Influence of Salt Stress on Different Pepper Genotypes: Ion Homeostasis, Antioxidant Defense, and Secondary Metabolites

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Abstract: Salt stress, one of the most serious abiotic stresses, leads to a reduction in plant growth, development, and yield in many parts of the world. The purpose of this study was to determine the morphological, physiological, and biochemical salinity tolerance of nine local Turkish pepper genotypes and one variety. Greenhouse plants at the four-leaf stage were subjected to salt stress by adding a total of 150 mM NaCl to the nutrient solution over three days. The growth parameters, ion regulation, photosynthetic pigments, and antioxidative enzyme activities were investigated, as were the malondialdehyde, flavonoid, and phenolic contents. The growth parameters, K^+ and Ca^{2+} contents, and total chlorophyll and carotenoid contents decreased under salt stress. Conversely, the Na^+ and Cl^- contents and the total flavonoid and phenolic compounds increased under salt stress in all of the genotypes; lipid peroxidation also increased in all genotypes. Antioxidant enzyme activities, however, increased more under salt stress in the tolerant genotypes than it did in the less tolerant plants. The results show that genotypes BIB-6 and BIB-8 were more salt tolerant than the other genotypes and have high potential as genetic material in future breeding programs.

Keywords: *Capsicum annumm*, Ion regulation, Malondialdehyde, Salinity, Superoxide dismutase.

INTRODUCTION

Although both biotic and abiotic stressors can reduce crop yield, abiotic stresses are the primary inhibitor; they limit potential production by up to 70% [1]. Salinity is a significant abiotic stress factor that threatens agriculture in both arid and semiarid environments and affects over 20% of the world's irrigated land [2]. Salt stress results in alteration of a plant's biochemical, physiological, and morphological responses and thus reduces growth, yield, biomass, and quality. Under salt stress, plants often experience water relation disturbances and develop a buildup of toxic ions. In response to salinity-induced osmotic stress, plants develop an osmotic stress tolerance by accumulating organic osmolytes or ions or both in order to maintain water absorption going on [3]. In addition, salt-tolerant plants may exclude toxic ions (Na^+, Cl^-, etc.) to the apoplast or sequester them in the vacuole in order to avoid saline-induced toxicity [3, 4]. Salinity causes excessive accumulation of reactive oxygen species (ROS), which may result in lipid peroxidation, protein oxidation, enzyme inactivation, or in damage to interactions with other essential plant cell components or to DNA [5]. High concentrations of salt may result in stomatal closure, which reduces the availability of carbon dioxide in the leaves and causes carbon fixation inhibition, resulting in exposure of chloroplasts to high levels of excitation energy [4, 5]. This leads to increased generation of ROS (including hydroxyl radical, hydrogen peroxide, singlet oxygen, and superoxide) [5]. In order to minimize the toxic effects caused by ROS, plants possess various kinds of enzymatic antioxidants, including ascorbate peroxidase (APX), catalase (CAT), glutathione reductase (GR), and superoxide dismutase (SOD); they also utilize nonenzymatic antioxidants (*e.g.*, ascorbate, carotenoids, reduced glutathione, proline, amides, gamma-aminobutyric acid, glycine betaine, and tocopherol) [4].

Pepper is considered one of the most important crops in the world and has been classified from moderately sensitive to sensitive to salt stress. However, there are also variations within the pepper species regarding salt tolerance and sensitivity [6, 7]. Screening plant species for salinity tolerance or the genetic potential to develop tolerance are promising approaches to developing salt-tolerant commercial cultivars. Thus, this study attempts to identify the tolerance level and biomarkers of various pepper genotypes, which will undoubtedly help breeding programs produce new genotypes with enhanced salt tolerance. These plants, because of their ability to overcome abiotic stresses, may be used to extend cultivated property in areas with a salinity problem [8]. This work evaluates the effects of salt stress on the

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morphological, physiological, and biochemical characteristics of plants and identifies the degree of salt tolerance of the studied genotypes.

