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Assessment of Salt Tolerance Ability among Different Accessions of Sunflower (Helianthus annuus L.)

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Abstract
Twenty sunflower (*Helianthus annuus* L.) accessions were evaluated against three different salinity levels. Triplicate factorial completely randomized design was followed. Salinity was developed with NaCl to achieve the final salinity levels of $3dSm^{-1}$, $6dSm^{-1}$ and $9dSm^{-1}$, whereas control contained tap water. Data of 60 days old ten seedlings from each entry was recorded and analyzed. Accessions G-36, G-61, A-23, A-6, and A-185 performed better in both controlled and saline conditions. These accessions showed better biomass production and high shoot and root growth by least concentration of Na^+ and higher concentration of K^+ and Cl^- in leaf sap resulting in better K^+ and Na^+.
Keywords: Halianthus annus, salinity, biomass, leaf sap

INTRODUCTION

Salinity either in soil or in water is one of the major obstacles in crop production throughout the world especially in arid and semi-arid regions. Saline soils remarkably reduce oil production potential and oil yield of sunflower [28]. The plants that grow in saline soils have diverse ionic compositions and a range in concentrations of dissolved salts. These concentrations fluctuate because of changes in water source, drainage, evapo-transpiration, and solute availability.

Soil salinity reduces water availability of plant roots via negative (low) osmosis potential, as well as decrease of germination dynamics of plant seeds by ionic toxicity of Na+ and Cl^- [21]. Salinity is also considered as a major abiotic stress and significant factor affecting crop production all over the world especially in arid and semi-arid region [15]. In spite of this extensive literature there is still a controversy regarding mechanisms of salt tolerance in plants.

Low quality irrigation water is one of the factors leading to decline sunflower productivity in Pakistan. Estimates show that about 70–80% of pumped water (67,842 million m^3) contains soluble salts and/or sodium ions (Na^+) levels above the permissible limits for irrigation water [18]. Each unit in EC_e above 4.8 dSm^{-1} resulted in yield reduction by 4.5% in sunflower [7]. Salt water was used by many investigators to study tolerance or sensitivity of many crops to salinity. Sunflower genotypes exhibit considerable genetic diversity for salinity tolerance, which can be exploited for the selection of salt tolerant material using optimum selection tools [3,13]. Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants.

To increase production from salt affected and normal soils there is a need to identify salt tolerant genotypes of potential oil seed crops. The situation necessitates a regular selection/ screening of new genotypes. Owing to the importance of sunflower as oil seed crop and the soil and climatic conditions under which it is by and large grown in Pakistan. The research work was conducted to study variation in salinity tolerance of sunflower and to identify the sunflower genotypes showing tolerance to salinity. It was a scientific attempt to understand the genetic behavior and response of different accessions of sunflower to tolerate salt stress at seedling stage. The information so obtained will be useful in formulating criteria for salt stress tolerance and high yield. The selected types could be used in hybridization program aimed at breeding for sunflower high yield under salt stress conditions. The availability of high yielding salt stress tolerant sunflower is perceived to attract farmers to use the land resources otherwise left fellow due to salt stress. Higher harvest of edible oil thus achieved certainly will have great economic impact on farmers and country.

MATERIALS AND METHODS:

The present study was conducted in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The research material was comprised of 20 sunflower accessions namely G-16, G-30, G-32, G-36, G-44, G-45, G-61, G-64, G-66, G-68, G-86, A-2, A-14, A-23, A-56, A-60, A-61, A-79, A-133 and A-185, developed by the Oilseed Research Program of the Department.

Experiment was conducted in a glass house with no control on humidity, temperature, and light, following triplicate factorial complete randomized design. The sunflower seeds were planted in iron trays filled with soil and sand with 1:1. The seeds were sown at the depth of 1.5 cm by maintaining row-to-row and plant-to-plant uniform distance of 2.5 cm each.

