Effect of Various Levels of Salinity on the Uptake of Macronutrients (N, P, K, Ca, and Mg) by the Roots and Shoots of Sunflower (*Helianthus annuus* L.) Hybrids

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Summary: 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 -14.03 bars on the uptake of macronutrients (N, P, K^+, Ca^{2+}, and Mg^{2+}) by 2 hybrids of sunflower (*Helianthus annuus* L.). Salinity levels were achieved by dissolving calculated amount of NaCl, Na_2SO_4, $CaCl_2$, and $MgCl_2$ (4:10:5:1) in half strength Hoagland culture solution. Results suggested that salinity significantly ($P<0.05$) and linearly increased the uptake of macronutrients (except K^+) both by roots and shoots. A significantly maximum amount of N, P, Ca^{2+} and Mg^{2+} for roots (16.80, 4.13, 40.03 and 10.28 g kg^{-1}) as well as for shoots (26.70, 3.95, 37.52, and 15.62 g kg^{-1}), respectively, were recorded in highest dose of salinity (-14.03 bars). This might be due to excess use of SO_4^{-2} over Cl^- ions in the culture media. Results further suggested that K^+ uptake both by roots and shoots were significantly reduced by applied doses of salinity. A maximum reduction in both root (19.82 g kg^{-1}), and shoot (10.52 g kg^{-1}) were observed where highest level of salinity (-14.03 bars) was applied. This inhibitory effect on K^+ uptake could be attributed to excess use of Na^+ salts in the growth media. Results also showed that, sunflower hybrid DO-728 accumulated more P & K^+ by their roots and shoots when compared with hybrid DO-730. Therefore, hybrid DO-728 could be rated as salt tolerant followed by hybrid DO-730 as salt sensitive. Results further suggested that based on grand mean values of all mentioned species of nutrients (including K^+), shoot exhibited 1.027 % increased uptake over their roots.

Introduction

Salinity is a major abiotic environmental factor that reduces plant growth and productivity throughout the world [1]. Approximately 23% of the world's cultivated lands are considered as saline and another 37% are sodic. Salinization is still expanding, posing a threat to sustainable agriculture development [2]. The soil having an EC > 2.0 mmhos cm^{-1} but SAR < 6 is said to be saline non-sodic, and if the same soil having SAR > 6 is said to be saline sodic. Salinity and alkalinity (sodicity) may develop in different area and associate with many soil-forming processes. Depending on the soil types and local environmental conditions, salinity and alkalinity influence the soil and the landscape diversely. Arid conditions contribute to salinization, but particularly to alkalinization; these also occur in semi-arid and semi-humid areas [3]. Salinity and / or alkalinity (sodicity) are serious problem throughout the world particularly in newly reclaimed areas (*e.g.*, Pakistan & Egypt) and the plants differ in their ability to grow under these conditions. It has been also estimated that salinity and water logging seriously affect one-half of all irrigated lands *i.e.*, 2.5 x 10^8 hectares. About 20 million hectares of land deteriorates to zero production each year [4]. This problem is more serious in the agriculture of South Asia and Southeast Asia [4, 5]. The recent figure for the extent of salt affected soils in Pakistan is 6.17 million hectares [6]. It includes both inland and coastal areas most of which are saline and not suitable for cultivation of conventional crops, forages, fuelwood and timber species.

Pakistan is the third largest importer of edible oil in the world. Sunflower is one of the major oil crops of the country, grown on an area of 506 thousand hectares with a total production of 755 thousand tons, and an average seed yield of 1492 kg ha^{-1}, which is far below than its potential yield [7].

Literature revealed that salinity stress caused accumulation of soluble sugars, free proline and soluble proteins in germinating seeds [8, 9], and plants fail to maintain the required balance of organic and inorganic constituents leading to suppressed growth and yield [10]. However, plant species respond differentially to the saline environment. Halophytes naturally grow even in soils where NaCl concentrations are > 250 mM. On the other hand, the large majorities of plant species are glycophytes and

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are easily damaged by salinity [11]. Many researchers even assessed significant inter- and intra-specific variation for salt tolerance both at germination and seedling growth stages [12].

