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Effect of NaCl Stress on Growth, Water Relations, Organic and Inorganic Osmolytes Accumulation in Sunflower (*Helianthus annuus* L.) Lines

Ahmad Heidari[1], Mahmoud Toorchi[1*], Ali Bandehagh[1] and Mohammad-Reza Shakiba[2]

[1] Department of Plant breeding & Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz 51664, Iran
[2] Department of Ecophysiology, Faculty of Agriculture, University of Tabriz, Tabriz 51664, Iran

[*]Corresponding author: mtoorchi@yahoo.com

Abstract:

Salinity is one of the important abiotic stresses that affect growth, physiology, biochemistry and molecules of plants. In this study, response of 12 sunflower (*H. annuus*) lines to NaCl salinity (0, 100 and 200 mM NaCl) was investigated in hydroponic culture system. Plant growth parameters, height, third leaf water status, relative membrane permeability (RMP), organic and inorganic osmolytes were measured 30 days after salinity induced. Among the lines, R_2, R_{56} and R_{50} showed significantly smaller reduction in growth parameters compared with B_{11}, B_{353}, B_{25} and B_{15} indicating that the former lines were more salt tolerant than the others. The line R_2showed less reduction in height and this result revealed that high correlation between height and growth parameters. Relative water content (RWC) was decreased under salinity stress and the lines not differed significantly in this water relation attribute. Leaf water potential (LWP) was increased under salinity but the lines showed contrary relation with growth parameters. Appears that LWP not efficient method to measured water status under greenhouse conditions. RMP in tolerant lines was lowest compared with other lines. Also, glycine betaine (GB) was enhanced under salinity stress but non-significant differences were observed among the lines for this compatibility solute. It seems GB had less important role in sunflower due to it was lowest osmolyte that accumulated under salinity condition. In tolerant lines proline was more accumulated compared with sensitive lines and it was 1.94 times further. The relationship between Na and K cations indicate that at least in sunflower, accumulation of K^+ dependent to Na^+ influx. In other words, the lines that accumulate high Na^+ was have more K^+ content and vice versa. Also, in this study, the K^+ content was increased under salinity but the K^+/Na^+ was decreased.

Key words: Glycine betaine, *Helianthus annuus*, LWP, NaCl, proline, RMP, RWC, salt stress, sunflower, water status.

1.0 Introduction:

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and the natural status of the environment. Increased salinisation of arable land is expected to have devastating global effects, resulting in 30% land loss within the next 25 years, and up to 50% by the year 2050 (Wang *et al.*, 2003). The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Ashraf, 1994b; Marschner, 1995; Zhu, 2003; Turan *et al.*, 2010). Salinity is known to adversely affect production of most crops worldwide (Hasegawa *et al.* 2000; Bayuelo-Jime´nez *et al.* 2002; Ashraf 2009).

Soluble salts at higher concentrations in growth medium cause hyperosmolality and imbalance of nutrients in most plants that harmfully decline plant growth (Zhu, 2003; Turan *et al.*, 2010). Many studies have shown that the height (jamil *et al.*, 2007; Rui *et al.*, 2009; Memon *et al.*, 2010), growth index (Bandeh-hagh *et al.*, 2008) and fresh and dry weights of the shoot and root system (Abdul Jaleel *et al.*, 2007; Ashraf and Ali, 2008; Shahbaz *et al.*, 2010) are affected negatively by changes in salinity concentration, type of salt present, or type of plant species. Numerous studies showed the affection of leaf area negatively by using different concentrations ofNaCl (Zhao *et al.*, 2007; Yilmaz and Kina, 2008; Rui *et al.*, 2009).

Under saline conditions, high accumulation of toxic ions such as Na and Cl takes place in the chloroplast (Jain *et al.*, 2001; Alvarez *et al.*, 2003; Munns, 2005; Munns *et al.*, 2006) and number of studies with different horticultural crops have shown that K^+ uptake is perturbed by salinity thereby resulting in reduced K^+/Na^+ ratio (Graifenberg *et al.*, 1995; Perez-Alfocea *et al.*, 1996). K^+ is very important to the cytosol ionic homeostasis maintenance in Na^+-stressed plants (Zhu, 2003). The K^+ ion plays a central role in OA, turgor maintenance, and in the stomata opening control of plants under physiological or stress conditions (Maathuis and Amtmann, 1999). However, high K^+/Na^+ ratio in plants under saline conditions has been suggested as an important selection criterion for salt tolerance (Ashraf, 1994b, 2002, 2004; Qian *et al.*, 2001; Reynolds *et al.*, 2005).

