Journal of Stress Physiology & Biochemistry, Vol. 15, No. 4, 2019, pp. 24-38 ISSN 1997-0838

ORIGINAL ARTICLE

OPEN ACCESS

Physiological and Biochemical Characteristics of Salt Stress Tolerance in Selected Varieties of Sunflower Under Various Treatments of Potassium, Zinc and Gibberellic Acid

Amin Ullah Jan [a, *] and Fazal Hadi [b]

[a] *Department of Biotechnology, Faculty of Sciences, Shaheed Benazir Bhutto University, Sheringal Dir Upper, Pakistan.*

[b] *Department of Biotechnology, Faculty of Biological Sciences, University of Malakand, Chakdara, 18800, Pakistan.*

*E-Mail: aminjan@sbbu.edu.pk

Received September 27, 2019

The effects of potassium, zinc and gibberellic acid foliar spray on salt stress tolerance, free proline, total phenolics, carbohydrates and chlorophyll contents in sunflower varieties were evaluated. Eight varieties of sunflower were grown in glass house and NaCl (150 mM) was added to roots alone and in combinations with KNO3, ZnSO4 and GA3 foliar spray. Different varieties were evaluated for salt tolerance through leaf disc assay (LDA). Various test solutions of NaCl were used to induce salt stress. T1 treatment (150 mM NaCl only) significantly decreased the greenness % in all of eight varieties of sunflower as compared to control C. The T2 treatment (150 mM NaCl + Potassium) enhanced the greenness % and maximum greenness % was observed in the SMH-0917 (76.00 %) followed by Ausigold-7 (75.50 %). The treatment T3 (150 mM NaCl + Zinc) and T4 treatment (150 mM NaCl + 100 ppm of GA3) retained the greenness % in leaf discs compared to control. The free proline and total phenolic contents significantly increased in all of eight varieties of sunflower when treated with K, Zn, GA3 and NaCl. Positively significant correlation was found between greenness % with proline (R^2 = 0.86) and total phenolics contents (R^2 = 0.83). Conclusively; salt tolerance could be increased by the application of K, Zn and GA3.

Key words: Sunflower, salinity, Potassium, Zinc, GA3, Proline, Phenolics

Plant are sessile organisms, frequently exposed to environmental stresses such as salinity, drought and low temperature which significantly reduced plant growth (Nasreen et al. 2013). Among the abiotic stresses, salinity is a major problem in the world that affects around 7 % of the world cultivated land area and significantly diminishes yield more than 50 % of major crops (Bray et al. 2000). Deposition of high salt concentration in soil, makes it difficult for the plant to up-take water, may lead to disturbing the water balance and reduced the metabolic process. Salinity affect leaf physiology, anatomy, protein synthesis, water relations, lipid metabolism and energy production (Parida and Das 2005). It can increase the ions toxicity, rate of respiration (Alavi and Ranjbar 2012), reduced biosynthesis of chlorophyll (Khan et al. 2009). Salinity induced osmotic stress and alter the production of reactive oxygen species (ROS) i.e. hydrogen peroxide (H_2O_2), singlet oxygen ($1O_2$), superoxide (O_2) and hydroxyl radical (OH). These ROS damage membranes, chloroplasts mitochondria and DNA by disrupting cellular structures (Mittler 2002).

Mineral nutrient plays an important role in increasing tolerance to salinity stress (Marschner 1995). Among the nutrients, potassium is an essential macro-nutrient and play a vital role in the adjustment of osmotic potential, water uptake, stomatal closure, activating enzyme system, enhance water use efficiency, protein synthesis and up taking nitrogen (Nguyen et al. 2002). Potassium turn on more than 60 different types of enzymes, formation of ATPs and yield under unfavorable condition (Umar and Moinuddin 2002; Pettigrew 2008). It greatly reduces the production of ROS and enhance the activities of antioxidant enzymes, i.e. catalase, superoxide dismutase and peroxidase (Cakmak 2000). Zinc is a micronutrient plays a fundamental role in bio-membrane integrity, protein metabolism, gene expression and metabolism (Cakmak 2000). It is a cofactor of several important enzymes, scavenging of reactive oxygen species (Marschner 1995) and diminishes the activity of membrane-bound NADPH oxidase which may leads to decrease the production of reactive oxygen species (Waraich et al. 2011). It minimizes the harmful effect of salinity and an important constituent of different enzymes, i.e. ribulose-1, 5-bisphosphate, carboxylase, glutamate dehydrogenase and superoxide dismutase (Brown et al. 1993). It provides stability to protein, DNA binding proteins and membranes (Aravind and Prasad 2004). Zinc play role in gene regulation, protein synthesis, and protection from oxidative damage and DNA transcription (Alloway 2008). Plant growth regulator (Gibberellic acid) playing an important role in the stress responses and adaptation. It stimulates seed germination, plant growth, development, stem elongation, leaf expansion, stem, seed yield and quality (Mohamed and Ismail 2011). Plant hormones play a key role in plant growth and development. GA3 plays a central role in different process of the plant such as increases root length, shoot length and seed development (Yamaguchi and Kamiya 2008). It improves the leaf area index, dry matter, process of cell division (Khan et al. 2002). GA3 also promote phloem loading, increases the amount of sucrose phosphate synthase and fructose-1, 6-biphosphatase. It reverse the inhibitory effect of salt, oxidative and heat stresses in the germination and seedling growth. GA3 may leads to increase the level of salicylic acid which is a plant hormone and plays a vital role in abiotic stress tolerances in plants (Khan et al. 2002).

During stress conditions, osmolytes production is started which are small neutral molecules provide stability and protection to macro-molecule of the plant cell (Hong-Bo et al. 2006). Proline is compatible osmolyte, play a central role in the drought and salinity tolerance and provide stability to the macromolecules, cell osmotic potential and detoxification of toxic ion (Ashraf et al. 2010). In adverse conditions, plants evolved a defense system which minimizes the formation of reactive oxygen species. It scavenges superoxide in cells (cytosol, chloroplasts, peroxisomes, mitochondria and the apoplast) leading to the formation of O_2 and H_2O_2. It also plays a central role in the neutralization of free radicals and the quenching of singlet oxygen during salinity. Plant cells have antioxidant systems which are consisting of low-molecular-weight antioxidants, such as a tocopherol, ascorbate, carotenoids and glutathione as well as antioxidant enzymes such as catalase, superoxide

dismutase, and ascorbate peroxidase (Noctor and Foyer 1998). The accumulation of carbohydrate is posited as an important trait for resistance. The carbohydrate is uses for the osmoregulation and maintenance of turgor pressure during stress conditions (Michael et al. 2014). Sunflower (*Helianthus annuus* L.) is the most important oil seed crop in the world which belongs to the family *Asteraceae*. Sunflower due to its significant share in vegetable oil production of Pakistan emerged as an economically important crop of the country. But its sensitivity to soil salinity, during the growing season constraints its seed yield with significant reductions. The aim of the present research work was to investigate the role of potassium, zinc and gibberellic acid in salinity tolerance in sunflower varieties.

MATERIALS AND METHODS

Plant material and growth condition

This research work was carried out at the Department of Biotechnology, University of Malakand. Seeds of eight sunflower varieties (Rising Sun, SMH-0907, Ausigold-7, SMH-0939, US-444, Hysun-33, SMH-0917 and HS-K6) were obtained from the Seed Bank of National Agricultural Research Center Islamabad (NARC) Pakistan. Seeds were germinated on MS media (Murashige and Skoog, Sigma-Aldrich) prior to transplantation into pots. Before seedling transplantation, medium size plastic pots filled with fertile soil and will irrigated. After one day of irrigation, healthy seedling were selected and transplanted into pots. Each pot was transplanted with a single seedling. The pots were shifted to the glass house and arranged in completely randomized design (RCD) at optimum temperature. The total number of pots used were = 8 varieties × 5 replicates × 5 treatments plus control = 240

Treatments application

When plant reached three to four leaf stage, foliar spray of these solutions (10 mg/L of KNO_3, 5 mg/L of KNO_3, 2 mg / of $ZnSo_4$, 1 mg / of $ZnSO_4$ and 100 ppm of GA3) were applied to the ariel parts of each plant. Four doses of the solution were applied at the interval of five days. During foliar spray, soil in pots was completely covered with polyene sheets to avoid drops access to soil. During plant growth all the irrigation, weeding and hoeing practice were carried out.

