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Effect of Salicylic Acid, Benzoic Acid, and *p*-Coumaric Acid on Growth, Chlorophyll, Proline, and Vitamin C of Salinity-Stressed Tobacco (*Nicotiana tabacum*)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors designed the study. Author AIHK wrote the protocol, performed the experiment, managed the experimental measurements and analysis of data, managed the literature searches and wrote the first draft of the manuscript with the guidance of author KSR. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Salinity is an important growth-limiting abiotic stress factor. Salicylic acid (S.A), Benzoic Acid (B.A) and *p*-coumaric acid (*p*-CA) are substances generally thought to play a crucial role in the enhancement of salt tolerance and regulation of growth and development in plants. This research was carried out to study the influence of Salicylic Acid (SA), Benzoic Acid (BA), and *p*-Coumaric acid (*p*-CA) on growth, contents of chlorophyll, proline and vitamin C in salinity-stressed tobacco plant (*Nicotiana tabacum*) grown *In vitro*. The results showed that Salinity (NaCl) stress impairs plant growth and inflicts severe physiological disorders. While supplementation of SA, BA and *p*-CA resulted in remarkable increases of all investigated biochemical components. Compared to the control, the high concentration of NaCl (150 mM) significantly reduced total fresh weight of tobacco. Total chlorophyll contents were remarkably increased by SA and BA treatments under 100 mM

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NaCl condition. The highest leaf and stem proline contents were recorded from the treatment of SA supplemented with 150 mM NaCl, while the lowest value was observed in *p*-CA + 0 mM NaCl treatment. Vitamin C contents were significantly increased by SA and BA treatments under 100 mM and 150 mM NaCl concentrations. SA and BA recovered the damage caused by high concentrations of salinity and enhanced salt tolerance in tobacco. Application of *p*-CA did not show significant differences compared to the control. This study suggests that SA and its derivatives could be used to overcome damages generated by salinity stress, enhance salt-tolerance and regulate plant growth and development.

Keywords: Benzoic acid; chlorophyll; proline; p-Coumaric acid; salicylic acid; salinity; tobacco; vitamin C.

1. INTRODUCTION

The influence of salinity on growth and development of plants has been a focus of research in plant biology for several decades because salt stress is a major environmental determinant limiting crop productivity and production [1]. Many researchers have reported on changing levels of physiological and biochemical parameters caused by salinity stress. The stresses generated by a high concentration of Salt lead to disturbance in growth and photosynthetic processes by causing changes in the accumulation of Na^+, Cl^- and nutrients, and disturbance in water and osmotic potential in plants [2]. Assimilation rate of photosynthetic CO_2 is also reduced by salinity. This reduction is partly due to a reduced stomatal conductance, both physiological and biochemical disorders created by salinity reduce crop production. In plants, toxic effects of salinity stress lead to metabolic changes such as reduction of chloroplast activity accompanied by decrease in photosynthetic rate which then leads to an increased reactive oxygen species. The decrease in photosynthesis may eventually cause reduction in both growth and yield [3]. It is estimated that at least 20% of all irrigated lands are salt-affected [4]. The huge areas of salt-affected lands on one hand and world population growth on the other have led plant scientists to the concept of enhancing salinity tolerance in plants by several approaches in order to meet the needs of ever growing world population. Proline and vitamin C are important biochemical components in plants. Proline plays a crucial role in the response of plants to salinity and other abiotic stresses [5]. Proline is also involved in flowering and development of plants [6]. Vitamin C is ubiquitous in plants, and serves a host of several physiological functions. It protects cells and organelles from oxidative damage, as well as improves the plant responses to environmental stresses like salt and drought [7]. Damages generated by salt stress can be alleviated or reduced either genetically or biochemically by application of chemical signaling molecules such as plant growth regulators. It is reported that application of signaling molecules like Salicylic Acid (SA) and its derivatives can enhance salt tolerance in plants by inducing protective effects of plants under salinity stress [8]. SA is considered as a naturally occurring molecule that can promote and develop plant growth by inducing plant tolerance against biotic and abiotic stresses such as salinity [9]. It also plays a crucial role in regulation of some plant physiological processes in plants like influences on growth and development, ion uptake, ion balance and transport and membrane permeability [10]. *p*-Coumaric acid is a hydroxycinnamic acid and it can be found in legumes. Both *p*-coumaric acid (*p*-CA) and benzoic acid (BA), are signaling molecules derived from phenylalanine [11], and has various physiological activities such as antimicrobial activity [12], cancer chemoprevention [13], antioxidant activity [14], and antimelanogenesis [15]. BA is a precursor of SA and produced by β-oxidation of cinnamic acid synthesized through phenyl propanoid metabolic pathway in plants [16]. Considerable results have been pointed concerning SA, BA and *p*-CA induced enhancements in salinity tolerance of many plants. SA improved growth and yield components of wheat plant [17]. SA and BA promote the generation of reactive oxygen species during salt stress thus play an important role in stress tolerance [18]. Despite numerous reports about the importance of signaling molecules such as SA, BA and *p*-CA, the role of *p*-CA, BA and SA in salt tolerance enhancement has not been intensively studied. Therefore, in the current research our aim is to study the influence of SA, BA and *p*-CA on salinity stressed tobacco cultivated in *In vitro* by investigating the physiological and biochemical parameters; fresh weight, chlorophyll, proline and vitamin C contents.