Field soil free from any salinity and sodicity hazards was collected. The mixture of sand and soil was air-dried, ground, passed through 2 mm sieve, and analyzed for chemical characters. The EC of soil was 1.23 dS

m^{-1} with saturation 25.7% and total soluble salt 17.7 me L^{-1}. Tap water was applied for irrigation for 15 days according to requirement. After germination, four salt (NaCl) levels of irrigation water were maintained i.e., Normal water (Tap water), 3 dsm^{-1}, 6 dsm^{-1}, 9 dsm^{-1}. The tap water comprised of EC 1.036 me L^{-1}, Na^{+} 3.83, Ca^{+} + Mg^{++} 6.53 me L^{-1} and total soluble salts 10.36 me L^{-1}. Sixty days old, ten randomly selected plants per entry were uprooted after through irrigation to facilitate the process of uprooting and data was recorded. Two lower leaves (from the basal node) and two upper leaves (from the top node) were collected. The collected samples were washed with tap water to remove the soil residues and then dipped instantly in distilled water for a short period. The samples were blotted dry, placed in polyethylene bags, marked with the spirit marker and stored in the deep freezer. Frozen leaf samples were thawed, after washing with distilled water the tissue sap was extracted in eppendorf tubes by using metal rod and immediately stored back in the deep freezer. After thawing the tissue sap at room temperature, it was centrifuged at the 6500 rpm for five minutes. The supernatant was analyzed for chloride, sodium, and potassium ions. Chloride ions in the tissue sap were determined using Sherwood chloride analyzer 926. The tissue sap was diluted as required with distilled water. Sodium and potassium ions were determined using Sherwood flame photometer 410. Data were subjected to statistical analysis by using complete randomized design with factorial classification [27] using MSTATC software. Treatment means were compared by LSD test.

RESULTS AND DISCUSSION

Shoot length (SL) is an important parameter used to determine the effect of salt stress on plants and can be used to evaluate the performance under saline conditions. On an average basis, differences in SL among all genotypes were significant at different NaCl concentration levels. Results showed that accession G-36 followed by G-66 and A-23 had maximum SL and accession A-60, A-2 and G-30 had minimum SL respectively at 0dsm^{-1}. At 3dsm^{-1}, accession A-23 followed by G-36 and G-64 had maximum SL and accession A-2 followed by A-60 and G-66 had minimum SL (Table-1). Table-2 showed maximum SL in case of accessions G-36 followed by G-45 and G-44 and minimum SL in case of accessions A-60 followed by G-133 and A-2 at 6dsm^{-1} and at 9dsm^{-1}stress level, accession G-86 followed by G-30 and A-14 had maximum SL and accession A-60 followed by A-2 and A-133 had minimum SL.

This study confirmed the results of Ghumman [9] who reported that SL and relative SL decreased with increase in salinity. Hussain and Rehman [10] found that SL of different sunflower genotypes decreased up to 19.5% with increase in salinity. Excessive accumulation of salts in cell wall modified the metabolic pathway; limit the cell wall elasticity and ultimately the SL. Further, the secondary cells appear sooner and cell wall becomes rigid. As a result, turgor pressure efficiency in cell enlargement declines. These processes may cause the shoot to remain smaller [20].

Many workers used root length (RL) as a criterion for assessing salinity tolerance in different crops. RL usually decreased with increase in salinity. In present study, on overall mean basis the RL decreased significantly with increase in salinity. Table-1 showed that accession A-133 followed by A-60 and A-185 had maximum RL and accession A-56 followed by A-2 and G-16 had minimum RL at 0dsm^{-1} and at 3dsm^{-1}, accession G-66 followed by A-2 and A-79 had maximum RL and accession G-32 followed by A-14 and A-44 had minimum RL. Accessions G-36 followed by A-2 and A-23 had maximum RL and accession G-45 followed by G-64 and G-30 had minimum RL at 6 dsm^{-1} and at 9dsm^{-1}, accession G-44 followed by G-36 and A-185 had maximum RL and accession A-133 followed by A-60 and G-31 had minimum RL (Table.2).