Salinity also presents several challenges to plant growth, including nutrient deficiencies and disorders [13, 14]. A number of laboratory and greenhouse studies have also shown that salinity decreases the concentration of Nitrogen [15-18] and Phosphorus in plant tissues [19], but the results of few others indicated that salinity either increased or had no effect on P uptake [18]. A large number of studies also demonstrated that salinity reduced nutrient uptake and accumulation or affected nutrient partitioning within the plant [20-24]. The differences in ion partitioning and the maintenance of higher nutrients such as K^+ and Ca^{2+} to Na^+ ratios, especially in young growing and recently expanded tissues, would appear to be important mechanisms contributing to the improved salt tolerance [25-26]. Studies also demonstrated that those sunflower are genotypes which uptake more Na^+ and produce more biomass, those genotypes tolerate the toxic effect of NaCl by ion inclusion while others by ion exclusion. Na^+ uptake is regarded as an important criterion for tolerance toward salinity [27]. While on the other hand for many salt sensitive plants, a major part of the growth inhibition is caused by excess Na^+ application [28]. Generally speaking that high Na^+ disturbs K^+ nutrition and when accumulated in cytoplasm, inhibits many enzymes of salt sensitive plants [29]. High level of Na^+ also inhibited the K^+ concentration and as a result of this, it caused an increase in Na^+ / K^+ ratio. This may causes disturbance in the ion balance in plant by an increase in the Na^+ uptake [30], and nutrient ion deficiency by disrupting K^+ nutrition [31]. Whereas, Manivannan *et. al.*, [32] revealed that K^+ and Na^+ contents of sunflower seedlings were increased in response to different level and kind of sodium salts when compared to control. Salinity is mostly caused by NaCl, producing defilement of soil particles resulting decrease in soil permeability and porosity. Saline conditions drastically change the environment of root aeration, osmotic potential of soil solution and normal equilibrium of the dissolved ions. Salinity dominated by Na^+ salts not only reduces Ca^{2+} availability but reduces Ca^{2+} transport and mobility to growing regions of the plant, which affects the quality of both vegetative and reproductive organs [21]. Research also revealed that in order to improve salt tolerance, it is important to explore inter-varietal or hybrid variation for salt tolerance, because all varieties even of the same crop does responded equally [33-34]. Therefore, in view of the above facts, a study was conducted to appraise the effect of different salinity regimes on the chemical aspect of two sunflower hybrids.

Results and Discussion

Results (Table-3) showed that in response to different treatments of salinity (A) all mentioned species of macronutrients (viz., N, P, K^+, Ca^{2+} and Mg^{2+}) of sunflower roots and shoots as well as hybrids (B) and their interactions too (A x B) exhibited statistically significant results ($P < 0.05$ and $P < 0.01$). Similar trend of results have also been reported by Achakzai [40, 41] in sorghum and maize seedlings subjected to various levels of water stress conditions.

Data (Tables-4, 5) showed that salinity treatments linearly and progressively increased the N and P uptake both by root and shoot of sunflower

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

Treatments	Amount of salts/L.				Molar Concentration (M)	Osmotic potential at 20 °C (bars)	EC mS/cm	pH
	NaCl	Na_2SO_4	$CaCl_2$	$MgCl_2$				
S_0	-	-	-	-	-	-	1.19	4.03
S_1	1.17	4.68	2.35	0.609	0.2	-4.67	9.54	4.40
S_2	2.34	9.36	4.70	1.220	0.4	-9.35	16.48	4.36
S_3	3.51	14.04	7.05	1.820	0.6	-14.04	22.38	4.30

Table-2: Amount of nutrients added in half strength Hoagland's solution.

Nutrients	Concentration	Amount of nutrients used (g L^{-1})
1. Macro-Nutrients		
$Ca(NO_3)_2$	5 mM	0.590
KNO_3	5 mM	0.252
$MgSO_4$	2 mM	0.246
KH_2PO_4	2 mM	0.136
$FeCl_3$	1 mM	0.0805
2. Micro-Nutrients		
H_3BO_3	0.029 ppm	0.0029
$MnCl_3.4H_2O$	0.018 ppm	0.0018
$ZnSO_4.7H_2O$	0.0022 ppm	0.00022
$CuSO_4.5H_2O$	0.0080 ppm	0.00080
$H_2MoO_3.H_2O$	0.0020 ppm	0.00020

Table-3: Analysis of variance (ANOVA) for nutrients uptake by two hybrids of sunflower (*Helianthus annuus* L.) subjected to various levels of salinity.