2. MATERIALS AND METHODS

2.1 Chemicals and Apparatuses

MS medium was purchased from Duchefa Biochemie (Haarlem, Netherland), while other chemicals were obtained from Sigma Chemical Co. [St Louis, MO, USA]. Refrigerator centrifuge (Kontron T-324), fraction collector (Bio-Rad 2110), ELISA microplate reader (Bio-Rad 680), UV-visible spectrophotometer (GeneQuant 100), were used in this experiment.

2.2 Growth Media

Plant growth medium of Murashige and Skoog 1962 [19] was supplemented with 10^{-5} mM SA, BA and *p*-CA treatments Kim and Roh 2014 [20] under four different concentrations of NaCl (0 mM, 50 mM, 100 mM and 150 mM) as interactive treatments in addition to the control treatment. The pH value was adjusted to 5.8 using pH meter. NaCl powder and 20 ml of the five-fold serial dilution of each of the three signaling molecules (SA, BA and *p*-CA) were combined with 180 ml of MS medium and poured in plant tissue culture glass jars. Culture glass bottles containing growth media were sterilized at 121ºC for 15 min using autoclave. Then jars were moved to the plant tissue culture room for planting.

2.3 Planting

In vitro cultivated tobacco stems were cut into 3 cm segments. Each three explants were cultivated on the culture medium. All culture jars were maintained at 28ºC under light for 16 hr. (800 uM/m2/s PFD) and dark for 8 hr. in aseptic condition for 50 days. Then measurements of the observed data were taken. The experiment was replicated three times.

2.4 Growth (Fresh weight) Determination

Tobacco growth was estimated by measuring fresh weights of tobacco roots, leaves and stems, and then total fresh weight of tobacco was calculated. To measure the fresh weight of tobacco, 50 days old tobacco plants were smoothly uprooted from the artificial medium and separated into roots, leaves and stems using sterilized forceps and scissors. Each of the three parts of the plant was put in petri dishes then fresh masses were measured using a digital balance. Total fresh weight was calculated by accumulating the fresh weight of the three separated parts of the plant.

2.5 Chlorophyll Content Determination

Chlorophyll was estimated by adopting the method described by Inskeep and Bloom 1985 [21]. Three fresh fully expanded leaves were extracted overnight with *N, N*-Dimethylformamide solution at 4ºC and then the extract was centrifuged at 8000x g for 5 min. The absorbance of the supernatant was spectrophotometrically read at 647 and 664.5 nm. The following equations were used to estimate the concentration of total chlorophyll from the supernatant.

$$\text{Total chlorophyll (mg/g fw)} = 17.90\ A_{647} + 8.08\ A_{664.5}$$

2.6 Tobacco Leaf and Stem Proline Contents Determination

Proline contents were determined according to the method described by Bates et al. [22]. Using liquid nitrogen tobacco leaf and stem samples were well powdered. 0.5 g of each sample was used for the measurement. Leaf and stem materials (0.5 g) were homogenized in 10 ml of 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 8,000x g for 5 min. 2 ml of the supernatant was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in test tubes. 6 ml of the mixture was placed in a water bath for 1 h at 100ºC. The reaction mixture was extracted with 4 ml toluene, and then cooled to room temperature, and the absorbance was read at 520 nm with a spectrophotometer apparatus. Appropriate proline standards were included for the calculation of proline in the samples.