Ghumman [9] found that in sunflower RL and relative RL decreased with increase in salinity. The RL of different sunflower genotypes reduced up to 9% when grown under saline conditions [10]. The reduction in RL in response to salinity may be due to Na^{+} and Cl^{-}, which affect root permeability and integrity due to the displacement of Ca^{+} from the plasma lemma, resultantly inhibition of root growth and RL. The saturation level and concentration of salts in the soil solution cause the dispersion of clay particles, which leads to clogging of soil pores and consequently results in reduction of soil permeability, porosity, and hydraulic conductivity, finally reduction in root development [2].

Fresh shoot weight (FSW) is another important parameter, which helps in identifying the performance of plants under saline conditions. In present study, with the increase in salinity level, FSW reduced significantly. At 0dsm^{-1}, accession G-36 followed by G-66 and G-68 had maximum FSW and accession A-2 followed by A-60 and G-30 had minimum FSW and accession A-79 followed by G-61 and G-64 had maximum FSW and accession A-60 followed by A-2 and A-185 had minimum FSW at 3dsm^{-1} (Table.1). According to Table.2, accession G-61 followed by G-36 and G-56 had maximum FSW and accession A-2 followed by A-185 and A-60 had minimum FSW at 6 dsm^{-1}. At 9dsm^{-1}, accession G-44 followed by G-32 and G-36 had maximum FSW and accession G-45 followed by G-64 and A-2 had minimum FSW.

Hussain and Rehman [10] found that FSW of sunflower reduced up to 21% due to increase in salinity. Reduction in FSW could be attributed to decrease water potential of rooting medium, due to high ion concentration; an initial growth inhibition in saline condition is related to osmotic effect. When salts accumulate

to toxic levels in leaves, the growth inhibition takes place, so ion toxicity of Na^+ and Cl^- could be the second reason for decreased FSW with increase salinity [11]. High Na^+ and Cl^- concentration in the rooting medium could have suppressed K^+, Ca^+ and NO_3 etc. and ultimately the growth [8]. Leaf senescence increases with salinity, which obviously reduces FSW.

Fresh root weight (FRW) can be used as a criterion for the determination of salinity tolerance and performance of different plants under salinity stress. It was observed in present study that FRW of all the genotypes decreased significantly with increasing salinity. Results showed (Table.1) that accession G-16 followed by G-36 and G-86 had maximum FRW and accession A-2 followed by A-68 and G-66 had minimum FRW at 0dsm-1. At 3dsm^{-1} accession A-68 followed by G-86 and G-185 had maximum FRW and accession G-32 followed by A-61 and A-60 had minimum FRW. According to results in table.2, accessions G-30 followed by G-23 and G-36 had maximum FRW and accession G-45 followed by G-16 and G-32 had minimum FRW at 6 dsm^{-1}. At 9dsm^{-1}, accession A-23 followed by G-61 and A-185 had maximum FRW and accession A-2 followed by A-14 and G-32 had minimum FRW.

Nawaz *et al,* [22] and Ghumman [9] noted that in sunflower FRW decreased with increase in salinity. The reduction in FRW may be due to the osmotic potential of salts in the soil solution which reduced cell water required from the soil solution [20].

Dry shoot weight (DSW) is an important criterion to observe the salt tolerance level of plants, which usually decreased with increase in salinity. In present study on an overall mean basis DSW found to show decreasing trend with increased salinity. Results showed that at 0dsm^{-1} accession A-23 followed by G-64 and G-36 had maximum DSW and accession G-44 followed by A-60 and A-79 had minimum DSW whereas accession G-36 followed by G-61 and G-16 had maximum DSW and accession A-60 followed by G-32 and G-30 had minimum DSW at 3dsm^{-1} (Table.1). At 6dsm^{-1}, accession G-61 followed by G-36 and A-185 had maximum DSW and accession G-31 followed by G-16 and G-45 had minimum DSW and accession G-61 followed by A-23 and G-36 had maximum DSW and accession A-133 followed by G-44 and G-30 had minimum DSW at 9dsm^{-1} (Table.2).

