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IONS ACCUMULATION, PROLINE CONTENT AND JUICE QUALITY OF SUGAR BEET GENOTYPES AS AFFECTED BY WATER SALINITY

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ABSTRACT

In arid and semiarid regions salinity affects physiology of most of the plants. Under salt stressed conditions, plants do not uptake required quantity of nutrient elements from soil solution; they rather uptake and accumulate larger quantity of toxic ions like Na^+, Cl^-, etc. Plants have various mechanisms to tolerate soil salinity. Among these mechanisms, K^+/Na^+ discrimination and synthesis of osmo-protectants is recognized as most effective ways to cope with salt-stress environment. However, variation exists among these genotypes. The goal of this study was to stress ten sugar beet (*Beta vulgare* L.) genotypes with 4, 8, 12 and 16 EC (dS m^{-1}) levels of saline water in field plots up to maturity and determine variations in Na^+, K^+ and Cl^- concentrations in the sap of fully mature leaves, proline (osmo-protectant) content of leaf tissues and juice quality. The genotypes included in the study were: California, Ernestina, Magnolia, Mirabella, Sandrina, SD 12970, SDPAK 03/06, SDPAK01/07, SDPAK 07/07 and SDPAK 09/07. The results indicated that the plants grown on soil receiving saline water of EC 4, 8, 12, and 16 (dS m^{-1}) salt solutions accumulated more Na^+ and Cl^- and less K^+. Hence they displayed lower K^+/Na^+ ratio in their leaves. Under salt-stress environment of soil receiving saline water of EC 8 and 16 (dS m^{-1}), sugar beet plants have synthesized more proline in their leaves. Among the 10 genotypes included in the study, four (California, SD PAK 09/07, SD PAK 03/06 and SD PAK 01/07) were found with less Na^+, more K^+ and lower K^+/Na^+ ratio, these genotypes were also able to exhibit more proline in leaves, under salt-stress environment, possibly to counter the osmotic effect of salts.

Keywords: Sugar beet, genotypes, proline, Na^+, K^+, K^+/Na^+.

INTRODUCTION

Salinity in soil or in water is a key factor that limits the growth, development and yield of field crops (Zhang and Shi, 2013; Wu *et al.,* 2015). The arid and semi-arid regions of the world are more prone to salinity, where more than 800 million hectares of land are salty in nature (Munns, 2002**;** FAO, 2005). One of the major factors of salinity development in these climates appears to be the result of usage of poor quality (mainly saline) groundwater for crops cultivation (Rajpar and Sial 1996, Bhatti *et al.,* 2016). The quality of such water is having with variable ranging from0.125 to 1% of commercial NaCl for useable to strongly saline (De Pascale and Barbieri, 1995). The data described by various researchers indicate that the field crops under such saline water environment poorly perform and give considerably low yields (Lee *et al*., 2002; Kim *et al.*, 2008; Feizi *et al.*, 2010). The plant grown under saline condition may be subjected to water-stress, ion toxicity and nutrient disorder (Feizi *et al.*, 2010; Bhatti *et al.,* 2016).

Ionic toxicity is one of the major adverse effects caused by salinity on plants (Munns and Tester, 2008; Ashraf and Foolad, 2013). Among toxic ions Na^+ and Cl^- have been reported as key ions in saline soils (Tester and Davenport, 2003; Munns *et al.,* 2006). Their effects are cumulative or individual in nature such as osmotic, ionic, nutrient imbalance and oxidative stress that leads to reduction of cell division and plant growth as well. The uptake and accumulation of large amount of Na^+ and Cl^- ions by plants under saline conditions could upset the activities of various enzymes and plant metabolism (Munns, 2002; De-lacerda *et al*., 2005**)**. The salt-stress environment also leads to produce large amount of reactive oxygen species (ROS) such as super oxide radicals, hydrogen peroxide and hydroxyl radicals in plants (Jiang and Zhang 2001 and Ahmad *et al.*, 2012).

