

Original Research

Identification and Morphologic Characterization of Some Salt Resistant Exotic Safflower (*Carthamus Tinctorius* L.) Lines During Seedling Growth

Sibel Day*, Nilüfer Koçak-Şahin

Ankara University, Faculty of Agriculture, Department of Field Crops,
06110 37/38 Fatih street Dışkapı Ankara, Turkey

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Abstract

Salinity is an increasing constraint in crop production. This abiotic stress has an impact on the life cycle of all plant species. It is well established that seed germination is vulnerable to soil salinity at a sowing depth of 1-5 cm. This study was conducted to investigate the impacts of NaCl on the germination performance of 10 exotic safflower lines (PI 248366, PI 243070, PI 237538, PI 613516, PI 601166, PI 613517, PI 613529, PI 250604, PI 613514, and PI 613460). Electrical conductivity (EC) values of NaCl solutions were 5, 10, 20, and 30 dS m⁻¹. Safflower lines were germinated with different NaCl treatments by measuring different morphologic parameters. The observations showed that seedling growth parameters were inhibited especially at 20 and 30 dS m⁻¹ NaCl treatments. It is clear that lines PI 248366 and PI 613516 had less diversity for the dry matter under dissimilar NaCl doses especially for 5 and 10 dS m⁻¹, which were the signs of their tolerance to salinity.

Keywords: salinity, oil crop, germination, industrial uses, natural dyes

Introduction

Safflower (*Carthamus tinctorius* L.) is a multipurpose crop cultivated for several industrial uses extraction of high-quality edible oil, obtaining natural dyes, medicinally important metabolites, use in the manufacture of cosmetics and use as ornamental plant. Its yellow to orange-red petals and stigmas are also used as natural dye in textile and wool industry [1, 2]. The seeds are rich in vegetable oil comprising oleic and linoleic acids [3]. Safflower is generally produced

in a very limited area compared to other oilseed crops. The cultivation area in Türkiye exceeds 15000 ha and the seed production is around 21000 tons [4]. Although, it is highly resistant to drought and salinity, its cultivation has not exceeded to more than 650 000 hectares of agricultural land in more than 20 countries with annual seed production exceeding 550 000 tons [5] due to many edaphic, socio-economic, and anthropological reasons. Sunflower is among the commonly cultivated oilseed crops in Türkiye [6, 7] with a place among the alternative crops like safflower and others whose cultivation is increasing due to changing irrigation regimes, plant diseases, pests, salinity and boron toxicity problems. Safflower has deep root growth which enables the use of deep

*e-mail: day@ankara.edu.tr

soil water; especially in areas suffering from salinity and it can also take part in crop rotation and phytoremediation to reduce salinity [8-11].

Salinity is becoming a major constraint globally due to using improper irrigation techniques and faulty cultivation methods [12]. Salt leaching from the soil profile, and the application of some organic or chemical compounds to reduce salinity for crops are labor-intensive, time-consuming, and costly solutions. Although safflower is considered a drought and salt tolerant plant species compared to other oilseed crops, yet its germination, emergence, and seedling development stages are highly sensitive to drought and salinity [13]. Crop cultivation in water-logged areas is seriously affected by the level of salinity in irrigation water quality that influences delayed emergence, low survival rate, irregular seedling establishment, and poor yield [14-16].

All plant species may have dissimilar developmental pathways to their response against resistance to salinity depending on the genetic makeup of the respective plant species; where seed germination and seedling growth stages are more susceptible to salt-induced osmotic effect, Na⁺ toxicity, oxidative stress, and nutrient shortage. Under these circumstances decrease in cell size due to adjustments in plant metabolism, and cell membranes are commonly observed [17-19]. These characteristics are more improved in some plants compared to others. Safflower tolerance to salinity is higher compared to other oilseed crops [20]. Identifying and selecting genotypes or lines are important for saline lands for sustainable crop production in these areas to decrease the uptake of salt ions for soil remediation is significantly important [21].

This diversity could be attributed to various complex biochemical, physiological, and genotypic interactions throughout the growth [22, 23].

