%. The levels of micronutrients like Zn and Mn are also affected by salt stress. Both Zn and Mn are important for the activity of antioxidant enzymes and defense mechanisms. Mn is also required for photosynthesis, protein synthesis, and enzyme activities, as well as for the synthesis of chlorophyll in plants [Kıran et al. 2019]. Under saline conditions, foliar application of SA increased the Fe, Zn, Mn, and Cu content by 3–140%, suggesting that application of SA to plant leaves could change the microelement concentration. Again, a 0.5 mM SA concentration was the most effective at increasing the levels of Fe, Cu, Mn, and Zn in lettuce plants under salt stress. Administration of SA also increased the levels of Fe, Mn, and Zn in canola plants under salinity [Rajabi Dehnavi et al. 2019], in agreement with our findings. Hussein et al. [2015] also reported that SA application increased the macro and micro ion levels in canola plants under salt stress. Mineral nutrient uptake requires functional cell membrane transporters; therefore, the improvement in uptake in response to SA could also reflect its protective effects against ROS-induced membrane damage.

These defense antioxidants act together to confer resistance to abiotic stressors that promote ROS production at various growth stages. SOD levels are a good criterion for indirect selection when looking for abiotic stress tolerance plant materials, and SOD has also been claimed to make plants more resistant to oxidative stress [Munawar et al. 2019]. CAT is responsible for detoxifying high levels of H_2O_2 in peroxisomes, and its activity is thought to be required to maintain redox balance during oxidative stress [Mohammadi et al. 2019]. The activities of SOD and CAT are also enhanced in response to salt stress. In the present study, SOD and CAT activity increased by 23% and 30%, respectively, in lettuce plants compared to the C group. Following SA application, the increases were 28–44% and 21–101%, respectively. H_2O_2 is detoxified in the chloroplast by APX, which uses AsA as a unique donor of electrons to reduce H_2O_2 to water. The antioxidant defense mechanism that involves GR drives the removal of H_2O_2 from chloroplasts and mitochondria [Kıran et al. 2019]. In the present study, the activities of GR and APX increased 15–126% and 8–37%, respectively, in salt-stressed lettuce treated with SA under salt stress. Several recent studies have

confirmed this effect of SA in the direct and indirect activation of antioxidant enzymes under various stress situations. According to Bose et al. [2017], SA triggers numerous pathways, including a battery of antioxidant enzymes, in the chloroplast to promote ROS scavenging and safeguard the photosynthetic apparatus. The effects of SA treatment on the antioxidant system were investigated by Lamnai et al. [2021], who discovered that the increased tolerance of strawberry plants to salt stress was likely associated with higher antioxidant enzyme activities, resulting in less ROS accumulation and fewer gene expression changes. Other investigations have indicated that exogenous SA administered to salt-stressed lettuce [Khalifa et al. 2016], pistachio [Jannesar et al. 2021], and lemongrass [Rehman et al. 2022] increases antioxidant enzyme activity. Taken together, the available evidence indicates that treatment of plants with SA improves their stress tolerance by lowering membrane oxidation, preserving cellular components, and reinforcing cell walls [Lamnai et al. 2021].

SA has been described as playing a crucial function in regulating plant defense signals in response to abiotic and biotic stresses. Under varied stress situations, an increase in the endogenous concentration of SA leads the fast buildup of ROS, resulting in oxidized proteins and cell death in infected tissues. SA can also produce stress tolerance that is highly dependent on the buildup of superoxide radicals and hydrogen peroxide, which are important mediators of hypersensitive response (HR) and PCD induction at high concentrations. In addition, SA-generated ROS may contribute to cellular redox equilibrium by regulating the production and activity of antioxidant enzymes at lower concentrations [Poor 2020]. On the other hands, a high SA concentration can result in the loss of mitochondrial integrity, the release of cyt c from mitochondria, and the generation of reactive oxygen species (ROS) and membrane lipid peroxidation [Van Aken et al. 2015, Poór et al. 2019, Poor 2020]. As seen in heat map cluster and PCA results (Fig. 2), it was observed that SA improved salinity tolerance and increased plant biomass at 100 mM by enhancing chlorophyll, K and Ca ions content, RWC, and total phenolic, and antioxidant enzymes (SOD, CAT, GR and APX). In addition, lower rates of Na, Cl ion accumulation, and MDA content were determined with SA application. However, it was observed that 1 mM SA application was not as effective as 0.50 mM SA application in this study.

CONCLUSIONS

The findings presented here show that saline conditions alter the growth, physiological attributes, and biochemical characteristics of lettuce plants (*Lactuca sativa* L.). Exogenous administration of SA, by contrast, enhances the resistance of these plants to salt stress. This enhancement can be ascribed to the ability of SA to (1) improve growth performance by promoting ion regulation and (2) minimize oxidative damage by activating SOD, CAT, GR, and APX. In lettuce, the most effective SA dosage was 0.5 mM in terms of reducing the harmful effects of salt on the various parameters examined. Our findings show that SA, a widely available substance, might be utilized in horticulture in a variety of ways to avoid crop losses due to salt stress. More investigations are required in the field to confirm the beneficial effects of exogenously applied SA on lettuce growth and physiology.

SOURCE OF FUNDING

This research received no external funding.

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