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Full Length Research Paper

An evaluation of safflower genotypes (*Carthamus tinctorius* L.), seed germination and seedling characters in salt stress conditions

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In order to study the effect of salinity stress on germination and early seedling growth of six safflower genotypes namely KM5, KM8, KM12, KM19, KM47 and Kose by using five concentrations of NaCl (0, -0.3, -0.5, -1 and -1.5 MPa) a factorial experiment was designed using Completely Randomized Design (CRD) with three replications in Biotechnology Laboratory, Islamic Azad University-Karaj Branch. Results of ANOVA showed that salt stress adversely affected the germination percentage, germination rate, shoot length, root length, seedling length, root/shoot length ratio, seed vigour, and germination index and mean germination time of all 6 genotypes of safflower, which demonstrates high diversity among genotypes that enabled us to screen salinity tolerant cultivar. At the highest salt level (-1.5 MPa), Kose produced maximum germination percentage and germination rate of all genotypes and they were considered as relatively tolerant. Best level of NaCl concentration in root length, shoot length, seedling length and seed vigour was -0.3 MPa. Seed vigour increased with increase in osmotic potential until -0.3 MPa but decreased in -0.5 MPa. Results of cluster analysis (Ward's minimum variance method) at the highest salt level (-1.5 MPa) classified all genotypes into three group. According to the obtained results, we found that Kose is the most resistant and KM5, KM8 and KM47 are the most sensitive genotypes.

Key words: Cluster analysis, germination indices, NaCl, seed vigour.

INTRODUCTION

Among various environmental stresses, soil salinity has become a critical problem worldwide due to its dramatic effects on plant physiology and performance (Golbashy et al., 2010). Salinity in soil or water is one of the major stresses and especially in arid and semi arid regions, can severely limit crop production (Shannon, 1998). Breeders seek to develop and identify cultivars that are more tolerant of salinity and water stress (Janmohammadi et al., 2008). Germination is generally considered to be the developmental stage that is most salt-sensitive, especially for crops exposed to hostile environments (Ashraf and Wahid, 2000).

Salinity impairs seed germination, reduces nodule formation, retards plant development and reduces crop yield (Greenway and Munns, 1980). Soil salinity may affect the germination of seeds either by creating osmotic potential external to the seeds preventing water uptake or through the toxic effects of Na+ and Cl- ions on germinating seed (Golbashy et al., 2010; Khajeh-Hosseini et al., 2003; Atak et al., 2006; Kaya et al., 2006).

Salinity delays the onset, reduces the rate and increases the dispersion of germination events, resulting in reduced plant growth and final crop yield (Ashraf and Foolad, 2005). Absence of germination in salinity soil is very often due to the high concentration of salt in the soil where the seeds are sown. The reason is that the salt solution moves upward, following the evaporation at soil level (Bernstein, 1974). Salt disturbs both germination and plant growth (Fowler, 1991). The main salt-induced physiological disorder is diminished seed imbibitions because of the low solute potential within the saline growth medium (Debez et al., 2004). Seed may be more sensitive to stress than mature plants because of exposure the dynamic environment close to the soil

surface. One of the commonest experiments in germination of the seeds is the application of NaCl. Seed response to salinity can be simulated by NaCl induced ionic stress in the germination experiments. Ionic stress is caused by a toxic accumulation of NaCl in plant tissues. Germination rates decrease with an increase in NaCl concentration (Murillo-Amador et al., 2002).

Thus, the salt-affected soils can be utilized by growing salt tolerant plants, whether halophytes or crops (Siddiqi et al., 2007). With this fact in mind, it is imperative to explore intra-specific (inter-cultivar) variation for salt tolerance of a crop by screening its available germplasm. For instance, a great magnitude of inter-cultivar variation for salt tolerance has been observed in different species such as wheat (Ashraf and McNeilly, 1988), lentil (Ashraf and Waheed, 1993), barley (Belkhodja et al., 1994), cotton (Ashraf and Ahmad, 1999), *Brassica napus* (Ulfat et al., 2007) and Safflower (Siddiqi et al., 2007). Safflower (*Carthamus tinctorius* L.) is one of the prospective oil-seed crops, because it yields about 32 to 40% seed oil (Weiss, 1983). However, due to its considerable salt tolerance compared with commonly grown oil-seed crops, it is usually cultivated in arid and semi-arid regions where soil salinity is one of the major threats to agriculture (Kaya, 2009).

