

ASSESSMENT OF BIOCHEMICAL AND IONIC ATTRIBUTES AGAINST SALT STRESS IN EGGPLANT (*Solanum melongena* L.) GENOTYPES

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ABSTRACT

Biotic and abiotic factors contribute in yield loss of horticultural crops, but the salinity is one of foremost concern. Salinity is dilemma in vegetable production in lowering quality and yield. Eggplant is a high value vegetable crop, playing important role in combating malnutrition and increasing income of farming community. Pot culture experiment was conducted to study the ionic and biochemical changes in eggplant genotypes (Saadia-tolerant and Black Beauty-sensitive) under different salinity levels (control, 3, 6, 9, 12 and 15 dS m⁻¹) of NaCl. The enzymatic activities such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and osmolytes [glycine betaine (GB) and proline] were substantially increased in both tested eggplant genotypes under salt stress. Tolerant genotype (Saadia) performed better than sensitive one for these variables. Whereas malondialdehyde contents (MDA) were maximum in sensitive (Black Beauty) with increasing salinity levels. Among the ionic traits, Na⁺ and Cl⁻ were enhanced while Ca²⁺ and K⁺ considerably reduced in response to increasing saline water stress. Whereas, tolerant genotype maintained the high concentration of beneficial ions (Ca²⁺ and K⁺) and the minimum amounts of lethal ions (Na⁺ and Cl⁻). The study elaborated that tolerant genotype showed positive enzymatic and ionic response against salinity stress levels.

Keywords: Antioxidants, biochemical, eggplant, GB, ionic changes, MDA, proline, salinity.

INTRODUCTION

Crop production is decreasing globally due to increased soil degradation, water scarcity, organic matter depletion, acidity, poor drainage, nutrient depletion and salinization (Roy *et al.*, 2014). Salinity is a global agricultural issue; as 20% of cultivated area and half of irrigated lands are saline in the world (Ghassemi *et al.*, 1995; Edelstein *et al.*, 2011). Countries like Australia, Egypt, China, United States of America, India and Pakistan have salinity problems ranging from 15 to 36% of irrigated lands (Schwabe *et al.*, 2006; Chaves *et al.*, 2009).

Initially, salt stress in eggplant imposes two effects: an osmotic stress that inhibits water intake and/or water efflux of root cells and ion toxicity of Na⁺ and Cl⁻ (Munns and Tester, 2008). At higher salinity level, eggplants accumulate Cl⁻ which causes chlorosis, necrosis and burning of leaf margins (Unlukara *et al.*, 2010). High accumulation of Na⁺ in leaves due to salinity also causes nutrient deficiency in eggplant (Tester and Davenport, 2003; Dekoum *et al.*, 2013). Nutrient deficiency is due to competitive interaction of ions or by changing the ion selectivity of membrane which results in salt induced calcium ion (Ca²⁺) and potassium ion (K⁺) deficiencies (Yasar *et al.*, 2006; Hakim *et al.*, 2014; Mustafa *et al.*, 2014).

Leaves of eggplant showed significant increase in MDA contents, electrolyte leakage, hydrogen peroxide contents, and hydroxyl radical and superoxide production at higher salinity levels (Ding *et al.*, 2012). The end product of lipid peroxidation (LPO) is MDA which is a potential indicator of oxidative damage to the cell membrane by ROS production (Jain *et al.*, 1987). Eggplant produces enzymatic and non-enzymatic ROS scavengers to cope with salt induced oxidative stress (Shaheen *et al.*, 2013). Salt stress increases SOD and CAT activities in eggplant to reduce the ROS damage (Dai *et al.*, 2009; Manar *et al.*, 2013). SOD enzyme transforms superoxide into hydrogen peroxide which is further scavenged by APX and CAT (Wu *et al.*, 2012). Wild genotypes of eggplant produce more antioxidant enzymes and less MDA under salt stress conditions, thus, high enzymatic activity means more tolerance to salinity (Yasar and Ellialtioglu, 2013).

