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Assessment of genetic diversity and establishment of selection criteria in Sunflower (*Helianthus annuus L.)* genotypes at seedling stage under saline environment

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Abstract

Salt stress reduces the capability of crops to up take water. The K^+ and Na^+ contents are critically important in plants; these may rise toxic levels. The present study is a scientific attempt to understand the genetic behavior and response of different sunflower genotypes to tolerate salt stress at seedling stage. Salt treatment was given at germination stage and seedling stage hydroponically. There was decrease in final germination percentage (FGP), radicle length, plumule length, K^+ ion concentration, shoot length, root length, shoot fresh weight, root fresh weight, total seedling biomass. An increase in time to start germination, time to 50% germination (T50), Na^+ contents, Na^+/K^+ ratio was recorded in an increase in salinity levels. Genotype A-84 proved as highly susceptible whereas A-11 found as tolerant to salt stress. Correlation analysis exhibited positive correlation between potassium ion concentration and plant biomass but negative association with sodium ion concentration. Plumule length showed positive correlation with plant biomass, potassium ion concentration and sodium ion concentration. Root fresh weight was positively correlated with plant biomass, sodium ion concentration and plumule length. There are various plant responses over time, to provide a conceptual framework will enable further progress in understanding of the molecular regulation of salt tolerance. The selected genotypes could be used in breeding programs aimed to breed for high yield salt tolerant sunflower varieties.

Keywords: Salinity, Sunflower, germination, seedling stage, hydroponic

Introduction:

Sunflower (*Helianthus annuus L.)* is a key oil seed crop worldwide (Khatoon *et al*., 2000). It is good source of vitamins A, D, E and K. Use to make margarine and cattle feed (Hussain *et al.,* 2006). Global warming is giving major challenges to scientist. Salinity is one of the most important stresses widely distributed in all types of lands in the world. Due to stress, highly reactive oxygen species (ROS) are produced. ROS disrupts metabolism by oxidative damage to nucleic acid, lipids and protein (Chen *et al*., 2011). Salt tolerance is critical at germination stage it reduced the rate and percentage of seed germination, reduce crop yields. It produces ion toxicity, water stress, damage to membrane, nutritional disorders, osmotic stress, reduction in the cell division, alteration of metabolic processes and slows the photosynthesis rate. Under saline environment, both K^+ and Na^+ cations have synergistic or antagonistic effects for translocation. (Flower *et al*., 1977)

The genetic potential of four different oilseed crop in saline environment has been studied by Gurrier (1983). Salt stress negatively effects the seedling parameters, as root length, shoot length, and root/shoot ratio and leaf area expansion (Kriedman *et al*.1983). In rice the deleterious effects of salinity were more pronounced on growth, yield components and paddy yield per plant (Undovenko1983). Correlation showed that plant height, stem thickness, head diameter and 1000 achenes weight. is strongly correlated with head yield (Caylak and Emiroglu 1984). Leaf size was decreased when it explores continuous to salt in the root zone (Munns *et al*., 1988). Yield of rice plant is positively correlated with height of plant and sodium content of shoot (Mahmood1994). Head diameter of sunflower was correlated with achene yield per capitulum (Kandil and El-Mohandes1989). K^+ maintains leaf osmolarity and improves photosynthetic rate of leaves (Cassman *et al*., 1990). K^+ fertilizer help in nutrient uptake, maintain level of cytoplasm and increase yield of wheat (Chow *et al*., 1992).

Higher salinity level significantly affected longest root, maximum shoot length and total plant dry weight in Sorghum (Azhar and Khan, 1997). Wide range of literature explains harmful effects of salt stress where higher Na^+ reduced translocation and uptake of K^+ but there is a little knowledge on K^+ fertilization improved growth and yield of plants in salt affected soils (Grattan and Grieve, 1994).

Emergence/germination of sunflower and maize was affected by the salinity. At early growth stage seedling plant showed symptoms of water stress by lowering leaf water potential, conductance of stomata and evapotranspiration (Katerji *et al*., 1994). Five genotypes of wheat plant to hypoxia and moderate salinity content. It showed that plant growth was negatively correlated with Na^+ and Cl^- concentration in sap of leaf on other hand K^+ was non-correlated with yield (Qurashi *et al*., 1994).

Fifteen genotypes of sunflower for salinity were examined at seedling stage. Shoot length, shoot fresh weight and shoot dry weight was badly affected at higher salt concertation growth reduced by 31% respectively. Root fresh weight increased up to 27% as compared to control environment. Root dry weight and root length were decreased 21% and 9% at higher salinity (Hussain and Rehman 1995).

