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Leaf Characteristics and Photosynthetic Pigments Response of Sunflower Hybrids toward Salt Stress

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Abstract: A pot culture experiment was conducted to study the effects of four different levels of salinity (having osmotic potential of 0.00; -4.67; -9.35 and -14.04 bars) on the leaf characteristics and photosynthetic pigments of four hybrids of sunflower (*Helianthus annuus* L.). A significant reduction in leaf area (2.34 cm), leaf water potential (-7.70 bars), relative water contents of leaf (48.20%), chlorophyll a (0.12 mg g^{-1}), chlorophyll b (0.05 mg g^{-1}), total chlorophyll (0.17 mg g^{-1}) and carotene contents (0.45 mg g^{-1}) were recorded in response to the highest salts concentrations (-14.03 bars). The hybrid DO-728 had the highest leaf area (12.74 cm), leaf water potential (-3.84 bars), relative water contents (72.49%), chlorophyll a (0.25 mg g^{-1}), chlorophyll b (0.17 mg g^{-1}), total chlorophyll (0.42 mg g^{-1}) and carotene contents (1.52 mg g^{-1}), while Suncross-843 the lowest ones by producing the same i.e., 8.26 cm, -7.52 bars, 55.78%, 0.20 mg g^{-1}, 0.12 mg g^{-1}, 0.32 mg g^{-1} and 1.35 mg g^{-1}, respectively. Results based on cumulative salinity tolerance index (CSTI, %) exhibited that hybrid Hysun-33 could be ranked as salt tolerant (66.51%) and Suncross-843 as salt sensitive (50.11%). However, DO-730 (60.49%) and DO-728 (53.20%) showed an intermediate salinity tolerance response. Therefore, on the basis of obtained results, sunflower hybrid Hysun-33 is recommended for saline and saline sodic areas.

Key words: Carotene · Chlorophyll a & b · Total chlorophyll · Salinity · Sunflower

INTRODUCTION

Salinity is a major abiotic environmental factor which affects large area of cultivated land in more than 100 countries [1]. Increased soil salinity adversely affects the growth of many crop plants and the continued salinization of agricultural land provides an increasing threat to global crop production [2]. Agricultural productivity is severely affected by soil salinity and every year more and more land becomes non-productive due to salt accumulation [3]. A global study of land use over 45 years found that 6% had become saline [4]. This problem is more serious in agriculture of the south and Southeast Asia [5, 6]. It is reported that 10% of the total arable lands of the world are affected by salinity [7]. In Pakistan 13% of the irrigated area is reportedly suffering from severe salinity problems [8] and out of the total area, approximately half is wastelands and is extremely saline and saline sodic in nature. Salinity is one of the most important environmental factors that cause reduction in plant growth, development and productivity worldwide. Salt stress changes the morphological, physiological and biochemical responses of plants [9, 10, 11, 12]. The effect of salinity on plant growth is mainly attributed to the decreased osmotic potential [13]. Salinity decreases leaf area [14, 15, 16, 17]; leaf water potential [18, 19]; and chlorophyll contents [20, 21, 22, 23].

The criteria used to appraise the salt tolerance potential of any plant species are morphological, physiological and biochemical in nature [24, 25, 26, 27, 28]. Physiological criteria include tissue ionic contents and photosynthetic rates [29, 30, 31]. There are many strategies to overcome the negative effects of salinity. A good strategy is the selection of cultivars and species for salinity [32]. Moreover, it is important to use a quick and reliable index of salt tolerance that will enable the screening of varieties [33, 34]. In the present study the effect of salinity on leaf growth and photosynthetic pigments of sunflower, under pot culture experiments was investigated in order to evaluate the adverse effects of soil salinity and also to screen out the salt tolerant varieties. Despite the negative impacts of salinity on several plant functions, many plant species or cultivars persist in saline environments. These plants have adapted

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a variety of mechanisms to alleviate the adverse impacts of salinity. The most common mechanisms include salt exclusion, salt excretion, succulence, osmotic adjustment [35, 36] and or membrane composition [37]. Researchers also underline the differences in salinity tolerance among plant species [27]. Researchers further revealed that relative to control, saline treatments led to a 17% biomass increase in halophytic diploid hybrid species (*Helianthus paradoxus*) while its glycophytic progenitors (*Helianthus annuus* and *Helianthus petiolaris*) suffered 19-33% productivity reduction [38]. According to a classification based on water stress day index, sunflower was determined as a moderately sensitive crop toward salinity [39]. Though, sunflower is low in salt tolerance but is somewhat better than field bean or soybean in this respect. Corn, wheat, rye and sorghum are rated medium and sugar beet and barley are high in salt tolerance [40].

