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VARIATION IN SEED GERMINATION AND SEEDLING GROWTH IN SOME DIVERSE LINES OF SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) UNDER SALT STRESS

EJAZ HUSSAIN SIDDIQI*, MUHAMMAD ASHRAF AND NUDRAT AISHA AKRAM

Department of Botany,
University of Agriculture, Faisalabad, Pakistan.

Abstract

To assess inter-cultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.), 10 available lines [Safflower-31, Safflower-32, Safflower-33, Safflower-34, Safflower-35, Safflower-36, Safflower-37, Safflower-38, Safflower-39 and Safflower-78] were screened at five levels (0, 60, 120, 180, and 240 m*M*) of NaCl at the germination and seedling stages. Salt stress caused a marked reduction in germination percentage and fresh and dry biomass of the seedlings of all 10 accessions of safflower. Maximum reduction was observed at higher levels (180 and 240 m*M*) of NaCl. Accessions Safflower-31 and Safflower-35 had higher germination percentage and seedling shoot dry weight than the other lines particularly at 180 and 240 m*M* of NaCl, but in contrast, Safflower-32, Safflower-33, Safflower-37, Safflower-38, and Safflower-78 were the lowest in germination percentage and shoot dry weight. Although a considerable magnitude of variation for salt tolerance was observed in a set of 10 available lines of safflower at the germination and seedling stages, it is not certain whether such variation exists at the later stages of growth. This needs to be further investigated.

Introduction

Of the various approaches known in the literature for overcoming salinity problems, biotic approach has gained a considerable ground due to being economical and efficient means of utilizing the salt affected soils (Ashraf & McNeilly, 1987; 1988; Yeo & Flowers, 1989; Al-Khatib *et al*., 1994; Ashraf, 1994). Thus, the salt-affected soils can be utilized by growing salt tolerant plants, whether halophytes or crops.

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 (Kingsbury & Epstein, 1984; Ashraf & McNeilly, 1988), lentil (Ashraf & Waheed, 1993), barley (Belkhodja *et al*., 1994), cotton (Ashraf & Ahmad, 1999) and *Brassica napus* (Ashraf *et al*., 1989; Ulfat *et al*., 2007).

Safflower (*Carthamus tinctorius* L.) is one of the prospective oil-seed crops, because it yields about 32-40% seed oil (Weiss, 1983). Its oil is widely utilized in industries mainly as edible and dying purposes (Knowles, 1958; Ashraf & Fatima, 1995). 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 (Maas, 1987). Although salt stress adversely affects the growth of safflower plants at all developmental stages (Francois & Bernstein, 1964; Kaya *et al.,* 2003; Jamil *et al*., 2006), varietal differences in salt tolerance of safflower have been observed at germination (Ghorashy *et al*., 1972), at adult (Ashraf & Fatima, 1995) as well as at both germination and adult growth stages (Francois & Bernstein, 1964).

*Corresponding author: ejazbotanist@yahoo.com

Keeping in mind the economic importance of safflower and the evidence that salt tolerance varies with the change in developmental stage of many crops (Akbar & Yabuno, 1974; Kingsbury & Epstein, 1984; Shannon, 1984, Ashraf & Waheed, 1990), the principal aim of conducting this study was to determine variation in degree of salt tolerance of 10 available diverse accessions of safflower, particularly at the germination and seedling stages.

Materials and Methods

An experiment was carried out in a growth room of the Department of Botany, University of Agriculture, Faisalabad, Pakistan to screen 10 safflower (*Carthamus tinctorius* L.) lines viz., Safflower-31, Safflower-32, Safflower-33, Safflower-34, Safflower-35, Safflower-36, Safflower-37, Safflower-38, Safflower-39, and Safflower-78. The seed of all the accessions was obtained from the Plant Genetic Resources Institute, National Agricultural Research Center, Islamabad, Pakistan. Four hundred seeds of each safflower line were surface sterilized in 5% sodium hypochlorite solution for 5 min and then carefully rinsed with distilled water to remove the traces of sterilizing agent. There were five different regimes of salt stress i.e., 0, 60, 120, 180 and 240 m*M* of NaCl. Twenty five seeds of each line were allowed to germinate in a Petri plate double lined with filter paper moistened with 10 mL of Hoagland's nutrient solution with or without appropriate levels of NaCl. The treatment solution in each Petri plate was changed every day by dripping out and adding fresh treatment solution. Germination started after two days of sowing, and when the radicle reached up to 5 mm in length a seed was considered germinated. The data for germination was recorded daily up to the termination of the experiment. Germination percentage was calculated using the following formula:

$$\text{Germination \%age} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

After fifteen days of the start of the experiment, plant seedlings were removed carefully from the Petri plates and separated into shoots and roots and fresh weights recorded. Fresh plant samples were oven-dried at 65 ^{o}C for one week and their dry weights measured.