2.7 Tobacco Leaf and Stem Vitamin C Determination

The content of tobacco leaf and stem vitamin contents were determined according to the method of Jagota and Dani 1982 [23]. Tobacco leaf extracts (0.5 ml) were added to 0.8 ml of 10% TCA and vigorously shaken and the mixtures were kept in ice box for 5 min and then centrifuged at 3000 x g for 5 min. 0.5 ml of this extract was then diluted to 2 ml with 1.5 ml distilled water. The 2 ml sample was added to 2 ml Folin phenol reagent was then vigorously shaken. Stored at room temperature for 10 min, then using microplate the absorbance was measured at 760 nm against distilled water as a blank and the vitamin C content was estimated

through the calibration curve of ascorbic acid. Unless specified otherwise, all aforementioned processes were done at 4ºC.

2.8 Statistical Analysis

The experiment was replicated three times. Results are presented as the mean ± Standard Error (SE). Significant differences were determined by one-way analysis of variance (ANOVA) using SPSS 12.0 software.

3. RESULTS

3.1 Fresh Weight (Growth)

Plant growth was significantly reduced when tobacco plants were subjected to high levels of salinity stress concentrations. Specially, fresh weights of tobacco root and stem were remarkably decreased in the presence of NaCl. The maximum reduction was observed from the treatment of 150 mM NaCl. As seen in Fig. 1, the application of SA and BA increased growth of salt-stressed tobacco, while salinity reduced the growth compared to the control. Results of tobacco growth in Fig. 1 were justified and confirmed by measuring fresh weights of tobacco roots, leaves and stems. Leaf fresh weight was increased by the exogenous application of both SA and BA supplemented with 50 and 100 mM of NaCl concentrations. While *p*-CA application didn't show significant differences in tobacco fresh weight compared to the control and salinity induced plants. However, maximum leaf fresh weight was observed in the treatment of BA and SA under 150 mM NaCl concentration as compared to control (Fig. 2).

3.2 Chlorophyll Contents

The chlorophyll contents were remarkably decreased in NaCl stressed plants. The maximum inhibition was observed when plants were exposed to the highest concentration of salt (150 mM NaCl), where the content of chlorophyll was reduced by 33.4% compared to the control, while supplementation of SA increased chlorophyll content in 100 mM NaCl induced plants. Compared to the control, the highest chlorophyll content was observed in SA treated plants under non-saline condition, while the lowest chlorophyll content was observed in 150 mM NaCl supplemented with *p*-CA treatment. Nevertheless, SA and BA treatments showed significant differences in non-saline, 50 mM and 100 mM NaCl conditions, while *P*-CA did not increase tobacco chlorophyll contents under all various concentrations of NaCl (Fig. 3).

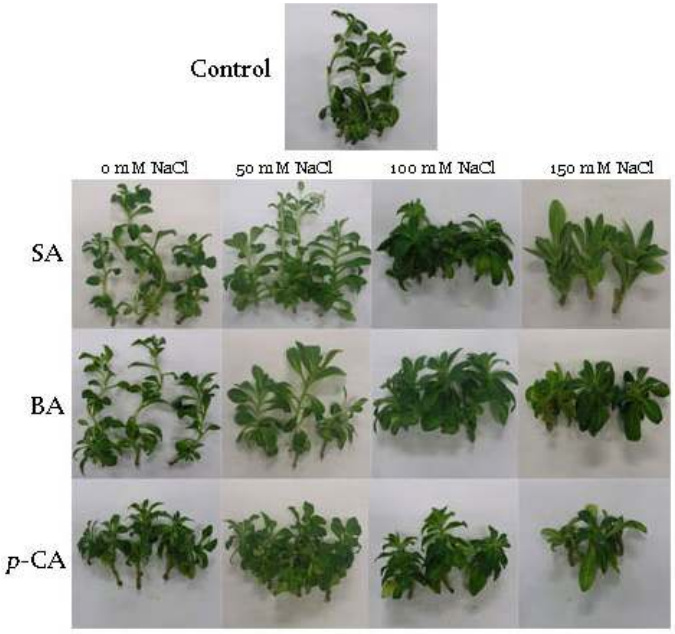

Fig. 1. Effects of SA, BA and *p*-CA on growth of salinity stressed-tobacco plants. Tobacco plants were maintained at 28℃ under light for 16 hr (800 uM/m2/s PFD) and dark for 8 hr