There has been variation in response of sunflower genotypes to salinity [41, 42]. For economical production in saline soils, it is crucial that many commercially released sunflower cultivars require to be tested for salinity using a rapid reliable screening method. As a result, in terms of development of salt tolerant plants or determination of suitable salt tolerant crops for a region (especially arid or semi-arid), selection and evaluation of salt tolerance of plants has a prime significance. Therefore, the main aim of the present study was to evaluate the effect of different level of salts (particularly Na^+ salts) on leaf characteristics and photosynthetic pigments and also to develop a rapid and easy screening method to choose salt tolerant sunflower hybrids prior to field trials.

MATERIALS AND METHODS

The certified seeds of four sunflower hybrids *viz.,* DO-728, DO-730, Hysun-33 and Suncross-843 were obtained from Agricultural Research Institute (ARI), Quetta. The above treatments were prepared by dissolving specified amount of NaCl, $Na_2SO_4.H_2O$, $CaCl_2$ and $MgCl_2$ (having ratio 4:10: 5:1) in half strength Hoagland culture solution [43] and as shown in Table 1. The osmotic potential of each salinity treatment was calculated by the following formula as described by [44]. The pH and conductivity of the treated solutions were also determined using AGB-400/UP pH/conductivity and temperature meter.

$$\Psi \text{ (bars)} = \frac{-218 \text{ x M x t}}{273}$$

whereas, M = Molar concentration of the desired solution and T = Absolute temperature + room temperature.

Plant growth studies of sunflower were carried out in plastic pots of standard size having drainage hole (plugged with blotting paper) on its bottom. Twelve pots were used for each hybrid and each of the salinity treatment was replicated three times. Therefore, the number of pots in total was 48. Every pot was filled with the same amount of thoroughly washed and moist sand. Then an equal amount of half strength Hoagland nutrient solution was also mixed with the moist and washed sand of each pot. Approximately uniform size and equal number of seeds were sown in each pot. They were then daily irrigated with equal amount of respective saline solutions. All pots were then arranged in a completely randomized design (CRD) on a Laboratory table for about 15 days. During germination, the temperature of the day was noted from 20-23°C and for the night it was in the range of 10-12°C. After the completion of germination, seedlings were thinned and left five in each pot. They were then transferred to a glass house. The day length of the seedlings was 13±1.5 hours and temperature in glass house during the month of July was in the range of 38-43°C. All agricultural practices were thoroughly made during the entire course of the study. After 8 weeks of seedling growth, a set of the resultant plants was harvested from each treatment/replicate and the following growth parameters were measured/ calculated:

Leaf Area: Leaf area was calculated by the fallowing formula of [44]:

$$A = K.L.W.$$

where A was the leaf area, K the correlation coefficient, L the length of the leaf and W the width of the leaf. K was calculated as:

$$K = S/X$$

whereas, X is the leaf length and S is the total leaf surface per unit of ground area.

Leaf Water Potential (LWP): LWP of sunflower hybrids were determined by Chamber Pressure (Model 615, PMS Instrument Co.). For this purpose we did select a healthy and fully exposed leaf from the plant of each treatment and replicate at 12:00 noon to 1:00 pm. We do placed a sandwich bag over the leaf to create a micro-environment and to protect the leaf from transpiration during the testing. Rolled the bag mouth closed around the petiole with plant cutter. Then we immediately insert the petiole

Table 1: Amount of salt added in one-liter solution of various salinity treatments