Salt-tolerant plants have mechanisms to stay away from the overproduction of these reactive molecules and to cope with salinity (Zhang and Shi 2013; Roy *et al*., 2014). Compartmentation of toxic ions (Na^+) into the vacuoles (Hajiboland and Joudmand, 2009, Abbas *et al.,* 2012) for osmotic adjustment and synthesis of highly water soluble compatible osmotica such as glycine, betaine, free proline, and low molecular weight sugars (Khafagi *et al*., 1996; Dhanapackiam and Ilyas, 2010) are few examples. Proline molecules help plants to maintain cell turger (Seki *et al*., 2007). Large quantity of proline is synthesized and accumulated in salt-tolerant plant species during the salt-stress and its accretion may play an important role for salinity stress adaptation (Mansour, 2000; Bavei *et al.,* 2011; Radic´ *et al.,* 2013). Proline accumulation has been reported during salinity

stress in various plants such as *hordeum vulgare* L. (Ueda *et al.,* 2007), *gossypium hirsutum* L. (Desingh *et al*., 2007) and *triticum aestivum* L. (Khan *et al*., 2009).

Sugar beet (*Beta vulgaris* L.) plant of *Chenopodiaceae* family is an important sugar crop after *saccharum offìcinarum* L. It is producing annually about 30% of sugar all over the world (Draycott, 2006**;** Hameed and Ghaffar, 2010). It is mostly cultivated in European countries, while its cultivation is being spread in the arid and semi-arid regions of the world like Iran and Pakistan. Sugar beet is a salt-tolerant crop (Jameel *et al.,* 2006; Wu *et al.,* 2015) however, variations may exist in genotypes (Pakniyat and Armion, 2007; Rajabi, 2010). These variations may depend on the concentrations and contents of toxic ions and osmoprotectants. This paper reports the results of the study conducted for assessment of variation in ion accumulation (Na^+, K^+, K^+/Na^+) and proline content of different sugar beet genotypes under water saline conditions.

MATERIALS AND METHODS

The experiment was conducted under randomized complete block design with split plot arrangement having three replications at National Sugar and Tropical Horticulture Research Institute (NSTHRI) Thatta, Pakistan. Composite soil samples were drawn from 0-15 and 15-30 cm depths before planting. The experimental soil was at 0-15 and 15-30 cm depths was silty clay loam in texture, low in organic matter content (0.71 to 0.88 %), normal in reaction (pH 7.42 to 7.60), non-saline (EC 1.25 to 1.60 dS m^{-1}) and non-sodic in nature, respectively. Land was prepared by different cultural operations. The plots size was 150 m^2 and ridges of one meter length and one meter apart were made. Sugar beet seed was planted on both sides of ridges. Plant to plant space of 20 cm was maintained. Ten sugar beet genotypes (California, Ernestina, Magnolia, Mirabella, Sandrina, SD-12970, SD PAK 03/06, SD PAK 01/07, SD PAK 07/07 and SD PAK 09/07) were included in the study. Phosphorus @120 kg P_2O_5 ha^{-1} and N @ ½ of the nitrogen (60 kg N ha^{-1}) were applied before soil preparation. Remaining ½ of the N was applied in two equal splits, i.e. 30 kg N $ha^{-1}$was applied 50 days after sowing (DAS) and the remaining at 120 DAS. Thinning was done after establishment of seedlings, while eradication of weeds done by hoeing.

Salt treatments: Sugar beet plants were tested against five salinity levels. In the control treatment plots, plants were continuously irrigated with canal water (EC of 0.45 dS m^{-1} and SAR 2.12); whereas in salt treatment plots, plants were receiving salt solutions of 4, 8, 12 and 16 EC (dS m^{-1}). Sodium chloride in irrigation water was added to establish desired salinity levels. The salt solutions were prepared by dissolving sodium chloride salts into canal irrigation water until the desired EC (dS m^{-1}) level was achieved. Salt-stress was initiated soon after establishment of seedlings (15 DAS) and continued up to the maturity of the crop. Canal water and salt solutions were applied @ 3 acre-inch irrigation level each time and over all seven irrigations were applied.