An experiment was conducted of two maize genotypes (B-73 and Mo-17) and their hybrids (YHS-202). With increasing salinity treatments, a progressive decreased in parameters occurred. MO-17 proved salinity tolerant genotype as compared to other (Lopez and Satti, 1996) (Sharif *et al*., 1999). Salinity determined the initial effect of NaCl salinity on sunflower plant. At higher salinity treatments, stomatal conductance decreased it produces negative water potentials as compared to the control. (Sohan *et al*., 1999)

The oil content in sunflower genotypes was also declined due to higher salinity (Wahid *et al*., 1999). The yield per plant had positive correlation with number of seeds per head, head length in diameter and 1000 seed weight at both genotypic and phonotypic level (Yasin and Singh, 2010). Under high salt stress, uptake of K^+ reduced in sugar beet and contents of K^+ decreased in shoot of sugar beet plants. Evaluated 51 inbred lines of sunflower observed a positive heterosis along with genetic advance for most of the achene's yield contributing parameters (Shehata *et al*.,2000).

The carbohydrates content, yield components, yield and uptake of nutrients in pearl millet get affected by Potassium and salt stress interaction affects (Heidari and Jamshidi 2010). Interaction between K^+ and salt stress in which K^+ deficiency impaired assimilation of carbon in PS I and PS II pathways of light reactions were impaired under salt stress condition (Qu *et al*.2011, 2012).

The leaf and canopy chlorophyll (Chl) contents were strongly related by leaf and canopy nitrogen (N) (Schlemmer *et al*., 2013). Salinity effect the maize during early growth stages and germination there was significant differences among root length in maize genotypes; while differences for all other measured traits were non-significant (Awad *et al*., 2014). Salt stress resulted in structural changes in vascular system of both root and stem (Farhana *et al*., 2014). Maize growth-related parameters like leaf length, leaf area, shoot and root dry weights were reduced due to increasing salinity levels (Akbari *et al*., 2015).

Following research focused to study the effects of salinity on sunflower genotypes at seedling stage under various treatments. Effect on growth parameters and production of reactive oxygen species in stress conditions. These studies were undertaken to develop a suitable method for screening of sunflower plants at early stage of growth for salinity tolerance and to assess the extent of genetic diversity present among sunflower genotypes.

Materials and Method

The experiment was carried out in research area of the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad. The plant material was compromised of 30 genotypes (Table 1) collected from the department of PBG, UAF. The accessions were planted and evaluated for various traits in following experiments.

Experiment growth conditions

Seeds of each genotype were sown in petri plates separately. Following four treatments levels were applied from day first. The healthy and uniform seeds of all genotypes were surface sterilized. Subsequently, the seeds were incubated in a sodium hypochlorite solution containing ~5% of active chlorine for 5 min followed by three washing steps in sterilized double-distilled water. The seeds were sown in petri dish and sand (for hydroponic) and placed in a culture room (at 25 °C, continuous light of 120 μmol m^{-2} s^{-1}).

Treatment 1 (Distilled water)
Treatment 2 ($5dsm^{-1}$)
Treatment 3 ($7dsm^{-1}$)
Treatment 4 ($8.5dsm^{-1}$)

After seven days of seed sowing treatments were applied, seeds were dried in room temperature for 4-5 days. Dried seeds were grinded with electric machine and digestion was made by following method given by wolf,1982. Solution were made of digested material with distilled water. Standards solutions with reagent grade salt NaCl were made. Na^+ concentration of digested material was recorded by using Sherwood 410 flame photometer. Similarly, K^+ concentration of digested material was recorded.

Morphological analysis

Material was sown in petri plates following two factors factorial CRD under control conditions. The data of following parameters related to germination were recorded.

1. Time to start germination (days):
2. Time to 50% germination (T50):

Following formula was obeyed to calculate time taken to 50% germination (T_{50}).

$$T_{50} = t_i + \left[\frac{\frac{N}{2} - ni}{nj - ni} \right] (tj - ti)$$

3. Final germination percentage (FGP)

   At the end of experiment final germination percentage was noted. By using method of International Seed Testing Association (2008).
4. Radicle length

   The seedlings were measured from each petri dish with help of scale to obtain radicle length during harvesting.
5. Plumule length

   The seedlings were measured from each petri dish with help of scale to obtain plumule length during harvesting.

Hydroponic experiment

Healthy seeds of each entry were sown following two factors factorial arrangement CRD in germination tray filled with distill water washed sand. At three leaf stage seedlings were transplanted in aquaculture. Hoagland solution were added as supplements. Seedlings were transplanted into four different tubs with single treatment in each. Each tub contained capacity of about 100-liter water. Tubes were pre-washed and polythene was used in inner side of tubs to control the leakage of water. Holes were made in four polystyrene sheets floating over half strength Hoagland's nutrient solution (Hoagland and Arnon,1950). Foam plugs were used for transplantation of seedlings into tubs. Aeration pump were used for proper aeration. After 4-5 days of room drying shoots were dried in oven for further analysis. Dried shoots were grinded and digested. The digested material was diluted by using distilled water. Standard solutions were made by using reagents NaCl and KCl, then samples were run on flame photometer Sherwood 410, and value of Na^+ and K^+ were recorded.