	Amount of salts, g L^{-1}.							
Treatments	NaCl	$Na_2SO_4.H_2O$	$CaCl_2$	$MgCl_2$	Molar Concentration (mM)	Osmotic potential at 20°C (bars)	EC mS/cm	pH
S_0	-	-	-	-	-	-	1.19	4.03
S_1	1.17	3.2	2.35	1.9	20	-4.67	9.54	4.40
S_2	2.34	6.4	4.70	3.8	40	-9.35	16.48	4.36
S_3	3.51	9.6	7.05	5.7	60	-14.04	22.38	4.30

into the Compression Gland to secure a good seal around the petiole. Thereafter, we insert the leaf and bag together down into the Chamber and then locked it. The instrument rate valve flow was also set at about 1 bar/2 seconds. Then we turn the control valve to Chamber and begin the flow of nitrogen into the Chamber. The LWP was then immediately noted when water came to the surface of the end of the petiole. Recorded the amount of pressure (bars) that required for pushing water from the centre of petiole out of the xylem vessels.

Leaf Relative Water Content (RWC): RWC was calculated by the formula as described by [45].

$$\text{RWC (\%)} = \frac{\text{Fresh Weight-Dry Weight x 100}}{\text{Turgid Weight-Dry Weight}}$$

For RWC measurements 10 leaf discs of 0.5 mm in diameter were punched from the leaf (three leaves per variety/replicate) weighed for their fresh weight and then floated on water for 24 h at 25°C under light. The discs were blotted dry, their turgid weight recorded and dried for 24 h at 80°C in an oven for determining their dry weight.

Chlorophyll and Carotene Contents: One gram of fresh leaves of each treatment was mashed in the presence of 5 ml distilled water in pestle mortar. This process was carried out in cold and dark conditions to avoid photo-oxidation of the primary photosynthetic pigments. Then volume was made up to 10 ml. An aliquot 0.5 ml was taken and 4.5 ml acetone (80% pure) was added for the extracting of pigments, then centrifuged and upper layer was collected. Its optical density (OD) was measured at three different wave lengths viz., 663, 645 and 480 nm for chlorophyll a, chlorophyll b and carotene contents, respectively. The absorbance was monitored using a spectrophotometer (Hitachi U-1100, Japan). The amount of these photosynthetic pigments was calculated according to the following formulae as suggested by [46].

Chl. a = $11.75\ A_{663} – 2.350\ A_{645}$
Chl. b = $18.61\ A_{645} -3.960\ A_{663}$
Carot. = $1000\ A_{480}$ -2.270 Chl.a - 81.4 Chl.b/227

Statistical Analyses: Data obtained were arranged in a two factor (salinity and variety) completely randomized design (CRD) and then statistically analyzed for two-way analysis of variance (ANOVA). Salinity was the main factor followed by hybrids as sub-factor. The number of replicates was kept three for each factor. Data was also analyzed for multiple comparison of means for the considered traits (i.e., leaf area, RWC, chlorophyll and carotenoid content) using computer software Statistix version 8.1 (2005). This data was then also used manually for the determination of salinity tolerance index (STI, %) by using the following formulae:

- STI (%) = Photosynthetic response of individual parameter in highest dose of salinity (-14.03 bars) x100 Photosynthetic response of individual parameter in control dose of salinity (0.00 bars)
- Cumulative STI (%) = Average response of all photosynthetic parameters in highest dose of salinity x 100

Average response of all photosynthetic parameters in control dose of salinity (0.00 bars).

RESULTS AND DISCUSSION

Results obtained for ANOVA (Table 2) showed that all mentioned leaf characteristics and photosynthetic pigments of the considered sunflower hybrids were significantly ($P<0.01$) different in response to different levels of salinity and they are in accordance with results obtained by other researchers [47, 48]. Results further showed that variation among hybrids and their interactions with salinity levels are also signification.

Area, Water Potential and Relative Water Contents of Leaf: Data showed that as salinity level intensifies, leaf area linearly decreased (Table 3). This reduction could attributed to accumulation of solutes in cells in order to maintain the cell volume and larger against dehydration. This phenomenon is also known as osmotic adjustment. A maximum reduction is obtained in salinity treatments having -14.04 bars osmotic potential.

