INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY ISSN Print: 1560–8530; ISSN Online: 1814–9596 19–1686/2020/23–4–795–800 DOI: 10.17957/IJAB/15.1354 http://www.fspublishers.org



Full Length Article

Salinity Induced Differential Growth, Ionic and Anti-Oxidative Response of Two Bell Pepper (*Capsicum annuum*) Genotypes

Akhtar Javed¹, Abid Mahmood¹, Zeeshan Ahmed^{2,3}, Tanvir Shahzad¹, Ghulam Sarwar⁴, Ghazala¹, Fakhar Mujeeb⁵, Anser Ali⁶ and Muhammad Ibrahim^{1*}

¹Department of Environmental Sciences & Engineering, Government College University Faisalabad 38000, Punjab, Pakistan ²Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Xinjiang, 830011, China

³Cele National Station of Observation and Research for Desert-Grassland Ecosystem, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Xinjiang, 848300, China

⁴Department of Soil & Environmental Sciences, University of Sargodha, Sargodha 40100 Punjab, Pakistan

⁵Soil Bacteriology Section, Ayub Agricultural Research Institute (AARI), Jhang Road, Faisalabad, Pakistan

⁶Department of Agronomy, Ghazi University Dera Ghazi Khan, Punjab, Pakistan

^{*}For correspondence: ebrahem.m@gmail.com

Received 02 November 2019; Accepted 19 December 2019; Published 13 February 2020

Abstract

Salinity poses a serious threat to vegetable crop production in terms of crop quality and yield reduction globally. This pot experiment was performed to evaluate the effect of different salinity levels (0, 25, 50, 75, 100 and 125 m*M* of NaCl) on seedling growth, antioxidant activity and ionic concentration of different ions in two bell pepper genotypes *viz.*, California Wonder (salt tolerant) and Green Beauty (salt sensitive). With increasing salinity levels from 0 to 125 m*M* bell pepper shoot fresh and dry weight, root dry weight, shoot and root lengths, leaf potassium (K⁺) and root sodium (Na⁺) contents decreased linearly in both genotypes. However, this decrease was more pronounced in genotype Green Beauty at 125 m*M* salinity level compared to California Wonder. In contrast, peroxidase, catalase, leaf proline, leaf Na⁺ and root K⁺ contents increased significantly with increasing salinity stress levels (0 to 125 m*M*) in both genotypes. Since magnitude of this increase was much higher in genotype California Wonder at 125 m*M* salinity level when compared to Green Beauty. Moreover, shoot fresh and dry weights, root dry weight and shoot and root lengths were positively correlated with leaf and root K⁺ contents and had negative correlation with leaf and root Na⁺ ions, respectively. Therefore, by maintaining better seedling growth with higher accumulation of catalase, peroxidase and proline contents as well as K⁺ and Na⁺ ion ratios at all salinity levels California Wonder better tolerated the salinity stress as compared to Green Beauty. © 2020 Friends Science Publishers

Keywords: Antioxidants; Bell pepper; Ionic modifications; Proline; Salinity

Introduction

Soil salinity is an important abiotic stress and is becoming a major problem globally because it is encountered in all climates (Evelin et al. 2019). It is estimated that approximately one billion hectares (ha) across 100 countries are facing salinity problem (FAO 2015). Furthermore, soil salinity is expanding with an estimated annual addition of 0.3-0.5 million ha of arable land with overall 20% decrease in crop production (FAO 2015). Therefore, ensuring global food security for mounting population with decreasing farmlands is the biggest challenge for modern agriculture. Salinity reduces crop productivity owing to ion toxicity, reduced growth, osmotic stress, photosynthetic imbalance, mineral deficiencies and combination of these effects (Rangani et al. 2016). In fact, salt stress may also affect seed germination, leaf water contents and nutrients uptake and ultimately reduces yield (Kim et al. 2014). The instant

response of salt stress appears in the form of reduced rate of leaf expansion due to increased salt concentration. Reduction in leaf area expansion due to high salinity levels causes about 80% reduction in plant growth while decrease in stomatal conductance brings about 20% reduction in plant growth (Parida and Das 2005). Salinity also causes oxidative damage to the plants through overproduction of reactive oxygen species (ROS). However, plants also have the ability to counter salinity problem by utilizing different mechanisms like growth plasticity, turgor maintenance, ion homeostasis, enhanced photosynthesis, better water use efficiency, scavenging of ROS through antioxidant enzymes and molecules along with production of phytohormones (Farooq *et al.* 2015).