Salt stress tolerance estimation through leaf disc assay (LDA)

Salinity tolerance estimation in selected sunflower verities was carried out through leaf disc assay. Different sodium chloride (NaCl) solution were prepared, i.e. (Ta) 0 mM, (Tb) 150 mM, (Tc) (250 mM and (Td) 350 mM. These solutions were added to each sterilized Petri plate. MS salts (4 gm/L) was taken and dissolved in the distilled water, adjusted the pH (5.8) and then autoclaved. The MS salts were poured into plates in aseptic condition under laminar flow. Five replicate were used for each variety and treatments. Upper fully expanded fresh leaves from each plant was collected at the glass house and brought to the laboratory. Sample leaves were surface sterilized with 70% ethanol, kept 10 minutes with 10 % bleach followed by three time rinse with distilled water. Leaf disc of diameter (2 cm) was prepared in aseptic conditions (laminar flow) and transferred to sterilize petri plates containing test solution (NaCl) and MS media. Three discs per plates were added and three replicates for each variety and each treatment were used. All petri plates were incubated in the incubator at 25 ^{0}C under 16 h photoperiods. The effects of salinity on leaf discs was recorded after 5 days. Change in color of leaf discs was used to differentiate resistance strength. The color change was classified into (1) dark green (100% greenness), (2) light green (75 % greenness), (3) half light green half white (50 % greenness), (4) small amount of light green (25 % greenness) and (6) white (0% greenness).

Proline estimation

Proline estimation was carried out following the method of Bates et al. (1973). A fresh mature leaves were collected from experimental plants and properly washed with sterile distilled water. Samples (200 mg) were taken and crushed in 3 % sulfosalicylic acid. Centrifuged at 13000 rpm for five minutes, 300 µl of the supernatant was transferred to each test tube. Glacial acetic acid and (1 ml) and acid ninhydrin (1 ml) were added to each test tubes. The reaction mixture were boiled at 100^o C for 1 hour at water bath and then test

tubes were immediately dipped in ice to stop the reaction. One ml toluene was added to the reaction mixture and shake briskly for 20-30 seconds. The chromophore containing toluene was pipetted and the absorbance was read at 520 nm by spectrophotometer (Shimadzu UV-1700) using toluene for a blank. Three replicates were used for each sample.

Estimation of total phenolics contents

The total phenolics content in leaves of different varieties of sunflower were determined according to method of Malik and Singh (1980). Fresh mature leaves from each variety were collected and air dried at room temperature in shade. Samples (200 mg) were grinded into powder and transferred into ependorf tubes. Methanol (2 ml) was added to each sample and kept for four hours. Centrifuged for 6 minutes at 5000 rpm. The supernatant was transferred into fresh tubes. Methanol extract (0.5 ml) were mixed with 2.5 ml of 10 % FC reagent (Folin-Ciocalteau, Sigma) and kept for 5 minutes. Added 2.5 ml (7.5 % $NaHCO_{3)}$ and 2.5 ml distilled water and kept in dark for 1 hour. Blue color developed, then absorbance at 650 nm was measured. Reagent was used as blank. The concentrations of total phenolic contents in samples were calculated from the calibration plot and expressed as mg galic acid equivalent of phenol/g of sample.

Total carbohydrate contents estimation

The total carbohydrate contents in plants were determined according to the methods of Hedge and Hofreiter (1962). Fully mature leaves were collected from plants and dried. Then 200 mg sample were taken and added 10 ml water into test tubes and boiled for 1 hour in a water bath. 1 ml of extract was taken from the test tubes and 3 ml of 3 % Antheron reagent (3 mg Antheron dissolved in 100 ml of H_2So_4) were added to the extract and the mixtures were kept in water bath for 30 minutes at 100^0 C. Samples (2 ml) were taken in cuvettes and read the absorbance at 630 nm using glucose as a standard and the amount of carbohydrate present in the sample tube were calculated.

Analysis of chlorophyll contents

Chlorophylls a, b and total chlorophyll were determined according to the method of Arnon (1949). Fresh leaves from each verity were collected and 200 mg samples were taken from fully mature leaf. The samples were mixed with 2 ml (80 %) acetone and grind well. These mixtures were transferred in to eppendorf tubes and centrifuged at 10,000 rpm for five minutes. After centrifugation, the supernatant was removed and transferred into test tube. Acetone were added to sample test tube and reached the volume up to 6 ml. Absorbance of the samples was read at 645 nm and 663 nm using a spectrophotometer. The chlorophylls a, b and total chlorophyll were calculated by using the following formula:

Total chlorophyll (µg/ml) = 20.2 (A_{645}) + 8.02 (A_{663})

Chlorophyll a (µg/ml) = 12.7 (A_{663}) - 2.69 (A_{645})

Chlorophyll b (µg/ml) = 22.9 (A_{645}) - 4.68 (A_{663})

Statistical analysis

Analysis of variance was performed by GraphPad Prisim 5 and Minitab 15 and the mean values of three replicate plants within a variety along with standard error presented. Mean values were compared using Tukey's honestly significant difference test at $p \leq 0.05$. Correlations among different parameters were investigated using Excel fitting curve and values of the correlation coefficient for different levels of significance investigated according to Fisher and Frank (1948).

RESULTS

Effect of potassium nitrate, zinc sulphate and gibberellic acid on salt stress tolerance

The effect of different treatments of test solutions (NaCl) on the greenness % in sunflower varieties were presented in the (Fig. 1). Different concentrations of test solutions (NaCl) were used for the analysis of salinity tolerance in sunflower varieties through leaf disc assay (LDA). As the concentration of salt increases, leaf disc colour is also decreases and this change in colour is the indicator of salt resistance. The increase in the concentration of salinity and time of exposure decreases the greenness %. After incubation of four days, no such obvious differences were found, but at the time of exposure and intensity of salinity increased the greenness % were clearly decreased. Different treatments (potassium nitrate, zinc sulphate and gibberellic acid) showed significant ($p \leq 0.05$) effect on salinity tolerance in sunflower varieties as compared to Control C (Table 1). In Control C (without treatments),

the maximum salinity tolerance level was found in SMH-0917 (57.25 %) followed by Ausigold-7 (55.00 %) while the minimum was recorded in Rising Sun (41.25 %). The T1 treatment (150 mM Salinity), significantly ($p \leq 0.05$) reduced the greenness % in the leaves as compared to Control C. The T2 treatments (150 mM Salinity + 10 mg / L of KNO_3) significantly ($p \leq 0.05$) enhanced the salinity tolerance level as compared control C (Table 1). In T2 treatment, the maximum salinity tolerance level was observed in the SMH-0917 (76.00 %) followed by Ausigold (75.50 %) while minimum greenness % was found in HS-K6 (63.75 %). In T3 treatment (150 mM Salinity + 2 mg / L of $ZnSO_4$), the maximum tolerance level was found in the SMH-0917 (68.25 %) while the least tolerance was observed in the US-444 (55.00 %) (Table 1). Gibberellic acid foliar spray (100 ppm), significantly increased the salinity level in sunflower varieties as compared to Control C (Table 1). In T4 treatment (150 mM Salinity + 100 ppm of GA3), the highest salinity tolerance level was found in the SMH-0917 (65.00 %) followed by US-444 (63.75 %) while the minimum was noted in the Rising Sun (56.25 % Table (1). The overall effect of different treatments on salinity tolerance was presented in the (Fig. 1). The salinity tolerance level was strongly increased by potassium nitrate followed by gibberellic acid and zinc sulphate as compared to Control C.

Effect of NaCl, KNO_3, $ZnSO_4$ and GA3 on free proline production in root and shoot

Different treatments of NaCl, KNO_3, $ZnSO_4$ and GA3 significantly increased the free proline level in selected varieties as compared to Control C. In Control C (without treatments), the highest proline was found in the SMH-0917 (0.47 µg/mg, 0.42 µg/mg) while lowest proline level was demonstrated by Rising Sun (0.34 µg/mg, 0.28 µg/mg) root and shoot respectively. The T1 treatment (150 mM NaCl) significantly ($p \leq 0.05$) enhanced the free proline level as compared to Control C. The salt stress alter the production of free proline, to encounter the adverse effect. In T1 treatment the maximum proline was found in the SMH-0917 (0.51 µg/mg, 0.47 µg/mg) root and shoot respectively. The T2 treatment (150 mM NaCl + 10 mg/L of KNO_3) revealed a significant increase in the free proline level as compared to T1 treatment and Control C. In the T2 treatment, the highest free proline was observed in the SMH-0917 (0.68 µg/mg, 0.62 µg/mg root and shoot respectively) while the lowest was observed in the HS-K6 (0.56 µg/mg, 0.55 µg/mg) root and shoot respectively. The T3 treatment (150 mM NaCl + $ZnSO_4$) showed significant increased in proline level as compared to T1 treatment and Control C. The T4 treatments (150 mM NaCl + 100 ppm of GA3) demonstrated significant enhancement in the free proline level as compared to T1 and Control C. Overall potassium nitrate significantly increased the level of free proline level followed by zinc sulphate, GA3 and salt as compared to Control C (Table 2).