Table 2: Analysis of variance (ANOVA) for leaf characters and photosynthetic pigments of four varieties of sunflower (*Helianthus annuus* L.) subjected to various levels of salinity

	Sum of square			Mean square			F-value of variables at an error of 32				
Variables	Treatments (A)	Hybrids (B)	A x B	Treatments (A)	Hybrids (B)	A x B	(A)	(B)	A x B	CV (%)	Grand Mean
1. Leaf Area (cm)	1576.31	126.85	40.07	525.437	42.285	4.452	19437.2*	1556.97*	163.92*	1.57	10.487
2. Leaf Water Potential (bars)	102.326	89.539	11.009	34.1086	29.8463	1.2232	1316.19*	1151.72*	47.20*	-2.72	-5.9106
3. Relative Water Contents (%)	6423.21	1737.56	272.16	2141.07	579.19	30.24	55948.3*	15134.7*	790.20*	0.31	63.440
4.Chlorophyll "a" (mg g^{-1})	0.27949	0.02004	0.03436	0.09316	0.00668	0.00382	166.74*	11.96*	6.83*	9.95	0.2376
5.Chlorophyll "b" (mg g^{-1})	0.19208	0.03204	0.03443	0.06403	0.01068	0.0083	345.04*	57.55*	20.62*	9.69	0.1406
6. Total Chlorophyll (mg g^{-1})	0.92605	0.07369	0.12516	0.30868	0.02456	0.01391	293.63*	23.37*	13.23*	8.61	0.3765
7.Carotenoids (g L^{-1})	15.4970	0.5701	0.7451	5.16566	0.19004	0.08279	6341.47*	233.30*	101.64*	2.06	1.3833

*Data is highly significant at P<0.01

Table 3: Leaf characteristics and photosynthetic pigments response of sunflower hybrids (*Helianthus annuus* L.) subjected to various levels of salt stress

Salinity Treatments (bars)	Leaf Area (cm)	Leaf Water Potential (bars)	Relative Water Contents (%)	Chlorophyll 'a' (mg g^{-1} fresh weight)	Chlorophyll 'b' (mg g^{-1} fresh weight)	Total Chlorophyll (mg g^{-1} fresh weight)	Carotenoid contents (mg g^{-1} fresh weight)
0.00	18.068 a	-3.9942 a	79.285 a	0.3150 a	0.2225 a	0.5375 a	1.9358 a
-4.67	12.681 b	-5.0667 b	68.224 b	0.2925 a	0.1700 b	0.4600 b	1.7108 b
-9.35	8.862 c	-6.8833 c	58.054 c	0.2242 b	0.1183 c	0.3392 c	1.4392 c
-14.03	2.337 d	-7.6983 d	48.198 d	0.1188 c	0.0515 d	0.1694 d	0.4475 d
Sunflower Hybrids							
DO-728	12.740 a	-3.8391 a	72.486 a	0.2455 a	0.1740 a	0.4161 a	1.5158 a
DO-730	10.984 b	-5.6533 b	64.028 b	0.2525 a	0.1158 c	0.3650 b	1.2233 d
Hysun-33	9.968 c	-6.8283 c	61.473 c	0.2500 a	0.1575 b	0.4075 a	1.4417 b
Suncross-843	8.256 d	-7.5208 d	55.775 d	0.2025 b	0.1150 c	0.3175 c	1.3525 c
LSD (P<0.01)	0.1836	0.1798	0.2236	0.0265	0.0154	0.0362	0.0318

Mean values followed by the same letter(s) within a column of salinity and varieties are not significantly different (P<0.05) using LSD test

Table 4: Salinity tolerance index (STI, %) for leaf characteristics and photosynthetic pigments of four varieties of sunflower (*Helianthus annuus* L.) grown in high salt level as compared with control

Sunflower Hybrids	LA	LWP	LRWC	Chl. a	Chl. b	Tot. Chl.	Car.	*CSTI
DO-728	23.95	226.66	65.89	18.48	8.40	14.22	14.80	53.20
DO-730	10.57	203.76	61.49	74.49	18.83	27.90	25.88	60.49
Hysun-33	6.72	217.85	56.35	60.00	42.86	52.94	28.84	66.51
Suncross-843	7.90	156.46	58.53	40.74	27.78	35.56	23.78	50.11