Bell pepper (*Capsicum annuum* L.) is a member of the *Solanaceae* family (Penella *et al.* 2015) and it is traditionally used as vegetable in the world (Ali *et al.* 2017). Nutritionally it is rich in antioxidants containing polyphenols such as

To cite this paper: Javed A, A Mahmood, Z Ahmed, T Shahzad, G Sarwar, Ghazala, F Mujeeb, A Ali, M Ibrahim (2020). Salinity induced differential growth, ionic and anti-oxidative response of two bell pepper (*Capsicum annuum*) genotypes. *Intl J Agric Biol* 23:795-800

vitamin C, complete carotenoids, \beta-carotene, a-carotene (Nadeem et al. 2011). It is cultivated in Pakistan to produce 191.8 thousand tonnes of this plant on an area of 66500 hectares (Mehmood et al. 2017). Its production not only fulfills 88 percent of the country's requirements but also contributes to foreign exchange income (Zia 2006). Pepper is mostly cultivated in Punjab and Sindh where high levels of salinity affect its production (Khan 1999). According to an estimate approximately 21% of irrigated land in Punjab and Sindh is affected by salinity (Qureshi et al. 2007). Bell pepper is highly important cash food crop and its improved quality and production can generate high farm income (Mehmood et al. 2017). Bell pepper has been classified from moderately sensitive to sensitive under salinity and water stress conditions (Penella et al. 2015) and it is considered most susceptible to salinity during its seedling phase (Navarro et al. 2002). Excessive salts in soil solution or in root zone are transported with in the plant. These salts induce osmotic stress and ionic imbalances cause various biochemical and morpho-physiological abnormalities that ultimately leads to plant death (Pessarakli and Tucker 1988). If salinity problem is not managed properly in pepper it can become a major limiting factor affecting its production (Villa-Castorena et al. 2003). Therefore, there is a dire need to evaluate the salt tolerance potential of existing bell pepper genotypes. The comparison among different cultivars differing in their basic ability to cope with stress is quit useful in the evaluation of their salt stress tolerance potential. It will not only improve our understanding of the primary mechanisms responsible for salinity tolerance but it is also helpful to recognize the best salt tolerant cultivar (Akhtar et al. 2017). Screening of tolerant crops on the basis of morphological, biochemical, physiological and ionic responses may help to strengthen the breeding programs by recognizing the genotypes having higher yield and salt tolerance potential (Ashraf and Foolad 2007). Moreover, it is an easy way to screen salt tolerant cultivars under controlled conditions compared to field conditions and it is reported to be used in the screening of different crop cultivars (Akhtar et al. 2003). According to the best of our knowledge comparative analysis of salt sensitive and salt tolerant bell pepper genotypes is sparse. Therefore, this study was performed to elucidate the effects of salt stress on seedling growth, antioxidant activity and ionic concentration in two bell pepper genotypes viz., California wonder (salt tolerant) and Green Beauty (salt sensitive). The outcomes of this research will help in the identification of novel bell pepper cultivar with better salt tolerant potential to cultivate on salt affected soils under agro-climate of Pakistan.

Materials and Methods

Experimental details

This pot experiment was conducted under rain out structure in the GCU Faisalabad, Pakistan. Seeds of two Bell pepper genotypes viz., California Wonder (salt tolerant) and Green Beauty (salt sensitive) selected on the basis of previous experiments (Javed 2019) were collected from the Vegetable Research Institute (AARI), Faisalabad, Pakistan. The experiment was carried out in sand filled plastic pots (16 cm diameter $\times 16$ cm length). Each pot was wrapped with plastic bag and filled with 6 kg washed and fine sand. The Gravimetric method was used to estimate the water holding capacity of the sand (Bethlahmy 1952). Ten good and healthy seeds of both bell pepper genotypes were planted in each pot. Seven days after emergence five healthy seedlings were kept in each pot after thinning. To fulfill the nutrient requirement of the seedlings Hoagland's nutrient solution (Hoagland and Arnon 1950) was used. One month after sowing, bell pepper plants were exposed to different salinity levels (control, 25, 50, 75, 100 and 125 mM of NaCl). In splits, NaCl concentrations were given by raising 25 mM with an interval of two days until the necessary concentration was reached. For proper maintenance of salinity levels regular evaluation of salt levels were conducted by using an EC meter. The experiment was laid out in a completely randomized design with factorial arrangement having three replications.

Sampling and measurement of seedling growth related traits

Growth parameters including seedling shoot length (SL), root length (RL), seedling fresh (SFW) and dry weights (SDW), root dry weight (RDW), antioxidant activity, proline content and Sodium (Na⁺) and Potassium (K⁺) ion concentrations were determined 50 days after sowing.

Three seedlings from each pot were uprooted, cleaned and washed with distilled water to remove any sand or dirt particle. Seedlings were put in filter paper to remove any drop of water present on the surface of leaf or shoot. The seedlings were then cut into shoot and root parts and shoot and root lengths were measured separately with the help of a meter rod. Average data from each pot was recorded. Then SFW of three plants from each pot was recorded by using the digital balance and average was noted. After measuring the fresh weight, the three plants from each pot were taken into paper bags and then put in oven (Memmert-110, Schawabach) and dried out at 70°C for seven days to get constant weight. Then SDW and RDW were recorded on digital balance and average dry weight of each replicate was noted.