Effect of sodium chloride, potassium nitrate, zinc sulphate and gibberellic acid on total phenolic contents in root and shoot

Different treatments (Sodium chloride, Potassium Nitrate, Zinc Sulphate and Gibberellic Acid) significantly ($p \leq 0.05$) increased the total phenolic contents in all varieties of sunflower as compared to Control plants (Table 3). The T1 treatment (150 mM NaCl) increased the total phenolic contents in the root and shoot as compared to Control plants. In T1 treatment, the highest phenolic contents was observed in the SMH-0917 (0.61 µg/mg, 0.67 µg/mg root and shoot respectively). The total phenolic contents was significantly ($p \leq 0.05$) enhanced by T2 treatment (150 mM salinity + 10 mg/L of KNO_3) as compared to T1 treatment and Control C. In T2 treatment, the maximum phenolic contents were found in the SMH-0917 (0.82 µg/mg, 0.88 µg/mg root and shoot respectively). The T4 treatment (150 mM NaCl + 100 ppm of GA3) increased the total phenolic contents as compared to T1amd Control C. In T4 treatment, the maximum total phenolic content was found in the SMH-0917 (0.67 µg/mg, 0.73 µg/mg) root and shoot respectively (Table 3). Overall potassium nitrate treatment significantly increased the total phenolic contents followed by GA3 and zinc sulphate as compared to Control C.

Table 1. Effect of different treatments (KNO_3, $ZnSO_4$ and GA3) on salt (NaCl) tolerance in selected varieties of sunflower. Data showed the mean value of greenness % under various treatments of NaCl test solution. Control is compared with treatments in each column. ± denote standard deviation and different letter represent the significant difference.

Treatments	Rising Sun	SMH-0907	Ausigold-7	SMH-0939	US-444	Hysun-33	SMH-0917	HS-K6
Control C (without treatments)	41.25 + 1.02^{c}	46.25 + 1.02^{d}	55.00 + 2.00^{c}	41.75 + 2.51^{d}	53.25 + 3.12^{d}	50.75 + 0.56^{c}	57.25 + 2.00^{d}	51.00 + 2.31^{c}
T1 Treatments (150 mM NaCl)	33.75 + 1.45^{d}	36.25 + 2.00^{e}	45.00 + 2.12^{d}	31.75 + 1.11^{e}	43.25 + 2.45^{e}	40.00 + 2.04^{d}	47.25 + 1.31^{e}	40.75 + 1.89^{d}
T2 Treatment (150 mM NaCl + 10 mg / L of KNO_3)	65.50 + 2.03^{a}	73.00 + 3.12^{a}	75.50 + 2.32^{a}	73.75 + 2.56^{a}	68.75 + 3.11^{a}	74.75 + 2.51^{a}	76.00 + 3.21^{a}	63.75 + 2.98^{a}
T3 Treatment (150 mM NaCl + 2 mg /L of $ZnSO_4$)	57.50 + 2.09^{b}	55.00 + 2.11^{b}	59.00 + 2.34^{b}	55.25 + 0.98^{c}	50.00 + 2.09^{c}	62.25 + 2.11^{b}	68.25 + 4.32^{b}	62.50 + 1.11^{b}
T4 Treatment (150 mM NaCl + 100 ppm of GA3)	56.25 + 1.21^{b}	52.50 + 1.11^{c}	59.75 + 2.11^{b}	60.00 + 1.23^{b}	63.75 + 4.11^{b}	62.75 + 1.67^{b}	65.00 + 2.10^{c}	59.75 + 1.45^{b}

Table 2. Effect of Potassium Nitrate (KNO_3), Zinc Sulpahte ($ZnSO_4$) and Gibberellic Acid (GA3) on free proline (µg/mg) production in root and shoot in selected sunflower varieties. Control is compared with treatments in each column. ± denote standard deviation and different letter represent the significant difference.

Treatments	Rising Sun		SMH-0907		Ausigold-7		SMH-0939		US-444		Hysun-33		SMH-0917		HS-K6	
	Proline (µg/g) ± SD		Proline (µg/g) ± SD		Proline (µg/g) ± SD		Proline (µg/g) ± SD		Proline (µg/g) ± SD		Proline (µg/g) ± SD		Proline (µg/g) ± SD		Proline (µg/g) ± SD	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Control C (without treatments)	0.34 ± 0.05^{e}	0.28 ± 0.03^{e}	0.40 ± 0.02^{d}	0.34 ± 0.09^{d}	0.45 ± 0.01^{d}	0.39 ± 0.08^{e}	0.34 ± 0.02^{d}	0.29 ± 0.08^{e}	0.43 ± 0.01^{e}	0.35 ± 0.02^{d}	0.44 ± 0.01^{d}	0.37 ± 0.02^{e}	0.47 ± 0.01^{e}	0.42 ± 0.05^{d}	0.42 ± 0.01^{e}	0.35 ± 0.07^{d}
T1 Treatment (150 mm salinity)	0.38 ± 0.01^{d}	0.32 ± 0.05^{d}	0.41 ± 0.04^{d}	0.35 ± 0.11^{d}	0.48 ± 0.04^{c}	0.42 ± 0.12^{d}	0.42± 0.12^{c}	0.34 ± 0.11^{d}	0.47± 0.02^{d}	0.41± 0.08^{c}	0.51 ± 0.03^{c}	0.44± 0.03^{c}	0.51 ± 0.08^{d}	0.47± 0.04^{c}	0.46 ± 0.03^{d}	0.41± 0.06^{c}
T2 Treatment (150 mm salinity + K)	0.60 ± 0.07^{a}	0.54 ± 0.08^{a}	0.62 ± 0.05^{a}	0.56 ± 0.13^{a}	0.64 ± 0.06^{a}	0.58 ± 0.05^{a}	0.63 ± 0.05^{a}	0.55 ± 0.09^{a}	0.59± 0.03^{a}	0.51± 0.11^{a}	0.64 ± 0.06^{a}	0.56 ± 0.01^{a}	0.68 ± 0.09^{a}	.62 ± 0.05^{a}	0.56 ± 0.04^{a}	0.55 ± 0.05^{a}
T3 Treatment (150 mm salinity +Zn)	0.44 ± 0.04^{c}	0.38 ± 0.09^{c}	0.43 ± 0.08^{c}	0.37 ± 0.12^{c}	0.51 ± 0.09^{b}	0.44 ± 0.04^{c}	0.43 ± 0.03^{c}	0.38 ± 0.06^{c}	0.45 ± 0.04^{c}	0.40 ± 0.11^{c}	0.45 ± 0.08^{d}	0.41 ± 0.06^{d}	0.55 ± 0.04^{c}	0.48 ± 0.09^{b}	0.53 ± 0.02^{b}	.45 ± 0.01^{b}
T4 treatment (150 mm salinity + GA3)	.46 ± 0.11^{b}	0.40 ± 0.06^{b}	0.48 ± 0.01^{b}	0.42 ± 0.09^{b}	0.52 ± 0.04b	0.47± 0.02^{b}	.54 ± 0.05^{b}	0.46 ± 0.04^{b}	0.55 ± 0.01^{b}	0.48 ± 0.04^{b}	0.49 ± 0.01^{b}	.47 ± 0.02^{b}	0.57 ± 0.05^{b}	.49 ± 0.01^{b}	0.50 ± 0.01^{c}	.46 ± 0.01^{b}
Percentage Increase in Proline Over Control	11.76 %	14.29 %	2.50 %	2.94 %	6.67 %	7.69 %	23.53 %	17.24 %	9.30 %	17.14 %	15.91 %	18.92 %	8.51 %	11.90 %	9.52 %	17.14 %
Percentage Increase in Proline in T2 Over T1	57.89 %	68.75 %	51.22 %	60.00 %	33.33 %	38.10 %	50.00 %	61.76 %	25.53 %	24.39 %	25.49 %	27.27 %	33.33 %	23.40 %	21.74 %	34.15 %
Percentage Increase in Proline in T3 Over T1	15.79 %	18.75 %	4.88 %	5.71 %	6.25 %	4.76 %	2.38 %	11.76 %	4.26 %	2.44 %	11.76 %	6.82 %	7.84 %	31.91 %	15.22 %	9.76%
Percentage Increase in Proline in T4 Over T1	21.05 %	25.00 %	17.07 %	20.00 %	8.33 %	11.90 %	28.00 %	35.29 %	17.02 %	17.07 %	3.92 %	6.82 %	11.76 %	2.13 %	8.70 %	12.20 %

Table 3. Effect of Potassium Nitrate (KNO_3), Zinc Sulpahte ($ZnSO_4$) and Gibberellic Acid (GA3) on total phenolic contents (µg/mg) production in root and shoot in selected sunflower varieties. Control is compared with treatments in each column. ± denote standard deviation and different letter represent the significant difference.