Extraction of leaf sap for Na^+ and K^+ analysis: The concentration of Na^+ and K^+ was determined in the sap of the top fully mature leaves using by Flame-photometer (Jenway PFP-7); the sap was extracted through the method of Gorham (1984) using micro-centrifuge.

Analysis of proline in leaf tissues: Proline content was determined in leaf tissues through the method of Bates *et al.* (1973). A leaf sample (1.0 g) was extracted with 10 ml of 3% sulphosalicylic acid. Extracts with 2 ml was kept for 1.0 h in boiling water by adding 2.0 ml ninhydrin and 2.0 ml glacial acetic acid, after which cold toluene (4.0 ml) was added. Then proline contents was determined by spectrophotometer at 520 nm and calculated as μg g^{-1} against standard proline.

Analysis of beet juice quality: The sugar beet crop was harvested at physiological maturity. Beet juice was extracted using cutter grinder (Model SCF-L4, Smith Crafts Fabricator, Pakistan). The juice was then analyzed for brix, polarity (pol %), and purity %. The brix % was estimated with the help of digital Refractometer (PR-101, ATAGO Co Ltd, Japan). The pol% was measured by polarimeter (Model AA-5 Series, Optical Activity, London), whereas impurities in beet juice were calculated with the following formula:

Juice Impurity (%) = 100-(Pol% in juice × 100/Brix % in Juice).

Recoverable sugar (R %) was determined using the following formula of Asadi (2007) :

R (%) = (Pol% in beet) – 0.5 (Brix% in beet - Pol% in beet).

Statistical analysis: The experiment was carried out in split plot arrangement under randomized complete block design (RCBD). The treatments were placed in main plots and genotypes in subplots. The collected data were compiled and Analysis of Variance (ANOVA) was performed Using 8.1 Statistical software. Mean value differences for all parameters were compared by LSD using Tukey's *t* test (Steel *et al*., 1997).

RESULTS

Na^+ and K^+ concentrations determined in the leaf sap and K^+/Na^+ ratio: The data given in Table 1-3 indicated that the water salinity sugar beet plants were able to accumulate more Na^+, less K^+ and displayed lower K^+/Na^+ ratio in the sap obtained from their top fully mature leaves. Averaged overall genotypes compared to

control, the plants grown in 4, 8, 12 and 16 EC (dS m^{-1}) treatment plots had 50, 85, 102 and 200 % more Na^+; 13, 15 , 38 and 39 % less K^+ and showed 38, 56, 67 and 82 % lower K^+/Na^+ ratio in leaf sap, respectively. Overall, salinity levels; the difference between genotypes was also highly significant ($p<0.05$). The genotypes SDPAK 07/07, Mirabella, Ernestina and Magnolia accumulated more Na^+, less K^+, and displayed lower leaf K^+/N^+ ratio. Comparatively the other four genotypes including California, SDPAK 09/07, SDPAK 03/06, and SDPAK 01/07 were found to accumulate less Na^+, more K^+ and displayed higher K^+/Na^+ ratio in their leaf sap.

Effect of water salinity on beet juice quality: It is evident from the results plotted in Fig-1&2 that the effect of increasing water salinity was to increase impurities in beet juice. Compared to unstressed/normal plants, more impurities were found in the juice extracted from salt stressed plants. The difference among genotypes for juice impurities was also significant ($p<0.05$), the genotype SDPAK 07/07, followed by SD-12970, Magnolia and Sandrina displayed more impure juice than SDPAK 03/06, followed by California and SDPAK 09/07. Maximum sugar recovery was observed from the sugar beet plants stressed with 4 and 8 EC (dS m^{-1}) solution, followed by un-stressed plants, while minimum sugar recovery was observed from the plants stressed with 16 EC (dS m^{-1}) salt solution. Averaged overall salinity levels, the genotype SDPAK 09/07 showed maximum sugar recovery, followed by California and SDPAK 03/06. On the other hand, minimum sugar recovery was observed in SD 12970 and SDPAK 07/07 genotypes.