MATERIAL AND METHODS

The experiments used nine pepper genotypes and a variety (BIB-10: Demre), the seeds of which were germinated in a 2:1 mixture of peat and perlite. 21 days after sowing (DAS), the seedlings were transferred to plastic pots (12 L) containing a 2:1 mixture of peat to perlite. They were then housed in a greenhouse with day/night temperatures of 26/18 ± 2 °C and a relative humidity of 65% ± 5. The seedlings were watered with nutrient solution, the composition of which (M) was as follows: $Ca(NO_3)_2$ $4H_2O$, $3.0x10^{-3}$; K_2SO_4, $0.90x10^{-3}$; $MgSO_47H_2O$, $1.0x10^{-3}$; KH_2PO_4, $0.2x10^{-3}$; H_3BO_3, $1.0x10^{-5}$; 10^{-4} FeEDTA, $MnSO_4$ H_2O, $1.0x10^{-6}$; $CuSO_45H_2O$, $1.0x10^{-}7$; $(NH)_6Mo7O_24H_2O$, $1.0x10^{-4}$; $ZnSO_4$ $7H_2O$, $1x10^{-4}$ [9]. Each pot contained four plants, and each experiment included four replications (16 plants of each genotype were used in each treatment) [10]. Starting at 37 DAS, a salt (NaCl) treatment was added to the irrigation solution in daily gradual increments of 50 mM NaCl; it reached 150 mM NaCl, the desired salt level, on the third day. The amount of water applied was calculated based on the ratio of water drained to water applied, and free drainage was utilized under the pots to prevent excessive salt accumulation. The water collected in the pots was measured with EC meters after each irrigation to ensure a constant dosage of EC. Both the salt-treated and untreated (control) plants were kept under these conditions for 27 days, at the end of which they were harvested for physiological examination (*i.e.*, fresh and dry shoot weights, the diameters, lengths, and number of leaves per plant, leaf area per plant, the amount of photosynthetic pigments, and the Na^+, K^+, Ca^{2+}, and Cl^- ion content) and biochemical analyses for total phenolic content (TPC), flavonoids, lipid peroxide content (malondialdehyde, MDA), APX, CAT, GR, and SOD. The third fully expanded leaf from each plant was used for the biochemical parameters.

Growth parameters: four randomly selected plants from the control and salt-stressed populations of each genotype were weighed with a digital balance (±0.0001 g) to obtain their fresh weights. Samples were then dried in an oven at 65°C for 48 h and reweighed to obtain dry weights. Leaf area was determined through use of a leaf area meter (CI BIO Science CI 202, CID, Camas, Washington, USA).

In order to determine ion content, leaf samples were dried in an oven at 65 °C. The concentrations of Na^+, K^+, and Ca^{2+} were determined using an Inductively Coupled Plasma Emission Spectrometer (ICP model Liberty 200, Varian Australia Pty. Ltd., Australia). Before the analysis, 50 mg of ground dry material was digested by adding 2 mL concentrated HNO_3 (65%) and 1 mL H_2O_2 (30%) for 30 min at 2600 kPa (80 psi) in a MDS-2100 microwave oven (CEM Corp., USA). After digestion, deionized water was added until each sample had a final volume of 25 mL. The Cl concentration in the dry tissue samples was determined using titrimetric analysis with silver nitrate ($AgNO_3$) according to the Mohr method [11].

The total phenolic content was determined using a Folin-Ciocalteu reagent. Gallic acid was used as a standard [12]. Colorimetric assay was used to establish the flavonoid content [13]. A mortar and pestle, along with an extraction buffer (5 mL) comprised of a potassium-phosphate buffer (50 mM, pH 7.6) and disodium ethylene diamine tetra acetate (0.1 mM), was used to extract the enzyme from 0.5 g of leaf tissue. After centrifugation of the homogenate at 15,000 × g for 15 minutes, the supernatant fraction was then used for the enzyme assay. The SOD, CAT, APX, and GR enzyme activities were determined according to Cakmak and Marschner [14]. The amount of MDA was determined via the thiobarbituric acid reaction, a measure of lipid peroxidation [15].

Regarding statistical analysis, the experimental plot design was randomized and included four replications. A comparison of the control and treated mean values was performed via the least significant difference test. Statistical significance was determined as $P < 0.05$ using JMP statistical software, ver. 5.1 (SAS Institute Inc., USA).