Statistical Analysis

The experiment was conducted by using CRD design with 3 replications. The data were analyzed using a statistical tool XLSTAT 2018. One-way analysis of variance was employed followed by Duncan's multiple range test to determine the significant differences among means of the treatments at 5% level of significance. All data were analyzed by means of a two-way ANOVA (NaCl treatments and genotypes). When a significant interaction between NaCl and genotypes was found, a Tukey's HSD was used to determine differences between means at $P \leq 0.05$.

Results

Ionic Analysis

Sodium ion

Statistical analysis showed significant differences among genotypes and highly significant differences were observed among treatments. High salinity level causes significant increase in Na^+ concentration in all genotypes. The genotype A-84 was found to be more sensitive to salinity, it accumulated highest Na^+ ion concentration and genotype A-11 showed maximum tolerance and by up taking lowest Na^+ ion concentration. The higher concentrations of Na^+ in leaves cause toxicity and lead to salt Injury. In hydroponic culture with increase in salinity plants showed increase in Na^+ ion uptake. At 7dSm^{-1}, A-84 proved to be sensitive genotype. At salt stress level 8.5 dSm^{-1}, genotypes have high Na^+ content. Genotype G-9 proved to be sensitive next to A-84 produced maximum Na^+ concentration. The concentration of Na^+ in the seeds increased significantly under all salinity levels. It might have disrupted the osmotic balance of the cells thus inducing water deficit. (Fricke *et al*, 2004).

K^+ ion concentration (mol/m^3)

The K^+ concentration observed in sunflower genotypes behaved same in all treatments. It showed significant differences among genotypes and highly significant differences were observed among treatments. Increase in salinity at each level caused significant decrease or increase in K^+ concentration in all genotype. At salt stress 7 dSm^{-1}, all genotypes proved to be sensitive except genotype G-9. At level 8.5 dSm^{-1}, all the genotypes showed marked increase in K^+ concentration and most sensitive found genotype was A-87. A-87 genotypes showed decrease in K^+ concentration. Minimum K^+ ion concentration was obtained in A-11 at high salinity level. Genotype G-9 produced maximum K^+ concentration next to A-87. Minimum K^+ concentration was produced by the genotype A-11 in salt stress. The effect of high salinity (8.5dSm^{-1} NaCl) was more pronounced than the effect of low salinity (5dSm^{-1} NaCl).

Effects of salt stress on Na^+/ K^+ ratio

Significant differences among genotypes a were observed. With increase in salinity, all genotypes showed increase in Na^+/K^+ ratio significantly in each level. Minimum Na^+/K^+ ratio was obtained in control condition and it increased significantly when salinity was increased in treatment levels. Genotype with high value of sodium ion content was found to be more sensitive to salinity. All pair wise comparisons of Na^+/K^+ ratio for genotypes and treatment clearly showed that within specific salinity level genotypes behave similar, significant differences were observed among all levels of salinity. As salinity levels increases in shoots, Na^+/K^+ ratio also increases.

With rise in level of salinity level, all the genotypes behaved in a same way. Analysis of variance for germination (SUPPLEMENTRY Table 4.1) showed highly significant differences among treatments. Higher the salinity level more time the genotypes took to germinate. In control condition, minimum time to start germination was observed. Minimum time to start germination was given by the genotype was found to be tolerant genotype at this level. At 8.5 dSm^{-1}, all the genotypes showed marked delay in time to start germination; maximum time to start germination was taken by A-84 (8 days). Minimum time to start germination was taken

by the genotype A-11 (7 days). Genotype A-84 proved to be sensitive genotype at high level of salinity, as it took maximum time to start germination.

Genotype A-84 with high mean value (6.0833) was found to be more sensitive to salinity. Genotype A-11 with low mean value (4.8323) was found to be less sensitive to salinity. All pair wise comparisons of time to start germination for genotypes and treatment clearly showed that within specific salinity level genotypes behave similar, significant differences were observed among all levels of salinity. By applying Tukey's test for pair wise comparison of time to start germination and treatment, it is concluded that at 8.5dsm^{-1} salinity level, all genotypes showed least germination, or took maximum time to start germination. At treatment level 7dsm^{-1}, genotypes showed less sensitivity towards salinity. At treatment level 5dsm^{-1}, genotypes showed better germination than other treatment levels. At normal condition in absence of salinity application genotypes showed good germination percentage. Means off all four treatments are significantly different from each other. Analysis of variance showed with increase in salinity, all genotypes showed more time to 50% germination. Minimum time to 50% germination was obtained in control condition and it increased significantly with increase in stress. In non-saline conditions, maximum time to 50% germination was obtained by genotype A-84 took on an average 6.33 days. Genotypes showed more time to 50% germination when salinity was applied at level 5 dSm^{-1}. Genotypes G-9 took maximum 6.67 days to 50% germination. Genotype A-11 took minimum 6 days to 50% germination at this level. As salinity increased, genotypes took more time to 50% germination. At 8.5 dSm^{-1}, maximum time to 50% germination were taken by A-84(8.67 days).