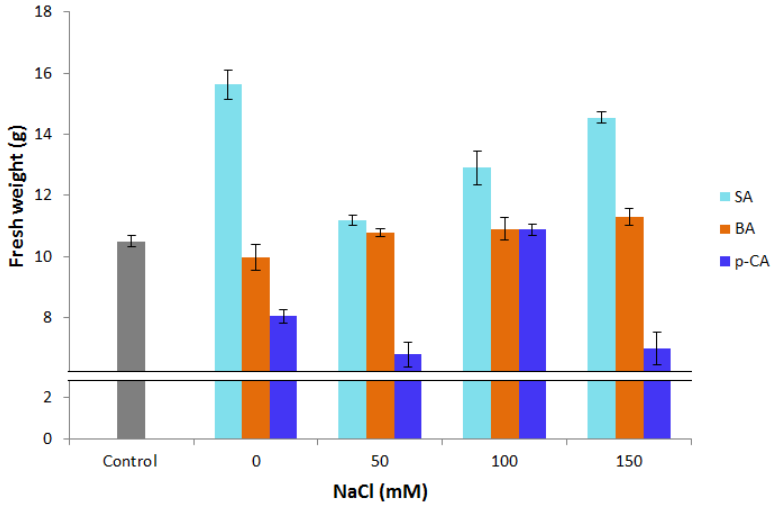

Fig. 2. Effects of SA, BA and *p*-CA on fresh weight of salinity-stressed tobacco plants. The bars indicate Standard Errors (SE)

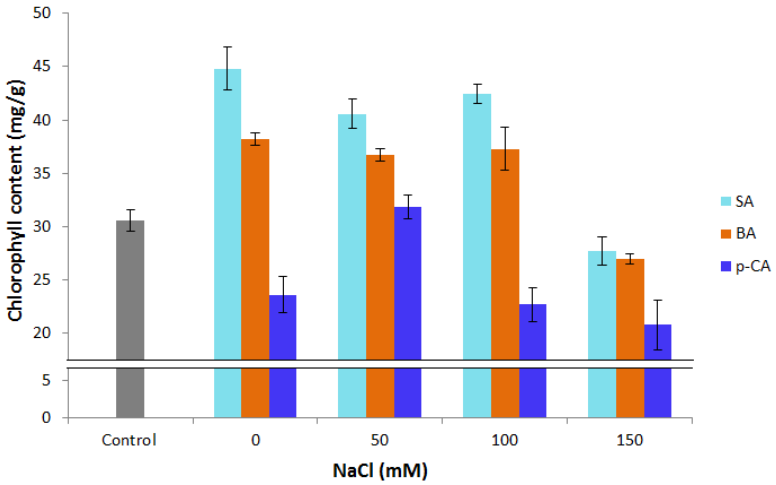

Fig. 3. Effects of SA, BA and p-CA on total chlorophyll content of salinity-stressed tobacco plants. The bars indicate Standard Errors (SE)

3.3 Proline Contents

Tobacco leaf and stem proline contents were hugely retarded by salt stress and increased by SA and BA supplementations. The stress of salinity significantly decreased tobacco leaf proline content at 0, 50 and 100 mM NaCl concentrations. Maximum reduction was observed at 100 mM NaCl concentration. Exogenous application of SA and BA increased proline content in 150 mM NaCl concentration. And the highest proline content was observed in treatment of 150 mM NaCl combined with 10^{-5} mM SA, which was 58% higher than the control. Application of *p*-CA did not increase leaf proline content of tobacco under both non-saline and saline conditions (Fig. 4). Tobacco stem proline content was significantly affected by salinity stress. Supplementation of SA remarkably increased tobacco stem proline content especially at 150 mM NaCl concentration which was higher than the control. BA treatment substantially increased proline content at 100 and 150 mM NaCl concentrations. Maximum proline content was recorded from the treatment of SA complemented with 150 mM NaCl. Both benzoic acid and salicylic acid improved tobacco salinity tolerance under elevated levels of NaCl as compared to the control. Whilst there was no significant differences observed in *P*-CA supplemented plants (Fig. 5).