The research has shown that in response to soil salinity, seedlings growth, leaves area, root biomass and shoot biomass have all been reduced (Redmann et al., 1994). Although salt stress adversely affects the growth of safflower plants at all developmental stages (Kaya et al., 2003; Jamil et al., 2006; Golbashy et al., 2010), varietal differences in salt tolerance of safflower have been observed at germination (Ghorashy et al., 1972), at adult (Ashraf and Fatima, 1995) as well as at both germination and adult growth stages (Francois and Bernstein, 1964). However, Kaya et al. (2006) reported that germination percentage was not influenced by NaCl level of 23.5 dsm^{-1}. Mohammed et al. (2002) reported that by NaCl levels germination percentage decreased and mean germination time increased proportionately.

The present study was therefore, conducted with the objectives to determine the response of safflower genotype to salinity stress at germination and seedling stages under controlled conditions. Moreover, NaCl was used for salinity stress induction in safflower.

MATERIALS AND METHODS

In order to study the effects of salinity stress on germination and early seedling growth in safflower genotypes, an experiment was conducted in factorial form, using a completely randomized design with three replications. In this experiment, six safflower genotypes inclusive KM5, KM8, KM12, KM19, KM47 and Kose were evaluated in five levels of salinity treatment (distilled water as control, -0.3, -0.5, -1 and -1.5 MPa) by using different NaCl concentrations. This experiment was carried out at Biotechnology Laboratory, Islamic Azad University- Karaj Branch.

The seeds were sterilized by soaking in a 5% solution of hypochlorite sodium for 5 min. After the treatment, the seeds were washed several times with distilled water. 25 seeds were put in each petridish (with 9 cm diameter) on filter paper moistened with respective treatment in 3 replications. The petridishes were covered to prevent the loss of moisture by evaporation. The petridishes were put into an incubator for 12 days at a temperature of 25°C and 65% relative humidity. Every 24 h after soaking, germination percentage and other traits were recorded daily. After 12 days of incubation, shoot length, root length, seed vigour and root to shoot ratio of germinated seeds was measured. Seeds were considered germinated when the emergent radical reached 2 mm length. Germination percentage, germination rate and seed vigour were calculated using the following formulas:

Formula 1: $GP = (SNG / SN0) \times 100\ \%$

where GP is germination percentage, SNG is the number of germinated seeds, and SN0 is the number of experimental seeds with viability (Close and Wilson, 2002; Danthu et al., 2003).

Formula 2: $GR = \sum Ni / \sum (ni \times Gi)$

where: GR: Germination rate; n: number of germinated seed on gth day and g: Number of total germinated seeds.

Formula 3: Seed vigour = [seedling length (cm) × germination percentage]

Analysis of variance was performed using standard techniques and differences between the means were compared through Duncan multiple range test ($P < 0.05$) using SAS release 9.1 (SAS, 2002) software package. All investigated traits were subjected to hierarchical cluster analysis using procedure ward's minimum variance method as a clustering algorithm using Stat Graphics Plus (Ver 2.1) software. Ward's minimum method is a hierarchical clustering procedure in which similarity used to join clusters is calculated as the sum of squares between the two clusters summed over all variables (Hair et al., 1998). It minimizes them within cluster sums of squares across all partitions.

RESULTS

Analysis of variance showed that, there were significant difference between genotypes, salinity stress levels and their interaction. The results of this study reveal that various concentrations of NaCl had a significant effect on the all measured traits (Table 1). The control showed clear genetically differences among the genotypes regards germination percentage, and such differences were statistically significant.

Germination percentage of all safflower genotypes was adversely affected due to the application of different levels (0, -0.3, -0.5, -1 and -1.5 MPa) of NaCl.

Also analysis of variance showed that, interaction effects was significant for all investigated characters except root to shoot length ratio and mean germination time.