Genotypes showed less final germination percentage (FGP) with increase of salt stress. Mean comparison of 30 sunflower genotypes against four salinity levels (SUPPLEMENTRY Fig. 4.2) showed that by increasing salinity levels, final germination percentage (FGP) decreased significantly in all genotypes. These results are in accordance with Idris and Siddig (2015) and Aliu *et al*., (2015). Increase in salinity stress level decreased radicle length significantly in all genotypes. Highly significant differences among treatments were observed. (Fig 3A) Minimum plumule length was obtained at high salinity level and it increased significantly when salinity was decreased in treatment levels. Specific salinity level genotypes behave similar, non-significant differences were observed among all levels of salinity. (Fig 3B).

Shoot length of 30 sunflower genotypes at three salinity levels was found to be decreased in all the genotypes with increase in NaCl levels. Analysis of variance (supplementary Table 4.3) treatments explained that increasing supply of salinity had significantly adverse effect on shoot length. In normal conditions A-57, A-17 produced maximum shoot length respectively among all genotypes. Genotype G-49 produced minimum shoot length. Shoot length decreased significantly among all genotypes at level 8.5dSm^{-1} NaCl. The genotype A-11 produce maximum length and genotype A-57 produce minimum shoot length at this salinity level. A similar trend was observed at level 7dSm^{-1} NaCl such as A-27 with good shoot length declared tolerant whereas G-91 declared as sensitive genotypes. A less pronounced decrease was observed at level 5dSm^{-1}NaCl where all genotypes showed significant decrease in shoots length. Minimum decrease among all genotypes was observed in A-11 while maximum decrease among all genotypes was observed in A-84. (Fig 3C) By applying Tukey's test it was found that means of all four treatments are significantly different from each other.

Root length was found to be decreased in all the genotypes with increase in salinity levels. Root length of 30 sunflower genotypes at four levels of salinity including control, showed decrease in value with addition of NaCl. Analysis of variance (supplementary Table 4.4) showed that increasing supply of NaCI had significantly adverse effect on root length. In normal conditions G-100 produced maximum root length among all genotypes followed by G-34. The root length decreased significantly among all genotypes at level 5dSm^{-1} NaCl. Genotype A-13 produced maximum root length among all genotypes in experiment while A-78 produced minimum root length. Decrease in root length was observed in all genotypes in experiment with increase in NaCI concentration. At level 7dSm^{-1} NaCl such as A-13 remained tolerant and G-10 was sensitive genotypes. At level 8.5dSm^{-1} NaCl the pronounced decrease was observed where all genotypes showed significant decrease in root length. Maximum decrease among all genotypes was observed in A-84 and minimum decrease was in A-11 (Fig 3D). Tukey's test declared that means of all four treatments were significantly different from each other.

Fresh shoot weight of 30 sunflower genotypes at all the four levels of salinity including control, showed decrease in value with addition of NaCl. Highly significant differences among treatments were observed in genotypes. In non-saline conditions A-17 produced maximum shoot fresh weight among all genotypes followed by A-57. The fresh shoot weight decreased significantly among all genotypes at level 5dSm^{-1} NaCl. Genotype A-17 produced maximum shoot fresh weight among all genotypes in experiment while G-49 produced minimum shoots fresh weight. Decrease in shoot fresh weight was observed in all genotypes in experiment with increase in NaCI

concentration. A similar trend was observed at level 7dS/m NaCl such as A-57 remained tolerant with fresh shoot weight and G-49 with shoot fresh weight remained sensitive genotypes. Genotype A-11 showed less decrease in shoot fresh weight at 8.5dS/m NaCl as compared to the other genotypes in experiment. Maximum decrease was observed in A-84 (Fig 3E).

Root fresh weight of 30 sunflower genotypes decreased with the increase in salinity stress level. Analysis of variance showed highly significant differences among treatments and genotypes. At normal salinity level, genotype A-11 produced maximum root fresh weight among all genotypes and genotype G-100 and G-34 produced minimum root fresh weight. The root fresh weight decreased significantly among all genotypes at level 5dS/m NaCl. Genotype A-11 produced maximum root fresh weight among all genotypes while G-34 and G-100 produced minimum root fresh weight. At level 7dSm^{-1} NaCl A-11 remained tolerant and G-100 and G-37 remained sensitive genotypes. A more pronounced decrease was observed at level 8.5dSm^{-1} NaCl where all genotypes showed significant decrease in root fresh weight. Minimum root fresh weight was observed in A-11, whereas maximum root fresh weight among all genotypes was observed in A-84 followed by, G-100 and G-34. (fig 3F)