Statistical analysis of data: A completely randomized design (CRD) with four replicates was used for setting up the experiment. The COSTAT computer package (*CoHort software*, Berkeley, USA) was used for working out analyses of variance of all variables. The least significance difference test (Snedecor & Cochran, 1980) was used to compare the means.

Results

Germination percentage of all safflower lines was adversely affected due to the application of different levels (60, 120, 180 and 240 m*M*) of NaCl. Germination percentage of all lines was markedly suppressed at higher levels (180 and 240 m*M*) of NaCl (Table 1; Fig. 1). Variation in the set of accessions was not possible to discern at lower external salt levels, however, accessions differed significantly at the two higher salt levels (180 and 240 m*M* of NaCl).

Table 1. Mean squares from analyses of variance (ANOVA) of the data for growth and germination percentage of safflower (*Carthamus tinctorius* L.) seedlings grown under varying levels of NaCl.

Source of variation	df	Shoot fresh weight	Shoot dry weight	Root fresh weight
Salt stress	4	46.58***	0.201***	2.28***
Lines	9	0.503**	0.006**	0.019ns
Salt x Lines	36	0.559***	0.004**	0.048***
Error	150	0.165	0.002	0.014
		Root dry weight	**Germination percentage**	
Salt stress	4	0.006***	21481.6***	
Lines	9	0.0002ns	119.3ns	
Salt x Lines	36	0.00012*	125.6ns	
Error	150	0.00008	105.14	

*, **, *** = significant at 0.05, 0.01, and 0.001 levels, respectively.
ns = non-significant.

Imposition of varying levels of NaCl markedly ($p \leq 0.001$) reduced the shoot fresh and dry weights of the seedlings of all 10 lines of safflower. However, maximum reduction in shoot biomass was observed at the highest level i.e., 240 m*M* of NaCl. A significant inter-cultivar variation was observed under salt stress. Of all lines, Safflower-31, Safflower-35 and Safflower-39 produced highest shoot fresh weight at all salt regimes, but lowest shoot fresh weight was recorded in Safflower-32, Safflower-37 and Safflower-38 while the remaining lines were moderate in this attribute. At the highest salt level (240 m*M*), Safflower-31 and Safflower-35 produced maximum shoot dry weight of all lines and they were considered as relatively tolerant (Table 1; Fig. 1).

A marked reduction in root fresh and dry weights of the seedlings of all strains of safflower was observed due to salt stress. The most effective levels in reducing these attributes were 180 and 240 m*M* of NaCl. A considerable variation in the 10 accessions was also observed with respect to root fresh and dry weights (Table 1; Fig. 2).

On the basis of shoot dry weight and germination percentage at the highest salt regime (240 m*M*), all safflower lines were classified into three different categories i.e., tolerant, moderately tolerant and sensitive. Accessions Safflower-31 and Safflower-35 were found to be tolerant, Safflower-34, Safflower-36, and Safflower-39 moderately tolerant, and Safflower-32, Safflower-33, Safflower-37, Safflower-38 and Safflower-78 salt sensisitive (Table 2 & 3).

Table 2. Ranking of 10 safflower (*Carthamus tinctorius* L.) lines on the basis of shoot dry weight under the highest level (240 m*M*) of salt.

Classes	Range of shoot dry weight (g/plant)	Line no.
Tolerant	>0.12	L1, L5
Moderately tolerant	0.08-0.11	L4, L6, L9
Sensitive	<0.08	L2, L3, L7, L8, L10

Table 3. Ranking of 10 safflower (*Carthamus tinctorius* L.) lines on the basis of germination percentage at the highest level (240 m*M*) of salt.

Classes	Germination percentage	Line no.
Tolerant	>25	L1, L5
Moderately tolerant	20-25	L2, L4, L6, L9
Sensitive	<20	L3, L7, L8, L10

Discussion

Screening of available germplasm of a crop is a feasible means of identifying salt tolerant cultivars or lines which could maintain a comparatively reasonable yield on salt affected soils (Kingsbury & Epstein, 1984; Ashraf & McNeilly, 1987). It is now well evident that some crops or cultivars if selected at a particular growth stage maintains their degree of salt tolerance consistently at other growth stages, while others do not maintain their degree of salt tolerance at different developmental stages. For the latter crops, it is advisable to assess degree of salt tolerance at each growth stage.