Treatments	Varieties															
	Rising Sun		SMH-0907		Ausigold-7		SMH-0939		US-444		Hysun-33		SMH-0917		HS-K6	
	Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD		Phenolic (µg/g) ± SD	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Control C (without treatments)	0.41 ± 0.06^{e}	0.49 ± 0.03e	0.45 ± 0.01^{e}	0.52 ± 0.11e	0.47 ± 0.06^{e}	0.53 ± 0.02d	0.42 ± 0.08^{d}	0.47± 0.01e	0.46 ± 0.01^{d}	0.51± 0.10e	0.47 ± 0.05^{e}	0.50± 0.03d	0.53 ± 0.08^{d}	0.57 ± 0.06d	0.46 ± 0.09^{d}	0.54± 0.05e
T1 Treatment (150 mm salinity)	0.50 ± 0.09^{d}	0.55 ± 0.08d	0.55 ± 0.11^{d}	0.60 ± 0.12d	0.56 ± 0.09^{d}	0.59 ± 0.6c	0.54 ± 0.05^{c}	0.54± 0.03d	0.59 ± 0.06^{c}	0.65± 0.12d	0.58 ± 0.09^{d}	0.64± 0.04c	0.61 ± 0.04^{e}	0.67 ± 0.09c	0.55 ± 0.08^{c}	0.60± 0.06d
T2 Treatment (150 mm salinity + K)	0.75 ± 0.04^{a}	0.85 ± 0.01a	0.74 ± 0.04 ^{a}	0.82 ± 0.10a	0.71 ± 0.04^{a}	0.77 ± 0.07a	0.72 ± 0.02^{a}	0.72± 0.02a	0.77 ± 0.04^{a}	0.82± 0.09a	0.78 ± 0.04^{a}	0.84± 0.12a	0.82 ± 0.12^{a}	0.88 ± 0.11a	0.74 ± 0.03^{a}	0.80± 0.04a
T3 Treatment (150 mm salinity +Zn)	0.56 ± 0.01^{c}	0.64 ± 0.02c	0.58 ± 0.07^{c}	0.63 ± 0.09c	0.61 ± 0.03^{c}	0.69 ± 0.02b	0.61 ± 0.01^{b}	0.61± 0.01e	0.62 ± 0.09^{b}	0.68± 0.07c	0.64 ± 0.01^{b}	0.68± 0.14b	0.66 ± 0.05^{b}	0.71 ± 0.14b	0.63 ± 0.06^{b}	0.68± 0.05c
T4 treatment (150 mm salinity + GA3)	0.59 ± 0.08^{b}	0.67 ± 0.06b	0.61 ± 0.02^{b}	0.67 ± 0.06b	0.64 ± 0.06^{b}	0.68 ± 0.11b	0.62 ± 0.09^{b}	0.64± 0.03b	0.63 ± 0.03^{b}	0.71± 0.04b	0.60 ± 0.03^{c}	0.66± 0.03b	0.67 ± 0.03^{b}	0.73 ± 0.05b	0.63 ± 0.04^{b}	0.71± 0.01b
Percentage Increase in TPC Over Control	21.95 %	12.24 %	22.22 %	21.15 %	19.15 %	11.32 %	28.57 %	14.89 %	25.53 %	27.45 %	23.40 %	28.00 %	15.09 %	17.54 %	19.57 %	11.11 %
Percentage Increase in TPC in T2 Over T1	50.00 %	54.55 %	34.55 %	30.16 %	26.79 %	30.51 %	33.33 %	33.33 %	30.51 %	26.15 %	34.48 %	31.25 %	34.43 %	31.34 %	34.55 %	33.33 %
Percentage Increase in TPC in T3 Over T1	12.00 %	16.36 %	5.45%	1.59 %	8.93%	16.95 %	12.96 %	12.96 %	5.08 %	4.62%	10.34 %	6.25 %	8.20 %	5.97%	14.55 %	13.33 %
Percentage Increase in TPC in T4 Over T1	18.00 %	21.82 %	10.91 %	6.35 %	14.29 %	15.25 %	18.52 %	18.52 %	6.78 %	9.23%	3.45%	3.13 %	9.84%	8.96%	14.55 %	18.33 %

Table 4. Effect of Potassium Nitrate (KNO_3), Zinc Sulpahte ($ZnSO_4$) and Gibberellic Acid (GA3) on total carbohydrate contents (µg/mg) in selected sunflower varieties. Control is compared with treatments in each column. ± denote standard deviation and different letter represent the significant difference.

Treatments	Rising Sun		SMH-0907		Ausigold-7		SMH-0939		US-444		Hysun-33		SMH-0917		HS-K6	
	CH_2O (µg/g)	A	CH_2O (µg/g)	A	CH_2O (µg/g)	A	CH_2O (µg/g)	A	CH_2O (µg/g)	A	CH_2O (µg/g)	A	CH_2O (µg/g)	A	CH_2O (µg/g)	A
Control C (without treatments)	0.90 ± 0.04^{e}	e	0.91 ± 0.02^{e}	e	0.99 ± 0.07^{e}	c	0.92 ± 0.13^{e}	ef	1.18 ± 0.09^{d}	b	1.20 0.05^{e}	b	1.24 ± 0.02^{e}	a	0.96 ± 0.01^{e}	d
T1 Treatment (150 mm salinity)	0.96 ± 0.09^{d}	e	1.09 ± 0.12^{d}	d	1.12 ± 0.09^{d}	c	1.01 ± 0.11^{d}		1.11 ± 0.04^{e}	cd	1.34 ± 0.03^{d}	b	1.42 ± 0.22^{d}	a	1.12 ± 0.09^{c}	c
T2 Treatment (150 mm salinity + K)	1.80 ± 0.05^{a}	c	1.78 ± 0.03^{a}	d	1.84 ± 0.12^{a}	b	1.54 ± 0.23^{a}	ef	1.45 ± 0.02^{a}	g	1.55 ± 0.04^{a}	e	1.87 ± 0.03^{a}	a	1.51 ± 0.01^{b}	
T3 Treatment (150 mm salinity +Zn)	1.70 ± 0.08^{b}	c	1.68 ± 0.13^{b}	d	1.81 ± 0.14^{b}	b	1.44 ± 0.04^{b}	g	1.39 ± 0.01^{b}	h	1.50 ± 0.06^{b}	f	1.84 ± 0.09^{b}	a	1.60 ± 0.06^{a}	e
T4 treatment (150 mm salinity + GA3)	1.12 ± 0.11^{c}	f	1.18 ± 0.04^{c}	e	1.21 ± 0.34^{c}	d	1.31± 0.09^{c}	c	1.21 ± 0.03^{c}	d	1.40 ± 0.11^{c}	b	1.45 ± 0.01^{c}	a	1.10 ± 0.02^{d}	g
Percentage Increase in CH_2O Over Control	6.67 %		19.78 %		13.13 %		9.78 %		5.93 %		11.67 %		14.52 %		16.67 %	
Percentage Increase in CH_2O in T2 Over T1	87.50 %		63.30 %		64.29 %		52.48 %		30.63 %		15.67 %		31.69 %		34.82 %	
Percentage Increase in CH_2O in T3 Over T1	77.08 %		54.13 %		61.61 %		42.57 %		25.23 %		11.94 %		29.58 %		42.86 %	
Percentage Increase in CH_2O in T4 Over T1	16.67 %		8.26 %		8.04 %		29.70 %		9.01 %		4.48 %		2.11 %		1.79 %	

Table 5. Effect of Salt (NaCl), Potassium Nitrate (KNO_3), Zinc Sulpahte ($ZnSO_4$) and Gibberellic Acid (GA3) on chlorophyll "a", chlorophyll "b" and total chlorophyll in sunflower varieties. Control is compared with treatments in each column. ± denote standard deviation and different letter represent the significant difference.