Khatoon *et al.* [17] reported that in sunflower DSW and relative DSW decreased significantly with increase in salinity. Hussain and Rehman [10] noted a reduction of 31% in DSW of different sunflower genotypes with increase in salinity. The decrease in DSW under saline condition was due to reduced growth because of decreased water uptake, toxicity of Na^+ and reduced photosynthesis [5]. The decrease in DSW with increase in salinity may be because of unbalanced nutrients, solute suction in toxic quantities and use of metabolites [4].

Dry root weight (DRW), showed significant differences among different accessions at various salinity levels. Results in table-1, stated that accession A-79 followed by A-185 and G-86 had maximum DRW and accession G-64 followed by A-68 and A-61 had minimum DRW at 0dsm^{-1}. At 3dsm^{-1} accession G-36 followed by A-14 and G-61 had maximum DRW and accession G-16 followed by G-64 and A-61 had minimum DRW. Accessions A-185 followed by G-36 and G-86 had maximum DRW and accession G-68 followed by A-61 and A-14 had minimum DRW at 6dsm^{-1} whereas at 9dsm^{-1}, accession G-86 followed by G-61 and G-36 had maximum DRW and accession G-66 followed by A-60 and A-79 had minimum DRW (Table.2).

These findings confirmed the results of Ghumman [9] and Afzal [1], who reported that DRW decreased with increase in salinity. Hussain and Rehman [10] expressed 21% reduction in DRW of different sunflower genotypes under saline conditions. The reduction in DRW under saline conditions was due to reduced growth because of decline in water uptake, toxicity of Na^+ and Cl^- in root cells [5]. Reduction in DRW was correlated with reduction in FRW. High Na^+ and Cl^- concentration in rooting medium could suppress the uptake of K^+, Ca^+ and NO^-_3 and ultimately the growth [8].

Regarding chlorophyll contents, results showed that at 0dsm^{-1}accession A-79 followed by G-68 and G-32 had maximum chlorophyll and accession G-36 followed by G-45 and G-64 had minimum chlorophyll. At 3dsm^{-1}, accession G-86 followed by G-68 and A-23 had maximum chlorophyll and accession G-30 followed by G-32 and A-16 had minimum chlorophyll (Table.1). Results depicted in table.2 stated that accession A-61 followed by A-185 and A-60 had maximum chlorophyll and accession A-133 followed by G-30 and G-32 had minimum chlorophyll at 6dsm^{-1} and Accession G-68 followed by A-185 and G-66 had maximum chlorophyll and accession G-16 followed by A-61 and G-36 had minimum chlorophyll at 9dsm^{-1}.

Sodium concentration (Na^+) in a plant is of a parameter and can be used to determine the tolerance and performance of a crop under salt stress; it usually increases with increase in NaCl level in the media. In present study, it is clear from the data that Na^+ concentration significantly increased with increase in salinity. At 0dsm^{-1}, accession A-23 followed by A-14 and A-61 had maximum Na^+ content and accession G-45 followed by G-61 and G-30 had minimum Na^+ content and accession A-56 followed by A-133 and A-61 had maximum Na^+ content and accession G-16 followed by G-61 and G-36 had minimum Na^+ content at 3dsm^{-1} (Table.1). Results of Table.2 said that accession A-133 followed by A-56 and A-60 had maximum Na^+ content and accession G-44 followed by A-79 and G-32 had minimum Na^+ content at 6dsm^{-1} and at 9dsm^{-1}, accession A-133 followed by A-

56 and A-60 had maximum Na^+ content and accession G-44 followed by G-32 and A-79 had minimum Na^+ content.

Nawaz *et al.* [22] reported that in sunflower sodium concentration increased significantly with increase in salinity. Tolerant plants maintain less Na^+ concentration in the leaves at high salinity level by efficient exclusion of Na^+. The increase in sodium contents in leaves with increase in salinity was attributed to the increased amount of sodium ion in rooting medium, passive Na^+ diffusion through damaged membranes, decreased efficiency of exclusion mechanism. Besides, this increase in sodium concentration could be due to greater uptake of sodium ion to build osmotic pressure. Sodium being a monovalent is very effective for osmotic adjustment. Efficient Na^+ exclusion is a good selection criterion for salt tolerance in sunflower and other glycophytes [22].