The main perspective and aim of the study is the screening of some exotic safflower lines to determine their germination and seedling growth characteristics under dissimilar levels of salinity.

Material and Methods

Seed Material

This study was carried out with 10 exotic safflower lines obtained from GRIN-Global (Germplasm Resource Information Network) and USDA including PI 613514, PI 613516 and PI 613517 (from Australia), PI 613529 (from China), PI 250604 (from Egypt), PI 248366 (from India), PI 613460 (from Iran) PI 243070 (from Jordan), PI 601166 (from Montana USA), and PI 237538 (from Türkiye). These seed lines were multiplied and all seeds belonged to the crop of 2020.

These lines were propagated under humid continental (CSa-Köppen Geiger) climatic conditions of Ankara, Türkiye.

Germination Experiment

Four replicates of 50 seeds from each exotic safflower line were sandwiched between three layered rolled filter papers soaked with 21 mL of respective test solutions to germinate. These were transferred to transparent plastic bags and incubated at 20±1°C in dark to germinate. The papers used in the test were replaced every 2 days to avoid salt accumulation. The seeds which had 2 mm protruded radicles were considered germinated and were counted daily for 10 days. The germination percentage calculation was conducted following Youseffi et al. [24]:

$$GP = \frac{NG}{NT} \times 100$$

Where

GP = Germination percentage

NG = Number of germinated seeds

NT = Total number of seeds

Mean germination time (MGT), the indication of germination speed was evaluated according to ISTA rules [25] using the following equation

$$\text{Mean Germination Time} = \frac{\sum(Dn)}{\sum n}$$

Where, n is the number of germinated seeds on day D, and D is the number of days from the beginning of the germination test.

Root length, shoot length, fresh and dry seedling weights of 10 randomly selected seedlings in each replication were measured after the 10th day. The samples were dried in an oven at 105°C for 2 h to determine dry weight of the samples in each replicate [26].

Experimental Design

The design of the experiment was based on a two factorial (10 × 5) experiment arranged in a completely randomized design with four replicates and 50 seeds per replicate. The main factor was exotic safflower lines and the sub-factor was NaCl levels. Data for germination percentage were subjected to arcsine transformation before ANOVA using MSTAT-C computer software (Michigan State University, 1991). The differences between the means were compared using Duncan's Multiple Range Test - DMRT (*p*<0.01).

All measured parameters of the lines exposed to dissimilar concentrations of NaCl levels were considered for grouping the lines. Principle Component and Cluster Analysis was performed by classifying the lines for salinity tolerance.

Results and Discussion

Germination percentage of the each lines showed non statistical differences after treatment with dissimilar NaCl doses ($F = 2.1361, df = 147, P = 0.0008$). The maximum and minimum germination of 96% and 68.5% was observed in line PI 613516 and PI 613514 in control and 30 dS m⁻¹ treatments in the same order. The line PI 613529 had the consistency among treatment and showed the minimum differences using salt concentration of 30 dS m⁻¹ compared to the control treatment (2.11 % decrease observed). However the decrease in the germination percentage was not statistically significant, with observed reduction in salt stress. That could be attributed to the increased osmotic pressure in the medium causing a decrease in water intake [27] which could be addressed as drought stress (related to limited water uptake) and interference of NaCl with the enzymes starting germination. Similar diminishing effects of increasing salinity levels on

seed germination have also been observed by other researchers in dissimilar plant species like sunflower, linseed and faba bean [28].

Genotype (line) × NaCl doses interaction showed statistically significant interaction on mean germination time ($F = 22.4946, df = 147, P = 0.0000$). Delayed Mean Germination Time (MGT) with increasing salinity stress was observed in all genotypes (Table 1). Pearson’s correlation performed between MGT and the seedling growth parameters (shoot length, root length, fresh weight, and dry weight) was negative (Table 4). While the MGT increased with high levels of NaCl, shoot length ($r = -0.7181, p = 0.001$), root length ($r = -0.7208, p = 0.001$), and fresh weight ($r = -0.7555, p = 0.001$) decreased. Among NaCl levels, 30 dS m⁻¹ had the highest retarding impact on MGT. The fastest germination at 30 dS m⁻¹ was observed using lines PI 243070 and PI 237538 which showed 2.91 and 2.99 days in germination respectively. Retarding effects of high levels of NaCl on germination is the result of drought

Table 1. Effects of treatment with dissimilar NaCl doses on germination percentage and mean germination time of some exotic safflower lines.