3.4 Vitamin C Content

Vitamin is one of the most important components. In the present study the three signaling molecules SA, BA and *p*-CA hugely increased tobacco leaf and stem vitamin contents under all different levels of salinity. Tobacco leaf vitamin C content was not significantly affected by the stress of salt. The applications of SA and BA treatments under 50 mM and 150 mM NaCl concentrations increased leaf vitamin C contents compared to the control. The maximum leaf vitamin C contents were recorded from the treatments of SA under 50 mM and 150 mM of NaCl, while the lowest content was observed in *p*-CA treatment under non-saline condition. The exogenous application of *p*-CA slightly reduced the contents of vitamin C in NaCl stressed plants (Fig. 6). However, Salt stress did not show significant reduction of stem vitamin content. When compared to the control, SA application increased stem proline especially at high concentrations of NaCl. Maximum stem vitamin C content was observed in the treatment of SA under both 100 mM and 150 mM NaCl concentrations. Compared to NaCl treated plants, *p*-CA did not show significant differences under all various levels of salinity except 100 mM NaCl (Fig. 7).

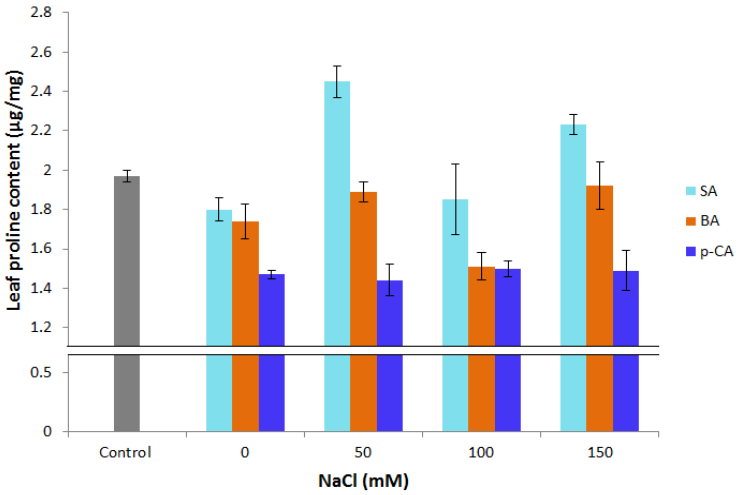

Fig. 4. Effects of SA, BA and *p*-CA on leaf proline contents in salinity-stressed tobacco plants. The bars mean Standard Errors (SE)

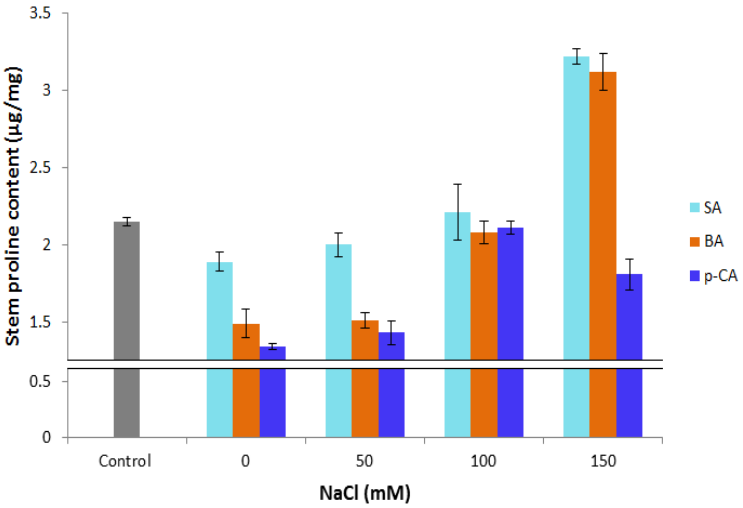

Fig. 5. Effects of SA, BA and *p*-CA on stem proline contents in salinity-stressed tobacco plants. The bars indicate Standard Errors (SE)