Determination of K^+ concentration in leaf sap is an important tool to identify salinity tolerance in plants; it decreases with the increase in salinity. In present study on an overall mean basis, the concentration of K^+ decreased significantly with increasing salinity. At $0dsm^{-1}$, accession A-60 followed by G-68 and G-32 had maximum K^+ content and accession G-30 followed by G-36 and G-64 had minimum K^+ content whereas accession G-32 followed by G-44 and G-45 had maximum K^+ content and accession A -2 followed by A -60 and A -14 had minimum K^+ content at $3dsm^{-1}$ (Table.1). Table.2 showed that at $6dsm^{-1}$, accessions A-56 followed by G-61 and G-44 had maximum K^+ content and accession A-23 followed by A-2 and G-64 had minimum K^+ content and at $9dsm^{-1}$, accession G-68 followed by G-66 and A-56 had maximum K^+ content and accession A-79 followed by A-61 and G-86 had minimum K^+ content.

The results are in accordance with the findings of Khalil [16] and Nawaz *et al.* [22] who reported decrease in K^+ concentration with increase in salinity.

Regarding Cl^- contents, results showed that accession G-30 followed by G-185 and G-68 had maximum Cl^- content and accession A-79 followed by G-86 and A-133 had minimum Cl^- content at $0dsm^{-1}$ and at $3dsm^{-1}$, accession G-68 followed by A-2 and A-56 had maximum Cl^- content and accession G-32 followed by A-14 and A-60 had minimum Cl^- content (Table.1). Accessions G-32 followed by G-36 and A-61 had maximum Cl^- content and accession G-44 followed by A-14 and A-133 had minimum Cl^- content at 6 d Sm^{-1} and at $9dsm^{-1}$, accession A-185 followed by G-36 and G-61 had maximum Cl^- content and accession G-44 followed by G-45 and A-133 had minimum Cl^- content (Table.2).

Results of table.1 showed that accession G-32 followed by G-30 and G-45 had maximum mortality % and accession A-185 followed by A-66 and A-79 had minimum mortality % at $0dsm^{-1}$ whereas at $3dsm^{-1}$, accession G-32 followed by G-66 and G-68 had maximum mortality % and accession G-14 followed by G-61 and G-45 had minimum mortality %. And results of Table.2 stated that accessions A-185 followed by A-2 and G-66 had maximum mortality % and accession G-44 followed by A-79 and G-16 had minimum mortality % at $6dsm^{-1}$ whereas at $9dsm^{-1}$, accession G-32 followed by A-133 and G-86 had maximum mortality % and accession G-30 followed by A-79 and G-68 had minimum mortality %.

Regarding root/shoot ratio, accession A-79 followed by G-86, A-185 had maximum root / shoot ratio and accession G-64 followed by A-61, and G-68 had minimum root / shoot ratio at $0dsm^{-1}$. At $3dsm^{-1}$, accession A-60 followed by G-96 and A-14 had maximum root/ shoot ratio and accession G-16 followed by G-64 and A-61 had minimum root/ shoot ratio (Table.1). Accessions G-32 followed by G-86 and A-60 had maximum root/ shoot ratio and accession G-68 followed by A-14 and A-61 had minimum root/ shoot ratio at $6dSm^{-1}$. At $9dSm^{-1}$, accession A-133 followed by G-86 and A-185 had maximum root/ shoot ratio and accession G-64 followed by G-66 and A-14 had minimum root/ shoot ratio (Table.2).