One of the most common stress responses in plants is overproduction of different types of compatible organic solutes such as proline and GB (Serraj and Sinclair, 2002). The organic solutes have been proven to be helpful in osmoregulation (Rodes and Hanson, 1993), enzyme activity (Mansour, 2000), detoxification of reactive oxygen species (Ashraf, 1994a) and protection of membrane integrity (Bohnert and Jensen, 1996). Of the quaternary ammonium compounds in plants subjected to salt stress, GB occurs most abundantly (Mansour, 2000). This organic compound is mainly localized in chloroplasts and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency (Robinson and Jones, 1986; Boucaud, 1991). Murata *et al.* (1992) reported that GB protects the photosystem II (PSII) complex by stabilizing the association of the extrinsic PSII complex proteins under salt stress. Proline, occurs widely in higher plants, accumulates in larger amounts than other amino acids in salt stressed plants (Ashraf, 1994b; Abraham, 2003).Proline regulates the accumulation of useable N, is osmotically very active (Ashraf, 1994a), contributes to membrane stability (Gadallah, 1999) and mitigates the effect of NaCl on cell membrane disruption (Mansour, 1998).

The aim of this study was to elucidate some key biochemical and physiological parameters in 12 sunflower lines, which may provide an insight into the mechanism of salt tolerance in sunflower under varying levels of NaCl stress.

2.0 Material and methods:

2.1 Plant Materials and Growth Conditions:

The experiment was conducted in hydroponic culture system (Fig. 1) under greenhouse conditions at Faculty of Agriculture, University of Tabriz. The experimental design consisted of 36 treatments replicated three times in a split plot design, with salinity as main factor and line as sub factor. Twelve sunflower lines namely R_{2}, R_{27}, R_{29}, R_{41}, R_{43}, R_{50}, R_{56}, B_{11}, B_{15}, B_{25}, B_{109} and B_{353} were subjected to three NaCl concentrations (0, 100 and 200 mM). Seeds were sterilized with sodium hypochlorite and germinated in petri dishes and seven day old seedling of uniform size were transferred into large sand tanks housed within an environmentally controlled greenhouse (15 h daily light, 600-800 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD), thermo period 25±5 °C day\night, and relative humidity 45\60% day\night). The tanks were sub irrigated and flushed four times daily with a modified Hoagland nutrient solution. NaCl stress was imposed 7 days after the seedlings were transferred.

2.2 Growth Parameter:

Thirty day after imposing salt stress, plants were harvested for growth measurement. After separation of shoots, the roots were carefully removed from the sand and washed with distilled water to remove any additional salt surface contamination and dried on absorbing paper, then, the height, fresh and dry weight was measured. Leaf area was recorded using a leaf area meter (Model LI-3100C, LI-COR Biosciences, USA). Average relative growth rate (RGR), absolute growth rate (AGR), net assimilation rate (NAR), leaf area duration (LAD) and relative leaf growth rate (RLGR) were estimated based on the recorded characters (Chaparzadeh *et al.*, 2003).

2.3 Relative Water Content (RWC):

The third fully expanded youngest leaf from top was taken and four leaf discs (1.0 cm diameter) of each leaf were sampled and immediately weighed fresh weight (FW). Then, they were immersed in distilled water in Petri dishes for 24 h at 4 °C in darkness and the turgid weight (TW) determined. The discs were dried in an oven at 70 °C for 24 h and the dry weight (DW) obtained. Then RWC was calculated as given below (Silveira *et al.*, 2003):

$$\mathbf{RWC\ (\%)} = \frac{(\mathbf{FW-DW})}{(\mathbf{TW-DW})}\times\mathbf{100}$$

2.4 Leaf water potential (LWP):

Leaf water potential was measured once on the third fully expanded youngest leaf from top, 30 days after imposing salt stress at 1:00 and 3:00 p.m. with pressure chamber (Turner, 1981).

2.5 Relative membrane permeability (RMP):

RMP of the leaf cells was determined as the extent of ion leakage following Yang *et al.* (1996). The third fully expanded youngest leaf from each plant was cut into three discs with1.0 cm diameter, and these freshly prepared discs, and these freshly prepared discs were placed into test tubes containing 10.0 ml deionized distilled water. After vortex the samples for 3 s, initial electrical conductivity (EC_0) of each sample was measured. The samples were then incubated at 4°C for 24 h and electrical conductivity (EC_1) measured again. The samples were then autoclaved at 120°C for 15 min and cooled to room temperature and electrical conductivity (EC_2) measured for the third time. The (RMP) was calculated using the following formula:

$$\textbf{RMP} = \frac{(\mathbf{EC1-EC0})}{(\mathbf{EC2-EC0})} \times \mathbf{100}$$

2.6 Organic Solutes Determination:

2.6.1 Glycinebetaine: Leaf GB contents were extracted and estimated by the method of Grieve and Grattan (1983). Leaf extracts prepared by vigorous shaking in 2 M H_2SO_4 were cooled and mixed with equal volume of periodide, vortexed and kept at 0-4 °C for 16 h. The mixture was centrifuged at 10000 g at 0 °C for 15 min and the supernatant was poured off. Crystals were dissolved in 1,2-dichloroethane and the absorbance was taken at 365 nm.

