J. Agr. Sci. Tech. (2021) Vol. 23(5): 1091-1103

Physiological and Biochemical Responses of Four Genotypes of Common Bean (*Phaseolus vulgaris* L.) under Salt Stress

Z. Azimychetabi[1] and M. Sabokdast Nodehi[1*]

ABSTRACT

This investigation was conducted to determine the impact of salinity stress on some physiological and biochemical parameters of common bean (*Phaseolus vulgaris* L.) genotypes. Three levels of NaCl (0, 100, and 200 mM) were applied to four common bean genotypes. Chlorophyll content, Relative Water Content (RWC) of leaves, Electrolyte Leakage Index (ELI), Na^+ and K^+ concentrations, the K^+/Na^+ ratio, Malondialdehyde (MDA) content, total protein content, and proline concentration were determined and compared. Moreover, the activity of some antioxidant enzymes, including: Catalase (CAT), Ascorbate Peroxidase (APX), Polyphenol Oxidase (PPO), and Guaiacol Peroxidase (GPX) were analyzed. Content of chlorophyll a, chlorophyll b and carotenoid decreased by increasing the intensity of salinity stress along with the SPAD value. RWC dropped and ELI incremented by augmenting salinity together with the K^+/Na^+ ratio. The results revealed that MDA and proline concentrations significantly increased under the mentioned conditions. Activities of antioxidant defense enzymes were altered notably and total protein content decreased under salt stress. Consequently, genotypes Jules and 201 were found as, respectively, tolerant and semi-tolerant genotypes during this experiment.

Keywords: Antioxidative defense, Chlorophyll fluorescence, Oxidative stress, Proline, Salinity tolerance.

INTRODUCTION

Salinity stress is one of the major environmental stresses that limit the productivity and geographical distribution of plants. Salt stress not only diminishes the expected yield of crops, but also affects metabolic processes in plants through impairment of cell water potential, ion toxicity, membrane integrity and function, and uptake of indispensable mineral nutrients (Arzani and Ashraf, 2016).

One of the crucial biochemical changes befalling when plants are subjected to salt stress is the accumulation of Reactive Oxygen Species (ROS), which can provoke metabolic disorders such as oxidation of membrane lipids, proteins, and nucleic acids. Malondialdehyde (MDA) is a final product of the polyunsaturated fatty acids peroxidation in the cell membranes. An increment in the number of free radicals leads to overproduction of MDA, which is commonly known as a marker of oxidative stress. An increase the in the concentration of antioxidant enzymes including superoxide dismutase (SOD), Ascorbate Peroxidase (APX), Polyphenol Oxidase (PPO), and Catalase (CAT) that are of great significance for maintaining the amount of ROS within the cell (De Azevedo Neto *et al.*, 2006; Abdelaal *et al.*, 2014). Guaiacol Peroxidase (GPX) is another member of the enzymatic antioxidants, which withdraws spare amount of H_2O_2 either under a normal condition or stress (Das and Roychoudhury, 2014).

[1] Department of Agronomy and Plant Breeding, College of Agriculture & Natural Resources, University of Tehran, Karaj, Islamic Republic of Iran.
*Corresponding author; E-mail: sabokdast@ut.ac.ir

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

By being exposed to stressful conditions and confronting additional production and accumulation of ROSs as a consequence, plants accumulate an array of metabolites. Proline, the only amino acid (actually imino acid containing a secondary amine group), acts as an excellent osmolyte, an antioxidant, and a signaling molecule. Thereby, it maintains osmotic balance, membrane integrity, and concentrations of ROS within a normal range to inhibit oxidative burst in plants (Szabados and Savouré, 2010; Bhagavan and Ha, 2015).

Common bean (*Phaseolus vulgaris* L.) is the most prominent sustenance legume globally. It is sensitive to high soil salt and even low soil salt levels, which can notably impair crop yield (Szabados and Savouré, 2010). Enhanced knowledge concerning various aspects of salt tolerance will be advantageous in the cloning of the genes involved in salt tolerance, development of transgenic plants, and better breeding programs (Arzani and Ashraf, 2016). Moreover, evaluation of plants' ability to cope with salt stress may be more accurate if we analyze a combination of biochemical and physiological parameters and carry out the experiment under controlled conditions (Mudgal *et al.*, 2010). Central to the entire discipline of environmental stresses is the concept of alternations in the diverse characteristics of the plant. Despite physiological and biochemical responses of plants under salt stress being an object of research for decades, there have been little studies regarding the comparison of changes in these traits in Iranian genotypes. The objective of the present study was to investigate the physio-biochemical responses of four Iranian common bean genotypes under salt stress, to distinguish the changes occurring during stress and how the plants manage to alleviate the unfavorable conditions.

MATERIALS AND METHODS

Plant Material and Salt Stress Treatment

The experiment was carried out in the Research Greenhouse of the Department of Agriculture and Plant Breeding of the University of Tehran, Karaj, Iran (25°C, 75% relative humidity), in 2019. The experiment consisted of three salinity levels of zero (control), 100, and 200 mM (Torche *et al.*, 2018) and the second factor consisted of four common bean Iranian genotypes (Jules, Naz, 201 and 278), afforded by Gene Bank of the Department of Agronomy and Plant Breeding, University of Tehran (Sabokdast *et al.*, 2017, 2019). Table 1 gives the details of the genotypes used for this study. Experimental units consisted of pots 40×35 cm, filled with a mixture of peat soil and sand. Soil Electrical Conductivity (EC) was 0.8 dS m^{-1}. After disinfection, five seeds were sown at a depth of 3 cm and were regularly irrigated. When the seedlings grew enough, we removed two extra seedlings, leaving three. In the five-leaf stage, salinity stress was initiated with sodium chloride and all the pots, except the control, were irrigated by adding 25 mM NaCl for adaptation. In the two subsequent weeks, the salinity treatments were applied. Distilled water was used for irrigation of the control pots. After 15 days, sampling for determination of the desired traits began.

Measurements

The relative chlorophyll content in leaves was measured by a portable chlorophyll meter SPAD-MINOLTA-502 from three spots of the leaves to differentiate common bean genotypes under salt stress (Amirruddin *et al.*, 2020). The photosynthetic pigments were extracted by 80% (v/v) acetone as a solvent as stated by Arnon Method (Mbadi *et al*., 2015) . RWC of leaves was calculated according to the following equation to assess the water status

Table 1. Details of the genotypes used for this study.