RESULTS

In comparison with controls, plants treated with NaCl showed reduced fresh and dry shoot weights, lengths, and diameters, diminished leaf numbers, and reduced leaf area average of genotypes by 50%, 43%, 38%, 22%, 36%, and 35%, respectively (Figure **1**). The pepper genotypes exhibited differences in their responses to salinity that were statistically significant: the fresh and dry weights were decreased by 12% and 28% in BIB-6 and by 19% and 13% in BIB-2; the decrease in fresh and dry weight reached 76% in genotype BIB-8 and 55% in genotype BIB-10. The decrease in shoot height and diameter also varied. These decreases were lowest in genotypes BIB-6

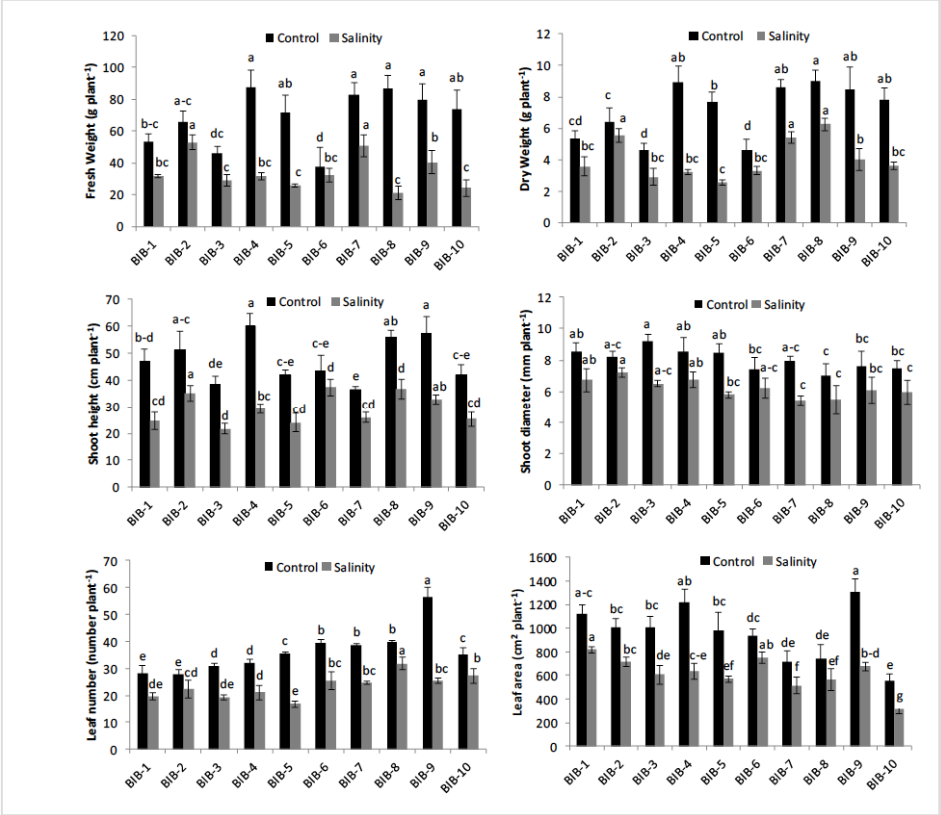

Figure 1: Change in the growth parameters of pepper genotypes grown under control and salt treatment. Each value is the mean of four replicates ± SD. For each parameter, different letters represent statistically significant differences at $P < 0.05$ according to LSD test.

(14%) and BIB-7 (28%) and were highest in BIB-4 (51%) and in BIB-1 (47%).

In comparison to the untreated plants, the average Cl^- and Na^+ contents in salt-treated plants increased by 1016% and 1686%, respectively (Figure **2**). Genotypes that were selective for toxic Na^+ were BIB-7 (increase of 658%), BIB-6 (increase of 1119%), and BIB-2 (increase of 1305%). Genotypes with the highest Na^+ contents and highest variation were BIB-9 (increase of 3493.33%), BIB-4 (increase of 3111.76%) and BIB-3 (increase of 1859.09%). The lowest Cl^- content was obtained in genotypes BIB-6 and BIB-8 (Figure **2**). Salt treatment also decreased K^+ and Ca^{2+} contents in the pepper genotypes when compared with the controls: while the K^+ and Ca^{2+} decreased 5%–11% in genotypes BIB-2, BIB-6, and BIB-8, the reduction ranged between 54%–69% in genotypes BIB-5, BIB-9, and BIB-10. Following salinity stress, BIB-6 and BIB-8 had significantly higher K/Na and Ca/Na ratios than did other genotypes (Figure **2**).

Total chlorophyll and carotenoid contents decreased with salinity. Among the ten studied genotypes, the smallest reductions were observed in BIB-8 (16% and 6% decreases) and BIB-6 (22% and 8% decreases) (Figure **3**). The sharpest declines were observed in BIB-4 (56% and 67%), BIB-5 (48% and 61%), BIB-9 (46% and 70%), and BIB-10 (41% and 67%).