2.6.2 Proline: Free proline contents were measured according to the method of Bates *et al.* (1973), 0.2 g of fresh leaf material was homogenized in 5 ml of 3% aqueous sulfosalicyclic acid and the residue was removed by centrifugation. Then,1.0 ml of the extract was mixed with 1.0 ml acid-ninhydrin and 1.0 ml of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C. The reaction mixture was extracted with 2.0 ml toluene, cooled to room temperature, and the absorbance was measured at 520 nm with a spectrometer (WPA model S2100).

2.7 Inorganic Ions:

Inorganic ions were determined following Ashraf *et al.* (2001). For the determination of Na^+ and K^+ contents, 10–100 mg of well-ground dry material of The third fully expanded youngest leaf from top was digested in 8.0 ml concentrated HNO3 (Merck), and the Na^+ and K^+ in the digests were determined with a flame photometer (Jenway PFP7).

2.8 Statistical Analysis:

Data were subjected to analysis of variance based on the statistical model of the used experimental design and mean comparison was done using LSD test.

3.0 Results and Discussion:

3.1 Growth Parameters:

The analysis of variance revealed the significant effects of salinity stress on total dry weight, height, leaf area and all the growth parameters. Significant differences were observed among lines for all the growth parameters. Dry biomass production and leaf area were more affected by 200 mM NaCl compared with 100 mM. Interactions between lines and salinity were non-significant for these treats. RGR, AGR, NAR, LAD and RLGR decreased in the stressed plants in comparison controls (Table 1).Among the lines, R_2, R_{56} and R_{50} showed significantly smaller reduction in RGR, AGR, NAR, LAD, RLGR compared with B_{11}, B_{353} and B_{15}, indicating that the former lines were more salt tolerant than the others. The RGR in B_{15}, B_{11}, B_{353}, R_{43} and B_{25} was inhibited by salinity, whereas in R_2, R_{50} and R_{56} only slight inhibition was observed in RGR due to salinity stress (Table 2).

The NaCl salinity reduced growth of the studied lines, and the extent of reduction was difference among the lines. The lines B_{11}, B_{353} and B_{15} showed higher growth reduction under salinity while this was lower in R_2, R_{56} and R_{50}. There were differences among lines with respect to growth parameters under salinity stress. RGR, AGR, NAR, LAD and RLGR in salt-tolerant lines were slightly reduced by salinity stress, whereas those of the other lines showed a larger reduction. NAR reduction reflects a decrease in the rate of photosynthesis (Cheeseman, 1988) or an increase in respiration (Schwarz and Gale, 1981). El-Hendawy *et al.* (2005) reported that under salinity stress; decrease in RGR of wheat was only related to photosynthetic rate, not to leaf area. In contrast, in a report of Chaparzadeh *et al.* (2003), RGR and dry matter production appear to be more dependent on LAR than on NAR. However, Zhao *et al.* (2007) reported that the RGR of studied genotypes was related to their photosynthetic rate and leaf area, suggesting that both leaf expansion and photosynthetic rate are the growth limiting factors under salinity conditions. Several studies reported the same trend in growth parameters under salinity in other plant species such as canola (Bandeh-hagh *et al.*, 2008), naked oats (Zhao *et al.*, 2007) and rice (Akita and Cabuslay, 1990).

Height measurements taken 30 days after salt induced showed that plants of all lines in the high-salt level were about 32% shorter than control plants (Table 1).The lines R_2 showed minimum reduction, when compared with control, whereas maximum reduction over control was recorded in B_{109} (Table 2). Height significantly decreased in salt-stressed plants. The inhibitory effect on plant growth was more effective when treated by 200 mM NaCl. It seems that reduction height due to decreasing turgor pressure in cells. El and Saffan (2008) reported that Osmotic effects of salinity might cause a stir in the water relations of plants, reduce turgor potential and decline growth due to stomatal closure and reduced photosynthesis.