Regarding root dry weight, analysis of variance showed highly significant differences among treatments and genotypes. Under normal condition, G-41 produced maximum root dry weight among all genotypes and genotype G-49 and A-78 produced minimum root fresh weight. The root dry weight decreased significantly among all genotypes at level 5dSm^{-1} NaCl. Genotype G-4 produced maximum root dry weight among all genotypes in experiment while A-78 produced minimum root dry weight. Decrease in root dry weight was observed in all genotypes with increase in NaCI concentration. A similar trend was observed at level 7dSm^{-1} NaCl, G-68 followed by G-41 declared as tolerant genotypes. A more pronounced decrease was observed at level 8.5dSm^{-1} NaCl where all genotypes showed significant decrease in root dry weight. Minimum root dry weight was observed in A-11 but maximum root dry weight among all genotypes was observed in genotypes A-84. (Fig 3H). In pair, wise comparison of root dry weight and treatment it is concluded that at 8.5dsm^{-1}, all genotypes showed least root dry weight. At treatment level 7dsm^{-1} genotypes showed less sensitivity towards salinity. At treatment level 5dsm^{-1} genotypes showed better root dry weight than other treatment levels. All treatment levels were significantly different for root dry weight of sunflower genotypes. Under normal condition, genotypes showed good root dry weight.

Shoot dry weight of 30 sunflower genotypes at four levels of salinity including control, showed decrease in value with addition of NaCl. Analysis of variance (Table 4.16) showed highly significant differences among treatments which explained that increasing supply of NaCl had significantly adverse effect on shoot dry weight. In normal conditions G-41 produced minimum shoot dry weight among all genotypes and genotype G-49 and A-78 produced maximum shoot fresh weight. At higher salinity level 8.5dms^{-1} A-11 produce less shoot dry weight while a-84 produces drier shoot weight. At treatment level 5dsm-1 genotypes show better root dry weight than other treatment levels. All treatment levels are significantly different for shoot dry weight of sunflower genotypes. (Fig 3G)
Total plant biomass of all the 30 sunflower genotypes showed decrease in value with increase in salinity stress level. Analysis of variance (supplementary table 4.5) showed highly significant differences among treatments which explained that increasing supply of NaCl had significantly adverse effect on total plant biomass, whereas significant differences were shown among genotypes. genotype A-11 produced maximum total plant biomass at all salinity levels while A-84 produced minimum total plant biomass among all genotypes in all the treatments. The genotypes were significantly different among each other in all treatments. Increase in salinity stress increased mortality of 30 sunflower genotypes. All mean wise comparisons of mortality percentage for genotypes showed that genotype A-11 showed high mortality percentage at all salinity levels while A-84 showed minimum mortality percentage among all genotypes, in all the treatments. The genotypes differed significantly in all treatments.

Total mortality of 30 sunflower genotypes at four levels of salinity including control, showed decrease in value with addition of NaCl. Analysis of variance showed highly significant differences among treatments which explained that increasing supply of NaCl had significantly adverse effect on total plant mortality, whereas significant differences were shown among genotypes. All mean wise comparisons of mortality percentage for Genotypes showed that genotype A-84 produced more mortality percentage at all salinity levels while A-11 produced minimum mortality percentage among all genotypes, in all the treatments.

Correlation studies

At salinity level 8.5dsm^{-1} results revealed (Table 3) that potassium ion concentration was negatively correlated with plant biomass. Mortality percentage was negatively correlated with plant biomass and potassium ion concentration. Sodium ion concentration showed positive correlation with potassium ion concentration and negative correlation with plant biomass and mortality percentage. Plumule length showed positive correlation with plant biomass and potassium ion concentration. It showed negative correlation with

mortality percentage and with sodium ion concentration. Root fresh weight was positively correlated with plant biomass, sodium ion concentration and plumule length whereas it was negatively correlated with potassium ion concentration and mortality percentage. Plant biomass, plumule length and root fresh weight showed positive association with root length. Radicle length showed positive association with plant biomass, potassium ion concentration, plumule length, root fresh weight and root length. It showed negative correlation with mortality percentage and sodium ion concentration. Root dry weight showed positive correlation with plant biomass, mortality percentage, root fresh weight and radicle length. Shoot fresh weight showed positive correlation with plant biomass, plumule length, root fresh weight, root length, radicle length and root dry weight. Shoot length found to be a positive correlation with plant biomass, potassium ion concentration, plumule length, root length, radical length and shoot fresh weight. Plant biomass, plumule length, root fresh weight, root length, radical length, root dry weight, shoot fresh weight and shoot length were positively correlated with shoot dry weight. Time to 50% germination showed positive association with plant biomass, potassium ion concentration, plumule length, root fresh weight, root length, radicle length, root dry weight, shoot fresh weight and shoot dry weight. Time to start germination showed positive association with plant biomass, root fresh weight, root length, root dry weight, shoot fresh weight and time to 50% germination. These findings are in accordance with Lopez and Satti, (1996) Mohammad *et al*. (2002), Mehdi *et al*. (2000) and Khatoon *et al*., (2000).