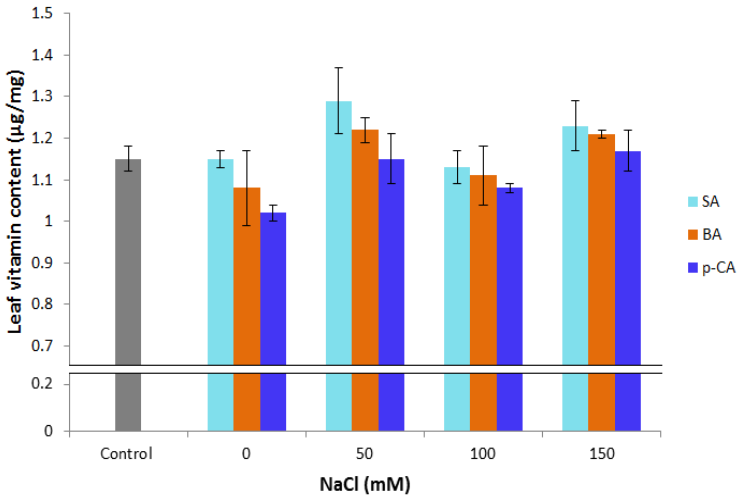

Fig. 6. Effects of SA, BA and *p*-CA on leaf vitamin C contents in salinity-stressed tobacco plants. The bars indicate standard errors (SE)

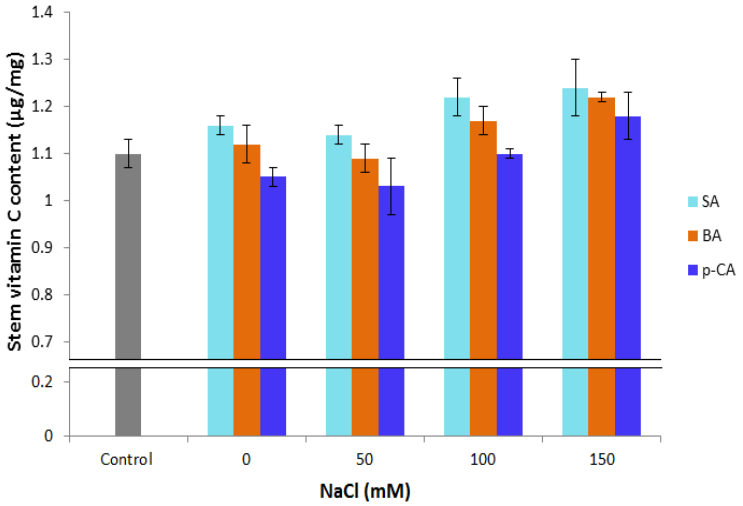

Fig. 7. Effects of SA, BA and *p*-CA on stem vitamin C contents in salinity-stressed tobacco plants. The bars indicate Standard Errors (SE)

4. DISCUSSION

Salinity is an important growth-limiting abiotic stress factor. SA, BA, and *p*-CA are substances generally thought to play a crucial role in the enhancement of salt tolerance in plants. The present research was conducted to study the influence of signaling molecules SA, BA, and *p*-CA on salt stressed tobacco physiological and biochemical characterizations. The results showed that by the increase in salinity there was decrease in all investigated parameters. Growth parameters (fresh weight of tobacco leaf, stem and root) were affected by high salt concentrations. These results are similar to those reported by Afzal et al. [24] who found that wheat fresh weight was reduced by salt stress. As well chlorophyll content, leaf and stem proline contents and vitamin contents of tobacco leaf and stem were also decreased in the presence of NaCl. Exogenous application of signaling molecules (SA, BA, and *p*-CA) had ameliorative impacts under saline conditions and enhanced tobacco salt tolerance. Tobacco plant growth, leaf and stem fresh weights were increased by SA and BA exogenous application under high concentrations of salt, 100 and 150 mM NaCl conditions. These results are in agreement with Gunes et al. [25] who observed high fresh weight by application of SA under salinity in maize plant.