Proline content (ug g^{-1}): Data pertaining to proline synthesis by ten sugar beet genotypes are depicted in Table-5. The effect of salinity, genotypes were significant ($p<0.05$), while interaction of salinity × genotypes remained non-significant ($p<0.05$). Results further revealed that the amount of proline was increased with increasing water salinity. Averaged overall salinity levels, SDPAK 03/06 had more proline, followed by SDPAK 01/07, SDPAK 09/07 and SD-12970, whereas; lower content of proline was observed in SDPAK07/07, Magnolia and Mirabella genotypes. The remaining genotypes were found to be intermediate in proline synthesis. Overall under salt-stress treatments response of SDPAK 03/07 and SDPAK 09/07 was better in terms of proline synthesis.

Table1. Sodium in sugar beet leaf sap (mol m^{-3}) under different water salinity levels.

Genotype	Salinity (EC dS m^{-1})					Mean
	Control	4	8	12	16	
California	478.3 d-f	608.7 c-f	792.8 c-f	758.5 c-f	884.1 b-f	704.4 B
Ernestina	261.3 f	763.3 c-f	1282.6 a-f	1347.8 a-f	1517.9 a-e	1034.6 AB
Magnolia	345.8 ef	605.1 c-f	644.9 c-f	857.5 c-f	1763.3 a-c	843.3 AB
Mirabella	411.1d-f	905.8 b-f	905.8 b-f	1050.7 b-f	2050.7 ab	1064.8 AB
Sandrina	791.5 c-f	593.0 c-f	983.1 b-f	1072.5 b-f	985.5 b-f	885.1 AB
SD-12970	557.5 d-f	576.1 c-f	601.4 c-f	688.4 c-f	1563.3 a-d	797.3 B
SDPAK 03/06	449.7 d-f	565.2 d-f	782.6 c-f	1008.7 b-f	1217.4 a-f	804.7 B
SDPAK 01/07	508.5 d-f	552.3 d-f	647.3 c-f	657.0 c-f	1285.0 a-f	730.0 B
SDPAK 07/07	536.2 d-f	942.0 b-f	1021.7 b-f	1299.5 a-f	2398.6 a	1239.6 A
SDPAK 09/07	378.2 d-f	968.6 b-f	1087.0 b-f	768.1 c-f	483.1d-f	737.0 B
Mean	471.8 C	708.0 BC	874.9 B	950.9 B	1414.9A	
	Salinity (S)		Genotypes (G)		S × G	
SED	91.46		129.35		289.25	
Tukey HSD (5%)	254.22***		418.65**		1192.4**	

, *=significant at 0.05 & 0.01%, respectively, ns=non-significant, df= degree of freedom

Table2. Potassium in sugar beet leaf sap (mol m^{-3}) under different water salinity levels

Genotype	Salinity (EC dS m^{-1})					Mean
	Control	4	8	12	16	
California	660.9	498.7	430.8	313.3	158.9	412.5 AB
Ernestina	272.8	277.7	329.0	235.0	207.4	264.4 B
Magnolia	421.2	396.0	286.3	182.3	142.4	285.6 B
Mirabella	291.6	277.8	282.0	236.4	165.2	250.6 B
Sandrina	410.2	425.2	235.0	166.6	148.1	277.0 B
SD-12970	471.5	410.2	286.3	232.9	140.7	308.3 B
SDPAK 03/06	726.7	475.8	371.7	284.1	239.3	419.5 AB

