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Growth, yield and ionic concentration of two sunflower (*Helianthus annuus* L.) genotypes exposed to brackish water irrigation

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Abstract

The effects of brackish water application were assessed on the growth, uptake of sodium (Na^+), potassium (K^+), water relations, membrane stability index, proline accumulation, and yield of two sunflower genotypes (SF-187 and hysun-33). Treatments of irrigation water with different EC_{iw}, SAR and RSC were T_1, control; T_2, EC: 8 dS m^{-1}; T_3, SAR: 16 (mmol $L^{-1})^{1/2}$; T_4, RSC: 4 meq L^{-1}, and T_5, EC: 8 dS m^{-1} + SAR 16 (mmol $L^{-1})^{1/2}$ + RSC 4 meq L^{-1}. Genotypes displayed a substantial variability for salinity and/or sodicity tolerance and salt tolerant behavior of SF-187 regarding, high K:Na ratio, increased water contents, higher membrane stability index and higher yield as compared to Hysun-33, was confirmed under various treatments. Saline-sodic water caused maximum reduction in plant growth and yield in both sunflower genotypes followed by saline water treatment. However, no significant differences were noted between high SAR (T_3) and high RSC (T_4) treatments. In conclusion, SF-187 exhibited some important features of salt tolerance that can be successfully exploited under brackish water irrigation.

Key words: Saline-sodic irrigation, sunflower, water relations, proline, yield

Introduction

In Pakistan, sunflower (*Helianthus annuus* L.) is grown on an area of 929 thousand acres with a production of 598 thousand tonnes achene yield and 227 thousand tonnes oil production (GOP, 2009). The total demand of edible oil in 2008-09 was 2.821 million tonnes against 684 thousand tonnes of local production (24% of total demand). Consequently, Pakistan has to spend major chunk of its foreign exchange reserves on the import of edible oil to fulfill the requirement (GOP, 2009). Sunflower is high yielding, non-conventional oilseed crop and has the potential to bridge up the gap between import and production. Sunflower is a crop that fits well in the local cropping system and is considered the most important cash crop in all parts of the country.

Due to high yield potential coupled with high oil contents, the sunflower has been recognized as a crop that can successfully meet future oil requirements. Sunflower seed contains about 42 percent high quality edible oil and is gaining popularity among consumers for its good cooking quality from health stand point. Additionally, sunflower meal is a rich source of crude protein, for feeding the livestock as a source of vegetable protein. Furthermore, it has better nutritional profile and low cost as compared to traditionally used cottonseed cakes (Zahid *et al.*, 2003).

Adverse factors including soil salinity and low quality irrigation water is a menace for plants, dipping average yield each year. Particularly, water demands for agriculture production are projected to rise, bringing increased competition between agriculture and other users. This situation is especially alarming in Pakistan which is moving from being a water stressed country to a water scarce country (Qadir and Oster, 2004). Hence irrigated agriculture is exposed to increasing pressure to expand the use of brackish waters for crop production. For this purpose, about 0.53 million tube wells are pumping about 49.91 million acre feet underground water in Pakistan (GOP, 2002). Estimates show that about 70-80% of pumped water contains soluble salts and/or sodium ion (Na^+) levels, above the permissible limits for irrigation water (Latif and Beg, 2004).

Combined evidence of many researchers has reflected that sunflower is a species moderately tolerant to salt stress being unaffected by soil salinity up to EC_e of 4.8 dS m^{-1} (Maas and Hoffman, 1977: Ayers and Westcot, 1985; Francois, 1996). Flagella *et al.* (2004) have found that each unit in EC_e above 4.8 dS m^{-1} resulted in yield reduction by 4.5%. Keeping in consideration that sunflower is a short duration crop requiring 3 to 4 irrigations; it can be successfully managed under scarce water resources. Luckily, sunflower genotypes possess considerable genetic diversity for salinity tolerance that can be exploited for the

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selection of salt tolerant material using optimum selection techniques (Ashraf and Tufail, 1995).

Keeping the above scenario in view, a pot experiment was conducted to assess the detrimental effects of brackish waters in two sunflower genotypes, pereviously tested and identified as salt sensitive and tolerant genotypes (Hussain *et al.*, 2008). The objective of this study was to investigate the physiological and ionic parameters along with yield and yield components in a salt tolerant genotype in comparison with salt sensitive genotype under brackish water irrigation.