Variables	F-value of variables at an error of 14			CV (%)
Root				
1. Nitrogen	276.079**	664.031**	67.5052**	3.44
2. Phosphorus	430.6088**	280.7777**	185.7851**	4.24
3. Potassium	4152.9428**	21909.3928**	9031.4004**	0.91
4. Calcium	19137.3893**	36755.0809**	3372.9627**	0.79
5. Magnesium	424.876**	1089.5001**	201.3849**	1.62
Shoot				
1. Nitrogen	1432.6283**	18.9024**	42.1125**	1.87
2. Phosphorus	162.7976**	398.7821**	6.9274**	4.00
3. Potassium	12566.8812**	5230.1917**	194.1271**	0.93
4. Calcium	131.9404**	55.2342**	11.2167**	5.48
5. Magnesium	6372.2879**	1291.6318**	1893.6511**	1.21

** Highly significant both at P<0.05 and 0.01. CV = coefficient of variation.

Table-4: Effect of salinity on the uptake of total nitrogen contents (g kg^{-1}) by root and shoot of two hybrids of sunflower (*Helianthus annuus* L.).

Hybrids	Salinity Treatments (bars) 0.00	-4.67	-9.35	-14.03	Mean
Root					
1) DO-728	13.6 c	15.9 b	16.4 b	17.5 a	15.85a
2) DO-730	5.3 e	09.4 d	13.3 c	16.0 b	10.99b
Mean	9.5 d	12.7 c	14.9 b	16.8 a	13.42
Shoot					
1) DO-728	12.7 g	16.9 e	26.3 b	27.2 a	20.78b
2) DO-730	16.1 f	18.2 d	25.4 c	26.2 b	21.47a
Mean	14.40 d	17.55 c	25.85 b	26.70a	21.13

LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the roots are 0.07832 and 0.084420, respectively.
LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the shoots are 0.07832 and 0.1087, respectively.

Mean values followed by the same letter(s) within right side column (varieties) and bottom row (treatments) of the Table are not significantly different (P < 0.05) using LSD test. Similarly, values followed by the same letter(s) within column and rows (varieties x salinity treatments) in the center of the Table are not significantly different from each other.

Table-5: Effect of salinity on the uptake of total phosphorus (g kg^{-1}) by root and shoot of two hybrids of sunflower (*Helianthus annuus* L.).

Hybrids	Salinity Treatments (bars) 0.00	-4.67	-9.35	-14.03	Mean
Root					
1) DO-728	2.70c	2.80c	3.37b	3.57b	3.11a
2) DO-730	1.27e	1.50de	1.80d	4.70a	2.32b
Mean	1.98c	2.15c	2.58b	4.13a	2.71
Shoot					
1) DO-728	3.03cd	3.20c	3.57b	4.60a	3.60a
2) DO-730	2.07g	2.10f	2.90e	3.30bc	2.59b
Mean	2.55d	2.65c	3.23b	3.95a	3.10

LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the roots are 0.0313 and 0.0450, respectively.
LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the shoots are 0.0030 and 0.0416, respectively.

Mean values followed by the same letter(s) within right side column (hybrids) and bottom row (treatments) of the Table are not significantly different (P < 0.05) using LSD test. Similarly, values followed by the same letter(s) within column and rows (hybrids x salinity treatments) in the center of the Table are not significantly different from each other.

hybrids. The performance of hybrid DO-728 was found better than that of DO-730. A maximum uptake of N and P in root (1.680 and 4.13 g kg^{-1}) and shoot (26.70 and 3.95 g kg^{-1}) was recorded in highest treatment of salinity (-14.03 bars), respectively. These findings in term of N uptake are also in line with Groenigen and Kessel [42], but are in contradiction with the findings obtained by Zalba and Peinemann [43]. They obtained high positive as well as negative correlation between salinity, NH_4^+ uptake and NO_3 concentration in shoot under saline conditions, respectively. This contradictory response could be attributed to different nature of the crops used. A number of laboratory and greenhouse studies have also shown that salinity decreases the concentration of N [15-17] and P in plant tissues [19].

Table-6 shows that salinity significantly and linearly decreased the uptake of K^+ both by roots and shoots of sunflower as compared with their respective control treatment. The shoot uptake performance of hybrid DO-728 was comparatively found better than that of DO-730. K^+ concentration in shoot were also found lesser than their respective roots even under the same treatment. A maximum reduction in K^+ both for root (19.52 g kg^{-1}) and shoot K^+ (10.52 g kg^{-1}) were obtained in highest dose of salinity (-14.03 bars). Though Na^+ uptake was not analyzed in the present study, but the reduction in K^+ uptake may be due to inhibitory effects of Na^+ as it was used in the growth medium of sunflower hybrids. This might have caused disturbance in the ion balance in the subjected plant organs. High Na^+ particularly disturbs K^+ nutrition and when accumulated in cell cytoplasm inhibits many enzymes. These findings are also in conformity with the results obtained by many researchers [21, 30, 44], but are in contradiction with the findings obtained by Manivannan *et al.*, [30]. They stated that K^+ and Na^+ contents of sunflower were increased in response to different level and kind of Na^+ salts when compared to control dose of salinity.