Correlation analysis showed that in the treatment 1 ($5dsm^{-1}$) positive correlation was observed between potassium ion concentration and plant biomass. Sodium ion concentration showed positive correlation with plant biomass and potassium ion concentration and negative correlation with mortality percentage. In the treatment 2 ($7dsm^{-1}$) plumule length showed positive correlation with plant biomass, potassium ion concentration and sodium ion concentration. Root fresh weight was positively correlated with plant biomass, sodium ion concentration and plumule length. Plant biomass, plumule length and root fresh weight showed positive association with root length. Radicle length showed positive association with plant biomass, potassium ion concentration, plumule length, root fresh weight and root length in the treatment 4 ($8.5dsm^{-1}$).

Discussion

Genotypic variation in comeback to salt stress has been reported in corn (Cramer *et al*., 1990). In this study, difference in response to salinity was experimental between sunflower genotypes. Though, some genotypes were tolerant or slightly tolerant during the seedling stage. Salinity stress harmfully affects plant growth and regulation. More sensitive stages are germination and seedling stage of plant life cycle than adult stage (Lianes et al., 2005, Ashraf et al., 1986). (Shalhevet, 1995). Without salt stress (Control: 0 mM NaCl) plant shoot length enhanced. High salinity levels from decreases shoot length. (Pessarakli & Kopec 2009, Mohammad et al. 1998, Gill & Singh 1989). The extreme accretion of salts in the cell wall, secondary cell appears and wall becomes rigid resultant to turgor pressure and cell enlargement declines. Salt stress effect roots severely. In result of salt stress roots remain shortened, increased weight due to uptake of ions from solution.

The above experimental results showed that the increased concentration of salt negatively affected the shoot fresh weight. Our result was also supported that higher salt concentration produced destruction of early seedling growth due to osmotic stress caused by ion imbalance, shoot fresh weight decreased, root fresh weight increased. Shoot dry weight of all sunflower genotypes decreased.

Salt stress effects the nutrient uptake Na^+ higher concentration disturbs the secondary metabolites. Regulation of potassium uptake and prevention of sodium entry. The genotypes having higher K^+/Na^+ ratio is the salt tolerant. In breeding programs assortment of genotypes with high K+/Na+ ratios are a significant approach to lessen growth declines in salted soils (Santa-Maria & Epstein, 2001).

Conclusion

A good screening method should be efficient, reproducible, reliable and simple. In this study, two screening methods were tested to regulate the reliability in response of several genotypes grown in control and three salt levels. Sunflower genotypes exhibited similar response to salt in both approaches shows that both of the techniques are effective for assessment of salt tolerance. As compared to petri dish method, hydroponic grown method is easy, simple and economical at early stage of growth for salinity tolerance. From the results it can be concluded that screening at the early stage of plant growth is a suitable and honestly reliable technique for determining differences with respect to salt tolerance of sunflower genotypes. It is recommended that the assessment and selection of pipe-line genotypes could offer great scope for the combination of high yield and stress resistance (salinity/drought). Our outcomes provide strategies for the assortment of salt tolerant sunflower genotypes and this information is relevant and very important to breeders and plant physiologists interested in improving salt tolerance of sunflower.

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Table 1: List of sunflower genotypes use in experiment

Sr. No.	Accessions	Sr. No.	Accessions
1	G-100	16	A-30
2	A-11	17	A-17
3	A-13	18	G-34
4	A-7	19	G-4
5	A-39	20	G-88
6	A-45	21	G-7
7	A-23	22	G-41
8	A-78	23	G-10
9	A-27	24	G-2
10	A-84	25	G-6
11	A-71	26	G-68
12	A-74	27	G-49
13	A-70	28	G-9
14	A-21	29	A-57
15	A-12	30	G-91

Table 2: F-values and probability levels of estimated traits in experiment

Seedling trait	SOV	F value	Seedling trait	SOV	F value
Na^+	Genotypes (G)	21.02**	**SFW**	Genotypes (G)	7.40
	Treatments (T)	5175.25**		Treatments (T)	55.63
	G×T	17.86**		G×T	1.06
K^+	Genotypes (G)	28.86**	**RL**	Genotypes (G)	11072.0
	Treatments (T)	1869.56**		Treatments (T)	1232983
	G×T	20.51**		G×T	1757.45
Na^+/K^+	Genotypes (G)	0.75	**RFW**	Genotypes (G)	8.60
	Treatments (T)	2.768		Treatments (T)	49.68
	G×T	0.56		G×T	0.89
TSG	Genotypes (G)	82.37	**DRW**	Genotypes (G)	10.70
	Treatments (T)	21363.3		Treatments (T)	44.44
	G×T	50.84		G×T	0.37
T50	Genotypes (G)	1.9*	**DSW**	Genotypes (G)	229.020
	Treatments (T)	199.37**		Treatments (T)	3925.984
	G×T	1.54*		G×T	113.102
FGP	Genotypes (G)	0.85NS	**TPB**	Genotypes (G)	31.88**
	Treatments (T)	49.03**		Treatments (T)	1143.79**
	G×T	0.83NS		G×T	10.14**
Rl	Genotypes (G)	4.8E+31	**MP**	Genotypes (G)	75.00
	Treatments (T)	3.5E+33		Treatments (T)	33552.5
	G×T	3.1E+30		G×T	42.69
PL	Genotypes (G)	1.8E+32	**SL**	Genotypes (G)	5351.82
	Treatments (T)	6.1E+33		Treatments (T)	267.672
	G×T	1.5E+31		G×T	435.94