Salt stress imposes various physiological and biochemical changes in eggplant. To cope with this stress, plants adopt several mechanisms at the cellular, metabolic and whole plant level. Such mechanisms include stress signaling, osmotic regulation, ion homeostasis, and antioxidant production. In fact, it is hard to find single criteria suitable to study the salt stress tolerance of eggplant. It is obvious that ionic and biochemical attributes are more helpful for better understanding of salt tolerance in eggplant as compared to the growth indicators (Ashraf, 2004).

Although salinity has great influence on the ionic and biochemical attributes of eggplant as reported earlier. The main focus of this investigation was to explore the ionic and biochemical differences of salt tolerant and sensitive eggplant genotypes under salt stress conditions.

MATERIALS AND METHODS

Materials and equipment: Seeds of eggplant (*Solanum melongena* (L.) c.v Saadia and Black Beauty}. The major equipment; centrifuge machine spectrophotometer and flame photometer were used in this research.

Planting and Treatment: Ten healthy seeds of both eggplant genotypes were sown in plastic pots comprising 7 kg of sand. Salt solutions at different concentrations (control, 3, 6, 9, 12 and 15 dS m⁻¹ of NaCl) were applied to develop salinity one month after sowing time. NaCl concentrations were provided in splits by increasing 3 dS m⁻¹ with two days interval till required concentration was attained. Half strength Hoagland solution was used as nutrient solution. Each pot containing 5 seedlings was considered as one replicate and four pots were considered one treatment. Fifty days after seed sowing leaf samples was collected and analyzed.

Proline determination: Protocols of Bates *et al.* (1973) was followed for proline determination. The proline concentration was calculated on fresh weight (FW) basis by using a standard curve established by Analar grade proline and calculated as follows:-

$$\mu\text{mole of proline g}^{-1} \text{ FW} = \left[\frac{\{(\text{mg of proline mL}^{-1}) \times (\text{toluene (mL)})\}}{(\text{sample(g)/5})} \right] / 115$$

Leaf glycinebetaine (GB): GB was measured following Grieve and Grattan (1983) procedure. One gram fresh leaf sample was shaken for 5 min in 10 mL toluene solution (0.5%) and filtered. Then filtrate (1 mL) was mixed with 2N H₂SO₄ (1 mL) and 0.5 mL of this blend was taken in a glass tube and potassium tri-iodide (0.2 mL) was added. Then 6 mL of 1-2 dichloroethane and ice cooled distilled water (2.8 mL) were mixed into the mixture. The higher aqueous layer was removed and optical density of the organic layer noted at 365 nm.

Malondialdehyde (MDA): Protocols of Cakmak and Horst (1991) was used for MDA estimation. One gram fresh leaf sample from all replicate was ground in 5 mL of TCA (1%) and centrifuged at 15,000 rpm (10 min) under cold environment. Mixture of TCA (3 mL) and TBA (0.5% thiobarbituric acid in 20% TCA) was further mixed with 0.5 mL of the supernatant. Mixture was incubated (58 °C) for 50 min in a shaking water bath. This reaction was terminated by setting the test tubes on ice and optical densities were taken at 532 and 600 nm.

Antioxidant enzymes: Fresh leaf tissue (0.5 g) was ground in a grinder. Then 5 mL of cool phosphate buffer (50 mM; pH 7.8) was supplemented to it. After that homogenate was vortex and centrifuged (15,000 rpm) at 48 °C for 15 min. The supernatant was isolated and used for antioxidants assays. SOD was measured following Giannopolitis and Ries (1977), by assessing the photo-reduction of nitro blue tetrazolium (NBT) by the enzyme. The reaction mixture was containing enzyme extract, NBT (50 mM), riboflavin (1.3 mM), methionine (13 mM), EDTA (75 nM) and phosphate buffer (20 mM) which were homogenized in a test tube. Then reaction mixture was exposed to white luminous light (15W lamp) at 80 mmol m⁻²s⁻¹ for 15 min. The OD was measured using spectrophotometer at 560 nm of each solution. Enzyme required to stop half of NBT photo-reduction was measured equivalent to one unit of SOD. The POD and CAT activities were estimated by following the method of Chance and Maehly (1955). The changes in absorbance were noted at 470 nm with 30 second interval. Variation in the absorbance per minute was assumed equivalent to one unit of POD activity. For the estimation of CAT activity, 3 mL of reaction solution was used containing 50 mM phosphate buffer (pH 7.8), 5.9 mM H₂O₂ and enzyme extract (0.1 mL). The fluctuations in absorbance were measured at 240 nm of the reaction solution with 20 second interval. A variation of 0.01 unit min⁻¹ in absorbance was assumed equal to one unit of CAT activity. The activity of these enzymes was measured and indicated on the basis of total protein.