Results shown in Table 7-8 exhibited that salinity significantly and linearly increased both the Ca^{2+} and Mg^{2+} contents of root and shoot. Statistically a maximum significant amount of Ca^{2+} (40.03 & 37.52 g kg^{-1}) and Mg^{2+} (10.28 & 15.63 g kg^{-1}) both for roots and shoots were recorded in highest dose of salinity (-14.03 bars), respectively. It was also noted that uptake of Ca^{2+} was greater in

Table-6: Effect of salinity on the uptake of total potassium (g kg^{-1}) by root and shoot of two hybrids of sunflower (*Helianthus annuus* L.).

Hybrids	Salinity Treatments (bars) 0.00	-4.67	-9.35	-14.03	Mean
Root					
1) DO-728	23.03e	31.03c	16.00f	1.60g	17.92b
2) DO-730	39.00a	26.03d	23.03e	38.03b	31.52a
Mean	31.02a	28.53b	19.52c	19.82c	24.72
Shoot					
1) DO-728	34.03a	26.00b	19.00d	12.00f	22.76a
2) DO-730	25.97b	21.00c	13.03e	9.03g	17.26b
Mean	30.00a	23.50b	16.02c	10.52d	20.01

LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the roots are 0.5538 and 0.0768, respectively.
LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the shoots are 0.0286 and 0.03989, respectively.

Mean values followed by the same letter(s) within right side column (varieties) and bottom row (treatments) of the Table are not significantly different (P < 0.05) using LSD test. Similarly, values followed by the same letter(s) within column and rows (varieties x salinity treatments) in the center of the Table are not significantly different from each other.

Table-7: Effect of salinity on the uptake of total calcium (g kg^{-1}) by root and shoot of two hybrids of sunflower (*Helianthus annuus* L.).

Hybrids	Salinity Treatments (bars) 0.00	-4.67	-9.35	-14.03	Mean
Root					
1) DO-728	31.00d	21.00e	36.60c	42.03a	32.66a
2) DO-730	6.00h	9.03g	16.03f	38.03b	17.27b
Mean	18.50c	15.02d	26.32b	40.03a	24.97
Shoot					
1) DO-728	22.03d	37.03a	39.07a	37.00a	33.78a
2) DO-730	17.30e	27.03c	32.03b	38.03a	28.60b
Mean	19.67c	32.03b	35.55a	37.52a	31.19

LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the roots are 0.0312 and 0.02977, respectively.
LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the shoots are 0.2982 and 0.4139, respectively.

Mean values followed by the same letter(s) within right side column (hybrids) and bottom row (treatments) of the Table are not significantly different (P < 0.05) using LSD test. Similarly, values followed by the same letter(s) within column and rows (hybrids x salinity treatments) in the center of the Table are not significantly different from each other.

shoot (31.19 g kg^{-1}) than their respective roots (24.97 g kg^{-1}). Differential hybrids response in term of Ca^{2+} and Mg^{2+} uptake was also observed. Significantly greater uptake of both divalent cations was observed in hybrid DO-728 than that of DO-730. Research revealed that NaCl salinity reduces Ca^{2+} and Mg^{2+} concentration in shoots of various barley genotypes. Therefore, present findings are not in line with those achieved by other researchers [19, 42]. In the present study the amount of Na_2SO_4 salt used were greater than NaCl salt, which might have encouraged not only the availability, but also increased the Ca^{2+} and Mg^{2+} transport and mobility to the growing regions of the sunflower.

Table-8: Effect of salinity on the uptake of total magnesium (g kg^{-1}) by root and shoot of two hybrids of sunflower (*Helianthus annuus* L.).

Hybrids	Salinity Treatments (bars) 0.00	-4.67	-9.35	-14.03	Mean
Root					
1) DO-728	09.33c	9.30c	10.00b	11.73b	10.09a
2) DO-730	5.40d	9.00c	9.17c	8.83c	8.10b
Mean	7.37c	9.15b	9.58b	10.28a	9.19
Shoot					
1) DO-728	7.30e	8.20d	8.83c	11.57b	8.97b
2) DO-730	7.00f	8.03d	8.20d	19.67a	10.73a
Mean	7.15d	8.12c	8.52b	15.62a	9.85

LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the roots are 0.048 and 0.02147, respectively.
LSD @ P < 0.05 and P < 0.01 both for varieties and treatments of the shoots are 0.023 and 0.032, respectively.