Leaf area = LA; Leaf water potential = LWP; Leaf relative water contents = LRWC; Chlorophyll a = Chl. a; Chlorophyll b = Chl. b; Total chlorophyll = Tot. Chl. and carotenes = Car. * Cumulative salt tolerance index (CSTI)

A significant (P<0.01) hybrid response is also noted. A maximum leaf area (12.74 cm) is noted for DO-728 and a minimum (8.26 cm) for Suncross-843. The earliest plant response of salt stress is a reduction in the rate of leaf surface expansion, followed by cessation of expansion as the stress intensifies [49, 50, 14, 15, 21, 16, 17]. Salinity induced osmotic stress is considered responsible for the reduced leaf area. Earlier researchers also obtained similar trend of response under salt stress [51]. Results based on salinity tolerance index (STI, %) of leaf area exhibited that among four sunflower varieties, DO-728 could be rated as salinity tolerant and Suncross-843 as salt sensitive (Fig. 1, Table 4). Significant genotype and/or salt treatment effects for leaf area was also reported by other investigators [14].

Salinity significantly reduced leaf water potential (Table 3). Statistically a maximum reduction in leaf water potential (-7.6983 bars) is obtained in highest dose of applied salinity (-14.03 bars). A significant hybrid response is also recorded and a maximum reduction is obtained for Sunncross-843 (-7.5208 bars) and minimum for DO-728 (-3.8391 bars). Research studies reported that exposure to high salt concentrations resulted reduction in predawn water potential (Ψ_w), osmotic potential at full turgor ($\Psi\pi$FT), osmotic potential at turgor loss point ($\Psi\pi$TLP), pressure potential (Ψp) and stomatal resistance in faba beans, olive, snap bean and sorghum crops [18, 52, 53, 19]. Therefore, present findings are strongly in support of the results enumerated by previous researchers. Results based on salinity tolerance index (STI, %) of leaf water potential deciphered that among four sunflower varieties, DO-728 could be ranked as salinity tolerant and Suncross-843 as salt sensitive hybrid (Fig. 1, Table 4).

Data obtained for relative water contents (RWC, %) of leaf enumerated that induced salinity levels significantly and linearly reduced it when compared with their respective control treatment (Table 3). Statistically a maximum reduction in RWC (48.20%) is obtained in highest dose of induced salinity (-14.03 bars).

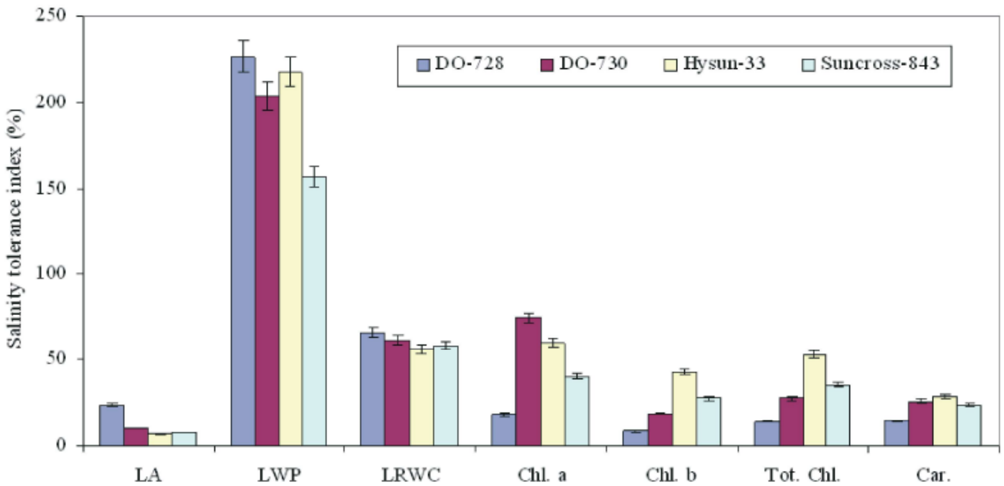

Fig. 1: Salinity tolerance index (%) for various growth parameters (viz., leaf area = LA; leaf water potential = LWP; leaf relative water contents = LRWC; chlorophyll a = Chl. a; chlorophyll b = Chl. b; total chlorophyll = Tot. Chl and carotenes = Car.) of four hybrids of sunflower (*Helianthus annuus* L.) influenced by highest level of salinity as compared with control level of salinity