Ion determination: Method of Allen *et al.* (1986) was followed for ion determination. Digestion mixture containing 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) from each replicate was digested separately in digestion mixture (2 mL). All flasks having plant leaf samples and digestion mixture were heated on a hot plate at 200 °C. Each digested sample was diluted up to 50 mL and used for ions (Na⁺, Ca²⁺ and K⁺) estimation using flame photometer (Jenway, PFP-7).

Cl⁻ determination: Distilled water (10 mL) was taken in a test tube and dried ground leaf material (0.1 g) from each replicate was added to it and then incubated it overnight at 25 °C. Test tubes were then heated (80 °C) till the volume in tubes remained half of the original volume. Distilled water was further added to every test tube to maintain the volume (10 mL) again after cooling. Cl⁻ concentration in the leaf extracts estimated using chloride analyzer (Model 926, Sherwood, Cambridge, UK).

Experimental design and statistical analysis: Experimental unit having four replications and six salinity treatments was designed in a Complete Randomized Design (CRD) with two factor factorial

arrangements. Analysis of variance and multiple comparison tests (Tukey test) were estimated with Statistix 8.1. Differences among treatments were considered significant at $p = 0.05$ after statistical analysis.

RESULTS

Antioxidants enzymes: The results for antioxidants (SOD, CAT and POD) activities were significant (Fig.1a,b&c) maximum enzymatic activities were recorded at 15 dS m⁻¹ and gradually decreased by 12, 9, 6, 3 and 0dS m⁻¹. The salt tolerant genotype (Saadia) exhibited higher enzymatic activities than sensitive genotype (Black Beauty) in response to increasing salt stress. The maximum enzymatic activities were recorded in Saadia at 15 dS m⁻¹ and minimum in Black Beauty in control.

