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MORPHO-PHYSIOLOGICAL CHARACTERIZATION OF SUNFLOWER GENOTYPES (*Helianthus annuus* L.) UNDER SALINE CONDITION

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Investigations on characterization of sunflower genotypes under different salinity (NaCl) levels were carried out at Saline Agriculture Research Centre, University of Agriculture, Faisalabad. Seeds of three sunflower genotypes (SF-187, S-287 and HYSUN-33) were sown in lysimeter and three salinity levels (control, 9 and 12 dS m^{-1}) were developed by using NaCl salt. Results revealed that salinity stress drastically affected the morphological, physiological and chemical attributes of all sunflower genotypes under all levels of salinity. Studies further exhibited that Hysun-33 was found leading genotype at high level of salinity (12 dS m^{-1}) by showing less reduction in all plant growth attributes i.e. plant height (46.58%), plant biomass (83.20), SPAD value (21.68%), flower diameter (41.80%), flower weight (78%), relative increase in plant height per day (13%), relative growth rate (53.33), Ca^{+2} (8%) concentration and K^+/Na^+ (65.60%), relative to percent of control treatment. The results of our experiment clearly indicated that HYSUN-33 can perform well followed by SF-187 and genotype S-287 is sensitive to salinity.

Keywords: Salt stress; sunflower, chlorophyll contents, RGR, K^+/Na^+ ratio

INTRODUCTION

Soil salinity is a big threat to world food production and is increasing progressively in many parts of the world, especially in arid and semi arid regions due to low rainfall, high temperature and high evapo-transpiration along with poor soil and water management practices (Azevedo Neto *et al*., 2006; Abdel Latef, 2010; Saqib *et al*., 2012).

It is estimated that about 7% of the land is affected by saline conditions. In the presents day about 77 million hectares out of 1.5 billion hectares are affected due to high salt concentration (Sheng *et al*., 2008).

The growth and production of crop under saline conditions is affected such as ion toxicity, low water absorption due to stress, photosynthetic inefficiency, and stomatal disturbance (Munns, 1993). Some crop species are sensitive under the low salt concentration, i.e. or their growth is inhibited so they are different in their growth response under saline conditions (Murphy and Durako, 2003). The excessive amounts of toxic ions like Na^+ and Cl^- create an ionic imbalance by reducing the uptake of beneficial ions such as K^+, Ca^{2+} and Mn^{2+} (Hajiboland *et al*., 2010). Salt stress that mainly effect the photosynthesis process (Hayat *et al*., 2010). Reduction in photosynthesis not only effect the opening and closing of stomata it also decrease the assimilation of CO_2 beside of these some non-stomatal factors also effected such as reduction in leaf area and green pigments (Misra *et al*., 1997).

The plants under saline condition adopt different mechanisms such as osmotic adjustment, i.e. by solute accumulation for reduction of cellular osmotic potential (Hasegawa *et al*., 2000) and oxidative stress tolerance (Farhoudi *et al*., 2012).

Salt affected soils are mostly identified by the higher concentration of exchangeable Na^+, as well as higher Na^+/K^+ ratios. When the plants are grown in these salt affected soils they accumulate a high concentration of Na^+ while it will be results in the reduction of Ca^+ and K^+ uptake. However even the small concentration of these nutrients is important for enzymatic activities, membrane stability and cell membrane (Wenxue *et al.*, 2003). The selective K^+ uptake and selective cellular K^+ and Na^+ compartmentalization and distribution in shoot are the actual mechanism for the maintenance of adequate K^+ in the plant under saline conditions (Ashraf, 2004; Chen *et al.*, 2007). The strategies used by the plants for maintaining the desirable K^+/Na^+ ratio in the cytosol are Na^+ efflux from the cell and utilization of Na^+ for the osmotic adjustment, regulation of the K^+ uptake and prevention of Na^+ influx (Zhu, 2002). High K^+/Na^+ ratios and K^+ vs. Na^+ selectivity in plants is one of the important selection criteria for the salt tolerance (Akram *et al.*, 2010).

The sunflower (*Helianthus annuus* L.) is the world's fourth largest oil-seed crop (Rodriguez *et al*., 2002) and Pakistan is the third largest importer of edible oil in the world (Anonymous, 2008). In Pakistan, there is a big threat of salinity due to its arid to semi-arid climate that leads to reduce the crop production. The sunflower yield was greatly reduced when it was grown under saline conditions (Ashraf, 2008; Hussain *et al*., 2012). The varietal differences and genetic potential are of the great practical interest for saline

area when introducing new crop varieties in that area. It will be highlight the demand of those varieties for their salt tolerance. Therefore, a lysimeter study was planned in to identify high yielding sunflower genotypes which has potential to tolerate salinity stress.