Lines	NaCl doses (dS m ⁻¹)				
	0	5	10	20	30
	Germination Percentage (%)				
PI 248366	82.50±2.52	85.50±5.74	83.50±1.91	78.00±6.92	76.50±3.78
PI 243070	91.50 ±5.74	90.50±4.12	89.00±2.00	85.50±5.74	87.50± 3.00
PI 237538	92.00 ±4.00	91.00±3.46	90.50±4.12	90.00±5.65	88.00±1.63
PI 613516	96.00±3.26	95.00±5.74	94.50±1.91	90.50±4.12	84.50±8.54
PI 601166	89.00±2.00	88.50±4.72	84.50±1.00	80.50±5.74	81.00±6.63
PI 613517	94.00±2.31	92.00±2.83	91.00±3.83	91.50±6.40	84.50±1.91
PI 613529	95.00±2.00	94.00±1.64	93.50±1.91	93.00±3.46	93.00±2.58
PI 250604	94.00±2.31	91.50±1.00	89.50±4.12	84.50±10.63	88.50±3.00
PI 613514	80.00±3.26	76.50±3.00	75.50±3.78	74.00±5.42	68.50±7.72
PI 613460	85.50±5.74	81.00±3.46	78.50±3.00	72.50±5.74	75.50±7.89
	Mean Germination Time (day)				
PI 248366	2.34±0.07 c	2.29±0.04 c	2.48±0.12 c	2.99±0.01 b	4.44±0.37 a
PI 243070	1.94±0.04 d	2.10±0.08 cd	2.18±0.06 c	2.41±0.07 b	2.91± 0.10 a
PI 237538	1.99±0.09 d	2.05±0.06 d	2.29±0.09 c	2.61±0.10 b	2.99±0.03 a
PI 613516	2.04±0.06 d	2.30±0.01 c	2.15±0.04 d	2.97±0.10 b	4.90±0.07 a
PI 601166	2.37±0.35 bc	2.20±0.01 c	2.09±0.01 c	2.72±0.05 b	3.83±0.14 a
PI 613517	2.02±0.02 d	2.29±0.33 cd	2.59±0.15 c	3.81± 0.073b	4.60± 0.26 a
PI 613529	2.00±0.00 c	2.13±0.04 c	2.14±0.06 c	3.66 ±0.31b	4.40±0.11 a
PI 250604	2.04±0.07 c	2.15±0.03 c	2.46±0.18 bc	2.82±0.05 b	4.61±0.44 a
PI 613514	2.00±0.00 c	2.15±0.05 c	2.65±0.15 b	2.76±0.14 b	3.71±0.9 a
PI 613460	2.00±0.00 d	2.18±0.16 d	2.55±0.45 c	3.08±0.54 b	3.66±0.24 a

All values shown by different letters in a row are significantly dissimilar using DMRT at $p < 0.01 \pm$ Standard deviation

Table 2. Effects of dissimilar NaCl doses on root and shoot length of some exotic safflower lines.