Materials and Methods

Plant culture and treatments

A pot experiment was conducted in a naturally lighted glasshouse at Saline Agriculture Research Centre, Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad. Plastic pots (28 cm diameter) lined with polyethylene were filled with 12 kg air dried loam soil (sand 38.36%, silt 46.36% and clay 15.28%) collected from the plow layer (0-20 cm) of normal experimental fields of the Agriculture Faculty, at the University of Agriculture, Faisalabad.

Table 1: Quality of different waters used for pot culture study

Characteristic	Unit	T_1 (Distilled water)	T_2 (Saline water)	T_3 (Sodic water)	T_4 (Alkaline water)	T_5 (Saline –sodic water)
EC	$dS\ m^{-1}$	-	8.0	1.5	1.5	8.0
$Ca^{2+} + Mg^{2+}$	$mmol_c\ L^{-1}$	-	43.0	1.43	3.87	24.25
Na^{+}	$mmol_c\ L^{-1}$	-	37.0	13.57	11.13	55.75
HCO_3^{-}	$mmol_c\ L^{-1}$	-	37.0	1.44	7.87	28.25
Cl^{-}	$mmol_c\ L^{-1}$	-	34.4	1.15	3.10	19.40
SO_4^{2-}	$mmol_c\ L^{-1}$	-	8.6	12.41	4.03	32.35
SAR	$(mmol\ L^{-1})^{1/2}$	-	8.0	16.0	8.0	16.0
RSC	$me\ L^{-1}$	-	-	-	4.0	4.0

Table 2: Amount of different salts ($g\ L^{-1}$) for saline and/or sodic water treatment

Treatment	$NaHCO_3$	Na_2SO_4	$CaCl_2.2H_2O$	$MgSO_4.7H_2O$
T_1	-	-	-	-
T_2	3.10	-	2.52	1.05
T_3	0.12	0.86	0.08	0.03
T_4	0.66	0.23	0.23	0.09
T_5	2.37	1.95	1.42	0.60

The experiment was arranged in a completely randomized design with three replicates. Achenes of two sunflower genotypes SF-187 (salt tolerant) and Hysun-33 (salt sensitive) were obtained from the Punjab Oil Development Board, Ayub Agriculture Research Institute, Faisalabad. Six sunflower seeds were sown per pot and seedlings were thinned to three of almost uniform size. Total five irrigation waters, having different EC_{iw}, SAR_{iw}, and RSC combinations were used for the study as shown in Table 1, while the amount of salts used is shown in Table 2. Distilled water was used for irrigation until eight leaf stage, after that brackish irrigation was initiated. Hence there was no salt stress at germination stage. Pots were irrigated according to crop requirement with respective brackish water while control plants were irrigated with distilled water throughout growth period.

Data regarding area of green leaves, membrane stability index, water potential, relative water contents, proline and ionic analysis were recorded at flower initiation while head size, 100 seed weight, and yield per plant were determined at the end.

Relative water contents and water potential

For the determination of relative water contents (RWC), fresh leaf samples (0.5 g) were weighed (FW) immediately after harvesting. The samples were then floated on distilled water for 4 hours. The turgid leaves were blotted rapidly to remove surface adhered water and then weighed to obtain turgid weight (TW). The leaves were then dried at 65°C for 48 hours and dried weight was obtained. The RWC were calculated on the basis of formula described by Weatherley (1950).

RWC = [(FW-DW)/ (TW-DW)] x 100

First fully expanded leaf was excised to determine the leaf water potential with Scholander type pressure chamber (Scholander *et al.*, 1965).

Leaf membrane stability index

Leaf membrane stability index (MSI) was determined according to method of Premachandra *et al.* (1990), as modified by Sairam (1994). Leaf pieces (0.2 g) were taken in test tubes containing distilled water in two sets. Test tubes in one set were kept in water at 40°C for 30 minutes and electrical conductivity of the water containing samples were measured (C_1) using a conductivity meter. Test tubes in the other set were incubated at 100°C in the boiling water for 15 minutes and their electrical conductivity was also measured as above (C_2). Membrane stability index was calculated and expressed on percentage basis by using the formula as given below:

$$MSI = [1-C_1/C_2] \times 100$$

Determination of Na^+ and K^+

Frozen leaf samples were thawed and crushed using a stainless steel rod with tapered end. The sap was collected in eppendorf tubes by Gilson pipette and centrifuged at 6500 rpm for 8-10 minutes. The supernatant sap was diluted as required by adding distilled water and sodium and potassium were determined using Sherwood 410 Flame photometer (Gorham *et al.*, 1984).