Na^+/K^+: Sodium/Potassium ratio; TSG: Time to start germination; T50: Time takes for 50% germination; FGP: Final germination percentage; Rl: Radicle length; PL: Plumule length; SFW: Shoot fresh weight; RL: Root length; RFW: Root fresh weight; DRW: Dry root weight; DSW: Dry shoot weight; TPB: Total plant biomass; MP: Mortality percentage; SL: Shoot length; * = Significant at 0.05%, NS = Non-significant **= Significant at 0.01%

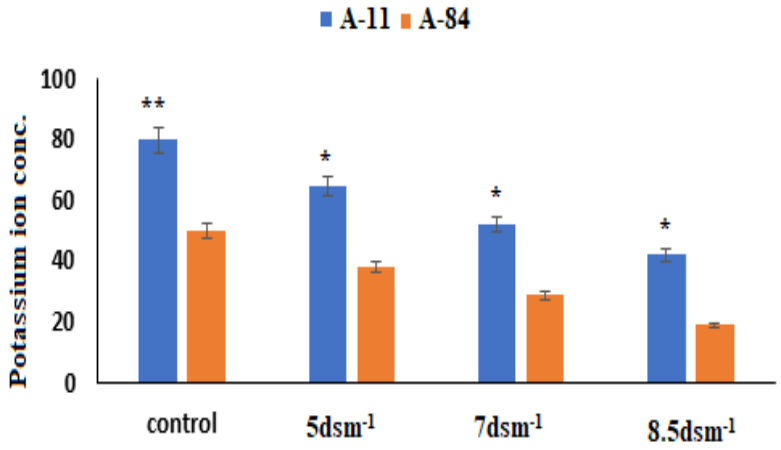


Fig1: Effects of different salinity levels on Na^+ ratio in sunflower genotypes observed at control, $5dsm^{-1}$, $7dsm^{-1}$ and $8.5dsm^{-1}$ NaCl salinity stress). Significant differences are showing by asterisks, according to T-test at P ≤0.01 and ≤0.05 significance levels.

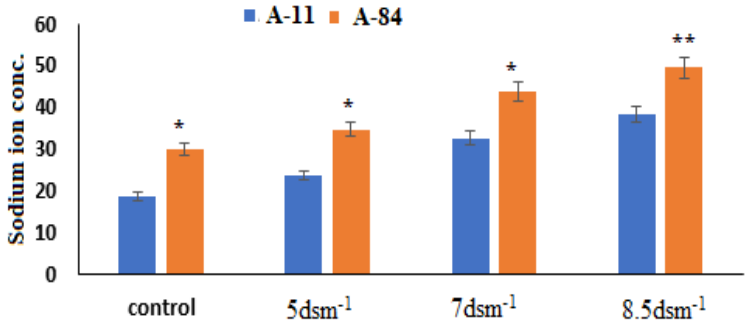


Fig2: Effects of different salinity levels on K^+ ratio in sunflower genotype observed at control, $5dsm^{-1}$, $7dsm^{-1}$ and $8.5dsm^{-1}$ NaCl salinity stress. Significant differences are showing by asterisks, according to T-test at P ≤0.01 and ≤0.05 significance levels.