In the present study, salt stress adversely affected the germination percentage, shoot and root fresh and dry weights of seedlings of all 10 lines of safflower and a significant variation in salt tolerance was observed among all the safflower lines with respect to seedling shoot biomass or germination percentage. For example, lines Safflower-31 and Safflower-35 were found to be tolerant, while Safflower-32, Safflower-33, Safflower-37, Safflower-38, and Safflower-78 sensitive to salt.

Ranking of the accessions was done using the data for germination percentage or seedling shoot dry weight at the highest salt level (240 m*M*), because this salt level was found very effective in discriminating the lines. In view of the ranking done at two initial growth stages, i.e., germination and seedling stage, it is evident that lines Safflower-31 and Safflower-35 were consistently tolerant at both growth stages. Similarly, lines Safflower-32, Safflower-33, Safflower-37, Safflower-38 and Safflower-78 were found to be sensitive consistently at the two growth stages. These results can be related to some earlier studies in which lines identified as salt tolerant at the earlier growth stages showed tolerance when tested at the later growth stages. For example, Ashraf *et al.* (1987) identified some salt tolerant lines of alfalfa at the initial growth stage which were found to be salt tolerant when tested at later growth stage. In the same way, Ashraf & Waheed (1990), while screening 133 accessions of lentil at the initial growth stages, found a significant amount of variation for salt tolerance in this set of germplasm. The salt tolerant lines identified at the initial growth stages maintained their degree of salt tolerance at the later growth stages.

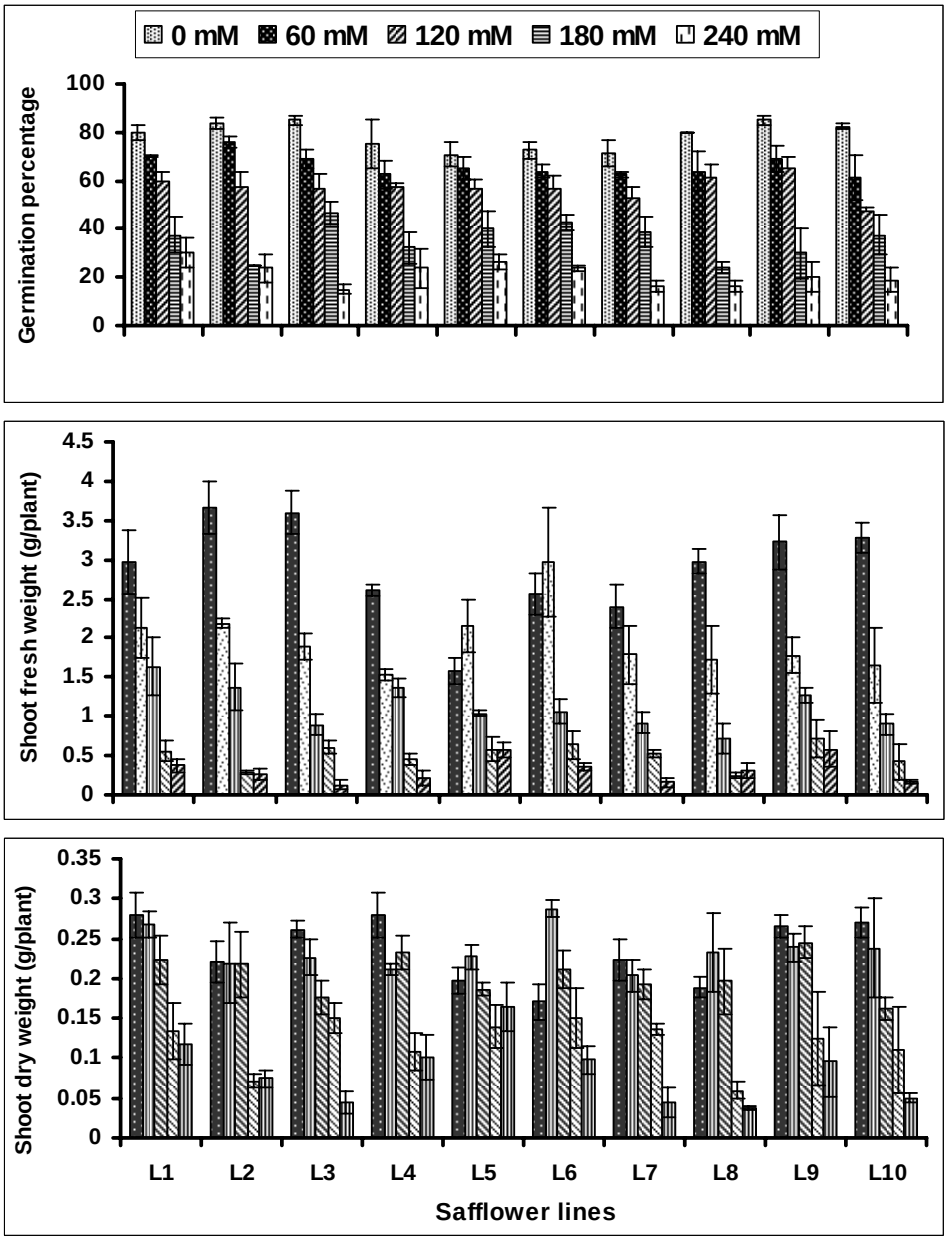

Fig. 1. Germination percentage, shoot fresh and dry weights of 15 day-old safflower seedlings subjected to control or different levels of NaCl.