3.2 Water Relations:

Leaf water content (RWC) decreased with increased NaCl concentration. However, the lines not differed significantly in this water relation attribute, and comparison among two salt levels (100 and 200 mM NaCl) indicates9 and 13% redaction relative control plants, respectively (Table 3).Relative to plants not exposed to NaCl, the leaf water potential (LWP) increased by 25% under treatment at 100 mM NaCl and subsequently increased by 35% at 200 mM NaCl (Table 3). In salt-stressed plants, LWP was less affected in lines B_{25}, B_{11} and R_2 while high effect observed in R_{50}, B_{15} and B_{353} lines Table 4). Analysis of variance revealed significant difference between control and salinity levels for RWC and LWP but among lines significant difference was observed only for LWP. In this study, the lines that have more growth and proline showed high and low LWP and the sensitive lines show not same procedure. It seems that LWP not efficient method to measured water status under greenhouse conditions. According to Mattioni *et al.* (1997), varieties, which accumulated more proline and free amino acids, recorded lower values of LWP, OP and more RWC percent than varieties, which accumulate lesser proline and free amino acid content. Siddique *et al.* (2000) reported that the cause of higher RWC in tolerant cultivars is ability to absorb more water from the soil and compensate transpiration was done from plant leaves.

3.3 RMP:

Salt stress significantly increased the relative membrane permeability of all 12 lines under salt stress (Table 3). However, highest RMP was observed in line B_{109}. In contrast, line R_{27}, B_{15}, R_{56}, B_{11} and R_2 was the lowest in membrane permeability under saline conditions. Interactions between lines and salinity was significant for this treat. The line R_{27} and B_{109} have lower and higher RMP respectively (Table 4). In this study, the lines that had highest growth parameters were had lowest RMP. Unlike drought, salinity stress is an intricate phenomenon which includes osmotic stress, specific ion effect, nutrient deficiency and this two stresses caused product reactive oxygen species (ROS) (Sairam *et al.*, 2002). Cell membrane damage caused by salinity in plants correlated with ROS (Sairam *et al.*, 2005). Plants have enzymes and antioxidant compounds to inhibit the ROS and the cultivars which able to synthesis this compounds are tolerant (Ashraf and Ali, 2008).

3.4 Organic solutes accumulation: significant differences were observed among the salt treatments for proline and GB accumulation in all the lines. Both proline and GB accumulation increased significantly in the leaves of all lines under saline conditions (Table 3). However, the lines differed significantly only in proline. Under saline conditions, highest proline accumulation was found in lines R_2 while B_{25}, B_{11}, $B_{109}$and B_{353} accumulate minimum proline. Increasing proline in R_2 (accumulate highest proline) was approximately 3.75 and 1.94 folds higher than that the control and B_{25} (accumulate lowest proline), respectively. In contrast to proline, all the lines had equal increase in GB content (Table 4).

The accumulation of nitrogen-containing compatible solutes including proline is known to function in osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals (Chookhampaeng, 2011).Many plants accumulate proline as a non-toxic and protective osmolyte under salinity, including mangrove (Parida*et al*, 2002), maize (Cicek and Cakirlar, 2002), sorghum (de Lacerda*et al*, 2005) and canola (Bandeh-hagh*et al*, 2008). However, a negative relationship was observed between proline accumulation and salt tolerance in tomato (Bolarin *et al.*, 1995) and soybean (moftah *et al.*, 1987) indicate proline in their leaves compared with the salt sensitive ones. Some authors argued that excessively high levels of proline accumulation may be a response to leaf damage (Bolarin *et al.*, 1995; De Lacerda*et al*, 2005) or may be a symptom of stress (Lutts *et al.*, 1999) when exposed to high NaCl concentration and that a higher level of proline accumulation is associated with salt sensitive plants. Proline accumulation in response to lower salt concentration may contribute positively to salt tolerance, whereas the high concentration in leaf tissues under high salinity treatment may be partly due to leaf damage. In our study, the line R_2 that had high growth and lower Na^+ content, was accumulate more proline in comparison with other lines.

The data showed that GB production under salinity conditions was increased significantly in comparison with control level. Significant difference was not observed between lines for their GB content. This finding was in agreement with the results reported in maize (Rodes *et al.*, 1989), barley (Grumet and Hanson) and canola (Bandeh-hagh *et al.*, 2008).Also, most investigations attest to positive effects of exogenous application of GB on plant stress tolerance (Iqbal and Ashraf, 2006;Iqbal *et al.*, 2005).