Treatments	Rising Sun			SMH-0907			Ausigold-7			SMH-0939		
	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Chlorophyll A	Chlorophyll B	Total Chlorophyll
Control C (without treatments)	3.31 ± 0.09 ^{c}	1.20 ± 0.05 c	4.53 ± 0.11 ^{d}	3.00 ± 0.01^{d}	1.60 ± 0.01^{a}	4.6 ± 0.11^{d}	2.56 ± 0.09^{c}	1.20 ± 0.04^{a}	3.77 ± 0.04^{d}	2.36 ± 0.14^{d}	1.01 ± 0.14^{b}	3.37 ± 0.11^{d}
T1 Treatment (150 mm salinity)	2.13 ± 0.02^{d}	1.00 ± 0.01 ^{c}	2.13 ± 0.13 ^{e}	1.74 ± 0.06^{e}	0.62 ± 0.04^{b}	2.36 ± 0.14^{e}	1.85 ± 0.08^{d}	1.16 ± 0.01^{a}	3.01 ± 0.02^{d}	1.00 ± 0.01^{d}	0.61 ± 0.08 ^{c}	1.61 ± 0.12 ^{e}
T2 Treatment (150 mm salinity + K)	6.93 ± 0.07 ^{a}	2.32 ± 0.04 ^{b}	9.25 ± 0.14 ^{a}	7.00 ± 0.03^{a}	1.47 ± 0.09^{a}	8.47 ± 0.18^{a}	5.99 ± 0.16^{a}	1.39 ± 0.01^{a}	7.38 ± 0.05^{a}	6.10 ± 0.14^{a}	2.16 ± 0.11^{a}	8.26 ± 0.11^{a}
T3 Treatment (150 mm salinity +Zn)	4.06 ± 0.05 ^{b}	3.50 ± 0.21 ^{a}	7.56 ± 0.12 ^{b}	6.35 ± 0.08^{b}	1.41± 0.01^{a}	7.76 ± 0.15^{b}	5.21 ± 0.22^{a}	1.29 ± 0.07^{a}	6.50 ± 0.07^{b}	5.43 ± 0.12^{b}	2.09 ± 0.02^{a}	7.52 ± 0.15^{b}
T4 treatment (150 mm salinity + GA3)	3.63 ± 0.03 ^{c}	1.57 ± 0.02 ^{c}	5.20 ± 0.11 ^{c}	4.92 ± 0.01 ^{c}	1.00 ± 0.03^{b}	5.92 ± 0.19^{c}	3.88 ± 0.01^{b}	1.00 ± 0.09^{b}	4.88 ± 0.11^{c}	4.62 ± 0.11^{c}	1.25 ± 0.01^{b}	5.87 ± 0.11^{c}
Percentage Reduction Over Control (T1-C)	35.65 %	16.67 %	52.98 %	42.00 %	61.25 %	48.70 %	27.73%	3.33 %	20.16 %	16.10%	59.41 %	29.08 %
Percentage Increase in K Over Salinity (T2-T1)	225.35 %	132.00 %	334.27 %	302.30 %	137.10 %	258.90 %	223.7 %	19.83 %	145.18%	122.63%	34.16 %	89.89 %
Percentage Increase in Zn Over Salinity (T3-T1)	90.61 %	250.00 %	254.93 %	264.94 %	127.42 %	228.81 %	181.6 %	11.21 %	115.95%	98.18%	29.81 %	72.87 %
Percentage Increase in GA3 Over salinity (T4-T1)	70.42 %	57.00 %	144.13 %	182.76 %	61.29 %	150.85 %	109.73 %	13.79 %	62.13 %	68.61%	22.36 %	34.94 %

Table 5. Continued

Treatments	US-444			Hysun-33			SMH-0917			HS-K6		
	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Chlorophyll A	Chlorophyll B	Total Chlorophyll	Chlorophyll A	Chlorophyll B	Total Chlorophyll
Control C (without treatments)	4.47 ± 0.05 ^{d}	2.33 ± 0.05 ^{b}	6.80 ± 0.05 ^{b}	2.60 ± 0.34 ^{c}	1.43 ± 0.87 ^{a}	4.03 ± 0.34 ^{c}	3.50 ± 0.66 ^{d}	0.99 ± 0.15 ^{a}	4.49 ± 0.31 ^{d}	2.79 ± 0.16^{d}	1.47 ± 0.03 ^{a}	4.26 ± 0.21 ^{d}
T1 Treatment (150 mm salinity)	2.90 ± 0.01^{e}	1.00 ± 0.06 ^{c}	3.89 ± 0.08 ^{c}	1.50 ± 0.05^{d}	1.00 ± 0.89 ^{b}	2.50 ± 0.12^{d}	2.00 ± 0.12 ^{e}	0.50 ± 0.51 ^{a}	2.50 ± 0.14 ^{e}	2.11 ± 0.04 ^{d}	1.00 ± 0.09 ^{a}	3.11 ± 0.11 ^{e}
T2 Treatment (150 mm salinity + K)	6.10 ± 0.76 ^{a}	3.20 ± 0.02^{a}	9.30 ± 0.11 ^{a}	4.02 ± 0.09 ^{a}	2.10 ± 0.33 ^{a}	6.12 ± 0.32^{a}	7.20 ± 0.21 ^{a}	1.50 ± 0.09 ^{a}	8.70 ± 0.15 ^{a}	6.80 ± 0.05^{a}	2.00 ± 0.05 ^{a}	8.80 ± 0.11 ^{a}
T3 Treatment (150 mm salinity +Zn)	5.00 ± 0.22 ^{b}	1.70 ± 0.03 ^{c}	6.70 ± 0.55 ^{b}	3.30 ± 0.34 ^{b}	2.00 ± 0.87 ^{a}	5.30 ± 0.11 ^{b}	6.01 ± 0.14 ^{b}	1.50 ± 0.08 ^{a}	7.51 ± 0.23 ^{b}	5.00 ± 0.04 ^{b}	1.96 ± 0.01^{a}	6.96 ± 0.12 ^{b}
T4 treatment (150 mm salinity + GA3)	4.90 ± 0.19 ^{c}	1.50 ± 0.06 ^{c}	6.40 ± 0.90 ^{b}	3.00 ± 0.22 ^{b}	1.90 ± 0.22^{a}	4.90 ± 0.65^{c}	4.00 ± 0.32^{c}	1.00 ± 0.01 ^{a}	5.00 ± 0.14 ^{c}	4.10 ± 0.21 ^{c}	1.50 ± 0.09^{a}	5.60 ± 0.09 ^{c}
Percentage Reduction Over Control (T1-C)	35.12 %	57.08 %	42.79 %	42.31 %	30.07 %	37.97 %	42.86 %	49.49 %	44.32 %	24.37 %	31.97 %	27.00 %
Percentage Increase in K Over Salinity (T2-T1)	110.3%	220.00 %	139.07 %	168.00 %	110.00 %	144.80 %	260.00 %	200.00 %	248.00 %	222.27 %	100.00 %	182.96 %
Percentage Increase in Zn Over Salinity (T3-T1)	72.41 %	70.00 %	72.24 %	120.00 %	100.00 %	112.00 %	200.50 %	200.00 %	200.40 %	136.97 %	96.00 %	123.79 %
Percentage Increase in GA3 Over salinity (T4-T1)	68.97 %	50.00 %	64.52 %	100.00 %	90.00 %	96.00 %	100.00 %	100.00 %	100.00 %	94.31 %	50.00 %	80.06 %