Salt-induced oxidative stress was confirmed through measurement of MDA level, which is indicative of membrane lipid peroxidation (Figure **3**). A significant increase in its content was averaged by 14.9 µmol g^{-1} fresh weight (205% increase) relative to the controls. The highest increase was shown in genotypes BIB-9, BIB-3, and BIB-10 at 374%, 337%, and 305%, respectively. The salt-triggered MDA content was lower in genotypes BIB-6, BIB-7, and BIB-8 than in other genotypes; it ranged between 80%–124%.

Under salt stress, total phenolic and flavonoid contents were increased by 32% and 31%, respectively (Figure **3**). The highest production of these nonenzymatic antioxidants was shown in genotypes BIB-8 (58% and 62%) and BIB-6 (55% and 40%), whereas their levels remained between 1% and 4% in genotypes BIB-4, BIB-9, and BIB-10. The activities of the antioxidant enzymes (APX, CAT, GR, and SOD) are presented in Figure **4**. The maximum mean values were obtained from the salt-stressed BIB-8 genotype.

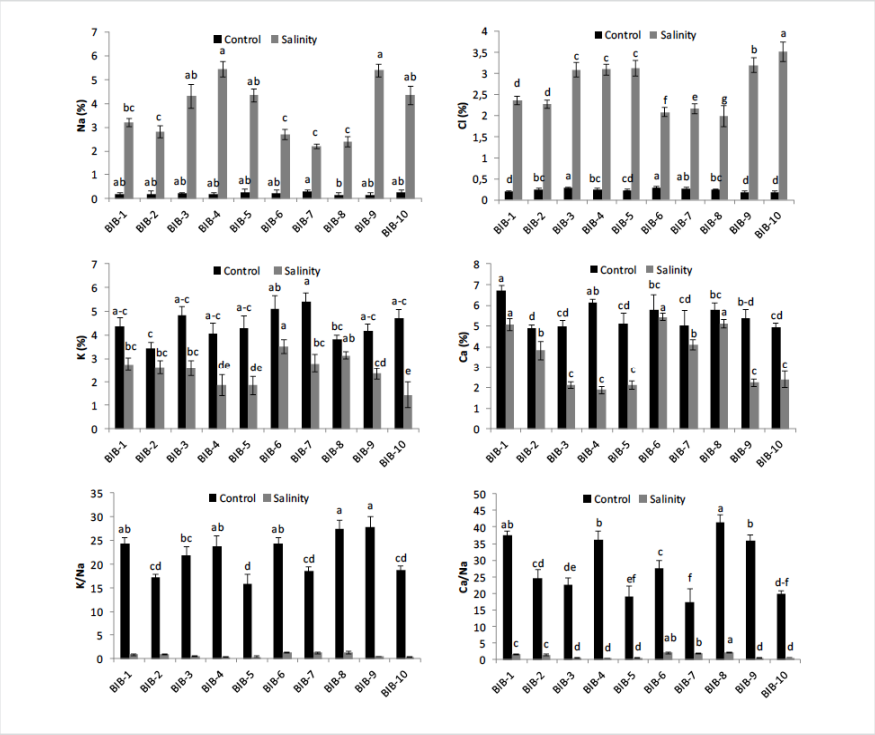

Figure 2: Change in Na, Cl, K, Ca, K/Na, and Ca/Na content of pepper genotypes grown under control and salt stress. Each value is the mean of four replicates ± SD. For each parameter, different letters represent statistically significant differences at P <0.05 according to LSD test.