Table 1.The means of growth parameters at increasing NaCl concentrations

NaCl (Mm)	**RGR (mg mg^{-1} day^{-1})**	**AGR (g plant^{-1} day^{-1})**	**NAR (mg cm^{-2} day^{-1})**	**LAD (m^2 day^{-1})**	**RLGR (cm^2 cm^{-2} day^{-1})**	**Height (cm)**
Control	0.149 ±0.002 a	0.232 ±0.015 a	2.707 ±0.122 a	0.466 ±0.034 a	0.107 ±0.003 a	82.861 ±2.350 a
100	0.134 ±0.002 (89)† b	0.157 ±0.007 (67) b	2.370 ±0.091 (87) b	0.317 ±0.018 (68) b	0.088 ±0.003 (82) ab	68.527 ±1.805(82) b
200	0.127 ±0.002 (85) b	0.118 ±0.005 (50) b	2.216 ±0.083 (81) b	0.253 ±0.017 (54) b	0.082 ±0.003 (76) b	56.638 ±1.832 (68) c
Salt effect	*	**	*	*	*	***

*P<0.05; **P<0.01; ***P<0.001. RGR, relative growth rate, AGR, absolute growth rate, NAR, net assimilation rate, LAD, leaf area duration, RLGR, relative leaf growth rate, respectively. † Value of parentheses is the mean reduction (% of control) of growth parameters. Amounts that at least have one similar letter have not significant difference.

Fig.1. Sunflower lines 15 days after treatment with 200 mM NaCl. Plants were grown in sand and irrigated with Hogland's solution.

Table 2.The means of growth parameters of salt-treated sunflower lines and their mean reduction (% of control) under salt stress

NaCl (Mm)	Line	RGR (mg mg^{-1} day^{-1})	Mean reduction	AGR (g $plant^{-1}$ day^{-1})	Mean reduction	NAR (mg cm^{-2} day^{-1})	Mean reduction	LAD (m^2 day)	Mean reduction	RLGR (cm^2 cm^{-2} day^{-1})	Mean reduction	Height (cm)	Mean reduction
100	R2	0.151 ±0.004	98.6 a	0.143 ±0.001	91.6 ab	1.877 ±0.014	98.1 a	0.390 ±0.014	93.0 ab	0.103 ±0.005	99.0 a	80.33 ±3.38	91.8 a
	R27	0.139 ±0.008	90.2 ab	0.153 ±0.043	70.1 bc	2.964 ±0.401	100.5 a	0.225 ±0.081	59.2 de	0.070 ±0.011	69.3 bc	73.00 ±1.52	84.8 ab
	R29	0.141 ±0.010	94.6 ab	0.118 ±0.033	73.2 abc	2.009 ±0.462	93.2 a	0.261 ±0.029	72.5 bcd	0.089 ±0.005	89.8 ab	57.33 ±1.45	86.0 ab
	R41	0.129 ±0.008	86.0 ab	0.160 ±0.028	61.7 c	2.482 ±0.141	76.0 a	0.336 ±0.046	68.0 cde	0.107 ±0.006	83.5 abc	67.33 ±4.97	79.2 ab
	R43	0.143 ±0.010	86.1 ab	0.184 ±0.054	55.7 c	2.520 ±0.235	77.6 a	0.374 ±0.115	62.5 de	0.104 ±0.011	88.8 ab	68.00 ±9.07	80.7 ab
	R50	0.139 ±0.002	93.2 ab	0.159 ±0.010	91.9 ab	1.933 ±0.147	93.0 a	0.388 ±0.019	88.3 abc	0.092 ±0.003	85.1 abc	68.33 ±5.84	84.0 ab
	R56	0.131 ±0.006	94.2 ab	0.167 ±0.021	96.5 a	2.019 ±0.181	98.2 a	0.397 ±0.031	98.5 a	0.095 ±0.005	99.8 a	76.00 ±9.64	79.1 ab
	B11	0.124 ±0.003	81.5 b	0.188 ±0.028	63.9 c	2.257 ±0.195	71.0 a	0.387 ±0.080	62.8 de	0.084 ±0.009	63.1 c	77.00 ±4.16	80.4 ab
	B15	0.115 ±0.008	81.5 b	0.119 ±0.016	58.9 c	2.525 ±0.479	74.0 a	0.187 ±0.006	63.1 de	0.065 ±0.005	65.6 bc	59.83 ±6.93	86.7 ab
	B25	0.126 ±0.002	89.3 ab	0.129 ±0.012	59.4 c	2.374 ±0.177	93.3 a	0.257 ±0.040	61.1 de	0.089 ±0.010	89.0 ab	72.33 ±5.17	86.4 ab
	B109	0.143 ±0.011	99.0 a	0.178 ±0.010	62.4 c	3.018 ±0.321	101.9 a	0.272 ±0.013	55.1 de	0.087 ±0.009	87.8 abc	58.33 ±1.66	72.6 b
	B353	0.126 ±0.003	81.2 b	0.186 ±0.029	58.6 c	2.460 ±0.295	89.6 a	0.328 ±0.094	48.8 d	0.070 ±0.011	66.6 bc	64.50 ±5.25	81.9 ab
200	R2	0.145 ±0.005	94.7 ab	0.134 ±0.003	85.8 a	1.949 ±0.154	101.8 a	0.366 ±0.018	87.3 a	0.108 ±0.003	103.8 a	69.00 ±0.57	78.8 a
	R27	0.139 ±0.002	90.2 abc	0.090 ±0.010	41.2 c	2.619 ±0.661	88.8 ab	0.173 ±0.062	45.5 cd	0.076 ±0.007	75.2 bcde	50.00 ±1.73	57.9 c
	R29	0.132 ±0.007	88.5 abcd	0.092 ±0.012	57.8 bc	2.020 ±0.279	93.7 ab	0.189 ±0.012	52.5 cd	0.073 ±0.006	73.7 bcde	48.66 ±6.22	72.9 ab
	R41	0.133 ±0.004	88.6 abcd	0.152 ±0.015	58.6 bc	2.614 ±0.276	80.1 ab	0.311 ±0.020	62.9 bc	0.110 ±0.013	85.9 abc	58.00 ±10.53	68.2 abc
	R43	0.125 ±0.012	75.3 d	0.120 ±0.044	36.3 c	1.975 ±0.176	60.8 b	0.300 ±0.114	50.1 cd	0.097 ±0.013	82.9 abcd	58.00 ±7.76	68.9 abc
	R50	0.146 ±0.004	97.9 a	0.141 ±0.005	81.5 ab	1.891 ±0.260	91.0 ab	0.377 ±0.031	85.8 a	0.098 ±0.002	90.7 abc	59.00 ±2.64	72.5 abc