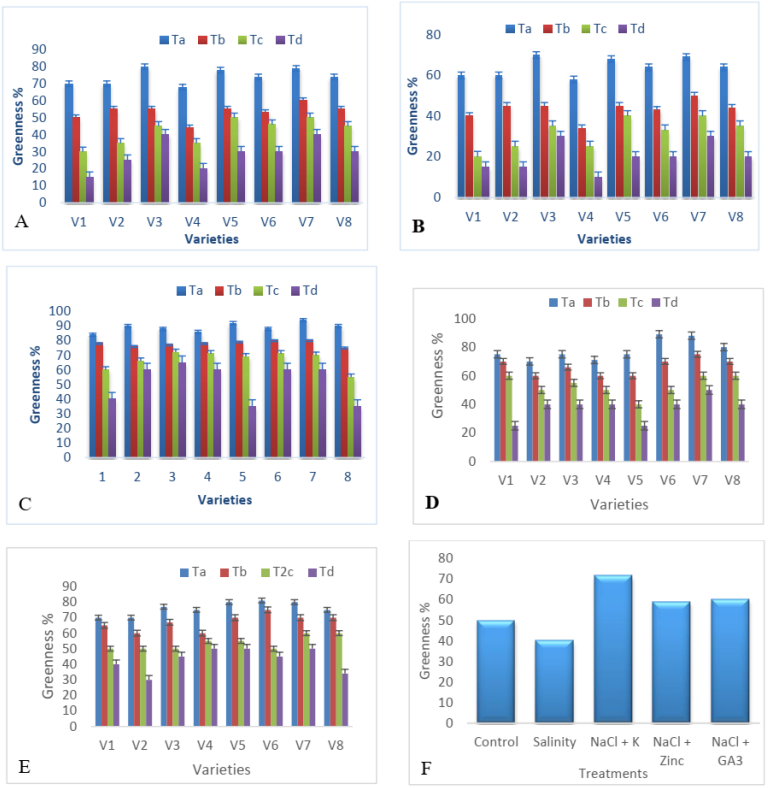

Figure 1. (A-F): Effect of different treatments of potassium nitrate on the greenness % in leaf discs of sunflower varieties under NaCl test solution, i.e. Ta = control without NaCl, Tb = 150 mM NaCl, Tc= 250 mM NaCl and Td = 350 mM NaCl. (A) Control plants without treatments (B) 150 mM NaCl added to soil (C) 150 mM NaCl added to soil + Potassium Nitrate foliar spray. (D) 150 mM NaCl added to soil + Zn foliar spray (E) 150 mM NaCl added to soil + GA3 foliar spray (F) Overall effect of different treatment on salt tolerance (mean value of all varieties). The bar show standard error.

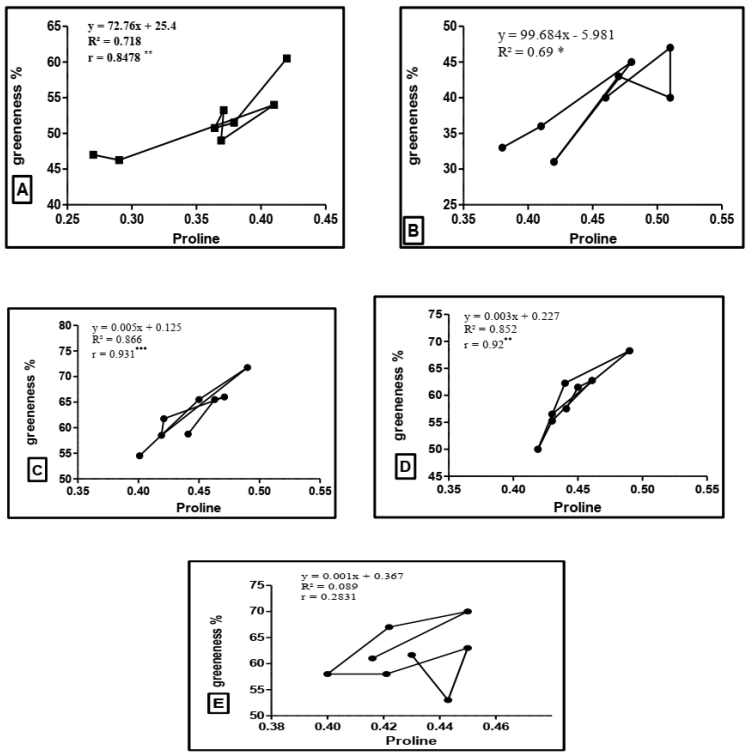

Figure 2. Correlation between greenness % and proline under various treatments of KNO_3, $ZnSO_4$ and GA3 (A) Control plants without treatments (B) 150 mM NaCl added to soil (C) 150 mM NaCl added to soil + 10 mg/L of KNO_3 foliar spray (D) 150 mM NaCl added to soil + 2 mg/L of $ZnSO_4$ foliar spray (E) 150 mM NaCl added to soil + 100 ppm of GA3 of foliar spray.

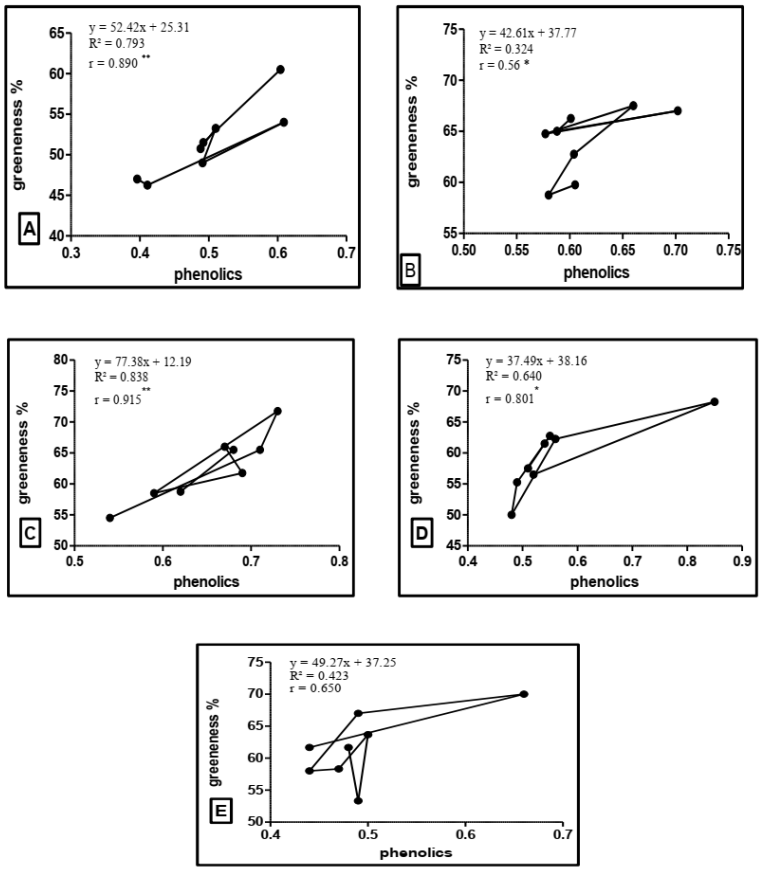

Figure 3. Correlation between greenness % and total phenolic contents under various treatments pkpof KNO_3, $ZnSO_4$ and GA3 (A) Control plants without treatments (B) 150 mM NaCl added to soil (C) 150 mM NaCl added to soil + 10 mg/L of KNO_3 foliar spray (D) 150 mM NaCl added to soil + 2 mg/L of $ZnSO_4$ foliar spray (E) 150 mM NaCl added to soil + 100 ppm of GA3 of foliar spray.

Effect of NaCl, KNO_3, $ZnSO_4$ and GA3 on total carbohydrate contents

Different treatments of potassium nitrate (KNO_3), zinc sulphate ($ZnSO_4$) and gibberellic acid increased significantly ($p \leq 0.05$) the total carbohydrate contents as compared to Control plants (Table 4). The T1 treatments (150 mM NaCl) enhanced the total carbohydrate content in selected sunflower varieties as compared to Control plants. Salt stress, increased synthesis of carbohydrate contents and other metabolites which contribute in salinity tolerance. The T2 treatment (150 mM NaCl + KNO_3) improved the production of total carbohydrate contents as compared to T1 treatment and Control plants. In T2 treatment, the maximum total carbohydrate contents were revealed by SMH-0917 (1.87 µg/mg) followed by Ausigold-7 (1.84 µg/mg) while the minimum was observed in the US-444 (1.45 µg/mg). The total carbohydrate contents was significantly boosted by T3 treatment (150 mM NaCl + Zn) as compared to T1 treatment and Control plants. The maximum total carbohydrate contents was observed in SMH-0917 (1.84 µg/mg) followed by Ausigold-7 (1.81 µg/mg) Table 4.