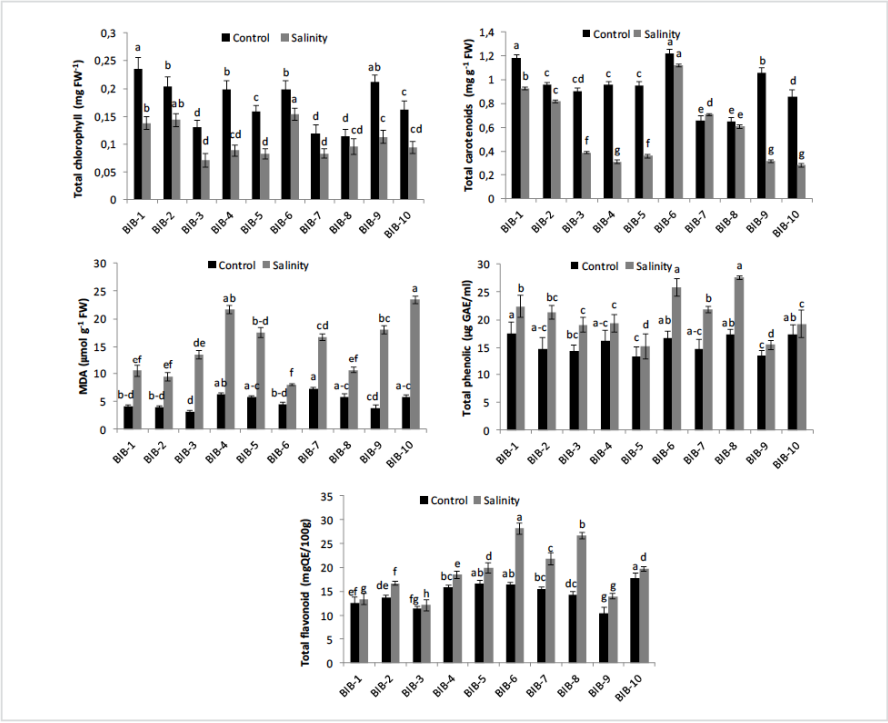

Figure 3: Change in total chlorophyll and carotenoids, MDA, total phenolic and flavonoid contents of pepper genotypes grown under control and salt stress. Each value is the mean of four replicates ± SD. For each parameter, different letters represent statistically significant differences at P <0.05 according to LSD test.

SOD, CAT, GR, and APX activity in plants treated with saline increased by 323%, 356%, 454%, and 267%, respectively, when compared with the controls.

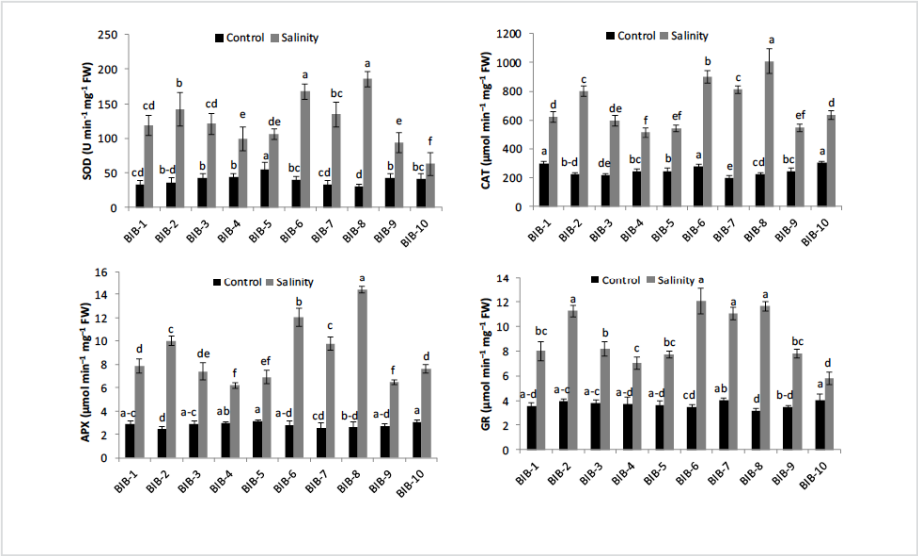

Figure 4: Change in SOD, CAT, APX and GR activities of pepper genotypes grown under control and salt stress. Each value is the mean of four replicates ± SD. For each parameter, different letters represent statistically significant differences at $P < 0.05$ according to LSD test.

DISCUSSION

Twenty-seven days of salt stress significantly affected the growth characteristics of the ten studied pepper genotypes; in accord with previous publication, the response varied by genotype [8]. The lowest reduction in the measured growth parameters (highest relative salt tolerance) was observed in the BIB-6 and BIB-8 genotypes. In contrast, BIB-4, BIB-9, and BIB-10 exhibited the greatest reduction. High salinity affects two main mechanisms in plants. One, it disturbs the capacity of roots to extract water from the soil (*i.e*. osmotic stress), which in turn results in a reduction of growth parameters such as fresh mass. Two, high concentrations of toxic ions in cells result in an inhibition of many physiological and biochemical processes, such as nutrient uptake and assimilation as well as photosynthesis, which reduces plant growth, development, odds of survival [16].