Names of the genotypes	Accession
Jules	Jules
201	65-071-339
Naz	Naz
278	65-071-453

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

of plants (Pieczynski *et al*., 2013):

$$RWC\ (\%) = \frac{(fresh\ weight\ -\ dry\ weight)}{(turgid\ weight\ -\ dry\ weight)} \times 100$$

The electrolyte leakage was measured as described previously by Nazeri *et al.* (2012) to evaluate the cell membrane permeability of the plants. The Malondialdehyde (MDA) content was measured by thiobarbituric acid reaction utilizing a UV–Vis spectrophotometer (UV2550, Shimadzu Corporation, Kyoto, Japan) as explained before to determine lipid peroxidation under stress (Nazeri *et al*., 2012). Enzyme extraction and the supernatant collection for the determination of CAT (EC 1.11.1.6), APX (EC 1.11.1.11), GPX (EC 1.11.1.7) and PPO (EC 1.14.18.1) activity was conducted according to Bradford method at 4°C (Bradford, 1976). CAT activity was measured using the assay mixture formed of 0.1 mL of the enzyme extract, 0.1 mM phosphate buffer (pH 7.5), 0.1 mM EDTA, and 0.3 % H_2O_2. The absorbance was measured at 240 nm. CAT activity was expressed as µmol.mg^{-1} protein (Bates *et al.*, 1973). APX activity was measured. A reaction solution containing sodium phosphate buffer (50 mM, pH 7), ascorbate (0.5 mM), EDTA (0.1 mM), H_2O_2 (1.2 mM), and 100 mL enzyme extract was prepared. Absorbance was measured at a wavelength of 290 nm. GPX activity was measured employing 50 µL of supernatant added to 3 mL of the reaction mixture (3 µL of Guaiacol and 10 µL of H_2O_2 (30%) solved in 3 mL of sodium phosphate buffer, pH 7). Differences in absorbance were read at 470 nm and the activity of guaiacol peroxidase was expressed in µmol.mg^{-1} protein. PPO activity was measured as described previously. The reaction solution contained M pyrogallol, phosphate buffer, and 0.1 mL enzyme extract. Absorbance was measured at a wavelength of 420 nm (Nazeri *et al*., 2012). Proline was extracted as described before, and measured based on the proline standard curve and expressed in microgram per gram fresh mass (µg g^{-1} FW) (Bates *et al*., 1973). Total soluble protein content was measured according to Coomassie Brilliant Blue G-250 method (Bradford, 1976). For the analysis of Na^+ and K^+, leaf samples were dry ashed at 550°C for 5 hours and, subsequently, dissolved in 0.5M HCl and brought up to 50 mL with distilled water. Concentrations of Na^+ and K^+ were determined by a flame photometer (Jenway, UK) (Habibi, 2017).

Statistical Analysis

The experiment was carried out as factorial arrangement based on randomized complete block design with four replications. SAS 9.2 software was used for data analysis, and Duncan's multiple range test was used for mean comparisons.

RESULTS

The results of analysis of combined variance for physiological (Table 2) and biochemical traits (Table 3) showed that the effect of salinity, genotype and their interaction on all measured traits were significant at 1% probability level.

Four genotypes of common bean were exposed to three levels of salt stress. The results revealed the rise in the relative chlorophyll content and the amount of Chl a, Chl b, and carotenoid was observed along with augmenting the level of salinity stress up to 200 mM, excluding the carotenoid content of Jules genotype that increased in the first level of salt treatment and then decreased while applying 200 mM of salt. As shown in Figure 1, the highest drop in the content of Chl a and Chl b was observed in the Naz genotype, which decreased by approximately 57% at the salinity stress level of 200 mM. In the same way, the most marked change was in the total chlorophyll content of Naz genotype, which dropped by 30%. Whereas, the total chlorophyll content of Jules, 201, and 278 genotypes fell by 15, 23, and 21%, respectively.

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

Table 2. Analysis of variance for salt stress effect on some physiological traits of common bean genotypes.

SOV		MS								
	df	Relative Water Content (RWC)	Electrolyte Leakage Index (ELI)	SPAD	Chla [a]	Chl b	Carotenoid	Leaf Na^+	Leaf K^+	Leaf K^+/Na^+
Genotype (G)	3	1408.35**	315.84**	390.63**	137.95**	100.70**	20.06**	444.01**	7341.92**	35.33**
Salt (S)	2	11913.33**	22554.60**	110.98**	296.06**	4191.44**	1040.63**	705.82**	8562-42**	99.277**
G × S	6	14.18**	88.51**	4.47**	5.70**	35.14**	9.26**	82.11**	613.00**	18.26**
Error	48	3.28	20.08	0.97	0.89	3.36	1.69	17.34	264.22	1.36**
CV%		1.62	0.69	2.99	3.43	6.40	9.06	17.81	15.96	22.70

[a] Chlorophyll.

Table 3. Analysis of variance for salt stress effect on some biochemical traits of common bean genotypes.

SOV	df	*MS*						
		Proline	MDA	Protein	CAT[a]	APX[b]	GPX[c]	PPO[d]
Genotype (G)	3	3.10**	5.72**	0.079**	148814.00**	74.86**	0.15**	0.0078**
Salt (S)	2	1538.76**	10.87**	3.48**	321081.66**	328.25**	0.95**	0.0057**
G × S	6	0.94**	0.63**	0.12**	15512.9**	18.22**	0.021**	0.0034**
Error	48	0.09	0.126	0.033	1054.55	082	0.006	0.00055
CV%		3.06	9.99	12.79	16.21	15.16	21.4	24.66

[a] Catalase; [b] Ascorbate peroxidase; [c] Guaiacol peroxidase; [d] Polyphenol oxidase.

The RWC decreased under salinity stress. The highest drop in RWC was in Naz genotype by 38% compared to the control. Genotype 278 showed a slight decrease in RWC (22% compared to 30% for Jules and 26% for genotype 201) (Figure 2).