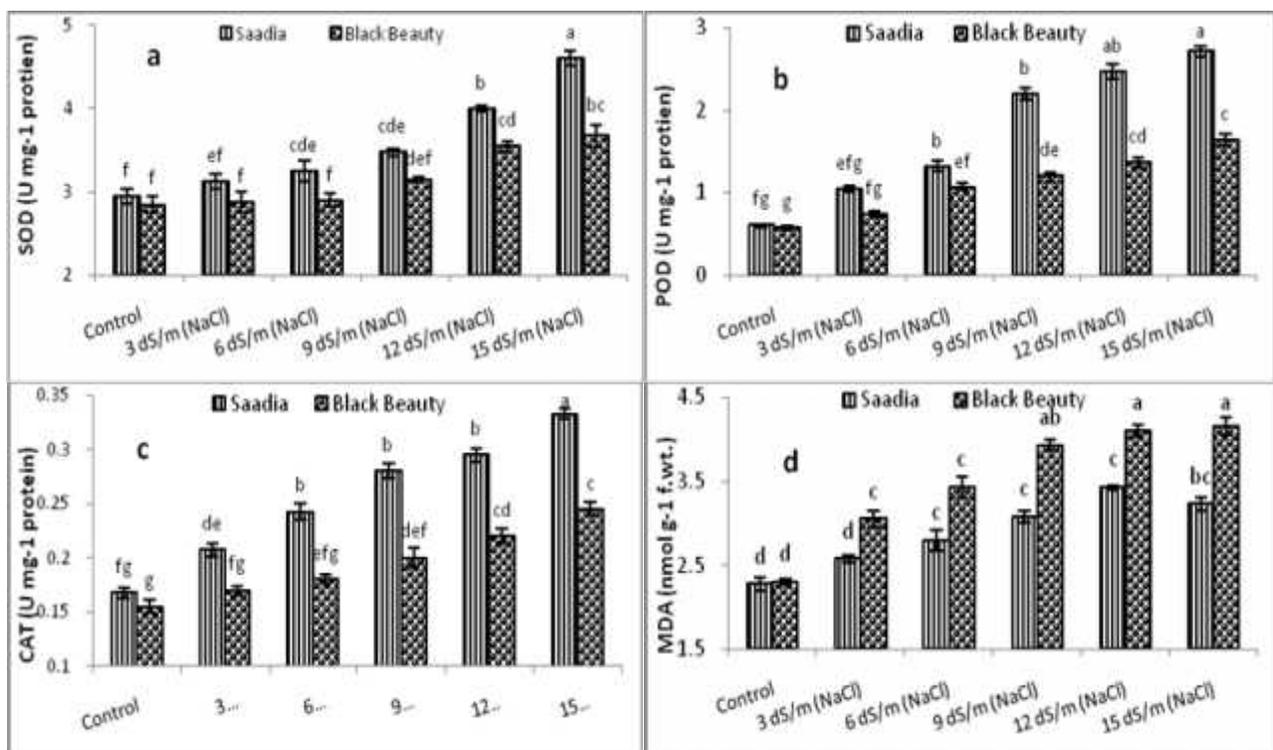
Malondialdehyde (MDA): MDA contents exhibited an increasing trend under salinity stress, whereas maximum was recorded at 12 dS m⁻¹ which was statistically similar with 15 and 9 dS m⁻¹ but expressively greater over 3 dS m⁻¹ and control (Fig. 1d). The salt sensitive genotype (Black Beauty) showed higher MDA contents than tolerant-Saadia. Maximum MDA contents were recorded in Black Beauty at 15 dS m⁻¹ and minimum in Saadia at control. Positive response of both eggplant genotypes was

observed in terms of MDA contents with increasing salinity stress of 15 dS m⁻¹.

Osmolytes: The proline contents of both genotypes increased significantly by increasing salinity stress; however maximum increase was observed at 15 dS m⁻¹ and gradually decreased by 12, 9, 6, 3 and 0 dS m⁻¹ (Fig. 1e). The salt tolerant genotype-Saadia accumulated significantly higher free proline as compared to salt sensitive genotype-Black Beauty. The maximum proline contents were recorded in salt tolerant genotype (Saadia) at 15 dS m⁻¹ and minimum in salt sensitive-Black Beauty under no stress.

The GB contents of both the genotypes differed significantly by salinity stress; positive response of both eggplant genotypes was observed in terms of GB contents with increasing salinity stress level of 15 dS m⁻¹ (Fig. 1f). The salt tolerant genotype-Saadia accumulated significantly higher GB as compared to salt sensitive genotype-Black Beauty. The maximum GB contents were quantified in Saadia at 15 dS m⁻¹ and minimum in Black Beauty at control.

Ionic analysis of leaf: Mean comparisons of salinity stress levels indicated significant effect on Na⁺ and Cl⁻ contents whereas, the maximum increase was observed under 15 dS m⁻¹, followed by 12, 9, 6, 3 and 0dS m⁻¹. The salt sensitive genotype (Black Beauty) absorbed maximum Na⁺ and