Proline determination

Extraction and determination of proline was performed according to the method of Bates *et al* (1973). Half gram fresh leaf material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and filtered through Whatman's no.2 filter paper. Two mililiter of filtrate was mixed with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C, followed by ice bath. The reaction mixture was extracted with 4 mL toluene and the chromophore containing toluene was aspirated, cooled to room temperature, and the absorbance was measured at 520 nm with a Shimadzu UV 1601 spectrometer. Appropriate proline standards were included for calculation of proline in the sample.

Results

Shoot Dry Weight and Leaf Area

Shoot dry weight and leaf area were selected as growth parameters (Figure 1 and 2). Biomass production is frequently used to assess seedling growth while leaf area closely relates to photosynthesis on which growth depends. The maximum values of these parameters were recorded in control while the minimum in case of EC-SAR-RSC water application (T_5) followed by T_2 in both genotypes. However, genotypic variations were conspicuous, whereby salt sensitive genotype (Hysun-33) displayed more reduction in biomass and leaf area as compared to salt tolerant genotype (SF-187) under all stress treatments. Moreover, the relatively mild salinity regimes of T_3 and T_4 treatments corresponded to a slight reduction in biomass production and leaf area as compared with the control (T_1).

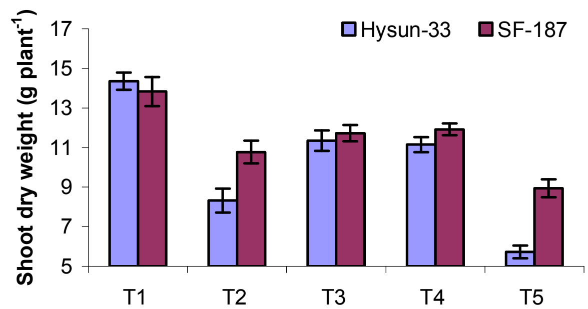

Figure 1: Effect of brackish waters on shoot dry weight of sunflower genotypes

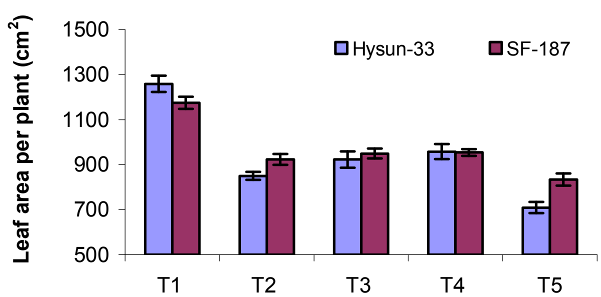

Figure 2: Effect of brackish waters on leaf area of sunflower genotypes

Each value is an average of three replications ± S.E. T_1 [Fit Water]; T_2 [EC (8 dS m^{-1}) Water]; T_3 [SAR (16 (mmol $L^{-1})^{1/2}$) Water]; T_4 [RSC (4 me L^{-1}) Water]; T_5 [EC (8 dS m^{-1}) + SAR (16 (mmol $L^{-1})^{1/2}$) + RSC (4 me L^{-1}) Water]

Ionic Contents

Applications of different brackish irrigation treatments have significant effect on Na^+ and K^+ concentration. As is evident from Figure 3, Na^+ contents of both genotypes increased with increasing salinity and/or sodicity of irrigation water. However, the extent of Na^+ increase was most steep in case of T_5 where high salinity was coupled with high sodicity of irrigation water. Likewise, there was a sharp and significant decrease in leaf K^+ contents in salt stressed sunflower genotypes as compared to non-stressed (Figure 4). The highest K^+ values were obtained in plants irrigated with T_1 followed by those in case of T_3, T_4, T_2 and T_5 in decreasing order. Moreover, SF-187 tended to maintain a higher K^+/Na^+ ratio (Figure 5) under all stress treatments compared to Hysun-33, indicating its salt tolerant behavior.