The increment of salinity from 0 to 200 mM increased ion leakage significantly. The lowest electrolyte leakage at 200 mM was for genotype Jules (75.33) and the highest for genotype Naz (97.60) (Figure 2).

As depicted in Figure 3., Na^+ content increased and K^+ content was lowered as a result of the treatment with higher concentrations of NaCl. By applying 100 mM sodium chloride, there was almost no change in the concentration of Na^+ for genotype 201. Jules showed the most intense addition of Na^+ in this level by 75%, yet the least increase at 200 mM level by 1.7%. There was a decrease in the amount of K^+ that corresponded with the increase in salt concentration. This reduction was noticeable in both NaCl concentrations for Jules genotype. The K^+/Na^+ ratio declined in NaCl treated plants. The highest value was observed in 100 mM NaCl treated Jules genotype by 58% and genotype 278 treated with 100 mM NaCl showed the lowest drop of 7%. Figure 4 gives information about the

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

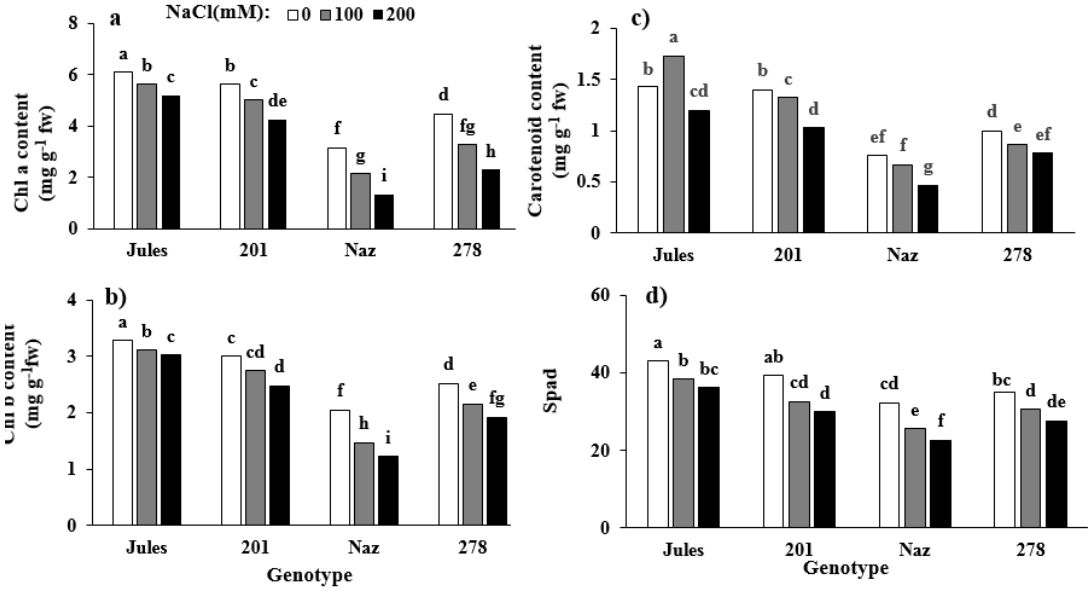

Figure 1. Chl (Chlorophyll) a (a), Chl b (b), carotenoid (c) and SPAD (d) content of *Phaseolus vulgaris* genotypes grown under salt stress conditions. Different letters represent significant difference at P≤ 0.05 according to Duncan`s test.

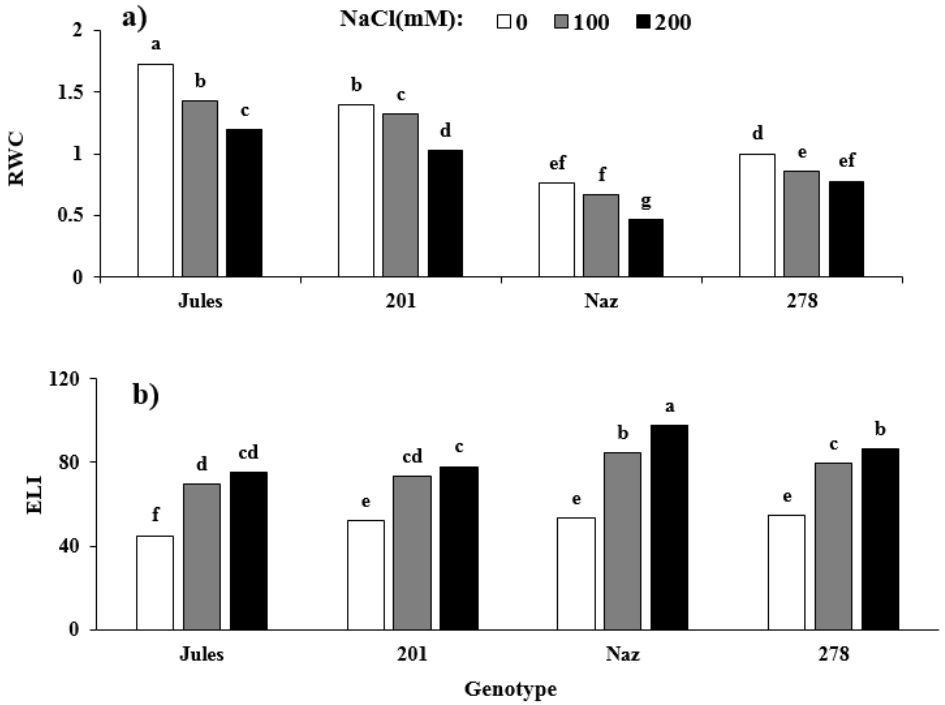

Figure 2. RWC (Relative Water Content) (a) and ELI (Electrolyte Leakage Index) (b) content of *Phaseolus vulgaris* genotypes grown under salt stress conditions. Different letters represent significant difference at P≤ 0.05 according to Duncan`s test.