Lines	NaCl doses (dS m ⁻¹)				
	0	5	10	20	30
	Root length (cm)				
PI 248366	3.20±0.00 b	5.68±0.79 a	3.95±0.43 b	1.90±0.42 c	0.98±0.50 c
PI 243070	5.33±1.72 a	5.35±1.03 a	5.28±0.25 a	1.98±0.69 b	1.28±0.12 b
PI 237538	3.63±0.71 b	2.90±0.27 bc	4.80±0.63 a	2.20±0.11 c	1.25±0.18 d
PI 613516	5.90±1.07 a	4.98±0.54 a	5.01±0.22 a	3.18±0.15 b	1.30±0.00 c
PI 601166	5.93±0.89 a	5.20±0.48 a	5.48±0.73 a	2.15±0.65 b	1.15±0.10 b
PI 613517	5.95±0.52 a	5.58±0.19 a	4.00±0.50 b	3.58±0.05 b	1.35±0.06 c
PI 613529	6.58±0.51 b	7.77±0.69 a	7.44±0.31 ab	3.17±0.19 c	1.17±0.50 d
PI 250604	6.93±0.33 a	7.68±1.35 a	5.13±0.94 b	3.15±0.37 c	1.40±0.08 d
PI 613514	7.00±0.32 a	5.93±1.04 a	3.10±0.00 b	3.05±0.57 b	1.15±0.06 c
PI 613460	5.88±1.09 a	6.40±0.6 a	6.88±0.52 a	4.75±1.49 a	1.08±0.05 b
	Shoot length (cm)				
PI 248366	4.35±0.37 a	4.78±0.19 a	3.73±0.29 b	1.20±0.08 c	1.75±0.17 c
PI 243070	5.15±0.19 a	4.43±0.22 b	3.93±0.09 c	1.30±0.00 d	1.10±0.81d
PI 237538	4.28±0.46 a	3.43±0.32 b	3.28±0.33 b	1.65±0.09 c	1.08±0.38 c
PI 613516	5.75±0.50 a	4.53±0.12 b	3.93±0.38 b	1.43±0.40 c	1.90±0.11 c
PI 601166	5.73±0.37 a	4.75±0.30 b	3.93±0.25 c	1.23±0.15 d	1.05±0.06 d
PI 613517	5.63±0.39 a	4.60±0.16 b	3.63±0.33 c	2.83±0.12 d	1.28±0.07 e
PI 613529	5.31±0.14 a	4.60±0.06 b	4.75±0.36 b	1.86±0.19 c	1.70±0.05 c
PI 250604	5.55±0.26 b	6.60±0.46 a	4.35±0.17 c	2.98±0.05 d	1.55±0.10 e
PI 613514	5.30±0.27 b	5.80±0.40 a	2.85±0.58 c	1.95±0.58 d	1.20±0.00 e
PI 613460	5.50±0.24 a	5.53±0.47 a	4.18±0.12 b	2.28±0.19 c	1.30±0.14 d

All values shown by different letters in a row are significantly dissimilar using DMRT at $p < 0.01$; ±Standard deviation

and accumulated Na⁺ ions in the treated seeds inflicting damages to the seed metabolites' activities during germination.

Root length was statistically affected by genotype (line) × NaCl doses interaction ($F = 9.78$, $df = 147$, $P = 0.0000$). NaCl enhanced root length of exotic safflower lines up to 5 dS m⁻¹ while it was dramatically decreased at 20 and 30 dS m⁻¹. Line PI 248366 induced minimum root length (0.98±0.50 cm) at 30 dS m⁻¹ NaCl treatment. Some of the lines (PI 248366, PI 243070, PI 613529, PI 250604, PI 613460) showed an increase in root length at 5 dS m⁻¹ NaCl treatment compared to the control treatment. A decrease in root length with the lowest NaCl treatment compared to the control treatment was determined in PI 237538, PI 613516, PI 601166, PI 613517, PI 613514 (Table 2). Especially root length values of the PI613460 in control, 5, 10, and 20 dS m⁻¹ reflected statistical similarity with representation by the same letters according to Duncan Multiple Range Test.