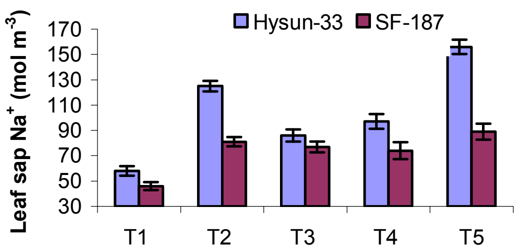

Figure 3: Effect of brackish waters on Na^+ concentration in leaf sap of sunflower genotypes

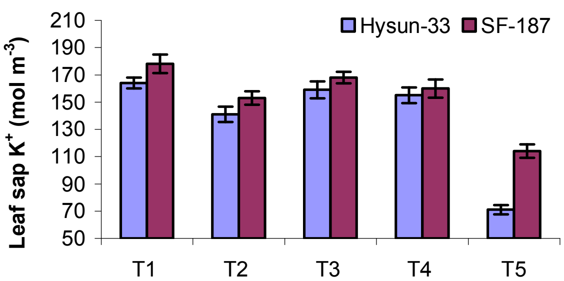

Figure 4: Effect of brackish waters on K^+ concentration in leaf sap of sunflower genotypes

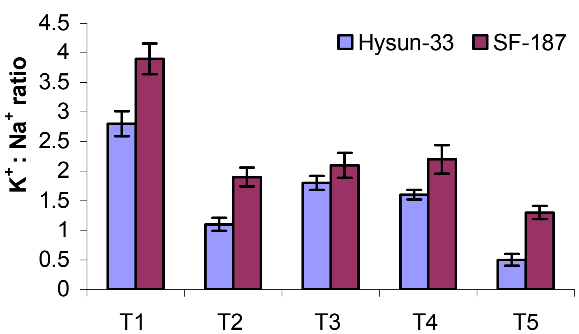

Figure 5: Effect of brackish waters on K^+: Na^+ ratio in leaf sap of sunflower genotypes

Each value is an average of three replications ± S.E. T_1 [Fit Water]; T_2 [EC (8 dS m^{-1}) Water]; T_3 [SAR (16 (mmol $L^{-1})^{1/2}$) Water]; T_4 [RSC (4 me L^{-1}) Water]; T_5 [EC (8 dS m^{-1}) + SAR (16 (mmol $L^{-1})^{1/2}$) + RSC (4 me L^{-1}) Water]

Relative water contents and water potential

Data regarding relative water contents and water potential are presented in Figure 6 and 7, respectively. Irrigation with water of high EC, SAR and RSC resulted in a trend of decreasing relative water contents of both genotypes, and minimum values were recorded in case of T_5, which were 54.9 and 65.3% in salt sensitive and tolerant genotype respectively. The maximum leaf water potential of -0.7 MPa was achieved in both genotypes under control treatment while it decreased progressively by increasing salinity/sodicity in irrigation water. The mean Ψ_s values of treatments T_2 and T_5 were -1.8 and -2.1 MPa significantly lower than T_1, respectively for Hysun-33. However, in SF-187 under saline and saline sodic treatments, the corresponding Ψ_s values were relatively higher than Hysun-33.

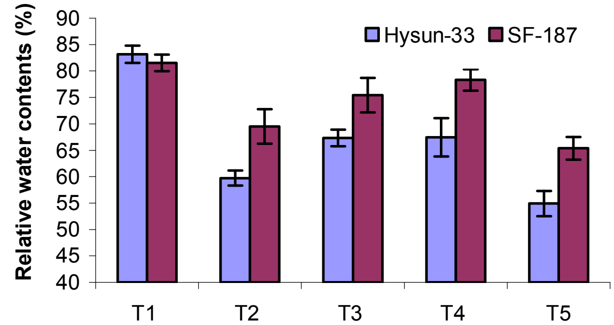

Figure 6: Effect of brackish waters on relative water contents of sunflower genotypes