In support of our interpretation, genotypes BIB-3, BIB-4, and BIB-9 had the highest Na^+ accumulation, while BIB-6 and BIB-8 showed the lowest Cl^- content under salt stress; these findings confirm the potential salt tolerance of the latter two genotypes. Salt induced a significant Na^+ and Cl^- influx in the salt-sensitive genotypes, which most probably caused their decreased growth [4, 5, 17]. Although salt stress reduced the uptake of K^+ and Ca^{2+} in the studied pepper genotypes, the reduction of both beneficial elements was lower in BIB-6 and in BIB-8. Higher uptake and accumulation of K^+ in the presence of salinity is regarded as a sign of increased tolerance to salt. This is because K^+ plays an important role in stomatal aperture mechanics, osmoregulation, and the prevention of Na^+ influx into roots and shoots [18]. Increased uptake of K^+ led to a higher K/Na ratio in response to NaCl treatment in BIB-6 and in BIB-8; this ratio has also been reported as a determinant of salt tolerance [19]. Calcium plays an important role in several physiological plant processes, such as cell membrane protection, ion transport, and the translocation of carbohydrates and proteins as well as their storage during seed formation [18]. Plants with the capability to take up more Ca^{2+} from the growth medium, therefore, have higher Ca/Na ratios, a trait that is also correlated with successful adaptation to saline environments [18, 20].

Salt treatment led to an overall reduction in the photosynthetic pigments, chlorophylls, and carotenoids in the ten pepper genotypes. The decreased chlorophyll content might be due to salt-induced increases in the activity of the chlorophyll degrading enzyme, chlorophylase [21]. Carotenoid content is also an aspect of salt tolerance; carotenoids play a critical role in light harvesting and oxidative damage protection by deactivating singlet oxygen, satisfactorily meeting the chlorophyll excited triplet state, and enhancing carotenoid synthesis in order to reduce photo damage caused by the salt-induced arrest of cell division [22, 23]. In the genotypes BIB-6 and BIB-8, the chlorophyll and carotenoid contents were less affected by the NaCl treatment.

Salt stress results in the formation of ROS that cause irreversible lipid and protein damage. Levels of lipid peroxidation, an indicator of oxidative stress, were

lower in BIB-6 and BIB-8 than in the other genotypes under salt stress. These decreased levels may be imputed to varieties as a result of their genotypic ability to scavenge ROS and protect against oxidative damage [24]. This assumption is in agreement with the higher antioxidant molecules and enzyme activities, discussed below, that were observed in both genotypes.

In general, phenolic compounds in plants are produced through the phenylpropanoid pathway, and they can be induced by environmental stresses and elicitors [10]. Mansori *et al*. [25] reported that polyphenols represent a large family of plant secondary metabolites and that they may act as antioxidants to protect against oxidative stress. Therefore, the observed increase in the total phenolic and flavonoid contents after salt stress, which was most marked in the BIB-6 and BIB-8 genotypes, can be explained by enzyme activation linked to their biosynthesis and may be an aspect of the salt tolerance of those genotypes.

A direct consequence of salinity stress in plants is the induction of antioxidant enzyme activities to minimize the damage caused by ROS [26]. Increased accumulation of ROS pose a challenge to the plant cells by inducing the peroxidation of lipids and proteins, the breakage of nucleic acids, limited efficiency of enzymes, and programmed cell death, which altogether eventually results in complete cell death [27, 28]. For plants to combat these adverse effects and minimize ROS levels in response to salt stress, they elevate antioxidant enzymatic expression and activity (including SOD, GR, POX, APX, and CAT) as well as produce enhanced levels of nonenzymatic compounds [29]. Several studies have demonstrated the relationship between tolerance to salinity and increased antioxidant enzyme activity [4, 30]. The activities of SOD, APX, GR, and CAT were more highly elevated in the BIB-6 and BIB-8 genotypes (177%–530%) than in the other salt-treated genotypes. It appears that BIB-6 and BIB-8 induce antioxidant enzymes more efficiently in order to mitigate oxidative stress and lipid peroxidation, which consequently reduces the growth inhibition triggered under saline conditions. Likewise, Mishra *et al*. [31] proposed that a higher antioxidant redox status and regulated elevation of the levels of GR, GPX, CAT, APX, and SOD activities could function as important predictive factors of tolerance to salinity amongst Indica rice seedlings. Further, Hand *et al*. [32] indicated that salt-tolerant pepper cultivars induce the antioxidative enzyme system more efficiently in response to salinity stress.

In conclusion, salt stress caused stunted growth, reduced photosynthetic pigment, elevated Na^+ and Cl^+ concentrations, and increased oxidative stress in the ten studied pepper genotypes. However, these harmful effects were much lower in the genotypes BIB-6 and BIB-8. Additionally, these two genotypes exhibited higher uptake amounts of K^+ and Ca^{2+}, enhanced levels of total phenolic compounds and flavonoids, increased induced antioxidant enzyme activity, and lower MDA content. Overall, these responses established BIB-6 and BIB-8 as the most salt-tolerant genotypes. This suggests their potential for cultivation under salt stress as well as their suitability for use as germplasm material in future pepper breeding programs.