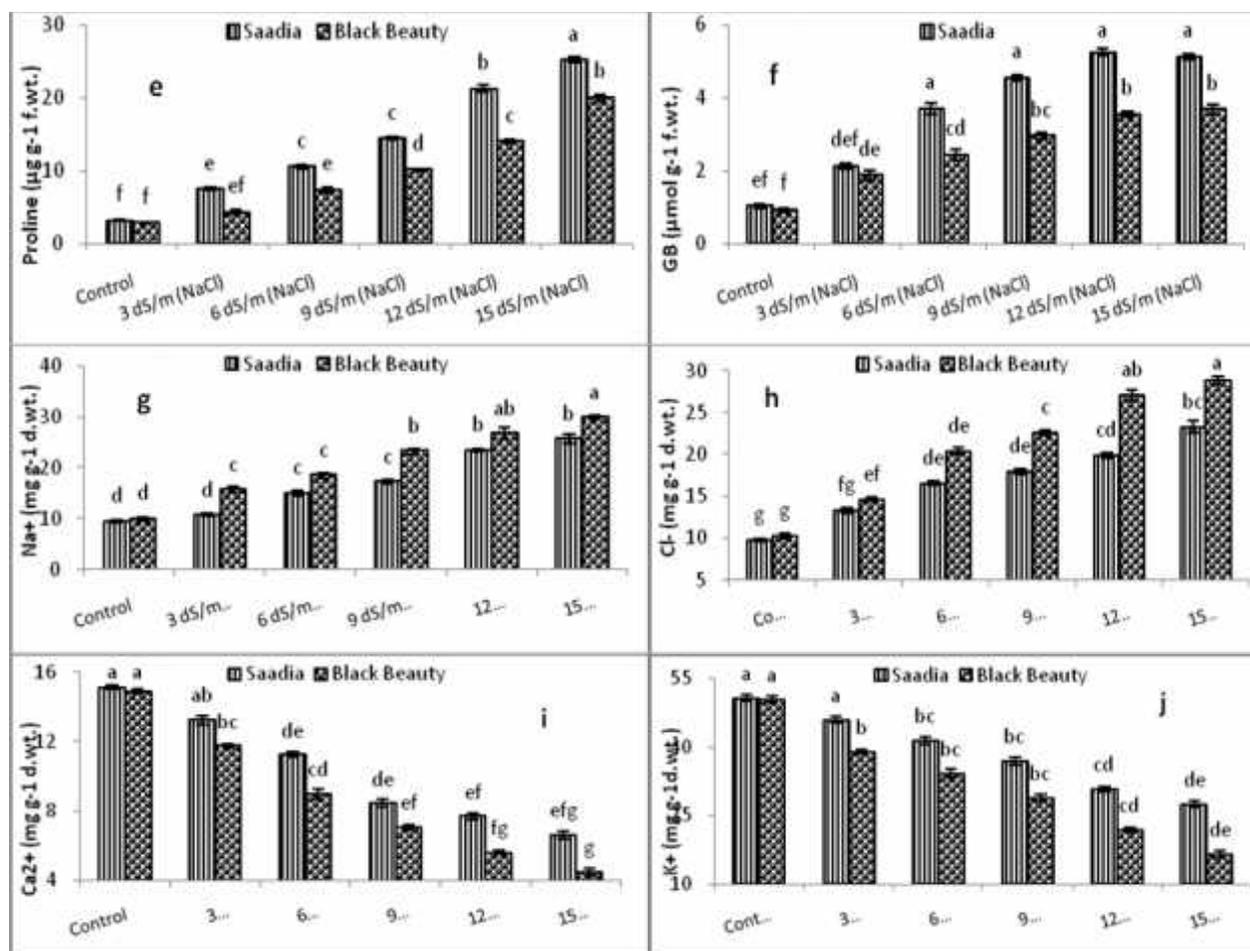


Figure 1. Biochemical and ionic attributes of eggplant genotypes under different salt stress levels: (a) SOD, (b) POD, (c) CAT, (d) MDA, (e) Proline, (f) GB, (g) Na⁺, (h) Cl⁻, (i) Ca²⁺, (j) K⁺.

Cl⁻ contents in its leaf than that of salt tolerant genotype (Saadia). The maximum leaf Na⁺ and Cl⁻ absorbance was recorded in Black Beauty at 15 dS m⁻¹ and minimum in Saadia at control. Positive response of both eggplant genotypes was observed in terms of leaf Na⁺ and Cl⁻ absorbance with increasing salt stress (Fig. 1 g&h).

A substantial reduction in Ca²⁺ and K⁺ contents was noted with the increase of salinity stress, the maximum reduction was observed under 15 dS m⁻¹ followed by 12, 9, 6, 3 and 0 dS m⁻¹. The genotype Saadia maintained significantly higher Ca²⁺ and K⁺ contents than that of Black Beauty. The maximum Ca²⁺ and K⁺ contents were recorded in genotype-Saadia at control (no salinity) and minimum in Black Beauty at 15 dS m⁻¹. Poor response of both eggplant genotypes was noted for Ca²⁺ and K⁺ contents with higher salinity stress (15 dS m⁻¹) (Fig. 1 i&j).