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

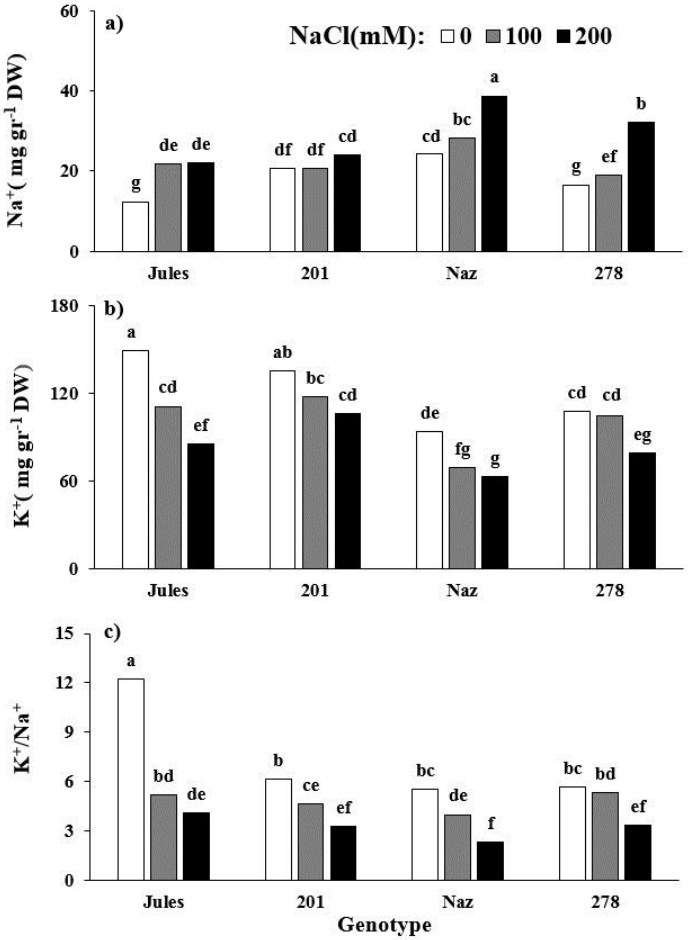

Figure 3. Na^+ (a), K^+ (b) content and Na^+/K^+ (c) ratio of *Phaseolus vulgaris* genotypes grown under salt stress conditions. Different letters represent significant difference at $P \leq 0.05$ according to Duncan`s test.

variation in proline, MDA and total protein content during salt stress. There was a considerable change in proline content of all genotypes when increasing salt concentration. Jules genotype's total proline content, at around 19.5 µmol g^{-1} FW, is larger than other genotypes while treating by 200 mM NaCl. With increasing salinity, MDA content enhanced significantly. It is noticeable that Naz genotype accumulated the highest amount of MDA (5.22 nmol g^{-1} FW) at 200 mM salt stress level. Whereas, genotype 201 showed a moderate change in MDA amount. The obtained results indicate that there was an inverse relationship between salt concentrations and protein content. At 200 mM salt stress level, Jules genotype showed the least significant dip in the protein content of the leaves by 20.9%. All other genotypes reached a similar level of approximately 1 mg mL^{-1}.

According to the results shown in Figure 5, the stressed common beans demonstrated elevated levels of antioxidant enzymes. It is noticeable that in all but one of the four antioxidant enzymes, Jules genotype had the highest jump in content compared to other genotypes in medium and higher concentrations of NaCl. CAT activity almost quadruplicated, APX quintupled and PPO approximately trebled in Jules genotype leaves in 200 mM salinity compared to the control. Although GPX content was significantly higher in Jules genotype control plants, its increase rate was about

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

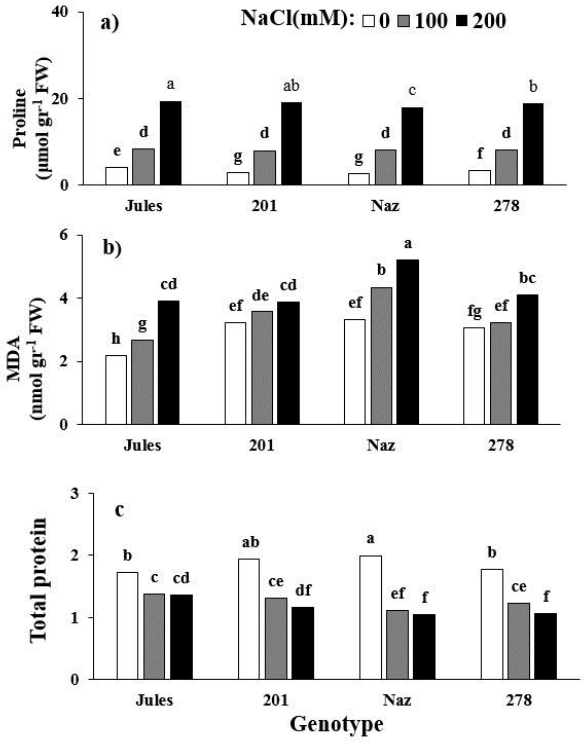

Figure 4. Proline (a), MDA (Malondialdehyde) (b), and total protein content (c) of *Phaseolus vulgaris* genotypes grown under salt stress conditions. Different letters represent significant difference at P≤ 0.05 according to Duncan`s test.