R56	0.128 ±0.006	92.0 abc	0.137 ±0.015	79.1 ab	1.960 ±0.337	95.3 ab	0.329 ±0.015	81.6 ab	0.090 ±0.003	94.6 ab	63.00 ±6.02	65.6 abc
B11	0.120 ±0.006	78.9 cd	0.136 ±0.004	46.2 c	2.443 ±0.217	76.9 ab	0.247 ±0.035	40.0 d	0.079 ±0.016	59.3 de	62.66 ±7.75	65.5 abc
B15	0.106 ±0.007	75.1 d	0.080 ±0.010	39.6 c	2.309 ±0.302	67.7 ab	0.133 ±0.010	44.9 cd	0.059 ±0.014	59.5 de	49.33 ±7.83	71.4 abc
B25	0.110 ±0.004	78.0 cd	0.098 ±0.004	45.1 c	2.580 ±0.202	101.4 a	0.142 ±0.015	33.8 d	0.053 ±0.010	53.0 e	54.33 ±5.04	64.9 abc
B109	0.116 ±0.007	80.5 bcd	0.114 ±0.010	40.0 c	2.156 ±0.160	72.8 ab	0.220 ±0.022	44.6 cd	0.073 ±0.013	73.7 bcde	51.16 ±5.08	63.6 bc
B353	0.123 ±0.008	79.3 cd	0.124 ±0.022	39.1 c	2.072 ±0.080	75.5 ab	0.247 ±0.053	36.8 d	0.070 ±0.006	66.6 cde	56.50 ±7.85	71.8 abc

All abbreviations and symbols are same as in table 1

Table 3.The means of water relations, organic and inorganic solutes at increasing NaCl concentrations

NaCl (Mm)	RWC (%)	LWP (-MPa)	RMP (%)	Proline (μg g^{-1} FW)	Glycine betaine (μg g^{-1} DW)	Na (mg g^{-1} DW)	K (mg g^{-1}DW)	K/Na
Control	77.998 ±1.318 a	1.425 ±0.037 a	43.531 ±2.196 a	87.337 ±2.311 a	1.781 ± 0.080 a	16.107 ±0.359 a	33.000 ±1.063 a	2.090 ±0.085 a
100	71.667 ±1.214 (91)† b	1.785 ±0.031 (125) b	56.283 ±1.988 (129) b	147.621 ±11.309 (169) b	2.061 ± 0.101 (115) a	20.260 ±0.781 (125) b	36.930 ±0.855 (111) b	1.897 ±0.072 (90) a
200	68.382 ±1.452 (87) c	1.932 ±0.033 (135) c	70.702 ±2.249 (162) c	195.231 ±14.631 (223) c	2.760 ± 0.133 (154) b	27.272 ±1.509 (169) c	39.452 ±1.165 (119) b	1.585 ±0.085 (75) b
Salt effect	***	**	***	***	**	**	**	**

*P<0.05; **P<0.01; ***P<0.001. RWC, relative water content, LWP, leaf water potential, RMP, relative membrane permeability, respectively. † Value of parentheses is the mean change (% of control). Amounts that at least have one similar letter have not significant difference.