Table 1. Correlation coefficients among biochemical and ionic attributes of eggplant genotypes under salinity stress. **, *: r² significant at 0.01, significant p 0.05.

	POD	CAT	GB	Proline	MDA	Ca	K	Na	Cl
SOD	0.8448**	0.8734**	0.8177**	0.9068**	0.3507*	-0.6146**	-0.5735**	0.6490**	0.5696**
POD		0.9638**	0.9583**	0.9081**	0.3731**	-0.6788**	-0.5427**	0.5866**	0.5397**
CAT			0.9268**	0.9069**	0.3295*	-0.6370**	-0.5048**	0.5517**	0.5114**
GB				0.9186**	0.5462**	-0.7995**	-0.6676**	0.7010**	0.6636**
Proline					0.5752**	-0.8147**	-0.7471**	0.7968**	0.7432**
MDA						-0.9104**	-0.9370**	0.8967**	0.9322**
Ca							0.9563**	-0.9488**	-0.9637**
K								-0.9713**	-0.9799**
Na									0.9645**

Correlation coefficients: Correlation coefficients were highly significant for most of the ionic and biochemical attributes studied in this experiment (Table 1). Among the positive and highly significant correlations were SOD, POD, CAT with GB, proline, Na⁺ and Cl⁻; MDA with POD, GB, proline, Na⁺ and Cl⁻; Ca²⁺ with K⁺. Ca²⁺ and K⁺ correlated highly significant and negative with POD, CAT, SOD, GB, Proline, MDA, Na⁺ and Cl⁻.

DISCUSSION

Salt stress caused an ionic effect which results into oxidative stress. Production of reactive oxygen species (ROS) is a sign of oxidative stress. Superoxide radical initiates a series of reactions that generates ROS, which interrupts the metabolic process of cell by oxidative degradation of nucleic acids, proteins and lipids (Munns and Tester, 2008). ROS also damage the cell membranes by LPO in plants under saline regimes (Wu *et al.*, 2012). MDA is end product of LPO and is used for oxidative damage determination (Hajlaoui *et al.*, 2010). Though, salinity stress positively influenced the LPO in both the tested eggplant genotypes, but the maximum LPO was noted for salt sensitive Black Beauty than tolerant Saadia (Figure 4.2.5). The maximum MDA contents in sensitive genotype is the indication of maximum oxidative injury while, the tolerant genotype resisted the ROS generation, so presented less MDA and consequently minimum LPO. Findings of present study proved that LPO has strong negative relationship with salt tolerance. Outcomes of current study are in agreement with the reports of Yasar and Ellialtioglu (2013) regarding the LPO in eggplant under salt stress regimes.

Plants have developed an antioxidant defense system based on many antioxidant enzymes such as SOD, POD and CAT etc. to reduce the oxidative loss under stressed surroundings especially salt stress (Masood *et al.*, 2006). Antioxidant system of plant keeps the ROS to at less noxious level inside the cell. The salinized plants of the tested genotypes displayed an improved values of antioxidant enzymes (SOD, CAT and POD) than control plants, but the maximum enzymatic activities were recorded in tolerant genotype (Saadia) while it was minimum in sensitive (Black Beauty) (Fig. 1 a,b&c) in present investigation.

Higher antioxidant enzymes (AOE) activities in tolerant Saadia showed that it is well adapted to the saline environment by decreasing the ROS. Whereas, Black Beauty failed to adjust itself under salinity stress because of less AOE activities, resulting in high ROS production which lead to the maximum LPO and the minimum photosynthesis. A strong correlation was found between AOE activities and salt stress tolerance in this study. Results of this study confirmed the findings of Hegazi *et al.* (2014). Several former reports are also in agreement

with the findings of current investigation (Colville and Smirnov, 2008; Dai *et al.*, 2009).