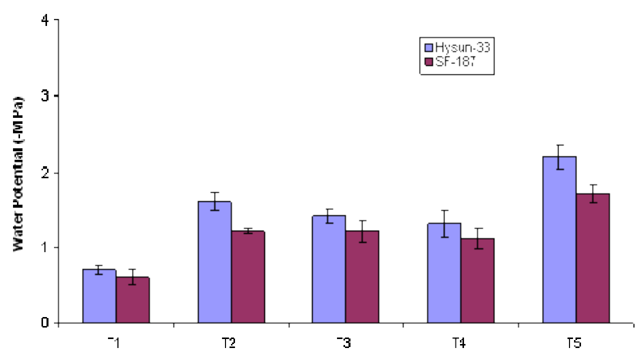

Figure 7: Effect of brackish waters on water potential of sunflower genotypes

Each value is an average of three replications ± S.E. T_1 [Fit Water]; T_2 [EC (8 dS m^{-1}) Water]; T_3 [SAR (16 (mmol $L^{-1})^{1/2}$) Water]; T_4 [RSC (4 me L^{-1}) Water]; T_5 [EC (8 dS m^{-1}) + SAR (16 (mmol $L^{-1})^{1/2}$) + RSC (4 me L^{-1}) Water]

Proline and membrane stability index

In both genotypes, salt stress stimulated proline accumulation and the results in Figure 8 demonstrate that the degree of increase also tended to be higher with upsurges in salinity and sodicity of irrigation water, the highest being in T_5. Similarly, the extent of proline accumulation was markedly higher in salt tolerant genotype (SF-187) under all stress treatments and it increased most steeply under T_5 followed by T_2. Membrane stability index is an evident indicator of plant resistance against cellular membrane damage due to salt stress. It is quite evident from Figure 9 that plasma membranes were damaged more seriously in salt sensitive genotype as compared to salt tolerant genotype and furthermore, saline sodic irrigation (T_5) had more intensifying impact on maintenance of membrane integrity in both genotypes.

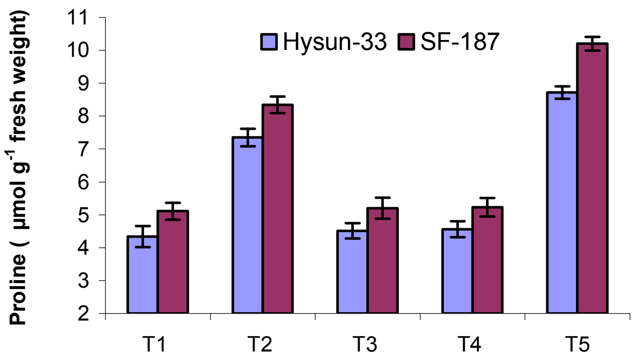

Figure 8: Effect of brackish waters on proline contents of sunflower genotypes

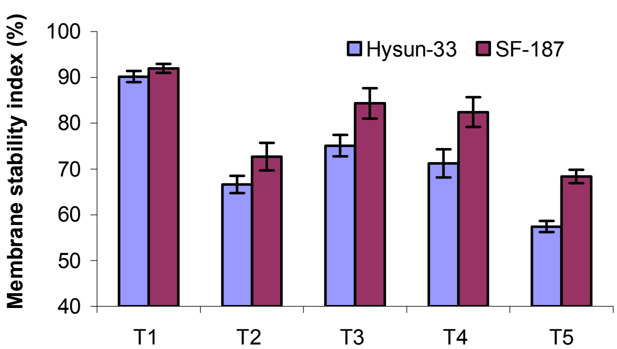

Figure 9: Effect of brackish waters on membrane stability index of sunflower genotypes

Each value is an average of three replications ± S.E. T_1 [Fit Water]; T_2 [EC (8 dS m^{-1}) Water]; T_3 [SAR (16 (mmol L^{-1})$^{1/2}$) Water]; T_4 [RSC (4 me L^{-1}) Water]; T_5 [EC (8 dS m^{-1}) + SAR (16 (mmol L^{-1})$^{1/2}$) + RSC (4 me L^{-1}) Water]

Yield and yield attributes

Significant differences in head diameter, 100 seed weight and yield were observed between irrigation treatments as well as genotypes. Achene yield, head diameter and 100 seed weight progressively decreased with increasing irrigation water salinity/sodicity and showed a similar trend for both genotypes (Figure 10, 11 and 12). In salt sensitive (Hysun-33) genotype, reduction in achene yield compared to control under T_2, T_3, T_4 and T_5 was 37, 23, 24, and 55%, respectively. While in salt tolerant genotype (SF-187) achene yield reduction under T_2, T_3, T_4 and T_5 was 19, 17, 18, and 35%, respectively, as compared to control.