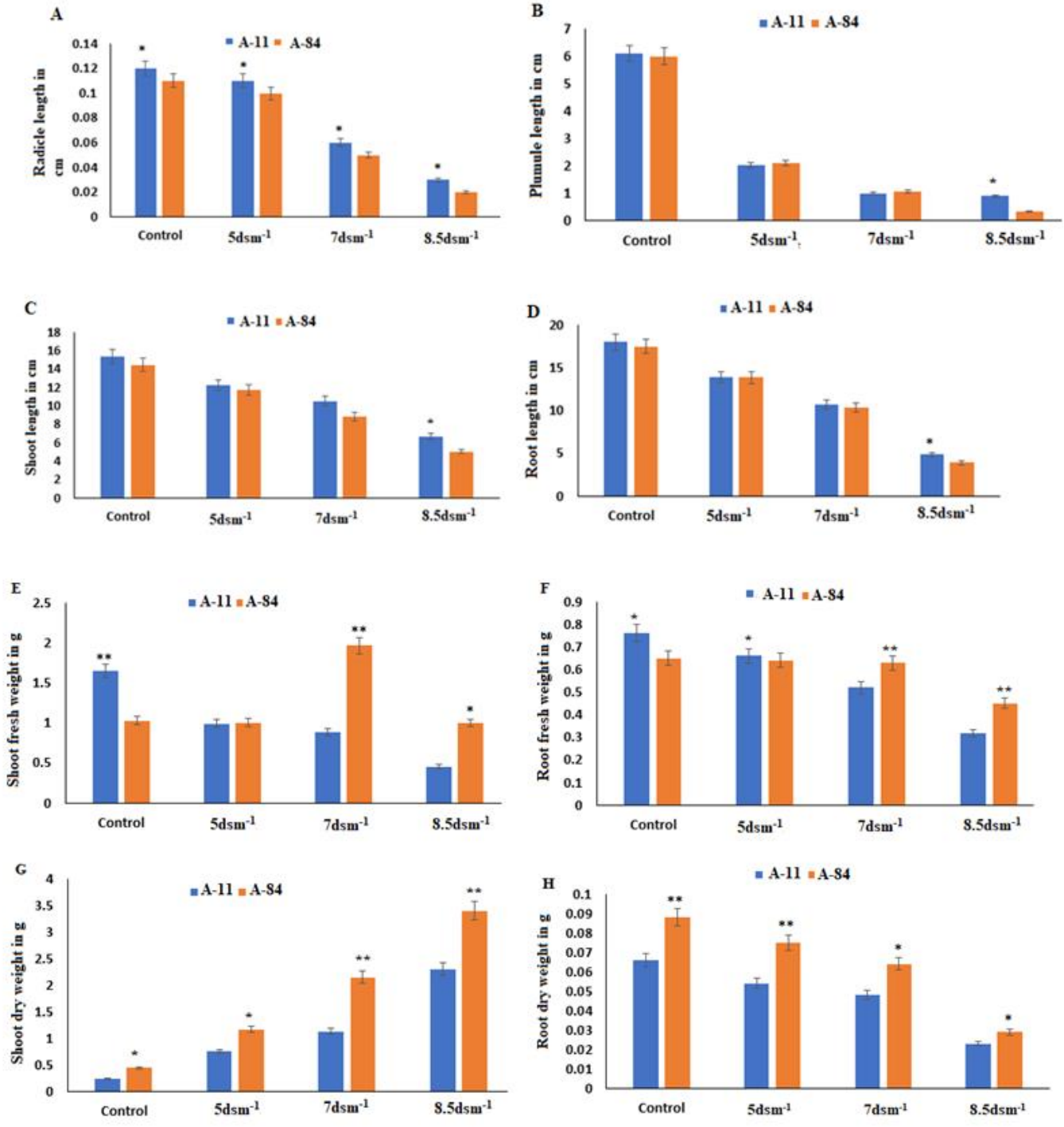


Fig 3: Effect of salinity on radical length (A), plumule length (B), shoot length (C), root length (D), shoot fresh weight (E), root fresh weight (F), shoot dry weight (G) and root dry weight (H) of sunflower genotypes at seedling stage in solution-culture method. Significant differences are showing by asterisks, according to T-test at P ≤0.01 and ≤0.05 significance levels.

Table 3: Correlation of growth parameters due to effect of 8.5dsm^{-1} NaCl

Traits	Biomass	Ptassium (K)	Mortality %	Sodium (Na)	Plumule length	Root fresh weight	Root length	Radicle length	Root dry weight	Shoot fresh weight	Shoot length	Shoot dry weight	Time to 50% germination
Ptassium (K)	-0.0319												
Mortality %	-0.1079	-0.1708											
Sodium (Na)	-0.0024	0.5983	-0.0996										
Plumule length	0.2788	0.1484	-0.2030	-0.0552									
Root fresh weight	0.5846	-0.0358	-0.0853	0.1293	0.2237								
Root length	0.1409	-0.2816	-0.1785	-0.0646	0.2553	0.0072							
Radicle length	0.4395	0.0451	-0.1542	-0.1227	0.6744	0.4990	0.2655						
Root dry weight	0.2217	-0.5025	0.0995	-0.5981	-0.0492	0.3073	-0.0681	0.1792					
Shoot fresh weight	0.9936	-0.0293	-0.1041	-0.0205	0.2686	0.4895	0.1505	0.4031	0.1956				
Shoot length	0.2546	0.0869	-0.1330	-0.0494	0.6967	0.1816	0.2547	0.6484	-0.0507	0.2484			
Shoot dry weight	0.2978	-0.1043	-0.1881	-0.0760	0.5105	0.4890	0.4739	0.7736	0.1606	0.2522	0.5019		
Time to 50% germination	0.2968	0.0910	-0.3113	-0.0579	0.2372	0.4055	0.0248	0.0250	0.1692	0.2627	-0.0022	0.1140	
Time to start germination	0.1812	-0.0992	-0.2929	-0.1641	-0.1726	0.0567	0.3703	-0.1175	0.0553	0.1869	-0.0267	-0.1011	0.2154