

## Determination of Germination Parameters of Safflower (*Carthamus tinctorius* L.) Cultivars Under Salt Stress

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Germination stress index,  
Abiotic stress

**Abstract:** The present study was carried out to characterize germination capacity of 10 safflower cultivars under saline conditions. Five salt (NaCl) levels (0, 60, 120, 180, 240 mM) were used to test safflower seeds. Germination of seeds was counted every day for 14 days and germination percentage, Timson's germination index, mean germination time, mean germination rate and germination stress index were calculated. Cultivar and salinity treatments were important for all parameters; however mean germination time and mean germination rate interactions were not important. Germination percentage, Timson's germination and germination stress indices decreased significantly with increased salt concentrations. However, mean germination time increased with higher salt concentrations. The most significant reductions in germination percentage were observed at 180 and 240 mM salt concentrations. Correlation coefficients were all important for germination indices. Based on germination indices, Leed and FO2 were more sensitive to salt stress at germination stage and Royal was more resistant than the other cultivars tested. Germination percentage, Timson's germination index and germination stress index were better to assess germination capacity of safflower cultivars under salt stress conditions. Mean germination time and rate were not suitable to assess differences in germination parameters of the cultivars under stress conditions.

## Aspir (*Carthamus tinctorius* L.) Çeşitlerinin Tuz Stresi Altında Çimlenme Parametrelerinin Belirlenmesi

### Anahtar Kelimeler

Çimlenme endeksleri,  
Çimlenme stres indeksi,  
Abiyotik stres

**Özet:** Bu çalışma, tuz stresi koşulları altında 10 aspir çeşidinin çimlenme kapasitesini belirlemek için yapılmıştır. Beş farklı tuz (NaCl) konsantrasyonu (0, 60, 120, 180, 240 mM) aspir tohumlarının çimlenme kapasitesini test etmek için kullanılmıştır. Tohum çimlenmeleri 14 gün boyunca her gün sayılarak çimlenme yüzdesi, Timson's çimlenme indeksi, ortalama çimlenme zamanı, ortalama çimlenme oranı ve çimlenme stres endeksleri hesaplanmıştır. Çeşit ve tuz konsantrasyonlarının etkileri tüm çimlenme parametreleri için önemli olarak bulunurken, ortalama çimlenme zamanı ve ortalama çimlenme oranlarının interaksiyon etkileri önemsiz bulunmuştur. Çimlenme yüzdesi, Timson's çimlenme ve çimlenme stres endeksleri artan tuz konsantrasyonları ile önemli ölçüde azalmıştır. Ancak, yüksek tuz konsantrasyonu seviyelerinde ortalama çimlenme zamanı artmıştır. Çimlenme parametreleri arasında hesaplanan korelasyon katsayılarının tamamı önemli çıkmıştır. Çimlenme yüzdesindeki en önemli azalma 180 ve 240 mM tuz konsantrasyonlarında gözlenmiştir. Çimlenme indeksi parametrelerine göre, Leed ve FO2 çeşitleri çimlenme aşamasında tuz stresine karşı daha hassas ve Royal çeşidi ise test edilen diğer çeşitlere göre daha dayanıklı olarak bulunmuştur. Çimlenme yüzdesi, Timson's çimlenme indeksi ve çimlenme stres indeksi tuz stresi altındaki aspir çeşitlerinde çimlenme kapasitesini değerlendirmek için uygun iken; ortalama çimlenme zamanı ve çimlenme oranı uygun bulunmamıştır.

## 1. Introduction

Soil salinity is a major problem of agricultural lands, and it is caused by accumulation of salts in the soil through over watering and evaporation, irrigation with saline water and lack of adequate drainage systems. It is especially problematic in arid and semi-arid parts of the world [1, 2]. Soil salinization affects approximately 800 million hectares of agricultural land in the world. Over 1.5 million hectares of land is affected by salinity and alkalinity in Turkey [3].