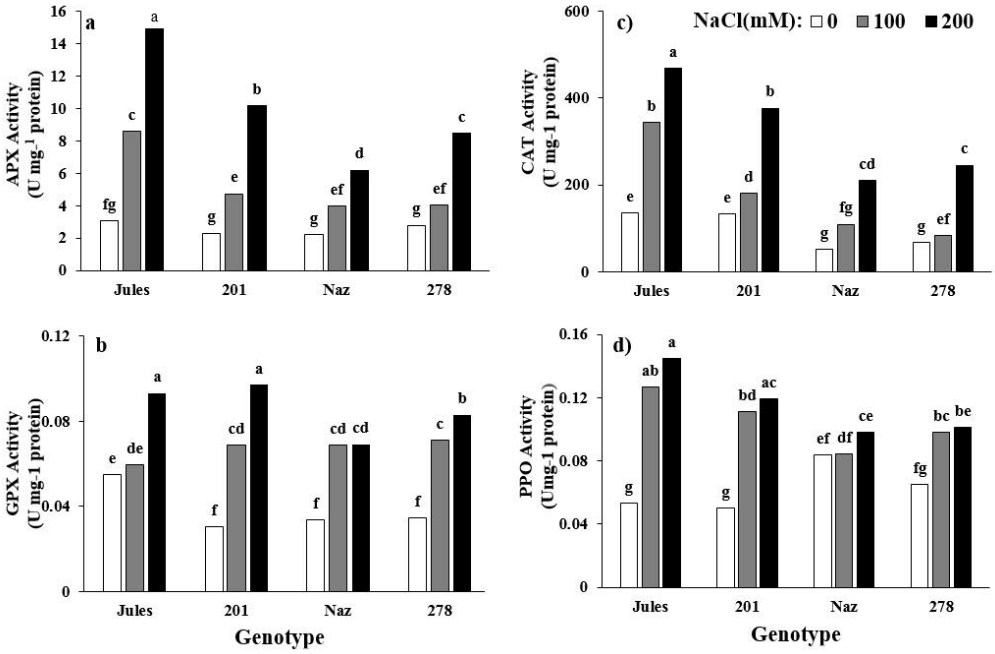

Figure 5. APX (Ascorbate Peroxidase) (a), GPX (Guaiacol Peroxidase) (b), CAT (Catalase), (c) and PPO (Polyphenol Oxidase) (d) content of *Phaseolus vulgaris* genotypes grown under salt stress conditions. Different letters represent significant difference at P≤ 0.05 according to Duncan`s test.

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

72%, while in other genotypes, the change was over 100%. CAT activity in the control and stressed leaves of genotype 201 was significantly higher than those of 278 and Naz genotypes. Besides, salt- induced alteration in the content of APX quadruplicated in genotype 201 whereas NaCl stress resulted in an approximately threefold increase in APX content of genotypes Naz and 278. PPO activity went up from 0.05 to 0.119, 0.065 to 0.101 and, 0.083 to 0.098 μmol mg^{-1} in genotypes 201, 278, and Naz, respectively.

DISCUSSION

Aerobic metabolism results in the generation of Reactive Oxygen Species (ROS). The reduction of molecular oxygen by high-energy exposure or as a consequence of electron-transfer chemical reactions produces these extremely reactive products. Scientific evidences indicate that ROS in plants have two contrary roles. Firstly, they can act as signaling molecules under favorable growth condition (240 μM s^{-1} $O_2^{\bullet -}$ and a steady-state level of 0-5 μM H_2O_2 in chloroplasts), and secondly their enhanced production under stresses can cause cell toxicity (up to 720 μM s^{-1} $O_2^{\bullet -}$ and a steady-state level of 5-15 μM H_2O_2). At these excessive levels, they cause oxidative damage to biomolecules leading to the peroxidation of lipids that accumulate MDA in the cells, injury to the cell membrane that results in an increase in electrolyte leakage index, oxidation of proteins and reduction of total protein content, DNA damage, down-regulation of enzymes, initiation of Programmed Cell Death (PCD) pathway and eventually apoptosis. According to the aforementioned roles, the cell must be capable of balancing the amount of ROS so that neither their deficiency nor their abundance leads to damage. Non-enzymatic and enzymatic antioxidants scavenge or detoxify the additional amount of ROS in the cell. Stressful conditions can upset this important balance, and the cell tries to maintain itself by up-regulating the production of non-enzymatic components such as tocopherol, carotenoids, and phenolic compounds coupled with enzymatic defense system including CAT, APX, GPX, and PPO. The preservation of a high antioxidant capacity to keep the toxic ROS under control or within limits has been linked to developed tolerance of plants to the environmental stresses (Sharma *et al.*, 2011).

Soils that contain a substantial concentration of salt can affect plants adversely by ion toxicity and disturbing osmotic adjustment (Torabian *et al.*, 2016).

In this study, we employed two genotypes of common bean, Jules, and Naz, that are tolerant and sensitive to salt stress, respectively. We also applied treatments to two other genotypes of common bean, namely, 201 and, 278, that have been proved by field experiments (Sabokdast *et al.*, 2019) to be tolerant and sensitive to drought stress to evaluate if they are suitable candidates for avoiding salinity stress.

Cultivating crops in soils with higher salt accumulation results in lower chlorophyll and protein contents. NaCl toxicity initially targets chlorophyll that leads to restricting photosynthesis and growth (Pourbabaee *et al.*, 2016). This reduction may be considered a direct result of the heightened activity of “chlorophyllase” that is a chlorophyll-degrading enzyme. This degradation can be mitigated by the activity of antioxidant enzymes (Yadav *et al.*, 2019). Jules and 201 genotypes by exhibiting higher Chl content as well as enhanced activity of antioxidant enzymes can be a proof of the derived conclusion. There are other suggestions including the destruction of the photosynthetic apparatus, removal of the photosynthetic pigments, and limitations of chlorophyll synthesis due to a reduction in ALA (5-Aminolevulinic Acid) content (a precursor of protochlorophyllide, which converts to chlorophyll by the light). Exogenous ALA has proven to be effective on lightening adverse effects of salt stress in cucumber *(Cucumis sativus* L.) seedlings by

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

fixing the photosynthetic apparatus and enhancing the chlorophyll biosynthesis pathway (Radi *et al.*, 2013; Wu *et al.*, 2018) . In the leaves, the disruption of Chl and the resulting depigmentation associates with the drop in the leaf greenness. Both Jules and 201 genotypes that are tolerant of salt and drought stress, respectively, showed lower decreases in SPAD. In accordance with the present results, a previous study on Chinese castor bean (*Ricinus communis* L.) have demonstrated that salt stress mitigates pigments contents and Chlorophyll fluorescence (Li *et al.*, 2010).

RWC decreased under salt stress, especially in susceptible genotypes. This can be due to the impairment of leaf turgor potential that leads to the loss of water in the leaves and, consequently, affects photosynthesis (Çiçek and Çakirlar, 2002). The results obtained from research on five cultivars of sugar beet (*Beta vulgaris* L.) under salt stress support our results and are ascribed to be the consequence of the flaccidity (Ghoulam *et al.*, 2002). To avoid the loss of turgor, genotype 278 regulated leaf water content by osmotic adjustment more effectively than other genotypes.