SDPAK 01/07	589.7	491.9	649.5	414.5	357.5	500.6 A
SDPAK 07/07	473.5	373.6	282.0	227.9	145.3	300.48 B
SDPAK 09/07	596.6	538.4	666.6	658.1	356.1	563.2 A
Mean	491.5 A	416.5 AB	381.9 BC	295.1 CD	206.1 D	
	Salinity (S)		Genotypes (G)			S × G
SED	38.43		54.36			121.56
Tukey HSD (5%)	106.84***		175.94***			NS

Table3. K^+/Na^+ ratio in sugar beet leaf sap of different water salinity levels

Genotype	Salinity (EC dS m^{-1})					Mean
	Control	4	8	12	16	
California	1.4	0.88	0.58	0.56	0.18	0.72 AB
Ernestina	1.2	0.40	0.26	0.21	0.16	0.46 B
Magnolia	1.3	0.65	0.45	0.22	0.09	0.55 AB
Mirabella	0.83	0.39	0.34	0.27	0.08	0.38 B
Sandrina	0.52	0.74	0.35	0.17	0.15	0.38 B
SD-12970	0.85	0.71	0.50	0.33	0.09	0.50 B
SDPAK 03/06	1.6	1.0	0.52	0.29	0.23	0.73 AB
SDPAK 01/07	1.1	1.1	0.98	0.65	0.33	0.86 A
SDPAK 07/07	0.84	0.41	0.31	0.18	0.06	0.36 B
SDPAK 09/07	1.5	0.57	0.67	0.81	0.76	0.88 A
Mean	1.1 A	0.70 B	0.50 BC	0.38 CD	0.21 D	
	Salinity (S)		Genotypes (G			S × G
SED	0.08		0.12			0.27
Tukey HSD (5%)	0.23***		0.39***			NS

, *=significant at 0.05 & 0.01%, respectively, ns=non-significant, df= degree of freedom

Table4. Proline content in sugar beet leaf tissues (ug g^{-1}) under different water salinity levels

Genotype	Salinity (EC dS m^{-1})			Mean
	Control	8	16	
California	718.3	1289.0	1857.3	1288.2 BC
Ernestina	827.0	899.3	1395.7	1040.7 C
Magnolia	651.7	1097.7	1674.7	1068.0 C
Mirabella	785.7	837.0	1680.3	1101.0 C
Sandrina	1049.0	1397.3	1390.3	1278.9 BC
SD-12970	1065.0	1530.7	1973.7	1523.1 BC
SDPAK 03/06	1632.0	2359.0	2446.0	2145.7 A
SDPAK 01/07	1798.3	1659.0	1639.0	1698.8 AB
SDPAK 07/07	528.0	952.7	868.7	783.1 D
SDPAK 09/07	988.0	1460.7	2170.3	1539.7B C
Mean	1004.3 C	1348.2 B	1709.3 A	
	Salinity (S)	Genotypes (G		S × G
SED	90.14	164.46		285.05
Tukey HSD (5%)	217.17***	540.89***		NS