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REFERENCES

[1] Yamauchi Y. Integrated Chemical Control of Abiotic Stress Tolerance Using Biostimulants. In: Andjelkovic V, Eds. Plant, Abiotic Stress and Responses to Climate Change. London, UK: Intech 2018; pp. 133-143. https://doi.org/10.5772/intechopen.74214

[2] Wu GQ, Feng RJ, Li SJ, Du YY. Exogenous application of proline alleviates salt-induced toxicity in sainfoin seedlings. The Journal of Animal & Plant Sciences 2017; 27(1): 246-251.

[3] Mansour, MMF, Salama KHA. (2019). Cellular mechanisms of plant salt tolerance. In: Strategies and Functions, Giri B, Varma A, Eds. Microorganisms in Saline Environments: Strategies and Functions Springer, Switzerland 2019; pp. 169-210. Springer, Cham. https://doi.org/10.1007/978-3-030-18975-4_8

[4] Rady MM, Taha SS, Kusvuran S. Integrative application of cyanobacteria and antioxidants improves common bean performance under saline conditions. Scientia Horticulturae 2018; 233: 61-69. https://doi.org/10.1016/j.scienta.2018.01.047

[5] Parihar P, Singh S, Singh R, Singh VP, Prasad SM. Effect of salinity stress on plants and its tolerance strategies: a review. Environmental Science and Pollution Research 2015; 22 (6): 4056-4075. https://doi.org/10.1007/s11356-014-3739-1

[6] Aktas H, Abak K, Cakmak I. Genotypic variation in the response of pepper to salinity. Scientia Horticulturae 2006; 110(3): 260-266. https://doi.org/10.1016/j.scienta.2006.07.017

[7] Niu G, Rodriguez DS, Call E, Bosland PW, Ulery A, Acosta E. Responses of eight chile peppers to saline water irrigation. Scientia Horticulturae 2010; 126(2): 215-222. https://doi.org/10.1016/j.scienta.2010.07.016

[8] Serrano LL, Penella C, San Bautista A, Galarza SL, Chover AC. Physiological changes of pepper accessions in response to salinity and water stress. Spanish Journal of Agricultural Research 2017; 15(3): 15. https://doi.org/10.5424/sjar/2017153-11147

[9] Dasgan HY, Koc S. Evaluation of salt tolerance in common bean genotypes by ion regulation and searching for screening parameters. J. Food Agric. Environ. 2009; 7(2): 363-372.

[10] Kusvuran S, Dasgan HY. Effects of drought stress on physiological and biochemicalchanges in Phaseolus vulgaris L. Legume Research-An International Journal 2017; 40(1): 55-62. https://doi.org/10.18805/lr.v0i0.7025

[11] Kacar B, Inal A. Plant Physiology. Ankara: Nobel Press 2008.

[12] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in Enzymolog 1999; 299: 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

[13] Molina-Quijada DMA, Medina-Juárez LA, González-Aguilar GA, Robles-Sánchez RM, Gámez-Meza N. Phenolic compounds and antioxidant activity of table grape skin from northwest Mexico. CyTA-Journal of Food 2010; 8: 57-63. https://doi.org/10.1080/19476330903146021

[14] Cakmak I, Marschner H. (1992). Magnesium deficiency and highlight intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiology 1992; 98: 1222-1226. https://doi.org/10.1104/pp.98.4.1222

[15] Heath RL, Packer L. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry Biophysics 1968; 125: 189-198. https://doi.org/10.1016/0003-9861(68)90654-1

[16] Carillo P, Annunziata MG, Pontecorvo G, Fugg A, Woodrow P. (2011). Salinity stress and salt tolerance. In: Shanker A, Venkasteswarlu B Eds. Abiotic stress in plants mechanism and adaptations. Reijeka: InTech Press 2011; pp. 21-38. https://doi.org/10.5772/22331

[17] Liang W, Ma X, Wan P, Liu L. Plant salt-tolerance mechanism: A review. Biochemical and Biophysical Research Communications 2018; 495(1): 286-291. https://doi.org/10.1016/j.bbrc.2017.11.043

[18] Kusvuran S. Ion regulation in different organs of melon (*Cucumis melo*) genotypes under salt stress. International Journal of Agriculture & Biology 2012; 14 (1): 141-144.