Analysis of variance showed a statistically significant interaction between genotypes (lines) × NaCl doses interaction ($F = 17.3531$, $df = 147$, $P = 0.0000$) for shoot length. Reduction in shoot length due to increased NaCl doses was recorded and it was very evident in 20 and 30 ds m⁻¹ NaCl treatments. Shoot length had a positive correlation with root length, seedling fresh and dry weight which implied that with each increase in shoot length also positively increased the dry weight. The current results confirmed that higher salinity (20 and 30 dS m⁻¹ NaCl) had high harmful impacts on seedling root and shoot length compared to the trend of germination at other doses; which could be attributed to decreased cell division due to low plant metabolism due to accumulation of Na in and around the cells. This accumulation leads to differentiation in Na:Ca and K:Na percent in plant cells. Inhibited seedling growth related to high NaCl has also been reported in several plants like *Suaeda salsa* [29], *Helianthus annuus* L. [30, 31].

Table 3. Effects of dissimilar NaCl doses on seedling fresh and dry weight of exotic safflower lines.

Lines	NaCl doses (dS m ⁻¹)				
	0	5	10	20	30
	Seedling fresh weight (mg plant ⁻¹)				
PI 248366	190.00±14.14 a	202.50±12.58 a	150.00±8.16 b	80.00±8.16 c	52.50±5.00 d
PI 243070	250.00±27.08 a	222.50±12.58 a	185.00±10.00 b	80.00±8.16 c	60.00±0.00 c
PI 237538	208.75± 23.31a	181.50±23.10 ab	160.75±12.44 b	80.00±5.83 c	52.00±1.41 c
PI 613516	265.00±28.87 a	215.00±23.80 b	187.5±26.30 b	76.75±3.40 c	76.00±5.03 c
PI 601166	309.00±46.02 a	232.00±17.39 b	185.75±3.40 b	91.50±15.84 c	62.50±5.00 c
PI 613517	269.30±24.29 a	206.30±5.50 b	171.80± 5.31c	98.25±4.71 d	59.75±6.65 e
PI 613529	207.80±7.63 a	195.50±4.93 b	170.50±9.25 c	73.75±4.79 d	57.50±9.57 e
PI 250604	244.00±29.69 a	261.00±2.00 a	182.50±3.78 b	108.75±8.30 c	76.50±9.14 d
PI 613514	267.30±12.50 a	238.80±21.39 b	170.00±1.15 c	101.00±8.75 d	70.00±11.55 e
PI 613460	226.75±27.03 a	185.30±14.47 b	141.80±3.77 c	88.00±6.68 d	49.75±5.62 e
	Seedling Dry Weight (mg plant ⁻¹)				
PI 248366	20.75±1.50 a	20.00±0.00 a	20.00±0.00 a	8.25±0.50 b	5.25±0.50 c
PI 243070	21.25±0.50 a	20.25±0.50 a	17.00±1.41 b	7.50±1.00 c	4.00±0.00 d
PI 237538	17.75±1.26 a	15.50±1.00 b	14.75±0.96 b	6.25±0.50 c	3.25±0.50 d
PI 613516	22.75±2.06 a	20.25±2.50 ab	16.50±3.00 b	5.75±0.96 c	4.50±0.58 c
PI 601166	21.75±1.50 a	19.75± 1.50 a	13.50±0.58 b	7.25±0.96 c	5.00±0.00 c
PI 613517	21.25±2.06 a	19.50±0.58 a	15.25±0.50 b	6.75±0.96 c	3.75±0.50 d
PI 613529	16.50±0.58 b	18.00±0.81 a	14.75±0.50 c	5.50±0.58 d	4.50±0.58 d
PI 250604	20.75±2.22 a	18.25±0.50 b	13.50±0.58 c	8.25±0.50 d	5.75±0.50 e
PI 613514	20.00±0.82 a	20.00±0.82 a	14.00±0.00 b	7.50±0.58 c	5.50±0.58 d
PI 613460	17.75±2.50 a	14.75±0.96 b	11.75±0.50 c	8.50±1.00 d	4.50±0.58 e

Figures having a similar letter(s) in a row are not statistically and significantly dissimilar using DMRT at $p < 0.01$; ±Standard deviation

Table 4. Correlation among seedling growth parameters of some exotic safflower lines subjected to salinity stress.