ELI, the first symptom of cell injury, increases under abiotic stresses including salinity due to the deterioration of membrane integrity and irreversible injuries to the cell membranes. Malondialdehyde (MDA) is one of the final products of the lipid peroxidation and is the indicator for cell membrane damage (Sharma *et al.*, 2011). As the Naz genotype had the highest ELI, this genotype also accumulated the highest MDA content. This response was less notable in the tolerant than in the susceptible genotype. One reason regarding this can be the higher potential of the antioxidative system in tolerant genotypes to detoxify ROS and the ensuing subdued level of lipid peroxidation under salt toxicity. Similarly, responses have been reported from salt-tolerant and salt-sensitive wheat (*Triticum aesativum* L.) and broad bean (*Vicia faba* L.) cultivars to salinity that have a resemblance to our results (Radi *et al.*, 2013). The results of enhanced ELI in common bean plants under salt stress agree with the findings on the effects of salinity and copper on some physiological and biochemical traits of rosemary (*Rosmarinus officinalis* L.). Hejazi Mehrizi *et al.* (2012) reported that salinity caused cell membrane damage and resulted in higher electrolyte leakage rate in the leaves of stressed rosemary plants.

Two salt stress accumulating products include carotenoid, which is a nonenzymatic antioxidant, and proline, which is an osmoregulator under drought and saline stress (Sharma *et al.*, 2011). Besides the role played by carotenoids in the photosynthetic apparatus as light absorbers, they can act as ROS scavengers in the chloroplast. Carotenoids absorb excess energy and become excited. Nevertheless, they go back to their ground state by energy dispersal in the form of heat without causing the production of ROS themselves. Under environmental stresses, carotenoid content reduces and a side product called β-cyclocitral accumulates. According to the previous results reported in the literature, β-cyclocitral up-regulates the expression of $^1O_2$ sensitive genes in Arabidopsis (Gupta *et al.*, 2015). The negative effect of sodium chloride on the carotenoid content of Jules was lower, demonstrating that this genotype diminished the disturbance of the balance between the production of ROS and the quenching activities of carotenoid. The accumulation of intracellular free amino acids such as proline helps the plant to cope with environmental stresses more efficiently. As shown in Figure 4, proline levels have increased dramatically in all genotypes. However, the sensitive genotype Naz has shown the lowest proline content at 200 mM NaCl treatment. In bean plants, proline balances osmotic pressure by acting as an osmolyte. Under environmental stresses, besides its function as an antioxidant and a signaling agent, proline interacts with important macromolecules like enzymes to support the retention of their structure and function. In a study on the positive effects of

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

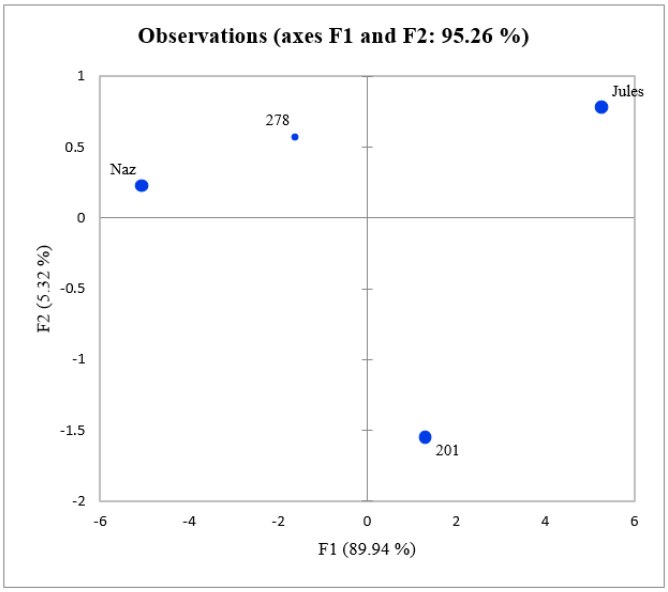

Figure 6. The biplot for the results of the performance of genotypes in different salinity levels

abscisic acid on common bean plants under salinity stress, proline content proliferated notably. This result indicates the substantial role of proline in counteracting salinity stress (Hayat *et al*., 2012; Radi *et al*., 2013; Shevyakova *et al*., 2013).

Function of proline as a molecular chaperon to maintain protein integrity under stress should therefore be taken into account too (Hayat *et al*., 2012). The results of this study showed that the proline concentration increased significantly by changing the salinity level from 100 to 200 mM. Consequently, it caused the change in protein content to be negligible by doubling the amount of salt in the soil. Decreased total protein in the plant can be attributed to its use as a source of nitrogen for osmotic adjustment. A diminution in protein synthesis and an increase in its degradation due to lack of access to amino acids can also be the cause of this reduction in content (Radi *et al*., 2013) . Previous studies have found similar results such as research on the effect of salinity on barley (*Hordeum vulgare* L.) (Wu *et al*., 2018).

Plants with higher K^+/Na^+ ratios are considered to be more resistant to salinity stress, therefore, low and high concentrations of Na^+ and K^+, respectively, in the plant parts under environmental stresses possibly improve salt stress tolerance in plants. Jules, as our tolerant genotype, had the highest ratio at all salinity levels. It also exhibited a lower amount of Na^+ (limited salt uptake from the soil) and higher K^+ content that is involved in osmotic regulation (Soleimani *et al*., 2010).

Antioxidants are first and foremost the imperative defense to encounter abiotic stresses. Enzymatic antioxidant defense includes various enzymes. Herein, CAT, APX, GPX, and PPO contents were measured. CAT catalyzes the disproportionation of H_2O_2, that is the main form of ROS, into water and oxygen in an energy-saving way. The glutathione-ascorbate cycle removes excessive hydrogen peroxide during stress and the first step in this cycle is accomplished by the help of APX, catalyzing H_2O_2 to water and oxygen with the help of a reducing agent, ascorbate. GPX has the same role in oxidative defense with the difference that the reductant in this redox chemical reaction is guaiacol. PPO is also another antioxidant enzyme during stress (Sharma *et al*., 2011; Gaur *et al*., 2014). Genotypes Jules and 201 presented the highest antioxidant enzyme contents respectively, in accordance with their ability to cope with salinity and drought stress tolerance. Similar trends could be seen in the results of other authors who investigated the alternation in the

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

antioxidant enzymes in common bean ('HRS 516' and 'RO21') under salt stress (Gama *et al*., 2009).