Effect of sodium chloride, potassium nitrate, zinc sulphate and gibberellic Acid on chlorophyll contents

Different treatments showed significant ($p \leq 0.05$) effect on chlorophyll contents in selected sunflower varieties (Table 5). The T1 treatment (150 mM NaCl), significantly reduced the concentration of chlorophyll “a”, chlorophyll “b” and total chlorophyll as compared to Control plants. The T2 treatment (150 mM NaCl + 10 mg/L of KNO_3), significantly increased the chlorophyll concentration as compared to TI treatment and Control plants. In T2 treatment, the maximum chlorophyll “a” concentration was found in the SMH-0917 (7.20 µg/mg) while minimum Chlorophyll “a” was observed in the Hysun-33 (4.02 µg/mg). The T3 treatment significantly enhanced the chlorophyll contents as compared to T1

treatment and Control plants. The exogenous application of GA3 along with salt stress (T4 treatment) significantly boosted the concentration of Chlorophyll in selected sunflower varieties. Overall salinity decreased the chlorophyll concentration, but the treatment of K, Zn and GA3 significantly increased the level of chlorophyll as compared to Control plants (Table 5).

Correlation between greenness % and free proline

The correlation between greenness % and free proline was shown in the (Fig. 2). In control plants (without treatments), highly significant ($p \leq 0.05$) positive correlation ($R^2 = 0.71$) was found between greenness % and free proline production Figure 2A. Potassium nitrate treatments showed highly significant ($p \leq 0.05$) positive correlation ($R^2 = 0.86$) between greenness % and proline level Fig. (2 B). T3 treatment (2 mg/L of $ZnSO_4$) showed highly significant ($p \leq 0.05$) positive correlation ($R^2 = 0.85$) between greenness % and proline level Fig. (2 D).

Correlation between greenness % and total phenolics contents

Figure 3 shows the effect of different treatments on greenness % and total phenolics content in selected sunflower varieties. In control plants (without treatments) high significant ($p \leq 0.05$) positive correlation ($R^2 = 0.79$) was found between greenness % and total phenolics content (Figure 3A). Potassium nitrate treatment showed significant ($p \leq 0.05$) positive correlation ($R^2 = 0.83$) between greenness % and phenolics content (Fig. 3 C). T3 treatment revealed highly significant ($p \leq 0.05$) positive correlation ($R^2 = 0.64$) between greenness % and total phenolic contents (Fig. 3 D).

DISCUSSION

Salinity significantly reduced the growth and yield of crops due to the presence of toxic ions in the root zone (Panda et al. 2003). It may cause ionic dis-equilibrium which leads to inactivation of important enzymes, ionic toxicity in tissues, oxidative stress and nutrient starvation. Salt stress induces over production of ROS (Nazar et al. 2011) that elicits DNA damage, inhibition of photosynthesis, lipid peroxidation, reduction in the rate of photosynthesis and disturbance in mineral nutrient status (Nazar et al. 2011). Sodium is the major cation that accumulated mainly in the roots and stems as salt concentration is increased (Netondo et al. 2004). In sunflower, potassium plays very important role in the salinity tolerance. It increased growth and minimize the effect of salinity (Safaa et al. 2013). Potassium is a good osmoticum and charge carrier in the plant and antagonistic relationship with sodium uptake (Kabir et al. 2004). In saline environment, K^+ can be used as a metabolite to control the turgor and avoid from osmotic shock (Kabir et al. 2004). It is an essential macro-element of the plant growth and plays a vital role in the several metabolic activities, i.e. the flow of water, nutrients and carbohydrates in plant tissue. Potassium can counteract the harmful effect of salinity and maintains the ions homeostasis. The insufficiency of Zn is acknowledged as one of the most serious micro-nutrient deficiency in plants grown on saline soils. Zn is a very important cofactor of many important enzymes, DNA-binding proteins and stabilizer of structural membrane, proteins (Aravind and Prasad 2004). The Zn availability to the plant in saline soil is very low and may lead to stunted growth and low yield of crop. The exogenous application of Zn can minimize the adverse effect of salinity, inhibit the absorption and translocation of Na^+ and Cl^- and alleviate possible injury in plants (Alpaslan et al. 1999). Our results are in line with findings of previous other researcher (Kamrani and Ardalan 2013). Gibberellic acid plays a key role in the induction of abiotic stress tolerance (Pedranzani et al. 2003). During salinity, the amount of gibberellic acid is decreased and the level of abscisic acid contents is increased (Ahmad 2009). Salinity significantly damaged metabolism, inhibit the growth and development of the plants. During salinity the content of DNA and RNA are decreased while GA3 treatment increased the content of DNA, RNA and growth of the plant under salt stress condition (Afzal et al. 2005). The exogenous application of gibberellic acid concentrations in crops could be a possible means of reversing the effects of salt stress. During stress condition proline production are increased which is a defensive mechanism of plants (Koocheki et al. 2004). Proline is a free amino acid; provide protection and stability to macromolecules of the cell by several mechanisms i.e. protect enzymes and maintains protein synthesis, plant stress tolerance, osmotic adjustment

and resistance increase against stress (Mattioli et al. 2009)**.** Potassium showed a significant effect on free proline level in different sunflower varieties. Our results are also in line with the findings of (Nobel 1991) which stated that potassium foliar application significantly increasing the free proline level. Zinc significantly increased the free proline content in plants. The ROS are produced during stress condition and zinc may act as a scavenger of ROS and provide protection to the cells (Weisany et al. 2012). According to Schat et al. (1997), zinc application significantly increased the free proline content in plants. Our results are contradictive with those of (Chaum et al. 2009) which stated that zinc foliar spray can reduced the production of proline content. The foliar application of plant growth regulator (GA3) showed non-significant effect on plants. GA3 has no or little effect on drought tolerance in plants. These findings are also supported by (Bakrim et al. 2007). Potassium different concentration showed significant effect on total phenolics content. Our findings was confirmed by other researcher (Sharafzadeh et al. 2011). Increase in the production of total flavonoid and total phenolics contents by potassium might be due to development of nonstructural carbohydrate (Ibrahim et al. 2012; Ghasemzadeh et al. 2010). It may be also due to the role of potassium, provoking the process of photosynthesis and translocation of photosynthete to different parts of the plant (Ghasemzadeh et al. 2010). Zinc foliar spray showed a significant effect on total phenolics content in sunflower varieties. The application of zinc sulphate significantly increased the total phenolics contents in plants (Razzaq et al. 2013). There was a positive significant correlation between greenness % and proline level. Our finding are also according with the previous findings (Chaum and Kirdmanee 2009). The accumulation of proline in the cell is very important for osmotic adjustment under adverse condition. Proline enhances the formation of hydrogen bonded water around protein and scavenger of free radicals (Ghoulam et al. 2002). A positive correlation was found between total phenolics content and salinity tolerance in sunflower varieties. A positive correlation was also reported by Ashraf et al. (2010). Salinity significantly reduced the chlorophyll content in the leaves due to reduction of stomata conductance, rate of transpiration, intercellular concentration of CO_2 and net photosynthesis (Lu et al. 2009). It also disturbs the protein synthesis, decrease in the absorption of magnesium (Ahmed, 1998), iron (Chougui et al. 2004) which influence chlorophyll structural component (Jaleel et al. 2008). Potassium plays a vital role in the elimination of toxic effect of salinity and increased the chlorophyll contents. The current results are in line with the previous studies (Sohrabi et al. 2008). Zinc maintains plasma membrane structural integrity, regulating the uptake of sodium ions (Na^+) and other toxic ions (Cakmak and Marschner 1988). It enhanced concentration of chlorophyll and photosynthesis under salinity stress. These results agree with early findings (Salama et al. 2012). The exogenous application of GA3 significantly boosted the concentration of chlorophyll. The current results are similar with the works of previous researcher (Shaddad et al. 2013). It is concluded that the adverse effect of salt can be significantly reduced with application of potassium followed by zinc and gibberellic acid. Salt stress badly damage the photosynthetic pigments in the plant cell and reduce the process of photosynthesis. The exogenous application of potassium, zinc and gibberellic acid enhanced level of free proline, total phenolic, chlorophyll contents and play vital role in the elevation of salt stress tolerance.

REFERENCES

Afzal I, Basra S, Iqbal A (2005) The effect of seed soaking with plant growth regulators on seedling vigor of wheat under salinity stress. *J Stress Physiol and Biochem* **1**: 6–14.