Similar results have been also reported by Misra and Saxena [26] and Khodary [27] in maize plant and El-Tayeb [28] in barely plant. This may indicate that exogenous application of SA and BA exhibited a significant increase in salinity tolerance. *p*-CA didn't show significant differences in fresh weight of tobacco plants and there are no previous findings. Chlorophyll content of NaCl-treated tobacco plants was significantly decreased below that of the control. Similarly, Dela-Rosa and Maiti [29] found an inhibition in chlorophyll biosynthesis in sorghum plants due to salinity stress. Application of SA and BA increased chlorophyll content under high salinity levels 100 mM and 150 mM NaCl. Whereas, in the presence of 10^{-5} mM SA and BA treatments the damage generated by salt stress was recovered. These results are in agreement with Zhao et al. [30] and Sinha et al. [31] who pointed out that chlorophyll and carotenoid contents of maize leaves were increased upon treatment with SA. SA is supposed to enhance the functional state of the photosynthetic machinery in plants by chlorophyll biosynthesis [32]. Previous author's results support our findings. Proline is an important osmolyte and one of the components of plant defiance system to counter stresses generated by salinity, increasing proline leads to increase in salinity stress resistance. Proline is accumulated as a final product and the source of proline is total protein and total amino acid [33]. Our data showed that the accumulation of proline increased in the stems of 150 mM NaCl stressed tobacco plants by application of SA and BA. These results are in agreement with Tasgin et al. [34] who reported that the proline accumulation by SA treatment increases in wheat, oat, bean and tomato, under salt and oxidative stresses. Similar results were reported by Desnigh and Kanagaraj [35] who observed that the more tolerant plants store more proline. Leaf proline content was also increased by application of SA and BA to tobacco plants subjected to high salinity level. The highest content of leaf proline was observed in the treatment of both SA and BA under 150 mM NaCl. Similar results were observed by Shakirova and Sakhabutdinova [36], who figured out that treatment of SA under salt stress increases proline and defensive proteins. In the present study both SA and BA didn't increase proline content under low salt levels, significant levels of proline were only reported under 150 mM NaCl. While exogenous application of *p*-CA didn't increase both stem and leaf proline contents under the four different NaCl levels. Proline was not detected in tobacco roots.

Tobacco leaf and stem vitamin C contents were not significantly affected by salinity. Compared to the control, the exogenous application of SA, BA increased stem and leaf vitamin C contents under all salinity levels. Maximum stem vitamin C amounts were observed in the treatments of SA combined with 100 mM and 150 mM NaCl, while the highest contents of leaf vitamin C were recorded from the treatments of SA and BA under non-saline and 50 mM NaCl. The application of *p*-CA did not increase tobacco leaf and stem vitamin C contents under both saline and non-saline conditions. These results are similar to those reported by Mahdi Javaheri et al. [37] who reported an increase of productivity, lycopene, and vitamin C content of tomato fruit and by SA application under salinity conditions. Almost all previously documented findings support our results. Vitamin C content could not be detected in tobacco roots.

5. CONCLUSION

SA, BA and *p*-CA, can be used to alleviate salinity toxicity in plants. And enhance salt-tolerance through increasing some important physiological and biochemical characteristics such as contents of chlorophyll, proline and vitamin C.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Greenway H, Munns R. Mechanisms of salt tolerance in nonhalophytes. Ann. Rev. Plant Physiol. 1980;31:149-190.
2. Kirst GO. Salinity tolerance of eukaryotic marine algae. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1989;40:21-53.
3. Parida AK, Das AB. Salt tolerance and salinity effects on plants. Rev. Ecotoxi. Environ. Safety. 2005;60:324-349.
4. Pitman MG, Läuchli A. Global impact of salinity and agricultural ecosystems, In: Läuchli A, Luttge U. (eds.), Salinity: Environment Plants Molecules. Kluwer