MATERIAL AND METHODS

***Plant material, growth and treatment condition*:** A lysimeter (1×1 m area) study was conducted at Saline Agriculture Research Centre, University of Agriculture, Faisalabad. Seeds of three sunflower genotypes (SF-187, S-287 and HYSUN-33) were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. Soil having pH= 7.56: EC_e= 4 dS m^{-1} and SAR= 8.075 (mmol $L^{-1})^{1/2}$ was used to fill the lysimeter. Two salinity levels (9 and 12 dS m^{-1}) were developed by mixing calculated amount of NaCl salt, whereas no salt was added in control treatment. Twenty seeds of each sunflower genotype were sown in each lysimeter and thinning was done fifteen days after germination to maintain nine plants per lysimeter. The recommended doses of nitrogen, phosphorus and potassium were added in the form of urea, DAP and SOP, respectively. Each lysimeter was irrigated with tap water having composition (EC= 0.95 dS m^{-1}; CO_3 \= absent; HCO_3^- = 4.9 me L^{-1}; Cl^-= 1.40 me L^{-1}; Na^+= 2.9 me L^{-1}; Ca^{2+} + Mg^{2+}= 6.4 me L^{-1}; SAR =1.62 ($mmol_c$ $L^{-1})^{1/2}$; TSS= 9.0 me L^{-1}) when required. The treatment was replicated four times using Completely Randomized Design (CRD) in factorial arrangement.

***Plant harvest*:** Plants were harvested at maturity stage. Two youngest fully expanded leaves of each genotype were separated at harvesting time and stored at freezing temperature to determine K^+, Na^+ and Ca^{+2} concentration in leaf sap. After measuring plant height through meter rod and shoot fresh weight was measured by weighing balance, plant samples were placed in oven at 65 ±5°C for 48 hours to determine plant dry weight.

***Growth analysis*:** SPAD value (chlorophyll content) of the leaves was determined by using SPAD instrument (Minolta, Japan), while leaf area was determined by using leaf area meter (Delta MK-2). Flower diameter was measured with the help of Vernier Calliper. Plant height was measured in centimeters from soil surface (stem base) to the base of capitulum (tip of plant) with a meter stick. Relative increase in plant height per day was calculate for each plant in each lysimeter and final calculations were made using formula proposed by Radford (1967), i-e.,

$$Log_e L_1 - Log_e L_2 / T_2 - T_1 \quad \text{(cm/day)}$$

Where, L_1 = Initial of preceding plant L_2 = Plant height of following harvest

Relative growth rate was determined using following formula.

$$RGR = Log_e W_2 - log_e W_1 / T_2 - T_1 \text{ (g/day)}$$

Where, W_1 = Initial dry weight (g), W_2 = Dry weight after `t' days (time interval between two harvest), T_1 = Days of preceding harvest, T_2 = Days of following harvest

***Ionic analysis*:** Frozen leaf samples were thawed and crushed using a stainless steel rod with tapered end. The sap was collected in other eppendorf tubes by Gilson pipette and centrifuged at 6500 rpm for 10 minutes. The supernatant sap was used for determination of Na^+ and K^+ concentration by using Sherwood 410 Flame photometer and calcium was determined through atomic adsorption spectrophotometer.

***Statistical analysis*:** All data presented in this experiment are means of four replicates and standard error (SE). Analysis of variance (ANOVA) was performed by using a statistical package, statistics 8.1. Also performed LSD test to find out the significance among the treatments.

RESULTS

***Effect of salinity on plant growth*:** The effect of salt stress on plant growth was evaluated by examining plant height and plant biomass Table 1. There was a marked decrease in plant height and plant biomass exposed to NaCl stress when compared with the control. At highest level of salinity (12 dS m^{-1}), Hysun-33, showed smaller reduction (46.58%) in plant height and plant biomass (83.20%) than SF-187 and S-287, suggesting its better tolerance against salinity stress.

The salt stress caused a significant ($P < 0.05$) decrease in leaf area and SPAD value Table 2. Maximum reduction in leaf area and SPAD value was observed at 12 dS m^{-1} as compared to the control in all sunflower genotypes.