Table 4.The means of water relations, organic and inorganic solutes of salt-treated sunflower lines and their mean increasing (% of control) under salt stress

NaCl	Line	LWP (-MPa)	Mean increasing	RMP (%)	Mean increasing	Proline (μg g^{-1} FW)	Mean increasing	Na (mg g^{-1} DW)	Mean increasing	K (mg g^{-1} DW)	Mean increasing
100 (Mm)	R2	1.512 ±0.035	116.6abc	49.004 ±2.401	128.8ab	199.354 ±16.137	254.0 a	15.740 ±1.092	100.2 a	33.450 ±2.559	112.1 a
	R27	1.776 ±0.119	130.5abc	59.236 ±1.491	92.5a	169.322 ±30.489	160.5ab	18.626 ±1.445	106.2 a	35.583 ±3.438	107.1 a
	R29	1.886 ±0.023	125.9abc	56.501 ±5.156	145.0ab	184.070 ±63.064	221.4ab	26.317 ±2.800	148.6ab	41.650 ±4.389	117.7 a
	R41	1.788 ±0.009	123.1abc	57.326 ±2.952	146.8ab	149.244 ±51.994	185.7 ab	18.891 ±1.312	130.9ab	35.616 ±3.956	112.9 a
	R43	1.700 ±0.051	128.0abc	54.223 ±7.168	149.8 b	102.866 ±11.307	136.3ab	17.394 ±1.020	102.0 a	35.200 ±4.909	115.1 a
	R50	1.788 ±0.135	141.4 c	58.564 ±6.571	132.5ab	159.771 ±35.105	164.1ab	18.673 ±1.292	117.1ab	42.483 ±2.425	124.3 a
	R56	1.847 ±0.068	131.3abc	76.955 ±1.895	118.3ab	208.312 ±48.927	206.6ab	18.798 ±1.831	123.8ab	35.983 ±1.569	133.5 a
	B11	1.572 ±0.193	105.4 a	46.061 ±6.873	120.0 ab	97.3416 ±8.8058	119.7ab	16.317 ±1.852	115.8ab	37.583 ±2.353	122.8 a
	B15	1.870 ±0.147	137.5bc	50.153 ±3.191	109.0 ab	202.395 ±58.502	224.6ab	21.325 ±2.134	133.1ab	34.950 ±0.986	101.8 a
	B25	1.898 ±0.016	110.7ab	48.073 ±4.301	123.8ab	88.0238 ±5.8608	106.1 b	19.983 ±2.674	125.2ab	37.266 ±1.443	96.1a
	B109	1.912 ±0.060	117.8abc	63.722 ±1.556	200.0 c	92.2168 ±5.2761	114.1 b	29.234 ±2.431	174.3 b	41.166 ±0.643	113.9 a
	B353	1.875 ±0.117	142.6 c	55.576 ±2.861	132.8ab	118.539 ±4.3142	128.8ab	21.824 ±1.099	129.0ab	32.233 ±0.674	93.1 a
200 (Mm)	R2	1.608 ±0.051	124.0ab	56.863 ±1.983	149.4abc	294.363 ±4.4408	375.1 a	16.444 ±1.150	104.7 a	34.866 ±3.578	116.8ab
	R27	1.884 ±0.053	138.5ab	75.904 ±1.668	118.5 a	261.801 ±72.135	248.1abc	19.874 ±0.735	113.3ab	34.983 ±4.564	105.3ab
	R29	2.050 ±0.056	136.8ab	68.535 ±1.835	175.9bcd	226.094 ±49.971	271.9abc	34.725 ±5.042	196.1 cd	40.950 ±1.029	115.7ab
	R41	1.930 ±0.145	132.9ab	67.773 ±3.533	173.5abcd	193.592 ±54.866	240.9abc	23.914 ±4.467	165.7abcd	39.816 ±3.923	126.2ab
	R43	1.852 ±0.145	139.4ab	54.138 ±2.103	149.6abc	126.892 ±20.101	168.1bc	26.208 ±4.050	153.7abcd	42.516 ±4.444	139.0 a
	R50	1.930 ±0.123	152.6 b	84.265 ±4.867	190.7 cd	185.306 ±38.045	190.3bc	23.244 ±1.220	145.7abc	46.366 ±0.917	135.7 a
	R56	1.801 ±0.060	128.0ab	84.799 ±2.709	130.3ab	282.473 ±49.274	280.2ab	30.373 ±6.592	200.1 cd	43.500 ±1.125	161.4 a
	B11	1.893 ±0.128	126.9ab	52.425 ±8.473	136.5abc	116.443 ±10.554	143.2 c	22.666 ±2.857	160.9abcd	35.750 ±1.365	116.8ab
	B15	1.967 ±0.045	144.6ab	59.948 ±1.380	130.2ab	249.877 ±69.188	277.3abc	34.444 ±5.729	214.9 d	42.600 ±3.744	124.0ab
	B25	2.077 ±0.045	121.1 a	87.066 ±3.536	224.2 de	123.664 ±3.2024	149.1bc	31.200 ±9.673	195.5 cd	25.716 ±2.708	66.3b
	B109	2.192 ±0.096	135.1ab	81.138 ±3.476	254.6 e	129.022 ±28.966	159.6bc	34.335 ±4.392	204.7 cd	44.483 ±1.369	122.9 ab
	B353	2.004 ±0.125	152.5 b	75.567 ±6.041	180.6bcd	153.248 ±13.113	166.6bc	29.842 ±3.310	176.4bcd	41.883 ±2.951	121.0ab