A significant hybrid response is also registered. A maximum reduction (55.785) is obtained for Suncross-843 and minimum (72.486%) for DO-728. Studies revealed that RWC significantly declined with increasing salt stress (i.e., 0.0 to 250 mM) or with the decrease in osmotic potential of NaCl salinity. Therefore, findings of RWC are strongly in agreement with the achievements of other researchers [19, 54]. Many early researchers reported that severe water stress conditions are caused by high salinity or drought, plant stop growing completely and accumulate solutes in cells to maintain the cell volume and turgor against dehydration. They further reported that RWC of the leaves decreased under drought stress [55, 56 57, 58]. Researchers also stated that plants preconditioned by salinity stress maintained a better leaf water status during drought stress due to osmotic adjustment and the accumulation of Na^+ and Cl^-. Results based on salinity tolerance index (STI, %) of leaf RWC showed that among four sunflower varieties, DO-728 could be rated as salt tolerant and Hysun-33 as salt sensitive (Fig. 1, Table 4).

Photosynthetic Pigments: The results of chlorophyll a, b and total chlorophyll plus carotene contents exhibited that as salinity level increased these photosynthetic pigments were linearly decreased (Table 3). Statistically maximum reduction for chlorophyll a (0.1188 mg g^{-1}), chlorophyll b (0.0515 mg g^{-1}), total chlorophyll (0.1694 mg^{-1}) and carotenes (0.4475 mg g^{-1}) are obtained in highest dose of salinity (-14.03 bars). A significant hybrid variation is also noted for each individual pigment. Sunflower variety DO-728 produced maximum photosynthetic pigments, while variety Suncross-843 produced the minimum concentrations. Researchers also revealed that at low salinity regimes, a slight decrease was noted in chlorophyll and carotene contents, but under high salinity conditions a significant reduction in the content of these photosynthetic pigments was observed by most of the researchers [59, 60, 61, 62, 63, 21, 64, 22, 65, 23]. Therefore, present findings about photosynthetic pigments are strongly in accordance with the results recorded by these researchers for various crops studied. This reduction in leaf chlorophyll and carotenoids under salinity could be attributed to the destruction of photosynthetic pigments and the instability of the pigment protein complex. Results based on salinity tolerance index (STI, %) of chlorophyll ‘a’ showed that among four sunflower varieties, DO-730 could be ranked as salt tolerant and DO-728 as salt sensitive. Whereas, the remaining two hybrids i.e., Suncross-843 and Hysun-33 exhibited an intermediate response. While both for chlorophyll ‘b’ and total chlorophyll as well as carotenoid contents, Hysun-33 is rated as salt tolerant followed by variety DO-728 as salt sensitive (Fig. 1, Table 4). Results also based on cumulative STI exhibited that hybrid Hysun-33 could be ranked as salt tolerant and variety Suncross-843 as salt sensitive. Whereas, remaining 2 hybrids *viz.,* DO-730 and DO-728 could be ranked as intermediate in respect of salinity stress (Table 4, Fig. 2).

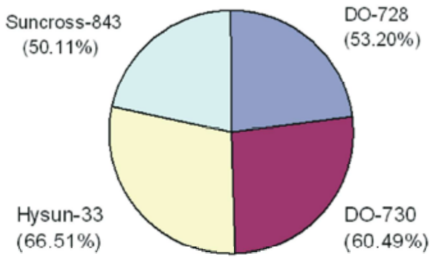

Fig. 2: Cumulative salt tolerance (%) for all leaf and photosynthetic attributes of four hybrids of sunflower of (*Helianthus annuus* L.) influenced by highest level of salinity as compared with control level of salinity

CONCLUSIONS

It can be concluded that as salinity level increased, leaf area, leaf water potential, relative water contents, chlorophyll a, b and total chlorophyll as well as carotene contents linearly decreased. Results based on cumulative salinity tolerance index (STI, %) showed that among the four sunflower varieties, Hysun-33 could be ranked as a salt tolerant (66.51%) and Suncross-843 as a salt sensitive one (50.11%), while DO-730 (60.49%) and DO-728 (53.20%) were in the middle, respectively.

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