Catalase (CAT) and peroxidase (POD) measurement

The activities of catalase (CAT) and peroxidase (POD) were calculated with some alteration by the Chance and Maehly (1955) procedure. The CAT reaction solution (3 mL) consisted of an enzyme extract of 50 m*M* phosphate buffer (pH 7.0), 5.9 m*M* H₂O₂ and 0.1 mL. Changes in reaction solution absorbance were recorded at 240 nm for every 20s.

As an absorbance change of 0.01 units per min, one-unit CAT activity was specified. It consisted of 50 mM phosphate buffer (pH 5.0), 20 mM guaiacol, 40 mM H_2O_2 and 0.1 mL enzyme extract. After every 20 s, variations in reaction solution absorption were calculated at 470 nm. As an absorbance change of 0.01 units per min, one-unit POD activity was assigned. Based on the protein content, the activity of each enzyme was articulated.

Free proline determination

To determine the free proline content in leaves Bates *et al.* (1973) method was followed. Fresh leaf sample of 0.5 g was thoroughly homogenized and mixed in 10 mL of 3% sulfosalicylic acid, then after filtration, two mL filtered sample was taken in a test tube and reacted to two mL ninhydrin solution. After adding two milliliters of glacial acetic acid in test tube, this sample mixture was heated at 100°C for one hour. After heating this sample was extracted with 4 mL toluene solution. The chromophore containing toluene was aspirated from the aqueous phase and absorbance was recorded on spectrophotometer at 520 nm. Toluene was used as a blank.

Sodium (Na⁺) and potassium (K⁺) ions determination

Method of Allen *et al.* (1986) for ion determination in leaf and roots was used. A mixture of 14 g of LiSO₄.2H₂O, Se (0.42 g), H₂O₂ (350 mL) and conc. H₂SO₄ (420 mL) was prepared. Dry leaf material (0.1 g) was digested separately from each template in a blend of digestion (2 mL). All flasks with leaf samples and mixture of digestion were heated at 200°C on a hot plate. Each digested sample was diluted to 50 mL and used to estimate the Na⁺, and K⁺ ion concentration using flame photometer (Jenway, PFP-7).

Statistical analyses

Fisher's analysis of variance technique using Statistix-8.1 software was used to statistically analyze the recorded data. Comparison among significant means was executed by employing least significant difference (LSD) test at 5% probability level (Steel *et al.* 1997). Microsoft excel program was used to calculate regression correlation among different traits.

Results

Growth traits

Seedling shoot fresh weight showed significant ($P \le 0.05$) individual as well as interactive effect of salt stress levels and bell pepper genotypes (Table 1). As the salinity levels increased from 0 to 125 m*M*, a linear decrease in shoot fresh weight of both genotypes was observed. The highest reduction in SFW was noted at 125 m*M* salinity level in salt sensitive genotype Green Beauty (Table 1). Imposition of salinity stress reduced the SFW by 11% (25 m*M*), 17% (50 m*M*), 27% (75 m*M*), 45% (100 m*M*) and 53% (125 m*M*) in California Wonder compared to control plants (Table 1). While salt sensitive genotype Green Beauty showed 15, 21, 36, 48 and 59% of reduction against 25, 50, 75, 100 and 125 m*M* salinity levels as compared to plants grown under normal conditions, respectively (Table 1). Nevertheless, both genotypes exhibited decrease in shoot fresh weight but the magnitude of decrease was higher in salt sensitive genotype as compared to salt tolerant genotype at all salinity stress levels.

A significant ($P \le 0.05$) individual effect of bell pepper genotypes and salt stress levels and their interaction was noted regarding shoot and root dry weights (Table 1). Both seedling shoot and root dry weights linearly decreased with increasing salt stress levels. Maximum reduction in shoot and root dry weights was recorded at 125 mM salinity level in sensitive genotype Green Beauty. Salt tolerant genotype California Wonder exhibited a reduction of (5, 24, 31, 40 and 49%), while sensitive genotype Green Beauty revealed 12, 28, 38, 47 and 67% reduction in SDW at 25, 50, 75, 100 and 125 mM salinity levels respectively as compared to non-stressed plants (Table 1). Similarly at 25, 50, 75, 100 and 125 mM salinity levels, RDW was decreased by 9, 28, 43, 57 and 67% in California Wonder, while this reduction was 19, 37, 53, 66 and 89% in genotype Green Beauty, respectively against normally grown plants (0 mM) (Table 1). In fact, at all salt stress levels, salt tolerant genotype California Wonder showed higher values of shoot and root dry weights when compared to salt sensitive genotype Green Beauty. Seedling shoot and root lengths depicted an individual significance of bell pepper genotypes and salt stress levels, as well as a significant ($P \leq$ 0.05) interaction between the two factors. Salt tolerant genotype California Wonder at all salinity levels (25, 50, 75, 100 and 125 mM) revealed a significant decrease in SL (8, 16, 21, 27 and 32%) and RL (2, 12, 19, 31 and 40%) respectively, as compared to control plants (0 mM) (Table 1). Likewise salt sensitive genotype Green beauty indicated a decline of 10, 19, 25, 31 and 41% in SL, while RL was reduced by 8, 18, 28, 37 and 52% at 25, 50, 75, 100 and 125 mM salinity levels respectively against control plants (Table 1). This reduction in shoot length and root length parameters was higher in Green beauty in comparison to California Wonder at all stress levels.