[19] Han Y, Yin S, Huang L, Wu X, Zeng J, Xiaohui L, Qiu L, Munns R, Chen Z, Zhang GP. (2018) A sodium transporter HvHKT1;1 confers salt tolerance in barley via regulating tissue and cell ion homeostasis. Plant Cell Physiology 2018; 59:1976-1989 https://doi.org/10.1093/pcp/pcy116

[20] Sönmez F, Gülser F. Effects of humic acid and $Ca(NO_3)_2$ on nutrient contents in pepper (Capsicum annuum) seedling under salt stress. Acta Agriculturae Scandinavica, Section B-Soil & Plant Science 2016; 66(7): 613-618. https://doi.org/10.1080/09064710.2016.1205654

[21] Sevengor S, Yasar F, Kusvuran S, Ellialtıoglu S. The effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidative enzymes of pumpkin seedling. African Journal of Agricultural Research 2011; 6(21): 4920-4924.

[22] Singh R, Upadhyay AK, Singh DP. Regulation of oxidative stress and mineral nutrient status by selenium in arsenic treated crop plant *Oryza sativa*. Ecotoxicology and Environmental Safety 2018a; 148: 105-113. https://doi.org/10.1016/j.ecoenv.2017.10.008

[23] Singh R, Upadhyay AK, Chandra P, Singh DP. (2018b). Sodium chloride incites reactive oxygen species in green algae Chlorococcum humicola and Chlorella vulgaris: implication on lipid synthesis, mineral nutrients and antioxidant system. Bioresource Technology 2018b; 270: 489-497. https://doi.org/10.1016/j.biortech.2018.09.065

[24] Talhouni M, Sönmez K, Kıran S, Beyaz R, Yıldız M, Kusvuran S, Ellialtıoğlu SS. Comparison of salinity effects on grafted and non-grafted eggplants in terms of ion accumulation, MDA content and antioxidative enzyme activities. Advances Horticulture Science 2019; 33(1): 87-95.

[25] Mansori M, Chernane H, Latique S, Benaliat A, Hsissou D, El Kaoua M. Seaweed extract effect on water deficit and antioxidative mechanisms in bean plants (*Phaseolus vulgaris* L.). Journal of Applied Phycology 2015; 27(4): 1689-1698. https://doi.org/10.1007/s10811-014-0455-7

[26] Amar T, Nourredine Y. Morphometric variability and biochemical analysis of growth seedlings under salt stress in tomato (*Lycopersicon esculentum* Mill.) cultivars. Molecular Plant Breeding 2016; 7(4): 1-9.

[27] Sharma C, Singh N, Pal K. The effect of salt stress on biochemicals of chilli at seedling level. International Journal Pharma. Prof. Research 2012; 3: 665-670.

[28] Butt M, Ayyub CM, Amjad M, Ahmad R. Proline application enhances growth of chilli by improving physiological and biochemical attributes under salt stress. Pakistan Journal of Agricultural Sciences 2016; 53(1): 43-49. https://doi.org/10.21162/PAKJAS/16.4623

[29] Wani AS, Ahmad A, Hayat S, Tahir I. Is foliar spray of proline sufficient for mitigation of salt stress in Brassica juncea cultivars?. Environmental Science and Pollution Research 2016; 23(13): 13413-13423. https://doi.org/10.1007/s11356-016-6533-4

[30] Roy PR, Tahjib-Ul-Arif M, Polash MAS, Hossen MZ, Hossain MA. Physiological mechanisms of exogenous calcium on alleviating salinity-induced stress in rice (*Oryza sativa* L.). Physiology and Molecular Biology of Plants 2019; 25(3): 611-624. https://doi.org/10.1007/s12298-019-00654-8

[31] Mishra P, Bhoomika K, Dubey RS. Differential responses of antioxidative defense system to prolonged salinity stress in salt-tolerant and salt-sensitive Indica rice (Oryza sativa L.) seedlings. Protoplasma 2013; 250(1): 3-19. https://doi.org/10.1007/s00709-011-0365-3

[32] Hand MJ, Taffouo VD, Nouck AE, Nyemene KP, Tonfack B, Meguekam TL, Youmbi E. Effects of salt stress on plant growth, nutrient partitioning, chlorophyll content, leaf relative water content, accumulation of osmolytes and antioxidant compounds in pepper (*Capsicum annuum* L.) cultivars. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 2017; 45(2): 481-490. https://doi.org/10.15835/nbha45210928

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