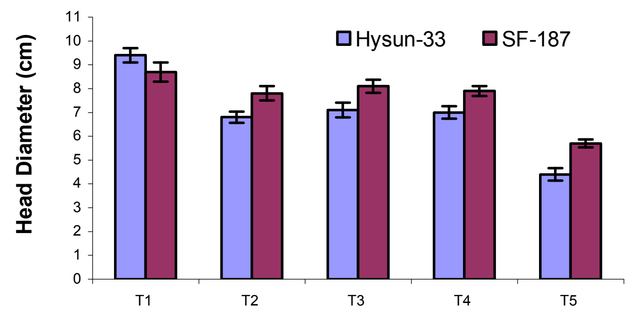

Figure 10: Effect of brackish waters on head diameter of sunflower genotypes

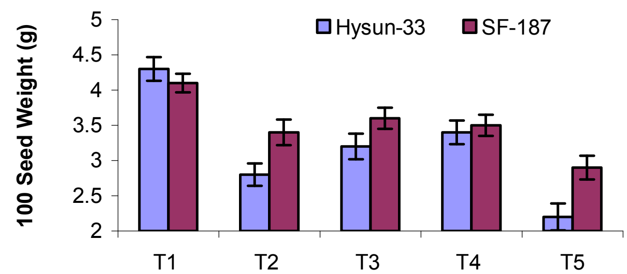

Figure 11: Effect of brackish waters on 100 seed weight of sunflower genotypes

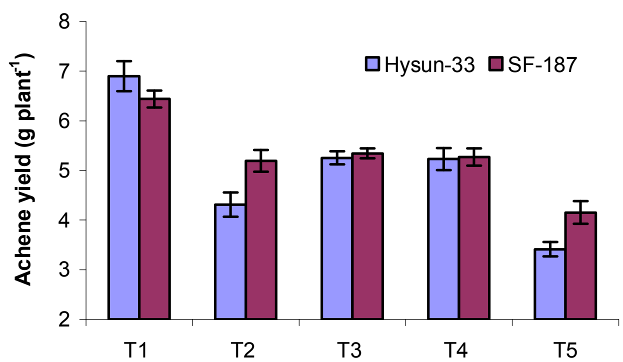

Figure 12: Effect of brackish waters on achene yield of sunflower genotypes

Each value is an average of three replications ± S.E. T_1 [Fit Water]; T_2 [EC (8 dS m^{-1}) Water]; T_3 [SAR (16 (mmol L^{-1})$^{1/2}$) Water]; T_4 [RSC (4 me L^{-1}) Water]; T_5 [EC (8 dS m^{-1}) + SAR (16 (mmol L^{-1})$^{1/2}$) + RSC (4 me L^{-1}) Water]

Discussion

The primary objective of this experiment was to evaluate growth and yield response of salt tolerant and sensitive genotypes (on the basis of previous experiment) under brackish irrigation in soil culture. Brackish water irrigation significantly reduced shoot dry weight, leaf area and yield in both genotypes, although the magnitude of reduction was much dependent upon genotypes and salt concentrations. Shoot dry weight is one of the most important traits for salt stress and for this reason, it is considered good criterion to assess the response of plants to salt stress (Jamil and Rha, 2004). Salinity reduced shoot dry matters of both sunflower genotypes; however, the decrease was more prominent in salt sensitive genotype (Hysun-33) than in salt tolerant genotype (SF-187). This finding was previously reported by Riaz *et al*. (2008) while working on different sunflower genotypes in solution culture. It is clear from our findings that the growth inhibition effect of saline-sodic water was stronger than that of saline or sodic water alone in both genotypes. Kurdali and Al-Ain (2002) also reported significant reduction in dry matter production of sunflower while using different levels of saline irrigation water in pot culture. The reason for reduction in shoot dry matter under salinity stress was chiefly because of Na^+ toxicity and its imbalances with other mineral nutrients like K^+ (Marschner, 1995) besides some other factors (Ghoulam *et al*., 2002; De Lacerda *et al*., 2003).

Munns (1993) pointed out that under moderate soil salinity; inhibition in growth patterns is primarily associated with a reduction in photosynthetic area rather than a reduction in photosynthesis per unit leaf area. Same was true for our experiment where stress conditions caused a significant reduction in leaf area as previously reported by other authors (Steduto *et al*., 2000). It is proposed that accumulation of high amounts of toxic salts like Na^+ in the leaf apoplasm leads to dehydration and turgor loss, and eventually death of leaf cells and tissues (Marschner, 1995).