Anti-oxidative defense system

Data regarding CAT and POD enzyme activity indicated a significant ($P \le 0.05$) individual as well as an interactive effect of bell pepper genotypes and salt stress levels (Table 2). A linear increase in CAT and POD enzyme activity was noted in both bell pepper genotypes with increasing salt stress levels from 0 to 125 m*M* (Table 2). An increase of 11, 17, 24, 32 and 46% in CAT activity, while 11, 15, 28, 35

| Salinity stress levels | Bell pepper genotypes | Shoot fresh weight (g/plant) | Shoot dry weight (g/plant) | Root dry weight (g/plant) | Shoot length (cm) | Root length (cm) |
|---------------------------|-----------------------|------------------------------|----------------------------|---------------------------|-------------------|------------------|
| Control | California Wonder | $7.37 \pm 0.89a$ | 3.58 ± 1.06a | $2.91 \pm 0.37a$ | $8.61 \pm 0.72a$ | $6.33 \pm 0.89a$ |
| (0 mM) | Green Beauty | $7.16 \pm 0.69a$ | $3.45 \pm 0.33a$ | $2.89 \pm 0.47a$ | $8.46\pm0.94a$ | $6.29 \pm 0.29a$ |
| 25 mM | California Wonder | $6.56\pm0.84b$ | $3.41 \pm 0.29a$ | $2.66\pm0.77b$ | $7.92\pm0.77b$ | $6.23 \pm 1.06a$ |
| | Green Beauty | $6.10 \pm 1.02c$ | $3.06\pm0.69b$ | $2.34 \pm 1.02c$ | $7.62 \pm 1.06c$ | $5.79 \pm 0.33b$ |
| 50 mM | California Wonder | $6.11 \pm 0.77c$ | $2.73 \pm 0.76c$ | $2.10 \pm 0.94d$ | $7.25 \pm 0.77d$ | $5.56 \pm 1.02b$ |
| | Green Beauty | $5.61 \pm 0.20d$ | 2.51 ± 1.06 cd | $1.82 \pm 0.89e$ | $6.89 \pm 1.07e$ | $5.17 \pm 0.36c$ |
| 75 mM | California Wonder | $5.39 \pm 0.69d$ | $2.47\pm0.20d$ | $1.65 \pm 0.77e$ | $6.81 \pm 0.94e$ | $5.11 \pm 0.84c$ |
| | Green Beauty | $4.56 \pm 0.33e$ | $2.15 \pm 0.36e$ | $1.35 \pm 0.69 f$ | $6.34 \pm 0.20 f$ | $4.54 \pm 0.29d$ |
| 100 mM | California Wonder | $4.06\pm0.84f$ | $2.13 \pm 0.29e$ | $1.25 \pm 0.29 f$ | $6.24\pm0.94f$ | $4.37 \pm 1.02d$ |
| | Green Beauty | $3.71 \pm 0.77g$ | $1.85\pm0.33f$ | $0.98 \pm 0.37g$ | $5.81 \pm 0.36g$ | $3.93 \pm 1.09e$ |
| 125 mM | California Wonder | $3.44 \pm 1.07h$ | $1.81\pm0.20f$ | $0.96 \pm 0.14g$ | $5.72 \pm 0.84g$ | $3.82 \pm 0.69e$ |
| | Green Beauty | $2.92 \pm 0.77i$ | $1.13 \pm 1.02 g$ | $0.56 \pm 0.17h$ | $4.98 \pm 1.06h$ | $3.02\pm0.77f$ |
| LSD values at 5% | 5 | 0.26 | 0.23 | 0.16 | 0.29 | 0.29 |

Table 1: Effect of different salinity stress levels on growth traits of two bell pepper genotypes

Means \pm SE values in each coulmn with different letters indicate that treatments are statistically different at P < 0.05