Parameters	Parameters					
	MGT	Germination Percentage	Shoot Length	Root Length	Fresh Weight	Dry Weight
MGT	-					
Germination Percentage	-0.0192	-				
Shoot Length	-0.7181**	0.0962	-			
Root Length	-0.7208**	0.0971	0.879**	-		
Fresh Weight	-0.7555**	0.0871	0.94**	0.8141**	-	
Dry Weight	-0.7811**	0.0709	0.9052**	0.7753**	0.9508**	-

** $p < 0.001$ level

The impact of genotype (line) × NaCl doses interaction on seedling fresh weight showed statistically significant differences ($F = 4.6382$, $df = 147$, $p = 0.0000$). Decrease in root and shoot length related to

increasing NaCl levels caused decline in seedling fresh weight. All lines gave highest fresh weight in control treatment except for line PI 248366. This genotype had its maximum fresh weight (of 202.50 ± 12.58 mg plant⁻¹)

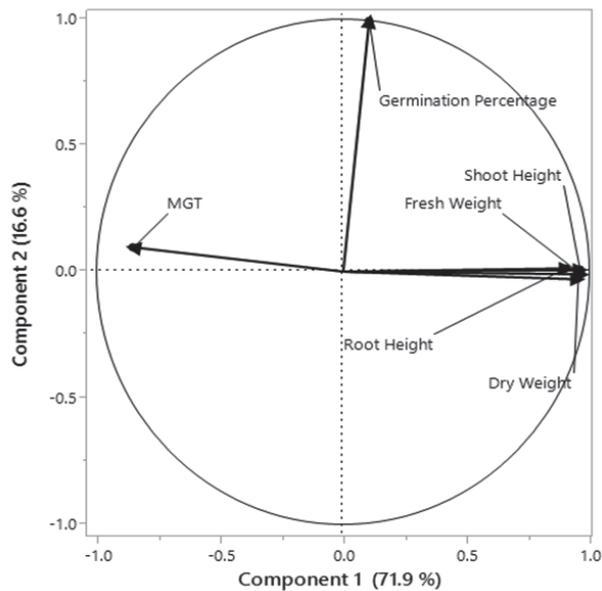


Fig. 1. Principal components 1 and 2 obtained from seedling growth data of exotic safflower lines subjected to dissimilar NaCl levels.

using 5 ds m⁻¹ NaCl. Among all lines, PI 601166 showed the highest fresh weight (309±46.02 mg plant⁻¹) in control (Table 3). The seedling fresh weight had positive correlation with seedling dry weight.

The seedling dry weight of exotic safflower lines was also affected statistically by genotype (line) × NaCl doses interaction ($F = 7.2300$, $df = 147$, $p = 0.0000$). Considering dry weight of exotic safflower lines they showed remarkable decrease using 20 and 30 ds m⁻¹ NaCl levels (Table 3).

High salinity levels decreased shoot and root length, seedling fresh and dry weight as has been observed in barley [32], and in tomato and cucumber [33].

Principal component 1 and 2 PCA results obtained from seedling growth parameters of these exotic safflower lines subjected to salinity are illustrated in Fig. 1. Component 1 and component 2 was 71.9 % and 16.6 % in this study respectively and their cumulative impact was 88.5 %. Visualization of data obtained from growth parameters clearly separated mean germination time from other parameters. Root length, shoot length, seedling fresh, and dry weight were grouped together in the biplot which suggests that these parameters had a positive correlation among themselves (Fig. 1).

Conclusively, it was noted that inhibitory impacts of salinity on seed germination and growth of plants could be observed easily and the promising lines could be used for identification and screening.

Conclusions

All the lines showed diversity among seedling growth parameters up to 30 ds m⁻¹. It is obvious that

2 lines (PI 248366 and PI 613516) used in this research had less diversity for the dry matter under dissimilar NaCl doses which is a sign of the tolerance to salinity. The study meets the aims of the research showing a successful screening of 2 lines against salt tolerance and could be recommended for soils having surface soil salinity up to ≤ 20 ds m⁻¹.

Conflict of Interest

The authors confirm and declare that they have no conflict of interest to disclose.

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