Table 1. Plant height (cm) and plant biomass (g $plant^{-1}$) of sunflower genotypes under different levels of salinity

Sunflower genotypes	Plant height			Plant biomass		
	S_0	S_1	S_2	S_0	S_1	S_2
SF-187	123.8±1.4	65.9±2.3 (46.77)	99.3±7.2 (40.95)	183.9±6.4	35.7±8.3 (80.60)	64±21.7 (75)
S-287	140.4±1.7	89.9±5 (39.13)	73.1±11.8 (32.77)	256±9.6	56.1±3 (78.09)	27.9±9.6 (69)
Hysun-33	147.7±1.0	70.2±3.7 (50)	75±7 (46.58)	166±11.3	23±11.7 (86.14)	56.9±13.1 (83.20)

Each value is an average of 4 replications ± SE (S_0: control, S_1: 9 dS m^{-1}, S_2: 12 dS m^{-1} and values in ( ) are % of control)

Table 2. Leaf area (cm^2) and SPAD value of sunflower genotypes under different levels of salinity

Sunflower genotypes	Leaf area			SPAD value		
	S_0	S_1	S_2	S_0	S_1	S_2
SF-187	513.1±81.0	231.7±13.5 (54.84)	237.5±53.1 (54)	44.8±0.3	39.7±1.2 (11.38)	41.8±0.6 (10.44)
S-287	907±94.5	350.3±35.5 (61.38)	219.7±49.6 (76)	51.2±1.0	46.4±0.5 (9.38)	40.1±0.54 (6.70)
Hysun-33	762.5±15.1	203.9±71.5 (73.26)	344.9±84.1 (74)	43.1±0.6	37.8±0.3 (12.30)	47.6±0.8 (21.68)

Each value is an average of 4 replications ± SE (S_0: control, S_1: 9 dS m^{-1}, S_2: 12 dS m^{-1} and values in ( ) are % of control)

Maximum leaf area (344.9 cm^2) and SPAD value (47.6) was recorded at control treatment in Hysun-33 genotypes while the lowest values for leaf area (219.7 cm^2) and SPAD value (40.1) was noted at highest salinity level in S-287 genotypes. Salt stress also exerted a drastic effect on flower growth and development by affecting its weight and diameter. Both salinity levels (9 dS m^{-1} and 12 dS m^{-1}) caused reduction in flower weight and flower diameter of all sunflower genotypes as compared to the control Table 3. When subjected to high salinity stress, genotype S-287 showed the largest flower diameter and flower weight reductions (4.7 cm and 9.0 g respectively) while genotype Hysun-33 showed the smaller flower diameter and flower weight reductions (41.80 and 78%, respectively).

There were significant ($P < 0.05$) effects of NaCl stress on relative increase in plant height per day and relative growth rate of three sunflower genotypes Table 4. All the three sunflower genotypes were significantly different to tolerate salt stress. The S-287 genotype displayed 5.56% and 25% reduction in relative increase in plant height per day and relative growth rate respectively at high level of salinity (12 dS m^{-1}) while Hysun-33 genotype showed less reduction in relative increase in plant height per day and relative growth rate ranging 13% and 53.33% respectively, as compared to S-287 genotype and SF-187 genotype at the same NaCl level.

***Effect of salinity on chemical attributes*:** Data regarding Na^+, K^+ and Ca^{+2} concentrations are depicted in Table 5 and 6. Significant differences were observed for concentrations of Na^+, K^+, Ca^{+2} and K^+/ Na^+ ratio in the cell sap of sunflower genotypes. Concentration of Na^+ differed significantly between control and 9 dS m^{-1} level of NaCl. By increasing salinity, a significant increase in Na^+ concentration was observed in each sunflower genotype. The

Table 3. Flower diameter (cm) and flower weight (g $plant^{-1}$) of sunflower genotypes under different levels of salinity

Sunflower genotypes	Flower diameter			Flower weight		
	S_0	S_1	S_2	S_0	S_1	S_2
SF-187	6.8±0.1	4.2±0.2 (38.24)	4.9±0.9 (32.88)	39.2±2.4	7.5±2.8 (80.87)	14.3±6.6 (74.25)
S-287	7.3±0.1	4.7±0.2 (35.62)	3.9±0.7 (25)	56.3±2.0	13.9±0.9 (75.31)	9±4.5 (63.25)
Hysun-33	6.7±0.3	4±0.4 (40.30)	5.1±0.7 (41.80)	41.3±2.9	4.5±3.7 (90)	14.5±4.2 (78)