Table 2: Effect of different salinity stress levels on ionic and biochemical traits of two bell pepper genotypes

| Salinity stress levels | Bell pepper genotypes | Catalase (Units g ⁻¹ FW) | Peroxidase (Units g ⁻¹ FW) | Free proline (µmol g ⁻¹ FW) | Leaf Na ⁺ (mg g ⁻¹ DW) | Root Na ⁺ (mg g ⁻¹ DW) | Leaf K ⁺ (mg g ⁻ ¹ DW) | Root K^+ (mg $g^{-1}DW$) |
|------------------------|-----------------------|-------------------------------------|---------------------------------------|---|---|---|--|-----------------------------|
| Control | California | $312\pm1h$ | $335\pm0.7 fg$ | $1.51 \pm 1 \mathrm{fg}$ | $1.68\pm0.3f$ | $0.86\pm0.4g$ | $21\pm0.9a$ | $20\pm0.8a$ |
| (0 mM) | Wonder | | | | | | | |
| | Green Beauty | $307 \pm 0.6h$ | $327 \pm 0.9 g$ | $1.44 \pm 0.9g$ | $1.72 \pm 0.9 f$ | $0.76 \pm 0.3g$ | $21 \pm 0.7ab$ | $20 \pm 1a$ |
| 25 mM | California | $347 \pm 1f$ | $372 \pm 0.6e$ | $1.76 \pm 0.4e$ | $1.92 \pm 0.7e$ | $1.11 \pm 0.3f$ | $20 \pm 0.9b$ | $17 \pm 0.3b$ |
| | Wonder | | | | | | | |
| | Green Beauty | $332 \pm 0.3g$ | $347 \pm 0.6f$ | $1.62 \pm 0.5 f$ | $2.16 \pm 1d$ | $0.90 \pm 0.2g$ | 17 ±.0.7c | $19 \pm 0.9a$ |
| 50 mM | California | 367 ± 0.7 de | $384 \pm 1e$ | $1.94 \pm 0.3d$ | $2.05\pm0.9d$ | $1.54 \pm 0.4d$ | $17 \pm 0.3c$ | $14 \pm 0.6c$ |
| | Wonder | | | | | | | |
| | Green Beauty | $353 \pm 1ef$ | $378 \pm 0.3e$ | $1.78 \pm 0.9e$ | $2.14\pm0.7d$ | $1.26 \pm 0.2e$ | $14 \pm 0.6d$ | $16 \pm 1b$ |
| 75 mM | California | $388 \pm 0.3c$ | $427 \pm 0.6c$ | $2.16\pm0.8c$ | $2.07\pm0.4d$ | $1.96\pm0.4c$ | $13 \pm 0.6d$ | $10 \pm 1e$ |
| | Wonder | | | | | | | |
| | Green Beauty | $373 \pm 0.9d$ | $403 \pm 0.6d$ | $1.98 \pm 0.9 d$ | $2.33\pm0.9c$ | $1.63 \pm 0.7d$ | $10 \pm 0.9e$ | $12 \pm 1d$ |
| 100 mM | California | $411 \pm 0.4b$ | $454 \pm 0.6b$ | $2.37 \pm 1b$ | $2.39 \pm 0.6c$ | $2.22 \pm 0.5b$ | $9 \pm 1f$ | $8 \pm 1 f$ |
| | Wonder | | | | | | | |
| | Green Beauty | $391 \pm 0.9c$ | $438 \pm 1c$ | $2.17 \pm 1c$ | $2.59\pm0.7b$ | $1.95 \pm 0.3c$ | $7 \pm 0.6g$ | $10 \pm 1e$ |
| 125 mM | California | 457 ± 1a | $492 \pm 0.5a$ | $2.73\pm0.9a$ | $2.66\pm0.40b$ | $2.71 \pm 0.4a$ | $7 \pm 01g$ | $5\pm0.8g$ |
| | Wonder | | | | | | | |
| | Green Beauty | $418\pm0.5b$ | $454 \pm 1b$ | $2.32\pm0.8b$ | $2.85 \pm 1a$ | $2.25\pm0.8b$ | $4 \pm 1h$ | $7\pm0.6f$ |
| LSD values at | 5% 13.77 | 13.04 | 0.13 0.14 | 0.11 1.17 | 1.29 | | | |

Means \pm SE values in each coulmn with different letters indicate that treatments are statistically different at P < 0.05

and 47% in escalation in POD activity was noted at 25, 50, 75, 100 and 125 mM salinity levels respectively against normally treated plants (0mM) in genotype California Wonder (Table 2). Corresponding to 25, 50, 75, 100 and 125 mM salt stress levels genotype Green Beauty showed an increase of 8, 15, 22, 27 and 36% in CAT activity, while POD activity was enhanced by 6, 15, 23, 34 and 39% respectively, as compared to non-stressed plants (Table 2). Although CAT and POD levels increased in both genotypes at all stress levels but this increase was more pronounced in salt tolerant genotype. Regarding leaf free proline content of bell pepper genotypes a highly significant ($P \le 0.05$) individual as well as interactive effect has been found between genotypes and salt stress levels (Table 2). Leaf free proline content increased with increasing salt stress levels from 0 to 125 mM salt stress level. California Wonder (Salt tolerant genotype) exhibited 17, 29, 44, 58 and 81% increase, while salt sensitive genotype Green Beauty showed 15, 27, 41, 55 and 65% increase in free proline contents against 25, 50, 75, 100 and 125 mM salinity levels respectively when

compared to control (Table 2). Salt tolerant genotype California Wonder showed better leaf free proline contents at all stress levels as compared to salt sensitive genotype Green Beauty.