The differences between the means (Genotypes and salinity stress levels) were compared by Duncan multiple range test and are shown in Table 2. It observed that, in all of genotypes there was a decrease in germination percentage due to salinity stress increment and maximum germination percentage was delayed. While in

Table 1. Analysis of variance of measured traits of safflower genotypes under salinity stress.

S.O.V	df	Germination percentage	Germination rate	Root length (mm)	Shoot length (mm)	Seedling length (cm)
Genotype	5	2325.237**	1995.124**	13.099**	28.818**	79.357**
stress	4	680.840**	1039.946**	29.224**	57.193**	167.838**
Genotype× stress	20	167.547*	85.336**	2.871**	3.143**	10.888**
error	60	83.944	23.827	1.053	0.834	3.003

S.O.V	df	Seed vigour	Root/Shoot length (mm)	Germination Index	Mean germination time (day)
Genotype	5	701732.309**	1.122**	2594.791**	1.597**
stress	4	1268344.148**	0.020ns	1043.178**	1.353**
Genotype× stress	20	93812.719**	0.140ns	110.124**	0.046ns
error	60	22757.840	0.149	44.722	0.033

*, **, ns: significant at 5%, 1% level and not significant, respectively.

Table 2. Mean comparison of main effects using Duncan multiple range test (at 5% probability level).

Genotype	Germination percentage	Germination rate	Root Length (mm)	Shoot length (mm)	Seedling length (cm)
KM12	76.443c	49.973c	3.021ab	4.080a	7.102a
KM19	69.334d	50.783bc	2.393ab	3.300b	5.693b
KM47	64.445d	43.133d	0.624c	0.316c	0.940c
KM5	86.667b	52.550bc	3.181a	4.073a	7.254a
KM8	69.778cd	54.139b	2.218b	2.869b	5.088b
Kose	97.333a	76.807a	2.830ab	2.888b	5.718b
Salinity stress (MPa)					
0	81.853a	60.774a	3.251a	4.402a	7.653a
-0.3	78.518a	60.452ab	3.757a	4.713a	8.471a
-0.5	81.112a	57.261b	2.565b	3.096b	5.662b
-1	78.518a	51.703c	1.797c	2.011c	3.809c
-1.5	66.666b	42.631d	0.518d	0.382d	0.901d

Genotype	Seed vigour	Root/shoot length (mm)	Germination index	Mean germination time (day)
KM12	579.480a	0.734ab	57.267c	2.032b
KM19	418.130b	0.776ab	51.867d	2.035b
KM47	62.170c	0.200c	45.600e	2.360a
KM5	662.620a	0.714b	66.067b	1.937bc
KM8	368.250b	0.757ab	53.600cd	1.888c
Kose	568.740a	1.038a	82.533a	1.360d
Salinity stress (MPa)				
0	651.900a	0.716a	64.889a	1.752c
-0.3	713.820a	0.677a	62.833ab	1.727c
-0.5	477.550b	0.717a	63.944ab	1.823c
-1	313.010c	0.744a	59.389b	1.981b
-1.5	59.870d	0.661a	46.389c	2.392a

Values in a column bearing different superscript are significantly different at 0.05 levels.

Table 3. Supplementary analysis of interaction effects.

Salinity level (MPa)	Germination percentage	Germination rate	Root length (mm)	Shoot length (mm)	Seedling length (cm)
0	624.578**	924.364**	4.087**	8.664**	20.842**
-0.3	981.697**	482.971**	11.001**	19.700**	59.314**
-0.5	365.936**	580.374**	6.213**	8.559**	28.480**
-1	626.218**	254.259**	3.081*	4.025**	13.234**
-1.5	396.999**	94.502**	0.199ns	0.444ns	1.037ns

Salinity level (MPa)	Seed vigour	Root/shoot length (mm)	Germination index	Mean germination time (cm)
0	245031**	0.140ns	739.289**	0.493**
-0.3	498372**	0.343ns	910.633**	0.384**
-0.5	227274**	0.153ns	462.989**	0.391**
-1	102251**	0.537**	541.922**	0.246**
-1.5	4054.980ns	0.508**	380.456**	0.269**

*, **, ns: significant at 5%, 1% level and not significant, respectively.

this experiment different genotypes had different response to the salinity stress. Among the safflower genotypes, Kose had the highest germination percentage and germination rate of 97.33% and 76.80 respectively.