Academic Publishers: Dordrecht, the Netherlands. 2002;3-20.
5. Kaneshiro ES, Holz GG Jr, Dunham PB. Osmoregulation in a marine ciliate, *Miamiensis avidus*. II. Regulation of intracellular free amino acids. Bio. Bull. 1969;137:161-169.
6. Mauro ML, Trovato M, De Paolis A, Gallelli A, Costantino P, Altamura MM. The plant oncogene rolD stimulates flowering in transgenic tobacco plants. Devel. Bio. 1996;180:693-700.
7. Conklin PL, Barth C. Ascorbic acid, a familiar small molecule intertwined in the response of plants to ozone, pathogens, and the onset of senescence. Plant Cell Environ. 2004;27:959-970.
8. Simaei M, Khavarinejad RA, Saadatmand S, Bernard F, Fahimi H. Interactive effects of salicylic acid and nitric oxide on soybean plants under NaCl salinity. Russian J. Plant Physiol. 2011;58:783-790.
9. Horvath E, Szalai G, Janda T. Induction of abiotic stress tolerance by salicylic acid signaling. J. Plant Growth Reg. 2007;26: 290-300.
10. Raskin I. The role of salicylic acid in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 1992;43:439-463.
11. Zang LY, Cosma G, Gardner H, Shi X, Castranova V, Vallyathan V. Effect of antioxidant protection by p-coumaric acid on low-density lipoprotein cholesterol oxidation. American J. Physiol. Cell Physiol. 2000;279:954-960.
12. Park SK, Park JC. Antimicrobial activity of extracts and coumaric acid isolated from Artemisia princeps var. orientalis. Korean J. Biotech. Bioeng. 1994;5:506-511.
13. Hudson EA, Dinh PA, Kokubun T, Simmonds MS, Gescher A. Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiol Biomark. Prev. 2000;9:1163–1170.
14. Ferguson LR, Zhu ST, Harris PJ. Antioxidant and antigenotoxic effect of plant cell wall hydroxycinnamic acids in cultured HT-29 cell. Mol. Nutr. Food Res. 2005;49:585-593.
15. An SM, Lee SI, Choi SW, Moon SW, Boo YC. *p*-Coumaric acid, a constituent of Sasa quelpaertensis Nakai, inhibits cellular melanogenesis stimulated by a-melanocyte stimulating hormone. J. Dermatol. 2008;159:292-299.
16. Ribnicky DM, Shulaev V, Raskin I. Intermediates of salicylic acid biosynthesis in tobacco. Plant Physiol. 1998;118:565-572.
17. Shakirova FM, Bezrukova MV. Induction of wheat resistance against environmental salinization by salicylic acid. Biol. Bulletin. 1997;24:109-112.
18. Borsani O, Valpuesta V, Botella MA. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol. 2001;126:1024-1030.
19. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-497.
20. Kim SA, Roh KS. Effect of p-coumaric acid, benzoic acid, and salicylic acid on the activity of glutathione reductase and catalase in in vitro grown tobacco plant. J. Life Sci. 2014;24:227-233.
21. Inskeep WP, Bloom PR. Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. Plant Physiol. 1985;77:483-485.
22. Bates L, Waldren RP, Teare, ID. Rapid determination of free proline for water-stress studies. Plant Sci. 1973;39:205-207.
23. Jagota SK, Dani HM. A new calorimetric technique for the estimation of vitamin C using folin phenol reagent. Anal. Biochem. 1982;127:178-182.
24. Afzal IM, Shahzad BN, Ahmad MF. Optimization of hormonal priming techniques for alleviation of salinity stress in wheat. Biol. Res. 2005;17:95-109.
25. Gunes A, Inal M, Alpaslan F, Eraslan E, Bagci G, Cicek N. Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. J. Plant Physiol. 2007;164:728-736.
26. Misra N, Saxena P. Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. Plant Sci. 2009;177:181-189.
27. Khodary SEA. Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. Intl. J. Agri. Biol. 2004;6:5-8.
28. El-Tayeb MA, Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Reg. 2005;45:215–224.

29. Dela-Rosa IM, Maiti RK. Biochemical mechanism in glossy sorghum lines for resistance to salinity stress. J. Plant Physiol. 1995;146:515-9.
30. Zhao HJ, Lin XW, Shi, HZ, Chang, SM. The regulating effects of phenolic compounds on the physiological characteristics and yield of soybeans. Acta Agro. 1995;21:351–355.
31. Sinha SK, Srivastava HS, Tripathi RD. Influence of some growth regulators and cations on inhibition of chlorophyll biosynthesis by lead in maize. Bull. Env. Contamin. Toxic. 1993;51:241-246.
32. Shi Q, Bao Z, Zhu Z, Ying Q, Qian Q. Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of *Cucumis sativa* L. Plant Growth Regul. 2006;48:127-135.
33. Sairam RK, Deshmakh PS, Saxena DC. Role of antioxidant systems in wheat genotype tolerance to water stress. Plant Biol. 1998;41:387–394.
34. Tasgin E, Atici O, Nulbantoglu B, Popova, LP. Effects of salicylic acid and cold treatment on protein levels and on the activities of antioxidant enzymes in the apoplast of winter wheat leaves. Phytochem. 2006;67:710-715.
35. Desnigh R, Kanagaraj G. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. Gen. Appl. Plant Physiol. 2007; 33:221-234.
36. Shakirova FM, Sakhabutdinova DR. Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci. 2003;164:317-322.
37. Mahdi J, Kambiz M, Alireza D, Fateme ZT. Effects of salicylic acid on yield and quality characters of tomato fruit (*Lycopersicum esculentum* Mill.). Agri. Crop Sci. 2012;16:1184-118.

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