Salinity affects many physiological processes in plants, such as sodium accumulation, reduction in water and nutrient absorption, production of reactive oxygen species leading to cellular damage, toxicity, water loss and decreased photosynthesis. As a result of these changes, plant growth is reduced and yields are decreased [4].

Safflower is an important oil seed crop and it has been grown for its oiliferous seeds and flowers since ancient times. Safflower is cultivated more than 60 countries with production area of more than 650.000 hectares around the world. Turkey ranks as the 8th largest producer and the safflower is cultivated more than 15.000 hectares with over 21 million tons of seed production [5]. Oil demand and consumption exceeds the production in Turkey and oil deficit covered by import of oils and oiliferous seeds. Therefore it is necessary to increase oil seed crop production in Turkey. In order to increase crop production, it is also necessary to alleviate the effects of environmental stress conditions on crops for increased yields [6].

Drought and salinity are the major causes of yield reduction in crop plants [2]. Therefore, it is necessary to investigate germination, growth and adaptation capabilities of crop plants required for food production under stress conditions. Safflower is known to be tolerant to drought and salinity stress and could be used as an alternative crop suitable to be grown under drought and saline conditions [7, 8]. Application of stress conditions and their timing are important screening factors to determine tolerant and susceptible genotypes. Germination and seedling development phases of safflower are the most sensitive development periods to salinity stress [4]. Therefore, the aim of the present study was to

evaluate diverse safflower cultivars under varying salt concentrations to determine their germination abilities and to calculate germination indices under salinity stress.

## 2. Material and Method

Seed of 10 safflower cultivars from 4 different countries were evaluated for their germination behaviors under different salt concentrations. Five safflower cultivars UC-1 (PI 572434), Royal (PI 537694), US-10 (PI 572414), Leed (PI 572436) and Gila (PI 537692) were from United States, FO2 (PI 506426) was from China, Lesaf 414 (PI 603206) and AC Sunset (PI 592391) were from Canada, Quiriego 88 (PI 537110) and San Jose 89 (PI 561703) were from Mexico. Cultivars oil contents ranged from 25% to 31% in field trails and they were linoleic type with the exception of US-10, which was oleic type [9].

Seed surface sterilization was carried out by soaking seeds in 1% sodium hypochlorite solution for 10 min. After sterilization, all seeds were rinsed under running tap water for 5 min and dried at room temperature. Seeds were sown in 15 cm wide petri dishes and 5 different concentrations (0, 60, 120, 180, 240 mM) of NaCl solution was added on filter papers. Control groups were wetted with distilled water. Germination tests were carried out in a germination cabinet under 25 °C for 14 days [10]. Each petri dish contained 40 seeds and germination tests were conducted with 3 replications. Number of germinated seeds was counted every day and germinated seeds were removed from petri dishes.

Germination data were logit transformed for normality of results. Germination percentage, mean germination time, Timson's germination index [11], mean germination rate [12] and germination stress index [13] were calculated according to formulas in Table 1. Results were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics 22.0 software (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test ( $p \leq 0.05$ ) was used to discriminate the differences between the means. To show the relationship between measured parameters, Pearson linear correlation analysis (heatmap correlation) was calculated using OriginPro software (version 2021, OriginLab, Northampton, MA).

**Table 1.** Formulas used to calculate different germination parameters in the study

Parameter	Formula for calculation	Description
Germination percentage	$(N_p / N_t) \times 100$	$N_p$ – the number of germinated seeds, $N_t$ – the total number of seeds
Timson's germination index	$\Sigma n$	$n$ – cumulative daily germination percentage for each day of the test
Mean germination time	$\Sigma(n_i \times d_i) / N$	$d_i$ – day, $n_i$ – number of seeds germinated at day $d_i$ , $N$ – total number of seeds germinated in the test
Mean germination rate	$1/T$	$T$ – mean germination time
Germination stress index	$S_i / K_i$	$S_i$ – germination percentage under stress condition, $K_i$ – germination percentage under non-stress condition

### 3. Results

Safflower cultivars were germinated under controlled conditions under 5 different salinity levels for 14 days and germination was recorded every day. Germination percentage and germination stress index were calculated at the end of the germination period. Daily counted germination values were used to calculate the other germination parameters. After germination tests, results were subjected to variance analysis and means were separated according to their importance levels. Germination results were used to calculate 5 germination parameters, and effects of cultivars, treatments and their interaction. Results of variance analysis were presented in Table 2. Cultivar and salt concentrations had significant effects on all germination indices ( $P < 0.01$ ). However interaction effects on mean germination time and mean germination rate was not important.