However, maximum reduction in germination percentage was observed at the highest level that is, -1.5 MPa of NaCl. At the highest salt level (-1.5 MPa), Kose produced maximum germination percentage and germination rate of all genotypes and they were considered as relatively tolerant.

Results of means comparison, using Duncan multiple range test, showed that germination percentage and germination rate were decreased by an increase in osmotic potential, while the maximum germination rate and percentage were obtained at 0 Mpa level (control treatment). Some studies referred that stress can contribute to improve germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao et al., 1999), enhancement of ATPase activity, RNA and acid phosphathase synthesis (Fu et al., 1988), also by increase of amylases, proteases or lipases activity (Ashraf and Foolad, 2005).

Imposition of varying levels of NaCl significantly reduced all measured traits of all 6 investigated genotypes.

Root length is one of the most important characters for salinity stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides an important clue to the response of plants to salinity stress. A marked reduction in root length, shoot length and seedling length of all genotypes of safflower was observed due to salt stress.

Among the genotypes, the longest root length was commonly determined in genotypes KM5, KM12, Kose and KM19 while KM47 gave the shortest root length. Generally, increasing salinity levels decreased root length, and KM5 genotype exhibited the greater performance in respect of root length. Result of this study showed that, shoot length diminished with increasing salinity levels in all genotypes (Table 2). The highest and the lowest seedling length were observed in KM5 and KM47 genotypes, respectively (Table 2).

The most effective levels in reducing these attributes were -1 and -1.5 MPa of NaCl (Table 3). Best level of NaCl concentration in root length, shoot length, seedling length and seed vigour was -0.3 MPa. Seed vigour increased with increase in osmotic potential until -0.3 MPa but decreased in -0.5 MPa (Figure1).

A significant inter-genotype variation was observed under salt stress. Of all genotypes, KM5, KM12 and Kose produced highest seed vigour at all salt regimes, but lowest seed vigour was recorded in KM47 while the remaining genotypes were moderate in this attribute.

Variation in the set of genotypes about root to shoot length ratio was not possible to discern at lower external salt levels, however, genotypes differed significantly at the two higher salt levels, that is, -1 and -1.5 MPa of NaCl (Table 3).

In addition, it was clearly determined that there were no statistical differences between measured genotypes at high salinity levels (-1.5 MPa) for root length, shoot length, seedling length and seed vigour traits (Table 3).

Cluster analysis was done using the data for all measured traits at the highest salt level (-1.5 MPa), because this salt level was found very effective in discriminating the genotypes. Results of cluster analysis (Ward's minimum variance method) showed that genotypes Kose was found to be tolerant, while KM5, KM8 and KM47 sensitive to salt (Figure 2).

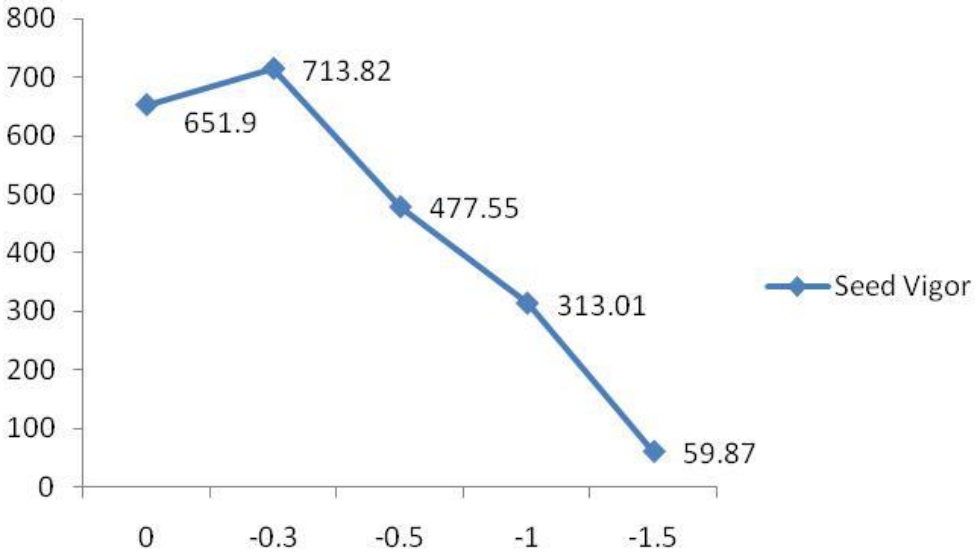

Figure 1. Seed vigour of safflower genotypes under different salinity stress.