All abbreviations and symbols are the same in table 3

3.5 Ionic Relations:

The presence of NaCl in the rooting medium induced an important increase in Na^+ concentration in the leaves of plants (Table 3). Lines R_{109}, R_{29} and R_{15}had considerably higher leaf Na^+ concentration than the other lines, especially R_2andR_{27} (Table 4). Opposite to most plants, the K^+ concentrations in leaves increased under saline conditions (Table 3). The line R_{56} had a higher K^+ content than that of the other lines especially B_{25}(Table 4).At cellular level, K^+/Na^+ ratio in leaves of control plants was higher than that of salt-stressed crop(Table 3). However, non-significant difference observed for K^+/Na^+ ratio. Therefore this ratio decreased in leaves in relation to salinity.

Ion effects have been considered to be related to salt tolerance (Cheeseman, 1988). In this study, salt tolerance was somehow correlated inversely with Na^+ accumulation. The same results were reported in leaves of barley and olive (James *et al.*, 2002). In contrast, in race and maize, salt tolerance of some individual does not correlated with leaf Na^+ concentrations (James *et al.*, 2002). The results also indicate that K^+ was the main inorganic osmolyte of sunflower which accumulate in large amount under saline conditions. This result opposite to most plants, such as canola (bandeh-hagh *et al*., 2008), sugar beet (Ghoulam *et al.*, 2005) and wheat (Yang *et al.*, 2009). Usually Na^+ concentrations are obviously higher than K^+ concentrations in the plants under salt stress. Shahbaz *et al.* (2010) reports that in sunflower plants non-significant difference observed in K^+ accumulation under 150 mM NaCl. Also, these results exactly parallel with Liu *et al.* (2010) for sunflower under 0, 50, 100 and 200 mM mixing two salts NaCl and Na_2SO4. The plants accumulated a large amount K^+ instead of Na^+, this not only reduced the water potential to achieve osmotic adjustment, but also reduced Na^+ toxicity (Munns, 2002). This result reflects a specific adaptability of sunflower under long-term stress(Liu *et al*., 2010). In our study, the line R_2 that accumulate minimum Na^+ was also had minimum change in K^+ content under salinity conditions. It seems that high accumulation Na^+act as signal role for more assembling K^+. A lower K^+/Na^+ ratio is an index of toxicity because Na^+ impairs the activity of K^+-requiring enzyme thus determining a low growth rate (Chaparzadeh *et al*., 2003). In this study, the K^+/Na^+ ratio was decreased with increasing NaCl concentration. Regarding to enhance the Na^+ and K^+ content and reduction in K^+/Na^+ ratio this is obvious that increasing in Na^+ was higher than K^+.

4.0 Conclusions:

The increase in Na^+ content, in response to elevated NaCl salinity, significantly inhibited all the studied sunflower lines growth by reduction total dry weight and leaf area. The RGR of R_2, R_{50} and R_{56} were slightly reduced by salinity, whereas the RGR of salt- sensitive lines were significantly reduced. The reduction of RGR appeared to be due to a decrease in NAR. Na^+content increased with the increased salinity level and Opposite to most plants, the K^+ concentrations in leaves increased under saline conditions. The lines that had minimum Na^+ also accumulate lowest K^+ in his leaves and vice versa in lines that had maximum Na^+ was had more K^+ content. Results for inorganic ions indicate especially Evolution in sunflower that maintenance K^+ upside under salinity stress. The amount of proline was increased in salt tolerant lines and it was very higher than GB and it showed that proline had major role and GB had less important role in sunflower under salt stress.

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