To conclude, by examining the osmotic as well as ionic toxicity induced by salinity on common bean, and different strategies of the plant to deal with this stress, it can be acknowledged that genotypes Jules and 201 can be suitable candidates for cultivation in soils with high salt content, while genotype 201 has been described as drought-resistant. Concerning the essential role of antioxidants in maintaining plant homeostasis while being exposed to biotic and abiotic stresses, it can be deduced that genotypes with higher antioxidant activity are physiologically and biochemically more capable of coping with abiotic environmental stresses and maintaining photosynthetic potential. Biplots are efficient tools for evaluating genotypes performance under different environmental factors (Yan and Frégeau-Reid, 2018). The biplot presented in Figure 6 suggests that the superior cultivars in this investigation that coped with salt stress were Jules and 201, tolerant and semi-tolerant, respectively.

REFERENCES

1. Abdelaal, K. A. A., Hafez, Y. M., Badr, M. M., Youseef, W. A. and Esmail, S. M. 2014. Biochemical, Histological and Molecular Changes in Susceptible and Resistant Wheat Cultivars Inoculated with Stripe Rust Fungus Puccinia striiformis *f. sp. tritici. Egypt. J. Biol. Pest Control*, **24:** 421–429.
2. Amirruddin, A. D., Muharam, F. M., Ismail, M. H., Ismail, M. F., Tan, N. P. and Karam, D. S. 2020. Hyperspectral Remote Sensing for Assessment of Chlorophyll Sufficiency Levels in Mature Oil Palm (*Elaeis guineensis*) Based on Frond Numbers: Analysis of Decision Tree and Random Forest. *Comput. Electron. Agric.*, **169:**105221.
3. Arzani, A. and Ashraf, M. 2016. Smart Engineering of Genetic Resources for Enhanced Salinity Tolerance in Crop Plants. *Crit. Rev. Plant Sci.*, **35:** 146–189.
4. Bates, L. S., Waldren, R. P. and Teare, I. D. 1973. Rapid Determination of Free Proline for Water-Stress Studies. *Plant Soil*, **39:** 205–207.
5. Bhagavan, N. V. and Ha, C-E. 2015. Chapter 3 - Amino Acids. In: "*Essentials of Medical Biochemistry",* (Eds.) Bhagavan, N. V and Ha, C. -E. Second Edition, Academic Press, San Diego.
6. Bradford, M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.*, **72:** 248–254.
7. Çiçek, N. and Çakirlar, H. 2002. The Effect of Salinity on Some Physiological Parameters in Two Maize Cultivars. *Bulg. J. Plant Physiol.*, **28:** 66–74.
8. Das, K. and Roychoudhury, A. 2014. Reactive Oxygen Species (ROS) and Response of Antioxidants as ROS-Scavengers during Environmental Stress in Plants. *Front. Environ. Sci.*, **2:**1–13.
9. De Azevedo Neto, A. D., Prisco, J. T., Enéas-Filho, J., De Abreu, C. E. B. and Gomes-Filho, E. 2006. Effect of Salt Stress on Antioxidative Enzymes and Lipid Peroxidation in Leaves and Roots of Salt-Tolerant and Salt-Sensitive Maize Genotypes. *Environ. Exp. Bot.*, **56**:87–94.
10. Gama, P. B. S., Tanaka, K., Eneji, A. E., Eltayeb, A. E., and El Siddig, K. 2009. Salt-Induced Stress Effects on Biomass, Photosynthetic Rate, and Reactive Oxygen Species-Scavenging Enzyme Accumulation in Common Bean. *J. Plant Nutr.,* **32:** 837–854.
11. Gaur, R. K. and Sharma, P. 2014. *Approaches to Plant Stress and Their Management.* Springer India.
12. Ghoulam, C., Fours,y A. and Fares, K. 2002. Effects of Salt Stress on Growth, Inorganic Ions and Proline Accumulation in Relation to Osmotic Adjustment in Five Sugar Beet Cultivars. *Environ. Exp. Bot*., **47:** 39–50.
13. Gupta, D. K., Palma, J. M. and Corpas, F. J. 2015. *Reactive Oxygen Species and Oxidative Damage in Plants Under Stress*. Springer International Publishing.
14. Habibi, G. 2017. Selenium Ameliorates Salinity Stress in *Petroselinum crispum* by Modulation of Photosynthesis and by Reducing Shoot Na Accumulation. *Russ. J. Plant Physiol*., **4:** 368–374.
15. Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J. and Ahmad, A. 2012. Role of Proline under Changing