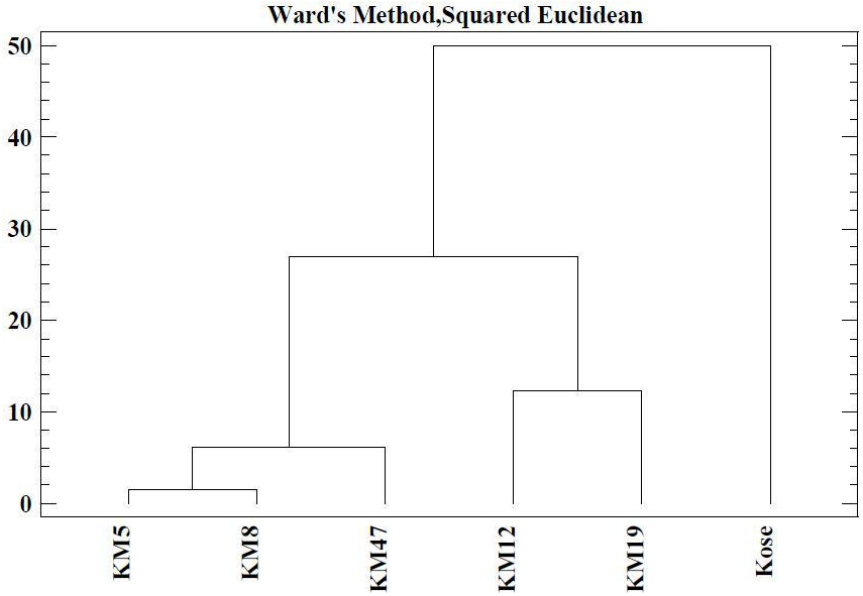

Figure 2. Cluster analysis of safflower genotypes under -1.5 level of salinity stress using Ward's minimum variance method.

Ajmal Khan and Weber (2006) found that, resistance to stress at germination stage and primary growth of seedling is independent from next growth stages and evaluation of stress tolerance need more experiment at next growth stages.

DISCUSSION

Screening of available germplasm of a crop is a feasible means of identifying salt tolerant genotypes or genotypes which could maintain a comparatively reasonable yield on salt affected soils (Ashraf and McNeilly, 1987). For the latter crops, it is advisable to assess degree of salt tolerance at each growth stage. In the present study, genotype Kose was found to be tolerant, while KM5, KM8 and KM47 sensitive to salt. Ranking of the genotypes was done using the data for all measured traits at the highest salt level (-1.5 MPa), because this salt level was found very effective in discriminating the genotypes. These results can be related to some earlier studies in which genotypes identified as salt tolerant at the earlier

growth stages showed tolerance when tested at the later growth stages.

Although a considerable magnitude of variation for salt tolerance was observed in a set of 6 available genotypes of safflower while screening them at germination stages, but a further study needs to be carried out to assess whether the genotypes marked as salt tolerant at the initial growth stages, maintain their degree of salt tolerance when tested as adult.

Conclusion

In the present study, salt stress adversely affected the germination percentage, germination rate, shoot length, root length, seedling length, and root to shoot length ratio, seed vigour, and germination index and mean germination time of all 6 genotypes of safflower and a significant variation in salt tolerance was observed among all the safflower.

Many researchers have reported similar results (Demir and Aril, 2003; Mauromicale and Licandro, 2002). Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to ability of seeds for best germination under unfavourable conditions, so necessity of evaluation of salinity resistance genotypes is important at primary growth stage. To find the best tolerant genotype to such conditions, taking all traits into account in this study, we found that Kose is the most resistant and KM5, KM8 and KM47 are the most sensitive genotypes.

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