Ionic compositions and balance

A highly significant ($P \le 0.05$) individual as well as interactive effect of bell pepper genotypes and salt stress levels has been noted regarding Na⁺ ion concentration in leaf and roots. Na⁺ ion concentration increased with increasing the level of salt stress in leaves and roots of both genotypes. Salt tolerant genotype California Wonder showed 14, 22, 23, 42 and 58% increase in leaf Na⁺ contents, while salt sensitive genotype Green Beauty revealed 26, 24, 35, 51 and 66% rise in leaf Na⁺ contents at 25, 50, 75, 100 and 125 mM salinity levels respectively in comparison to control plants (Table 2). In case of root Na⁺ contents California Wonder indicated 30, 79, 122, 159 and 215% increase, while Green Beauty displayed 18, 67, 115, 158 and 197% rise at 25, 50, 75, 100 and 125 mM salinity levels respectively against control

| Crop traits | CAT | Leaf K ⁺ | Root K^+ | Leaf Na ⁺ | Root Na ⁺ | POD | Proline | RL | SDW | SFW |
|----------------------|---------|---------------------|------------|----------------------|----------------------|--------|---------|-------|-------|-------|
| Leaf K ⁺ | -0.55* | | | | | | | | | |
| Root K ⁺ | -0.74* | 0.25* | | | | | | | | |
| Leaf Na ⁺ | 0.73* | -0.70* | -0.51* | | | | | | | |
| Root Na ⁺ | 0.90** | -0.39* | -0.68* | 0.69* | | | | | | |
| POD | 0.93** | -0.36* | -0.70* | 0.57* | 0.89** | | | | | |
| Proline | 0.97** | -0.56* | -0.71* | 0.74* | 0.89** | 0.93** | | | | |
| RL | -0.69* | 0.43* | 0.49* | -0.59* | -0.69* | -0.68* | -0.71* | | | |
| SDW | -0.43* | 0.14* | 0.13* | -0.36* | -0.38* | -0.42* | -0.42* | 0.31* | | |
| SFW | -0.73** | 0.41* | 0.32* | -0.71* | -0.72* | -0.71* | -0.73* | 0.50* | 0.71* | |
| SL | -0.76* | 0.54* | 0.63* | -0.71* | -0.74* | -0.66* | -0.76* | 0.65* | 0.22* | 0.47* |

Table 3: Correlation among growth traits, antioxidants and ions of bell pepper genotypes under salinity stress

*, ** significant at 0.05 and 0.01% probability levels, respectively

CAT: Catalase, POD: Peroxidase, RL: Root length, SL: Shoot length, SDW: Shoot dry weight, SFW: Shoot fresh weight

plants (Table 2). Hence, more roots and less leaf Na⁺ contents were noted in California Wonder as compared to Green Beauty at all salt stress levels.

Data pertaining to the effect of different bell pepper genotypes and salt stress levels on K⁺ ion concentration in leaf and roots indicated a significant ($P \le 0.05$) individual as well as interactive effect of two factors. A decline in the K⁺ ion concentration in leaves and roots has been noted in both bell pepper genotypes with the increase of salt stress in both leaves and roots. In case of California Wonder, leaf K⁺ contents revealed 6, 21, 39, 57 and 68% decline, while genotype Green Beauty showed 18, 36, 51, 67 and 80% reduction in leaf K^+ contents with respect to 25, 50, 75, 100 and 125 mM salinity levels respectively as compared to normally grown plants (Table 2). Regarding root K⁺ contents, California Wonder showed 11, 30, 37, 62 and 75 % decrease and genotype Green Beauty indicated 6, 20, 39, 51 and 63% decline at 25, 50, 75, 100 and 125 mM salinity levels respectively when compared to control plants (0 mM) (Table 2).

Correlation coefficients

Correlation coefficients between bell pepper genotype seedling traits, antioxidant activity, free proline and ion (Na⁺ and K⁺) accumulation are presented in Table 3. Correlation coefficients were highly significant for most of the ionic and biochemical attributes studied in this experiment. Among the positive and highly significant correlations were, POD, CAT, free proline, Na⁺ with POD, free proline, Na⁺ with K⁺ correlated highly significant and negative with POD, CAT, free proline and Na⁺, SL with RL, SDW with RDW and SFW with SDW (Table 3).