Plants have the potential to keep their water absorption ability under stressed conditions such as drought, salinity, cold and high temperature. Excessive salts in soil solution lead to salt induced osmotic stress and ion toxicity. High salts level also cause reduction in the water potential of soil solution and this lower water potential accelerates the synthesis of low molecular weight organic solutes for osmotic adjustment (OA) in the plants under saline environments (Bojorquez-quintal *et al.*, 2014). Eggplant genotypes suffered from osmotic stress under saline environments and salinized plants adopted OA mechanism by attaining different organic osmolytes (proline and GB etc.) in their tissues. Tolerant genotype (Saadia) with higher assimilation of proline and GB exhibited high salt tolerance potential because these organic osmolytes are involved in the maintenance of turgor potential under salinity stress which regulates the numerous metabolic processes inside plant body. Plants with maximum concentration of these osmolytes are high in OA potential. GB is a very important osmolyte that plays a vital role in cellular OA and GB performs its role in OA of leaf chloroplasts and protects the thylakoid membranes from the adverse effect of salinity stress (Chen and Murata, 2011; Kamil *et al.*, 2014).

Stressed plants of both the tested eggplant genotypes had high ratios of osmoprotectants (proline and GB) with respect to non-stressed plants (Figure 4.2.5). The salt tolerant genotype (Saadia) exhibited high amount of proline and GB contents while it was less in case of sensitive (Black Beauty). The maximum accumulation of osmoprotectants in tolerant genotype is the indication of efficient OA while the minimum increase in osmolytes in salt sensitive genotype is a sign of low OA potential under saline surroundings. It might be also the reason of high salt tolerance of Saadia than Black Beauty. A positive relationship was recognized between amount of osmoprotectants and salt tolerance. Similar findings have been reported by Kumar *et al.* (2006) and Shahbaz *et al.* (2013).

Salt stress also employed a substantial effect on various ionic traits of investigated eggplant genotypes. It was noticed that salinity stress elevated Na⁺ and Cl⁻ in leaves but a declining pattern was recorded in case of Ca²⁺ and K⁺. Both tolerant and sensitive genotypes displayed marked variations regarding ionic aspects. From the results it is evident that salt tolerant genotype (Saadia) displayed the lowest ratios of Na⁺ and Cl⁻ in its leaves while it was maximum in the leaves of salt sensitive (Black Beauty). Salt tolerant plants contain fewer amounts of toxic ions in upper parts by adopting a mechanism of deposition of toxic ions in to their roots (Dekoum *et al.*, 2013; Shaheen *et al.*, 2013). Less accumulation of leaf Na⁺ and Cl⁻ in tolerant genotype (Saadia) might be due to aforementioned reason.

Although both tested eggplant genotypes presented the considerable reduction in their K^+ and Ca^{2+} ions. But the tolerant genotype maintained the higher concentration of these ions in its leaves than sensitive one. Actually, an antagonistic effect subsists between Na^+ and beneficial ions (K^+ and Ca^{2+}) (Unlukara *et al.*, 2010; Farooq *et al.*, 2015), under this influence Na^+ inhibits the entry of essential ions from soil solution to the roots, resulting in the reduction of these ions in plant leaves. This antagonistic effect may be the cause of reduction in beneficial ions (K^+ and Ca^{2+}) in tested eggplant genotypes. Outcomes of present study are in accordance with the findings of Elwan *et al.* (2010) and Oliveira *et al.* (2011).

Conclusion: Salt tolerance potential of eggplant had direct link with the concentration of antioxidants and compatible organic solutes accumulations as Saadia (tolerant) exhibited maximum concentrations of these indicators. Salt tolerance potential had strong relationship with osmotic adjustment as salt tolerant (Saadia) cultivar demonstrated efficient osmotic adjustment than non-tolerant (Black Beauty) cultivar under saline environments. Sensitive (Black Beauty) accumulated high concentrations of harmful ions (Na^+ and Cl^-) whereas low concentration of beneficial ions (K^+ and Ca^{2+}) in leaves than tolerant (Saadia) cultivar. Hence, tolerant Saadia eggplant genotype had strong ability to cope with excessive salts in sand culture.

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