L1, Safflower-31; L2, Safflower-32; L3, Safflower-33; L4, Safflower-34; L5, Safflower-35; L6, Safflower-36; L7, Safflower-37; L8, Safflower-38; L9, Safflower-39 and L10, Safflower-78.

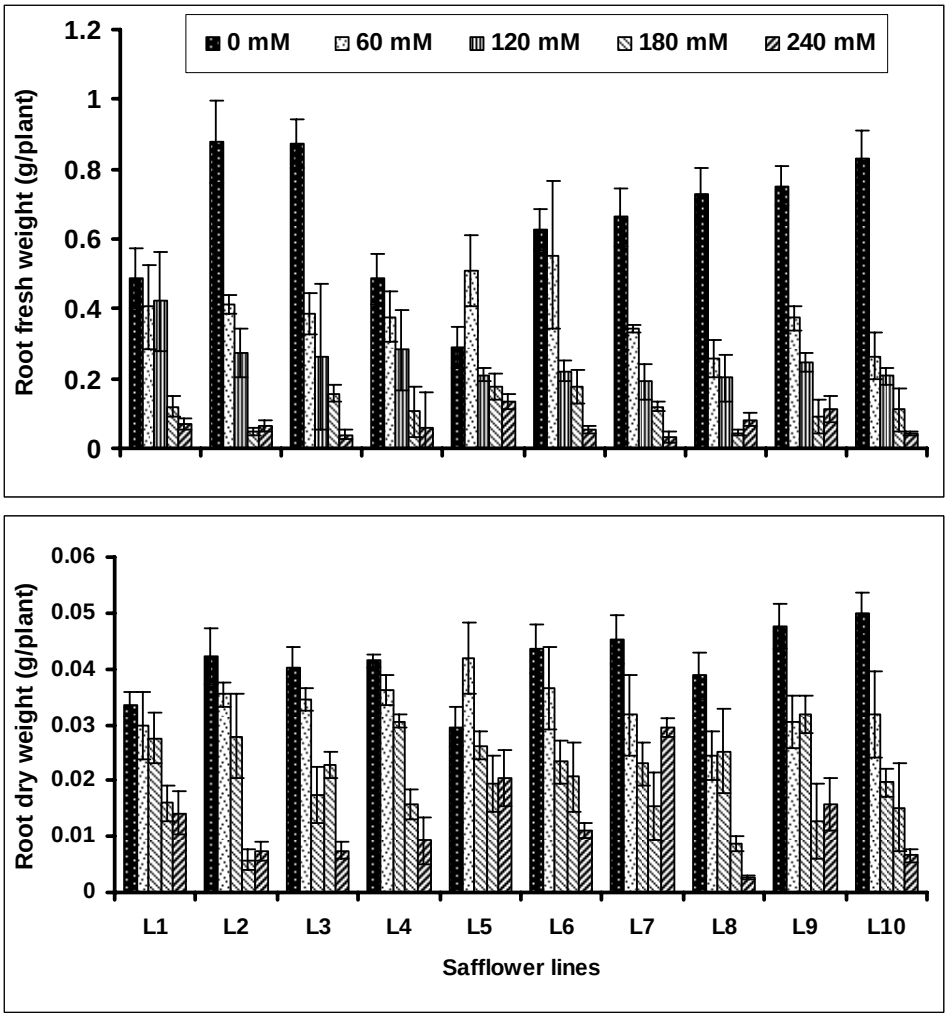

Fig. 2. Root fresh and dry weights of 15 day-old safflower plants subjected to control or different levels of NaCl.

L1, Safflower-31; L2, Safflower-32; L3, Safflower-33; L4, Safflower-34; L5, Safflower-35; L6, Safflower-36; L7, Safflower-37; L8, Safflower-38; L9, Safflower-39, and L10, Safflower-78.

Although a considerable magnitude of variation for salt tolerance was observed in a set of 10 available accessions of safflower while screening them at both germination and seedling stages, but a further study needs to be carried out to assess whether the lines marked as salt tolerant at the initial growth stages, maintain their degree of salt tolerance when tested as adult.

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