Discussion

Crop growth is influenced by change in salt level in growth medium and measuring crop differential response determines the salt tolerance level that assists in the varietal selection. In this study, the contrasting varietal response of pepper was measured in terms of growth, ionic composition and defense system (Tables 1 and 2). Growth traits like dry and fresh weights of shoot and root and their respective lengths were decreased with increasing salt stress depicting the negative correlation between growth and salt stress in both varieties. Actually, high salt concentration reduces the water potential of the growth medium thus, causing substantial decline in cell turgor to prevent cell elongation and cell division, hence minimizing the plant growth (Shahid et al. 2012). Similar growth trend has been reported by Ambede et al. (2012) in groundnut (Vigna subterranea) when subjected to supra-optimal saline environment. Moreover, higher accumulation of Na⁺ ions in leaves minimizes the photosynthetic capacity by decreasing the activity of rubisco and NADPH enzymes involved in photosynthesis, which might have caused the biomass production (Ashraf and Foolad 2007). Comparing performance of both pepper varieties lesser growth decline in California wonder could have been due to their better maintenance of cell turgor (Ashraf and Foolad 2007). Maintenance of better cell turgor by California Wonder can be correlated with higher K⁺ uptake and free proline accumulation and chlorophyll content in its leaves under salinity stress which is an important stress tolerance strategy (Huang et al. 2013; Kholghi et al. 2018).

Furthermore, California Wonder mediated the oxidative stress by employing antioxidant defense system to scavenge reactive oxygen species. Catalase and peroxidase were produced in its leaves more as compared to sensitive variety in the presence of salt stress (Table 2). These higher activities might decompose H_2O_2 into water thereby minimizing the damaging effects of H_2O_2 from the stressed cells and improves the oxidative capacity of plants enabling them to better tolerate the salt stress (Parida *et al.* 2004; Elgawad *et al.* 2016). So the California Wonder with defense system depicted higher salt tolerance through upregulation of CAT and POD activities under salt stress. The susceptibility or tolerance of a plant genotype depends on the relationship between ROS and antioxidant enzyme production under stress (Mittler 2002; Farooq *et al.* 2017).

It is a well-established fact that salt stress uplifts the Na⁺ concentration in different plant parts and declines the concentration of cations like Ca²⁺ and K⁺ (Husain *et al.* 2012, 2013). In the present investigation, salt tolerant genotype California Wonder showed higher leaf K⁺/ Na⁺ ratio compared to sensitive genotype. Maintenance of K⁺

ions uptake and preventing K^+ ion efflux from leaf cells, while avoiding Na⁺ and favoring efflux of Na⁺ ions from leaf cells are potential strategies undertaken by plants to achieve desirable $K^+/$ Na⁺ ratio in the cytosol to tolerate the salt stress (Wakeel *et al.* 2011; Hussain *et al.* 2013). Moreover, salt tolerant plants may retain more Na⁺ ions in the roots and limit their transport to the upper parts (leaves). It is an adaptation strategy to cope with salinity stress while salt sensitive plants lack this kind of adaptations (Shahid *et al.* 2012). In current research, overall the Green beauty could not compete with California wonder because of reduced K uptake, weaker defense system and growth traits and finally declared to be sensitive variety.

Conclusion

The genotype California wonder showed better growth performance by maintaining higher K^+/Na^+ ratio, enhanced anti-oxidative activity and greater free proline accumulation in the presence of slat stress as compared to Green beauty and proved to be salt tolerant.

Acknowledgements

The data presented here is a part of PhD thesis at GC University Faisalabad and authors highly acknowledge the support of HEC-NRPU 5635. Thanks are due to Dr. Abid Ali (UAF) for his comments and suggestion on earlier draft of this manuscript.

References

- Akhtar FM, A Hussain, F Ahmad, MA Kharal, I Khalid, M Latif, MU Jamshaid (2017). Screening of Pepper (*Capsicum annuum* L.) genotypes against salinity stress. *J Environ Agric Sci* 11:51–58
- Akhtar J, T Haq, A Shahzad, MA Haq, M Ibrahim, N Ashraf (2003). Classification of different wheat genotypes in salt tolerance categories on the basis of biomass production. *Intl J Agric Biol* 5:322–325
- Ali S, M Rizwan, MF Qayyum, YS Ok, M Ibrahim, M Riaz, MS Arif, F Hafeez, MI Al-Wabel, AN Shahzad (2017). Biochar soil amendment on alleviation of drought and salt stress in plants: a critical review. *Environ Sci Pollut Res* 24:12700–12712
- Allen SG, AK Dobrenz, MH Schonhorst, JE Stoner (1986). Heritability of NaCl tolerance in germinating alfalfa seeds. Agron J 77:90–96
- Ambede JG, GW Netondo, GN Mwai, DM Musyimi (2012). NaCl salinity affects germination, growth, physiology, and biochemistry of bambara groundnut. *Braz J Plant Physiol* 24:151–160
- Ashraf M, MR Foolad (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206–216
- Bates LS, RP Waldron, IW Teaxe (1973). Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
- Bethlahmy N (1952). A method for approximating the water content of soils. Eos Trans Amer Geophys Union 33:699–706
- Chance B, AC Maehly (1955). Assay of catalase and peroxidase. *Meth Enzymol* 2:764–775
- Elgawad HA, G Zinta, MH Momtaz, R Pandey, H Asard, W Abuelsoud (2016). High salinity induces different oxidative stress and antioxidant responses in maize seedlings organs. *Front Plant Sci* 7; Article 726
- Evelin H, TS Devi, S Gupta, R Kapoor (2019). Mitigation of salinity stress in plants by Arbuscular mycorrhizal symbiosis: current understanding and new challenges. Front Plant Sci 10; Article 470
- FAO (2015). Food and Agriculture Organization of the United Nations. Production Year Book, Rome, Italy