Each value is an average of 4 replications ± SE (S_0: control, S_1: 9 dS m^{-1}, S_2: 12 dS m^{-1} and values in ( ) are % of control)

Table 4. Relative increase in plant height day^{-1} (cm day^{-1}) and relative growth rate (g d^{-1}) of sunflower genotypes under different levels of salinity

Sunflower genotypes	Relative increase in plant height day^{-1}			Relative growth rate		
	S_0	S_1	S_2	S_0	S_1	S_2
SF-187	0.02±0.001	0.016±0.001 (11.11)	0.02±0.001 (8)	0.02±0.002	0.01±0.004 (35)	0.01±0.003 (33)
S-287	0.02±0.001	0.017±0.001 (8.76)	0.01±0.001 (5.56)	0.02±0.002	0.01±0.002 (45.45)	0.01±0.003 (25)
Hysun-33	0.02±0.001	0.013±0.001 (18.75)	0.04±0.001 (13)	0.02±0.002	0.01±0.003 (60)	0.02±0.001 (53.33)

Each value is an average of 4 replications ± SE (S_0: control, S_1: 9 dS m^{-1}, S_2: 12 dS m^{-1} and values in ( ) are % of control)

Table 5. Potassium and sodium concentration (mol m^{-3}) of sunflower genotypes under different levels of salinity

Sunflower genotypes	Potassium concentration			Sodium concentration		
	S_0	S_1	S_2	S_0	S_1	S_2
SF-187	43.7±1.9	42.5±2.1 (15.83)	45.2±6.0 (6.15)	7.4±1.8	14.7±5.6 (184)	22.5±4.6 (204)
S-287	53.7±3.6	50.4±2.3 (1.0)	43.3±4.9 (3)	5.4±1.3	14±6.9 (159)	25.6±4.4 (473)
Hysun-33	49.2±5.5	40.8±4.3 (15.83)	51.5±4.8 (17.07)	6.5±1.2	18.5±6.7 (98)	19.8±1.7 (204.6)

Each value is an average of 4 replications ± SE (S_0: control, S_1: 9 dS m^{-1}, S_2: 12 dS m^{-1} and values in ( ) are % of control)

Table 6. K^+/Na^+ ratio and calcium concentration (ppm) of sunflower genotypes under different levels of salinity

Sunflower genotypes	K^+/Na^+ ratio			Calcium concentration		
	S_0	S_1	S_2	S_0	S_1	S_2
SF-187	8.2±1.2	2.5±1.0 (69)	2.3±1.2 (61)	144.3±15.8	112.5±8.1 (22)	134.4±17. (4.21)
S-287	5.9±1.8	1.8±1.0 (69.50)	3.5±1.7 (57)	178.3±33.0	150.8±8.8 (25.42)	132.8±2.2 (2.68)
Hysun-33	9.3±0.8	1.5±0.2 (83.87)	3.2±1.2 (65.6)	138.1±21.7	88.8±17.9 (70)	170.8±15.6 (8)

Each value is an average of 4 replications ± SE (S_0: control, S_1: 9 dS m^{-1}, S_2: 12 dS m^{-1} and values in ( ) are % of control)

lowest Na^+ concentrations (19.8 mol m^{-3}) were observed in Hysun-33 genotype and the highest (25,6 mol m^{-3}) in S-287 genotype at both salinity levels. The trend in case of potassium was almost reverse, showing decreased K^+ concentration (3%) in all sunflower genotypes with increasing salinity. However, this decrease in potassium was more prominent in S-287 genotype as compared to Hysun-33 and SF-187 sunflower genotypes. Hysun-33 genotype was better in maintaining high level of K^+ at both salinity levels. The increasing uptake of Na^+ with increase in the salinity levels resulted in a decrease of K^+/Na^+ ratio Table 6. The highest potassium concentration (51.5 mol m^{-3}) at high salinity level resulted in maintaining higher K^+/Na^+ ratio (3.2) in Hysun-33 genotype, showing better performance under saline conditions. Data regarding Ca^{+2} concentrations Table 6 also show similar trend as in case of potassium, showing decreasing tendency of Ca^{+2} concentrations with increasing salinity level in all sunflower genotypes. High concentration of Ca^{+2} (170 mol m^{-3}) was observed in Hysun-33 genotype and lowest concentration of Ca^{+2} (132 mol m^{-3}) was noted in S-287 genotype at both levels of salinity.