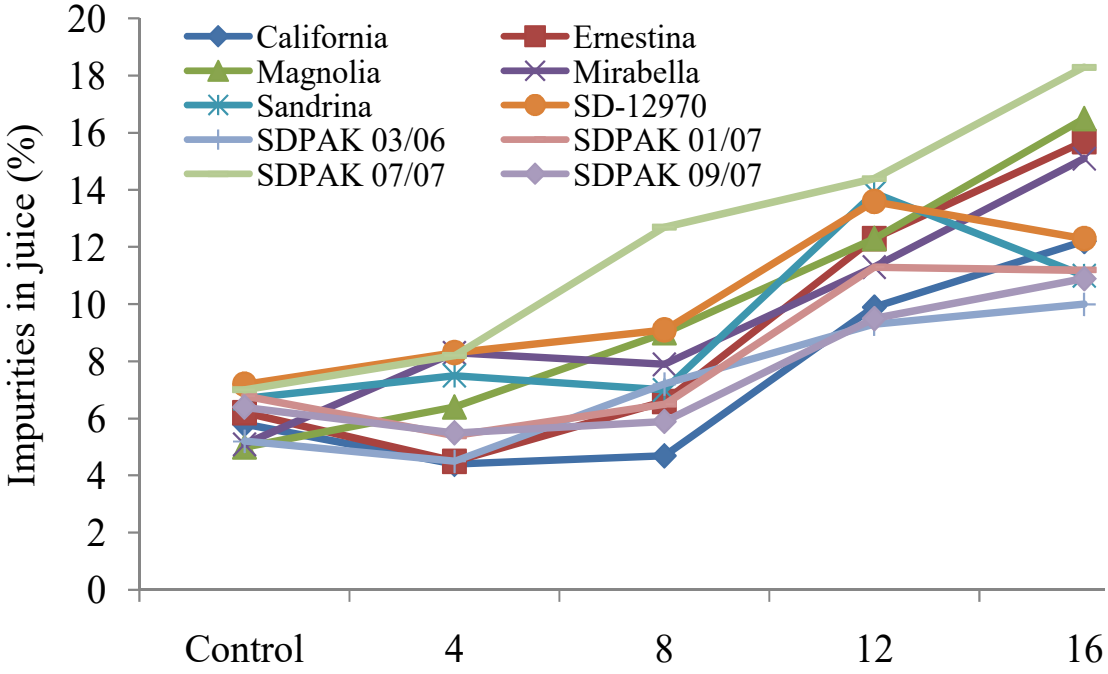

Fig. 1. Effect of water salinity on impurities in sugar beet juice

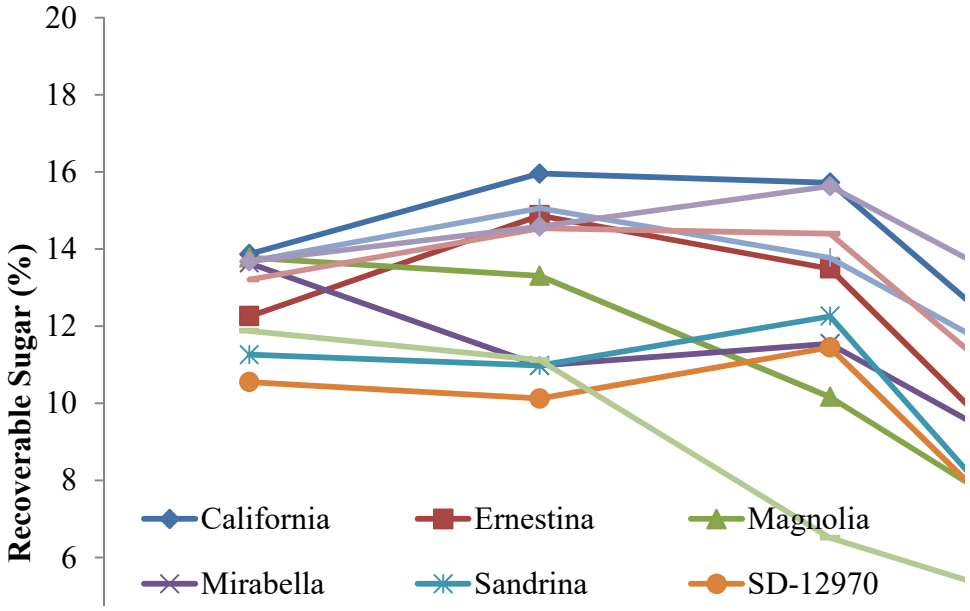

Fig. 2. Effect of water salinity on sugar recovery (%) from different sugar beet genotypes