Regarding K^+: Na^+ ratio, on an overall mean basis, K^+: Na^+ ratio decreased significantly with increase in salinity as compared to control. Accessions differed significantly for K^+: Na^+ ratio at all salinity levels. At $0dsm^{-1}$, accession G-68 followed by G-45 and A-60 had maximum K^+/Na^+ ratio whereas accession G-30 followed by A-23 and G-64 had minimum K^+/Na^+ ratio. At $3dsm^1$, accession G-61 followed by G-44 and G-32 had maximum K^+/Na^+ ratio and accession A-61 followed by A-2 and A-133 had minimum K^+/Na^+ ratio (Table.1). According to results presented in table.2, accession G-44 followed by G-68 and G-32 had maximum K^+/Na^+ ratio whereas accession A-133 followed by A-60 and A-23 had minimum K^+/Na^+ ratio at $6dsm^{-1}$ and at $9dsm^{-1}$, accession G-44 followed by G-68 and G-32 had maximum K^+/Na^+ ratio and accession G-86 followed by A-133 and A-2 had minimum K^+/Na^+ ratio.

Nawaz *et al.* [22] and Hussain and Rehman [10] noted that in sunflower K^+: Na^+ decreased with increase in salinity. Decreased K^+: Na^+ (4:1) ratio with increased salinity was due to displacement of Cu^{2+} from plasma lemma at high Na^+ concentration, resulting in loss of membrane integrity and efflux of cytosolic K^+ [6]. This K^+ leakage from the cell lowers the K^+:Na^+ ratio in the tissue [14]. K^+: Na^+ ratio decreased with increase in levels of salinity. K^+: Na^+ selectivity is an important criterion of salt tolerance [19], because tolerant varieties maintain high K^+: Na^+ ratio [12]. Potassium uptake by plant roots is often suppressed by sodium (Na^+) in the growth medium. High salinity conditions are mainly characterized by low nutrient on ion activities and extreme ratios of K^+/ Na^+ cause nutritional imbalances and restrict the plant growth. Plants with ability of accumulating

higher K^+: Na^+ ratio and maintaining lesser Na^+ and Cl^- contents in leaves possess more salt tolerance and show good growth under salt-affected conditions. Ionic imbalance of K^+/ Na^+ can also cause multiple metabolic problems and physiological malfunctioning within plant body [26].

At $0dSm^{-1}$, results showed that accession A-79 followed by A-23 and A-185 had maximum biomass and accession G-44 followed by A-60 and A-2 had minimum biomass. In addition, accession G-36 followed by G-61 and A-23 had maximum biomass and accession A-60 followed by G-32 and A-30 had minimum biomass at $3dSm^{-1}$ (Table.1). Results depicted in table.2 showed that accessions G-36 followed by G-45 and G-44 had maximum biomass and accession A-60 followed by A-133 and A-2 had minimum biomass at $6dSm^{-1}$ and at $9dSm^{-1}$, accession G-61 followed by A-23 and G-36 had maximum biomass and accession A-A33 followed by G-30 and G-44 had minimum biomass.

Rogers *et al.,* [25] indicated a reduction in an overall growth and biomass production of Lucerne (*Medicago sativa* L.) at high salinity level. Primavesi [23] has also documented poor biomass production under saline conditions. Hussain and Rehman [10] noticed a significant decrease in total biomass yield of *Prosopis juliflora*, *Casuarina equiselifolia* and *Eucalyptus camaldulesis* with an increase in soil salinity.

Osmolytes synthesis to withstand salinity stress utilizes much of carbon and reduces metabolites synthesis and thus ultimately biomass production is decreased and high concentration of salt in rooting medium cause unusual growth retardation. Overall growth retardation of seedlings at higher salinity/ sodicity levels has been reported by numerous researchers [24].

Conclusion

According to findings of this study fresh shoot weight, fresh root weight, chlorophyll contents, Na^+ content, K^+ concentration, Cl^- contents, root/shoot ratio, K^+:Na^+ ratio and total biomass are good standards for evaluation of response of plants at different levels of salt stress. Accessions G-36, G-61, A-23, A-6, and A-185 performed better in both controlled and saline conditions. These accessions showed better biomass production and high shoot and root growth by least concentration of Na^+ and higher concentration of K^+ and Cl^- in leaf sap resulting in better K^+ and Na^+.

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