Mean values followed by the same letter(s) within right side column (hybrids) and bottom row (treatments) of the Table are not significantly different (P < 0.05) using LSD test. Similarly, values followed by the same letter(s) within column and rows (hybrids x salinity treatments) in the center of the Table are not significantly different from each other.

Experimental

Present study deals with the effect of four different treatments of salinity (*i.e.*, S_0, S_1, S_2 and S_3) having osmotic potential of 0.0, -4.67, -9.35 and -14.03 bars on the nutrient uptake of sunflower (*Helianthus annuus* L.). The certified seeds of two hybrids of sunflower (viz., DO-728 and DO-730) were obtained from Agricultural Research Institute (ARI), Quetta. Both the hybrids are originated from USA and have the following morphological/ agronomical descriptions:- Their days to flower initiation is 91-96; days to flower completion is 98-103; days to maturity is 128-138; plant height is 161-172 cm; head diameter is 14.5-16.3 cm; seed yield is 2267-2945 kg ha^{-1}; 100 seed weight is 5.11-5.90 g and their oil contents ranges between 46.5-47.9%. The above salinity treatments were prepared by dissolving calculated amount of NaCl, Na_2SO_4, $CaCl_2$, and $MgCl_2$ (having ratio 4 : 10 : 5 : 1) in half strength Hoagland culture solution as explained by Machlis and Torrey [35] as shown in Table 1 and 2. The osmotic potential of each salinity treatment was calculated by the following formula as described by Ting [36].

$$\Psi \text{ (bars)} = \frac{-21.8 \times M \times T}{273}$$

where:
Ψ = Water potential in bars
M = Molar concentration of solution
T = Room temperature (°C) + Absolute temperature (K)

273 = Absolute temperature
-21.8 = Osmotic potential of one molar solution (bars).

The pH and conductivity of the treated solutions were also determined using AGB-400/UP pH/conductivity and temperature meter.

Plant growth studies of sunflower were carried out in standard size plastic pots having drainage hole on its bottom. Twelve pots were used for each variety, and each of the salinity treatment was replicated thrice. Every pot was filled with equal volume of thoroughly washed and moist sand. Approximately uniform size and equal number of seeds were sown in each pot. They were then daily irrigated with an equal amount *i.e.*, 50 mL respective salinity treatment. All theses 24 pots were then arranged in a completely randomized design (CRD) on a Laboratory table for about 15 days. After the completion of germination, seedlings were thinned and left five in each pot. They were then shifted to glass house. All agricultural practices were thoroughly made during the entire course of study. After 10 weeks of seedling growth, a set of the resultant plants were carefully harvested from each treatment/replicate. Their roots and shoots were manually separated, and washed them in tap water for three times, then in Decon and finally were rinsed with deionized water. Both root and shoot materials were dried in an oven at 80 °C for 24 h. They were then ground and digested using wet acid digestion method. The digested material *i.e.*, root and shoot were then separately analyzed for their macronutrients (N, P, K^+, Ca^{2+}, Mg^{2+}), following the procedures described by Richard [37].

Statistical Analyses of Data

The collected data on various macro-nutrients of the study were statistically analyzed for working out their analysis of variance (ANOVA). The MSTAT-C computer software package, version 1.3 was used for the purpose mentioned [38]. The significance of the differences among the pairs of salinity treatment means was evaluated by applying least significant test (LSD) at 5% level of probability [39].

Conclusions

It can be concluded that salinity significantly ($P < 0.05$) and linearly increased the uptake of macronutrients (except K^+) both by roots and shoots. A maximum significant amount of N, P, Ca^{2+} and Mg^{2+} for roots were recorded where highest level of salinity was applied (-14.03 bars). This might be due to excess use of SO_4^{-2} rather than Cl^- ions in the growth media. Results further suggested that K^+ uptake both by roots and shoots were significantly reduced by applied doses of salinity. This inhibitory effect on K^+ uptake could be attributed to excess use of Na^+ salts in the culture media.

Results also indicated that on the basis of hybrid mean values of roots and shoots, hybrid DO-728 showed 18.67 g kg^{-1} greater nutrients uptake over hybrid DO-730. Therefore, DO-728 could be rated as salt tolerant, and DO-730 as salt sensitive hybrids. Results further showed that based on grand mean values of all mentioned species of nutrients (including K^+), shoot exhibited 10.27 g kg^{-1} increased uptake over their roots.

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