DISCUSSION

Plant growth under saline conditions inhibits due to the two reasons: first, effect of ion-excess and second due to the water deficit (Munns *et al*., 2006). Every plant species has a different mechanism to cope with these effects (Munns, 2002). The result of present study clearly showed the reality of an exciting variation in tolerance to increasing NaCl levels. The sunflower genotypes responded varyingly under saline conditions. The salt stress reduced plant growth by affecting plant height, plant biomass, leaf area, chlorophyll contents, decreasing flower weight and diameter, reducing relative growth rate, altering K^+/Na^+ ratios and decreasing Ca^{+2} concentration in sunflower genotypes (Hussain *et al*., 2008; Akram *et al*., 2010; Okhovatian-Ardakani *et al*., 2010).

Results showed that NaCl stress caused a significant plant height and plant biomass reduction in all sunflower genotypes. Plant height and plant biomass reduction in S-287 genotype was higher than other two sunflower genotypes. The production of biomass in saline versus control conditions over a long period of time is related to salt stress also observed by Munns (2002). Under saline conditions reduction in total plant biomass and plant height due to the accumulation of Na^+ and Cl^- and osmotic stress. The salinity stress due to NaCl salt resulted in reduction of osmotic potential in nutrient solution and uptake of water in plant and finally dry matter production and plant height reduced (Shirazi *et al*., 2005).

The photosynthetic process under saline conditions reduced due to reduction in carbon uptake, lower stomatal conductance, inhibition in photochemical capacity, or combination of all these (Gaballah et al., 2006; Hussain *et al*., 2008). The growth of the plants is affected by leaf length will be short which decrease the growth intensity in central and distal portion (Bernstein *et al*., 1993). The reduction in leaf growth mainly affected by the effect of salinity (Lazof and Bernstein, 1998). Result of present study revealed that

chlorophyll contents (SPAD value) and leaf area was significantly decreased with increasing salinity. Hysun-33 genotype maintains better growth at all salinity level relative to SF-187 and S-287 sunflower genotypes. Some other researchers also observed the same results (Kader *et al.*, 2006).

The reduction in flower weight and diameter were also observed due to salt stress. Genotype S-287 suffered more reduction in flower weight and diameter while the smallest reduction was observed for genotype Hysun-33 suggesting that the former is the most salt-sensitive and the latter the most salt-tolerant. The decreased flower weight and flower diameter with increased salinity was also reported by Ahmed *et al.* (2005) and Di Caterina *et al*. (2007) in sunflower. Changes in relative increase in plant height per day and relative growth rate are a consequence of salt stress effects in intact plants. The growth inhibition effect of salt stress was stronger in S-287 genotype relative to other genotypes.

The dominant salt under saline condition is Na^+ which affects the cytoplasmic activity and is mostly taken up by the plant (Parvaiz and Satyawati, 2008) that results in the reduction of K^+ uptake which is a essential activator for many enzymes in cytosol (Tester and Dovenport, 2003; Okhovatian-Ardakani *et al*., 2010). The reasonable amount of Ca^{2+} and K^+ are required for the functioning of cell membrane, cell wall stabilization, ion transport regulation, selectivity and activity of the enzymes in cell wall (Rengel, 1992; Ashraf, 2004). Among the genotypes Hysun-33 genotype maintained higher K^+, Ca^{+2} and K^+/Na^+ ratio and lower Na^+ concentration at all salinity levels relative to SF-187 and S-287 genotypes. Some other researchers also observed same agreements about the (Hysun-33) which have a mechanism to select Ca^{2+} and K^+ in the presence of Na^+ which is a good character to maintain high K^+ and Ca^{2+} contents under the salinity stress (Esmaili *et al*., 2008; Akram *et al.*, 2010).

***Conclusion*:** On the basis of present investigations, it is concluded that salinity reduced plant height, plant biomass, leaf area, SPAD value, flower weight and diameter, relative growth rate, Ca^{+2} concentration and K^+/Na^+ ratio in all sunflower genotypes at both levels of salinity. However, Hysun-33 was found best performing sunflower genotype even in high salinity conditions and provided maximum plant height, plant biomass, leaf area, SPAD value, flower weight and diameter, relative growth rate, Ca^{+2} concentration and K^+/Na^+ ratio as compared to SF-187 and S-287. Therefore, the said promising genotype (Hysun-33) can be used in future breeding programme to develop salt resistant sunflower genotypes and can be recommended for cultivation on salt affected soil.

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