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

Environments: A Review. *Plant Signal. Behav*., **7:** 1456–1466.
16. Hejazi Mehrizi, M., Shariatmadari, H., Khoshgoftarmanesh, A. H. and Dehghani, F. 2012. Copper Effects on Growth, Lipid Peroxidation, and Total Phenolic Content of Rosemary Leaves under Salinity Stress. *J. Agr. Sci. Tech.*, **14:** 205–212.
17. Li, G., Wan, S., Zhou, J., Yang, Z. and Qin, P. 2010. Leaf Chlorophyll Fluorescence, Hyperspectral Reflectance, Pigments Content, Malondialdehyde and Proline Accumulation Responses of Castor Bean (*Ricinus communis* L.) Seedlings to Salt Stress Levels. *Ind. Crops Prod.*, **31:** 13–19.
18. Mbadi, S. H., Alipour, Z. T., Asghari, H. and Kashefi, B. 2015. Effect of the Salinity Stress and Arbuscular Mycorhizal Fungi (AMF) on the Growth and Nutrition of the Marigold (*Calendula officinalis*). *J. Biodivers. Environ. Sci.*, **6:** 215–219.
19. Mudgal, V., Madaan, N. and Mudgal, A. 2010. Bichemical Mechanism of Salt Tolerance in Plants: A Review. *Inter. J. Bot*., **6:** 136–143.
20. Nazeri, M., Maali Amiri, R., Mehraban, F. H. and Khaneghah, Z. 2012. Change in Antioxidant Responses against Oxidative Damage in Black Chickpea Following Cold Acclimation. *Russ. J. Plant Physiol.*, **59:** 183–189.
21. Pieczynski, M., Marczewski, W., Hennig, J., Dolata, J., Bielewicz, D., Piontek, P., Wyrzykowska, A., Krusiewicz, D., Strzelczyk-Zyta, D., Konopka-Postupolska, D., Krzeslowska, M., Jarmolowski, A. and Szweykowska-Kulinska, Z. 2013. Down-Regulation of CBP80 Gene Expression as a Strategy to Engineer a Drought-Tolerant Potato. *Plant Biotechnol. J*., **11:** 459–469.
22. Pourbabaee, A. A., Bahmani, E., Alikhani, H. A. and Emami, S. 2016. Promotion of Wheat Growth under Salt Stress by Halotolerant Bacteria Containing ACC Deaminase. *J. Agr. Sci. Tech*., **18:** 855-864.
23. Radi, A. A., Farghaly, F. A. and Hamada, A. M. 2013. Physiological and Biochemical Responses of Salt-Tolerant and Salt-Sensitive Wheat and Bean Cultivars to Salinity. *J. Biol. Earth Sci*., **3:** 72–88.
24. Sabokdast, M., Dashtaki, M. and Sasani, Y. 2017. Effect of Drought Stress on Some Agronomic Characteristics, Grain Yield and Its Components in Bean Genotypes. Iran. *J. Field Crop Sci*., **48:** 1201–1209.
25. Sabokdast, M., Dashtaki, M. and Sassani, Y. 2019. Evaluation of Responses of Common Bean (*Phaseolus vulgaris.* L) Genotypes to Drought Stress using Different Stress Tolerance Indices. *Iran. J. Field Crop Sci*., **50:** 1–9.
26. Sharma, P., Jha, A. B. and Shanker Dubey, R. 2011. Oxidative Stress and Antioxidative Defense Systems in Plants Growing under Abiotic Stresses. In: "*Handbook of Plant and Crop Stress*", (Ed.): Pessarakli, M. Taylor and Francis Group, PP. 90–124.
27. Shevyakova, N. I., Musatenko, L.I., Stetsenko, L.A. Vedenicheva, N. P., Voitenko, L. P., Sytnik, K. M. and Kuznetsov, Vl. V. 2013. Effects of Abscisic Acid on the Contents of Polyamines and Proline in Common Bean Plants under Salt Stress. *Russ. J. Plant Physiol*., **60:** 200–211.
28. Soleimani, A., Talaie, A. R., Naghavi, M. R. and Zamani, Z. 2010. Male Gametophytic and Sporophytic Screening of Olive Cultivars for Salt Stress Tolerance. *J. Agr. Sci. Tech*., **12:** 173–180.
29. Szabados, L. and Savouré, A. 2010. Proline: A Multifunctional Amino Acid. *Trends Plant Sci.*, **15:** 89–97.
30. Torabian, S., Zahedi, M. and Khoshgoftarmanesh, A. 2016. Effect of Foliar Spray of Zinc Oxide on Some Antioxidant Enzymes Activity of Sunflower under Salt Stress. *J. Agr. Sci. Tech*., **18:** 1013-1025.
31. Torche, Y., Blair, M. and Saida, C. 2018. Biochemical, Physiological and Phenological Genetic Analysis in Common Bean (*Phaseolus vulgaris* L.) under Salt Stress. *Ann. Agric. Sci.*, **63:** 153-161.
32. Wu, Y., Liao, W., Dawuda, M. M., Hu, L. and Yu, J. 2018. 5-Aminolevulinic Acid (ALA) Alleviated Salinity Stress in Cucumber Seedlings by Enhancing Chlorophyll Synthesis Pathway. *Front. Plant Sci.*, **9:** 635.
33. Yadav, S. P., Bharadwaj, R., Nayak, H. and Mahto, R. 2019. Impact of Salt Stress on Growth, Productivity and Physicochemical Properties of Plants : A Review. *Int. J. Chem. Stud.*, **7:**1793–1798.
34. Yan, W. and Frégeau-Reid, J. 2018. Genotype by Yield×Trait (GYT) Biplot: A Novel Approach for Genotype Selection Based on Multiple Traits. *Sci. Rep.*, **8:** 8242.

[DOR: 20.1001.1.16807073.2021.23.5.13.4]

پاسخ های فیزیولوژیکی و بیوشیمیایی چهار ژنوتیپ لوبیای معمولی (*Phaseolus vulgaris* L.)تحت تنش شوری

ز. عظیمی چتابی، و م. سبکدست نودهی

چکیده

این تحقیق به منظور تعیین تأثیر تنش شوری بر برخی پارامترهای فیزیولوژیکی و بیوشیمیایی لوبیا معمولی (*Phaseolus vulgaris* L.) انجام شد. سه سطح سدیم کلراید (۰ ، ۱۰۰ و ۲۰۰ میلی مولار) برای چهار ژنوتیپ لوبیای معمولی استفاده شد. در قدم بعدی میزان کلروفیل، محتوای آب نسبی (RWC)، شاخص نشت الکترولیت (ELI)، غلظت سدیم، پتاسیم و نسبت آن‌ها، میزان مالون‌دی‌آلدهید (MDA)، محتوای پروتئین کل و غلظت پرولین تعیین و مقایسه شد. علاوه بر این، فعالیت برخی آنزیم‌های ضداکسیدان کاتالاز (CAT)، آسکوربات پراکسیداز (APX)، پلی فنل اکسیداز (PPO) ، و گایاکول پراکسیداز (GPX) مورد بررسی قرار گرفت. محتوای کلروفیل و کارتنوئید همراه با میزان SPADبا افزایش شدت تنش شوری کاهش یافت. محتوای آب نسبی با افزایش شوری همراه با نسبت پتاسیم/سدیم کاهش یافت و شاخص نشت الکترولیت افزایش یافت. نتایج نشان داد که غلظت MDA و پرولین در شرایط ذکر شده بطور معنی‌داری افزایش یافته است. فعالیت آنزیم‌های دفاعی ضداکسیدانی به طور قابل‌توجهی تغییر یافت. محتوای پروتئین کل نیز تحت استرس شوری کاهش پیدا کرد. ژنوتیپ‌های جولز و ۲۰۱ در طول این آزمایش به عنوان ژنوتیپ‌های متحمل و نیمه‌متحمل تشخیص داده شدند.

[DOR: 20.1001.1.16807073.2021.23.5.13.4]