- Farooq M, M Hussain, A Wakeel, KHM Siddique (2015). Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agron Sustain Dev* 35:461–481
- Farooq M, N Gogoi, M Hussain, S Barthakur, S Paul, N Bharadwaj, HM Migdadi, SS Alghamdi, KHM Siddique (2017). Effects, tolerance mechanisms and management of salt stress in grain legumes. *Plant Physiol Biochem* 118:199–217.
- Hoagland DR, DI Arnon (1950). The Water-culture Method for Growing Plants Without Soil, Circular 347. The College of Agriculture, University of California, Berkley, California, USA
- HuangZ, L Zhao, D Chen, M Liang, Z Liu, H Shao, X Lon (2013). Salt stress encourages proline accumulation by regulating proline biosynthesis and degradation in Jerusalem artichoke plantlets. *PLoS One* 8; Article e62085
- Hussain M, HW Park, M Farooq, K Jabran, DJ Lee (2013). Morphological and physiological basis of salt resistance in different rice genotypes. *Intl J Agric Biol* 15:113–118
- Javed A (2019). Effect of salt stress on physiological, morphological and biochemical attributes of bell pepper and it's amelioration by silicon. *PhD Thesis*, GC University Faisalabad, Pakistan
- Khan GS (1999). Soil Salinity/Sodicity Status of Pakistan, pp: 59. Soil Survey of Pakistan, Lahore, Pakistan
- Kholghi M, M Toorchi, MR Shakiba (2018). An evaluation of canola genotypes under salinity stress at vegetative stage via morphological and physiological traits. Pak J Bot 50:447–455
- Kim K, SH Park, JC Chae, BY Soh, KJ Lee (2014). Rapid degradation of Pseudomonasfluorescens1-aminocyclopropane-1-carboxylicacid deaminase proteins expressed in transgenic Arabidopsis. FEMS Microbiol Lett 355:193–200
- Mehmood N, NA Abassi, IA Hafiz, I Ali, S Zakia (2017). Effect of Biostimulant on growth, yield and quality of Bell pepper CV. Yellow wonder. *Pak J Agric Sci* 54:311–317
- Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7:405–410
- Nadeem M, FM Anjum, MR Khan, M Saeed, A Riaz (2011). Antioxidant potential of bell pepper (*Capsicum annum* L.) A review. *Pak J Food Sci* 21:45–51
- Navarro JM, C Garrido, M Carvajal, V Martinez (2002). Yield and fruit quality of pepper plants under sulphate and chloride salinity. J Hortic Sci Biotechnol 77:52–57
- Parida AK, AB Das (2005). Salt tolerance and salinity effects on plants; a review. *Ecotoxicol Environ Saf* 60:324–349
- Parida AK, AB Das, P Mohanty (2004). Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. *J Plant Physiol* 161:531–542
- Penella C, SG Nebauer, S Lopéz-Galarza, AS Bautista, E Gorbe, A Calatayud (2015). Some rootstocks improve pepper tolerance to mild salinity through ionic regulation. *Plant Sci* 230:12–22
- Pessarakli M, TC Tucker (1988). Nitrogen-15 uptake by eggplant under sodium chloride stress. Soil Sci Soc Amer J 52:1673–1676
- Qureshi AS, PG McCornick, M Qadir, Z Aslam (2007). Managing salinity and waterlogging in the Indus Basin of Pakistan. *Agric Water Manage* 95:1–10
- Rangani J, AK Parida, A Panda, A Kumari (2016). Coordinated changes in antioxidative enzymes protect the photosynthetic machinery from salinity induced oxidative damage and confer salt tolerance in an extreme halophyte Salvado rapersica L. Front Plant Sci 7; Article 50
- Shahid MA, MA Pervez, RM Balal, T Abbas, CM Ayyub, NS Mattson, A Riaz, Z Iqbal (2012). Screening of pea (*Pisum sativum L.*) genotypes for salt tolerance based on early growth stage attributes and leaf inorganic osmolytes. *Aust J Crop Sci* 6:1324–1331
- Steel RGD, JH Torrie, DA Dickey (1997). Principles and Procedures of Statistics, Approach, pp: 178–182. McGraw Hill Co. New York, USA
- Villa-Castorena M, AL Ulery, EA Catalán-Valencia, MD Remmenga (2003). Salinity and nitrogen rate effects on the growth and yield of chile pepper plants. *Soil Sci Soc Amer J* 67:1781–1789
- Wakeel A, M Farooq, M Qadir, S Schubert (2011). Potassium substitution by sodium in plants. Crit Rev Plant Sci 30:401–413
- Zia MA (2006). Managing aflatoxin in chilli crop. *The Daily Dawn*: Economic & Business Review, July 31, 2006