Plants exposed to saline environment generally have higher concentrations of Na^+ and lower concentration of K^+ in their tissues (De Lacerda *et al*., 2003). In this study, accumulation of Na^+ and reduction in K^+ occurred under all brackish conditions, but with a substantial difference between genotypes. This could be attributed to the antagonism of Na^+ and K^+ at uptake sites in the roots or the effect of Na^+ on K^+ transport into xylem (Colmer *et al*., 2005). Hence, sunflower genotypes performed differentially under brackish water irrigation depending upon their ability to selectively absorb K^+ over Na^+ (Colmer *et al*., 2005). Although the mechanisms underlying these peculiarities have not been established, shoot Na^+ exclusion is well documented in many species, including sunflower (Ashraf and O'Leary, 1995), rice (Zhu. 2001) and wheat (Schachtman *et al*., 1989; Poustini and Siosemardeh, 2004). These results are also supported by the previous findings of Ahmed *et al*. (2005) who reported that sunflower genotypes were significantly different in their biomass production, ionic accumulation and yield attributes when grown under saline conditions. Water stress, an indirect consequence of saline- sodic irrigation was displayed by a substantial and sharp decline in leaf water potential and relative water contents. The observed decrease in water status of stressed plants may be attributed to unavailability of water in the soil or root systems, which are not able to compensate for the transpiration losses.

The permeability of the plasmamembrane is an evident index that reflects the degree of stress-induced injury to plants (Surjus and Durand, 1996). In general, plasma-membranes are exposed to severe injury with intensifying stress, leading to an increase in the electrolyte leakage rate. Our results also demonstrated that under saline-sodic water irrigation, membranes were injured more seriously as compared to saline or sodic irrigation alone. These results are supported by previous findings of Shi and Sheng (2005). Salt stress stimulated proline accumulation and extent of proline accumulation was dependent upon degree of stress applied in both genotypes. It is well established that proline has a protecting role in plant growth and productivity by reducing the production of free radicals and/or scavenging the free radicals (Jain *et al*., 2001). Mohamedin *et al*. (2006) reported that proline contents of sunflower plants grown on saline, saline alkali and alkali soils significantly increased when compared with the plants grown on non-saline soil. The fact that salt-sensitive genotype (Hysun-33) had tendency to accumulate more proline, in contrast to salt-tolerant genotype (SF-187), at various salt concentrations is corroborated by the results of Mutlu and Bozcuk (2005).

Saline and saline-sodic water irrigation had pronounced effects on crop yield and yield attributes like head diameter and 100 seed weight. Head diameter and 100 seed weight progressively decreased with increasing salinity/sodicity levels in irrigation water that ultimately reduced achene yield for both genotypes. However, salt sensitive genotype was rigorously affected by salt stress thus exhibiting more reduction under all stress conditions compared to salt tolerant genotype. The variability in achene yield decline in both genotypes could be attributed to differential rate of transport of Na^+ to shoot and selectivity for K^+ over Na^+ (Gorham, 1990). Higher accumulation of Na^+ might have damaged membrane integrity, disturbed water absorption, nutrient uptake, and activities of various enzymes (Wahid and

Ghazanfar, 2006). On the other hand, salt tolerant genotype (SF-187) exhibited strong affinity for K^+ over Na^+ and showed less decline in dry matter production, leaf area, K^+/Na^+ ratio and achene yield. Our findings are in good agreement with the results reported by Hebbara *et al*. (2003) who concluded that mean seed yields of different sunflower genotypes decreased from 17.41 to 15.53 q ha^{-1} as salinity increased from 4.0 to 6.0 dS m^{-1}.

Conclusion

Sensitive and tolerant behavior of selected genotypes was confirmed in pot culture under brackish water application. Reduction in growth and yield of sunflower genotypes under brackish water irrigation was due to toxicity of Na^+ and its imbalance with K^+. Maximum yield reduction was observed in case of T_5 (EC-SAR-RSC water) followed by T_2 (EC water) in both genotypes. Under mild stress (T_3 and T_4) both genotypes demonstrated almost similar response in terms of yield where no clear differences were discernable between two genotypes. However, under sodic water application, more yield was observed as compared to saline water, indicating that water resources with sodicity problem could be exploited for irrigation under the prevailing competing demands for freshwater.

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