Ahmed YMA (1998) Response of tomato plants to the salinity of irrigation water. Ph.D. Thesis, Fac. Agric. Zagazig Univ. Benha Branch

Alavi MH, Ranjbar GA (2012) Effects of different levels of salinity on germination, proline contents and a-, b- Chlorophylls in Rape seed. *Int J Agric and Crop Sci* **4(15)**: 1055-1059.

Alloway BJ (2008) Zinc in soils and crop nutrition. Second edition, published by IZA and IFA, Brussels, Belgium and Paris, France.

Aravind P, Prasad MNV (2004) Zinc protects chloroplasts and associated photochemical

functions in cadmium exposed Ceratophylum demersum L, a fresh water macrophyte. *Plant Sci* **16**: 1321–1327.

Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. *Plant physiol* **24**: 1-15.

Ashraf MA, Ashraf M, Ali Q (2010) Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents. *Pak J Bot* **42(1)**: 559-565.

Bakrim A, Lamhamdi M, Sayah F, Chibi F (2007) Effects of plant hormones and 20-hydroxyecdysone on tomato (Lycopersicum esculentum) seed germination and seedlings growth. *Afric J Biotechnol* **6 (24)**: 2792-2802.

Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205-207

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stress. In: Biochemistry and Molecular Biology of Plants. American Society of Plant Physiology, (Eds.): B. Buchanan, W. Gruissem, R. Jones. Rockville, MD, pp 1158-1203

Brown TJ, Churchill GA, Peter JP (1993) Improving the measurement of service quality. *J Retailing* **69 (1)**: 127-139.

Cakmak I (2000) Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *New Phytol* 146: 185–220.

Cakmak I, Marschner H (1988) Enhanced superoxide radical production in roots of zinc-deficient plants. *J Exp Bot* **39**: 1449-1460.

Chaum S, Kirdmanee C (2009) Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak J Bot* **41**: 87-98.

Ghasemzadeh A, Jaafar HZE, Rahmat A, Wahab PEM, Halim MRA (2010) Effect of different light intensities on total phenolics and flavonoids synthesis and anti-oxidant activities in young ginger varieties. *Int J Mol Sci* **11**: 3885–3897.

Ghoulam C, Foursy A, Fares K (2002) Effects of salts tress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environ Exp Bot* **47**: 39-50.

Hare PH, Cress WD (1997) Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regulation*. **21**: 79-102.

Hedge JE, Hofreiter BT (1962) Carbohydrate chemistry 17. Whistler, R.L. and Be Miller, J.N., Eds. Academic Press, New York.

Hong-Bo S, Xiao-Yan C, Li-Ye C, Xi-Ning Z, Gang W, Yong-Bing Y, Chang-Xing Z, Zan-Min H (2006) Investigation on the relationship of proline with wheat anti-drought under soil water deficits. *Colloidal Surface B* **53**: 113-119.

Kabir E, Karimand MA, Azad MAK (2004) Effect of Potassium on salinity tolerance of mungbean. *J Biol Sci* **4(2)**: 103-110.

Kamrani R, Ardalan MM (2013) A study on the growth and concentration of Zn, Mn and Na by two Wheat cultivars in response to application of Zn in natural saline soils. *Int Res J Applied and Basic Sci* **7(13)**: 1025-1030.

Khan NA, Mir R, Khan M, Javid S, Samiullah (2002) Effects of gibberellic acid spray on nitrogen yield efficiency of mustard grown with different nitrogen levels. *Plant Growth Regul* **38**: 243–247.

Khan, MA, Ansari R, Ali H, Gul B, Nielsen BL (2009) Panicum turgidum: a sustainable feed alternative for cattle in saline areas. *Agric Ecosys Environ* **129**: 542-546

Malik CP, Singh MB (1980) Extraction and estimation of amino acids and keto acids. In: Plant Enzymology and Histo-Enzymology. (Eds.): CP. Malik, MB. Singh, Kalyani Publishers, New Delhi-Lud Hana, India. pp. 286.

Marschner H (1995) Mineral nutrition of higher plants. 2nd Ed. London, Academic Press Inc

Mattioli MC, Wayne S, Sossin, Klann E, Sonenberg N (2009) Translational control of long-lasting Synaptic plasticity and memory. *Neuron* **61**: 10-34.

Mengel K, Kirkby EA (2001) Principles of Plant Nutrition, 5th edition. Kluwer Academic Publishers, London.

Michael JO, Leuzinger S, Christopher D, Philipson J,

Hector A (2014) Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate change* **4**: 710-714.

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* **7**: 405-410

Mohamed AN, Ismail MR (2011) Changes in organic and inorganic solutes of in vitro tomato cultivars under NaCl stress. *Aust J Crop Sci* **5(8)**: 939-944.

Nasreen S, Amudha J, Pandey SS (2013) Isolation and characterization of Soybean DREB3 transcriptional activator. *J Appl Biol and Biotech* **1(02)**: 009-012

Nazar R, Iqbal N, Syeed S, Khan NA (2011) Salicylic acid allevi-ates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mung-bean cultivars. *J Plant Physiol* **168**: 807–815.

Netondo GW, Onyango JC, Beck E (2004) Sorghum and salinity: Response of growth, water relations and ion accumulation to NaCl salinity. *Crop Sci* **44**: 797-805.

Nguyen HT, Nguyen AT, Lee BW, Schoenau J (2002) Effects of long-term fertilization for cassava production on soil nutrient availability as measured by ion exchange membrane probe and by corn and canola nutrient uptake. *Korean J Crop Sci* **47**: 108-115.

Nobel PS (1991) Physiochemical and Environmental Plant Physiology. Academic Press.

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. *Ann Rev Plant Physiol and Plant Mol Biol* **49**, 249-279.

Panda SK, Chaudhury I, Khan MH (2003) Heavy Metals Induce Lipid Peroxidation and affect antioxidants in wheat leaves Biologia Plantarum 46(2), 289–294

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: A review. *Ecotox Environ Saf* **60**: 324–349

Pedranzani H, Racagni G, Alemano S, Miersch O, Ram I, Taleisnik E, Machado-Domenech E, Abdala G (2003) Salt tolerant tomato plants show increased levels of jasmonic acid. *Plant Growth Regul* **41**: 149-158.

Pettigrew WT (2008) Potassium influences on yield and quality production of maize, wheat, soybean and cotton. *J Plant Physiol* **133**: 670 – 681.

Sohrabi Y, Heidari G, Esmailpoor B (2008) Effect of salinity on growth and yield of Desi and Kabuli chickpea cultivars. *Pak J Bio Sci* **11(4)**: 664-667.

Razzaq A, Ali Q, Qayyum A, Mahmood T, Ahmad M, Rashid M (2013) Physiological responses and drought resistance index of nine wheat (Triticum aestivumL.) cultivars under different moisture conditions. *Pak J Bot* **45**: 151-155.

Safaa R, Magdi T, Abde H, Fatma R (2013) Effect of Potassium Application on Wheat (Triticum aestivum L.) Cultivars grown under salinity stress. *World Applied Sci J* **26 (7)**: 840-850.

Sardoei AS, Shahadadi F, Vakili MA, Gholamshahi S (2014) Effects of gibberellic acid (GA3) on phenolics compounds and antiradical activity of marigold. *Int J Biol Sci* **4 (3)**: 80-84

Schat H, Sharma SS, Vooijs R (1997) Heavy metal induced accumulation of free proline in a metal tolerant and non-tolerant ecotype of Silene vulgaris. *J Plant Physiol* **101 (3)**: 477-482.

Sharafzadeh S, Esmaeili M, Mohammadi AH (2011) Interaction Effects of Nitrogen, Phosphorus and Potassium on Growth, Essential Oil and Total Phenolic Content of Sweet Basil. *Adv in Environ Biol* **5(6)**: 1285-1289.

Umar S, Moinuddin (2002) Genotypic differences in yield and quality of groundnut as affected by potassium nutrition under erratic rainfall conditions. *J Plant Nutr* **25**: 1549-1562.

Waraich EA, Amad R, Ashraf MY, Saifullah, Ahmad M (2011) Improving agricultural water use efficiency by nutrient management. *Acta Agriculturae Scandinavica, Section B–Soil and Plant Science* **61(4)**: 291-304.

Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Ghassemi Golezani K (2012) Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics J* **5(2)**: 60-67.

Yamaguchi S, Kamiya Y (2000) Gibberellin biosynthesis:

its regulation by endogenous and environmental signals. *Plant Cell Physiol* **41**: 251–252