DISCUSSION

Increase in leaf sap Na^+ with increasing salinity was the result of adding NaCl salt into water for application through irrigation. Under this environment, plants were possibly able to absorb passively more Na^+ ions through roots and transported to it leaf tissues (Tester and Davenport, 2003). Sugar beet genotypes SDPAK 01/07, SDPAK 09/07, California and SD-12970 accumulated low concentration of Na^+ depicting relatively more salt tolerant as compared to other genotypes. This might be due to better salt restriction mechanisms at root level in these genotypes over the other genotypes (Rajabi *et al*., 2010; Maathius *et al*., 2014**)**. The results are supported by Dadkhah and Grrifiths (2006), who reported that sugar beet variety P29 was salt tolerant due to accumulation of less Na^+ in leaf tissues under high saline water level (350 *mM*). As the potassium is an essential element, it plays an important role in plant metabolism, osmoregulation, and

maintenance of cell turgor and stimulation of photosynthetic activities (Maathius, 2009; Ashraf *et al.,* 2011; Deinlein *et al.,* 2014). Restricted K^+ uptake in sugar beet genotypes at higher water salinity levels indicated non-selective behavior of sugar beet genotypes for Na^+ over K^+. Hence, salinity decreased the uptake of K^+ and increased the uptake of Na^+ in sugar beet genotypes (Yildirim, 2008; Shokari and Maadi, 2009; Haq *et al*., 2013). However, in SDPAK 09/07 genotype up to EC 12 (dS m^{-1}) and in SDPAK 01/07 and Ernestina genotypes up to EC 8 (dS m^{-1}), the uptake of K^+ was higher than normal plants. It indicates better selection mechanisms of ions at root level by these genotypes. It has been widely demonstrated by various workers (Munns and Tester 2008; Wakeel *et al*., 2010; Zaki *et al*. 2014) that availability and uptake of K^+ by sugar beet plants changed under saline environment, mainly due to competition of Na^+ with K^+. The juice impurity in sugar beet plants can be the result of accumulation of higher concentration of absorbed Na^+ ions. These results are comparable with those of Abdel-Mawly and Zanou (2004); Darwish *et al.* (2005); Eisa *et al.* (2011) and Zaki *et al*. (2014), who also reported that high soil and water salinity deteriorates the beet juice quality. Under salt-stress environment, plants produce large quantity of reactive oxygen species (ROS), such as superoxide radicals, singlet oxygen, hydrogen peroxide and hydroxyl radicals (Kanwal *et al*., 2013; Hussain, 2015). In order to stay away from overproduction of ROS, salt-tolerant plants evolve some osmo-protectants, including polyoles, glycinebetaine and proline as well (Zaki *et al.,* 2012; Koyro *et al.*, 2013; Joseph, 2015). In this study proline content was determined in the plants grown in control, 8 and 16 EC waters (Table-5). The results of proline content of sugar beet indicated that plants grown in EC 16 dS m^{-1} accumulated more proline. Higher proline accumulation in SDPAK 03/06, SDPAK 01/07, SDPAK 09/07 and SD-12970 at high water salinity levels as compared to others genotypes suggested that different beet genotypes had variable ability to synthesize proline under stress conditions. More proline synthesis in leaf tissue of these genotypes made a better protection mechanism against oxidative damage to plants by maintaining a higher inherited and induced activity of antioxidant enzymes. Shehata *et al.* (2000); Ghoulam (2002) and Farkhondeh *et al.* (2012) reported that proline involved in improving salt tolerance by protecting against salinity stress.

Conclusion: Increasing water salinity levels significantly increased Na^+ and proline contents and decreased K^+ and K^+/Na^+ ratio in sugar beet leaf tissue. Consequently, sugar impurities were increased with increasing of water salinity levels. It is concluded from the present finding that ions and proline accumulation can be used to identify the sugar beet genotypes having potential to tolerate water salinity. The sugar beet genotypes California, SDPAK 09/07, SDPAK 03/06, SDPAK 01/07 were more tolerant to salinity and performed well at an EC 4 and 8 (dS m^{-1}). The better performance of these genotypes was linked with the accumulation of less Na^+, more K^+, high K^+/Na^+ ratio and considerable amount of leaf proline under salt-stress environment. Hence, these genotypes may be considered for cultivation under saline environments.

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