Germination percentage was higher for control groups for safflower cultivars. The lowest germination percentage was found in FO2 (73.3%) and the highest germination percentage was observed in Leed (89.2%) for the controls. Seeds subjected to germination test under different salt concentration exhibited reduced germination percentages than the control seeds. Increased salt concentrations from 60 mM to 240 mM caused reduced germination for all cultivars tested. Reduction in germination percentage was more severe for 180 and 240 mM salt concentrations, hence these salt concentrations started to differ safflower cultivars for their germination ability under salt stress. Leed and FO2 had the highest germination decline at 180 and 240 mM salt concentrations (Table 3). Gila, UC-1 and Lesaf 414 showed significant decline for germination percentage for 240 mM salt concentration as well.

Timson's germination index values based on germination data were similar to germination percentage. Higher germination index values were found for control seeds. As salt concentrations increased, germination index values started to decrease and the lowest germination index values were found in seeds germinated in 240 mM salt concentrations (Table 3).

Mean germination times of cultivars belong to control group were generally lower than 2 days and only 4

cultivars had mean germination times higher than 2 days. Increased salt concentrations affected Leed, Royal, AC Sunset and FO2 mean germination times more those of other cultivars. Salt stress increased mean germination times of cultivars. Even though Royal and AC Sunset germination percentages were not greatly reduced by increased salt concentrations, their mean germination times increased as much as Leed and FO2, and these cultivars had the highest increase in mean germination time in the study.

Mean germination rate ranged from 0.28 to 0.73 among the genotypes under different salt concentrations. Control groups had the highest mean germination rates in the study and the lowest mean germination rates were found in seeds germinated under high salt concentrations (Table 3).

Since control groups was not exposed salt stress conditions, it was not possible to calculate germination stress index for them. Seeds germinated under different salt concentrations showed different results for germination stress index depending on their germination capacity. Germination stress index was high at low salt concentrations; however, germination stress index values started to become lower as salt concentrations increased, showing germination of genotypes under salt stress was significantly affected by salt concentrations. The lowest germination stress index score was found in FO2 at 240 mM salt concentration.

The result of the correlation analysis for germination parameters were given in Fig 1. All the 10 coefficients are significant at the  $p \leq 0.001$  levels. Among the 10 significant coefficients, 6 were positively and 4 were negatively correlated with each other. Germination percentage was positively correlated to Timson's germination index (0.98), mean germination rate (0.43) and germination stress index (0.93) and negatively correlated to Mean germination time (-0.45).

### 4. Discussion and Conclusion

Salinization of agricultural soils is an important environmental stress factor that limit seed germination, plant growth and yield. In addition, salinization exacerbates soil conditions for plant growth by increasing soil sodium content and soil pH, creating suboptimal conditions for plant growth and

**Table 2.** Variance analysis of cultivars, treatments and their interactions on germination parameters of safflower seeds

Variables	DF	Mean of squares				
		Germination percentage	Timson's germination index	Mean germination time	Mean germination rate	Germination stress index
Cultivars	9	427.77**	128374.35**	2.60**	0.14**	0.12**
Treatments	4	2142.90**	699040.73**	4.20**	0.21**	4.09**
Interaction	36	74.95**	17853.73**	0.09 <sup>ns</sup>	0.005 <sup>ns</sup>	0.031**
Erör	100	11.46	2882.79	0.072	0.004	0.0001
CV (%)		5.97	7.87	12.34	13.32	1.98

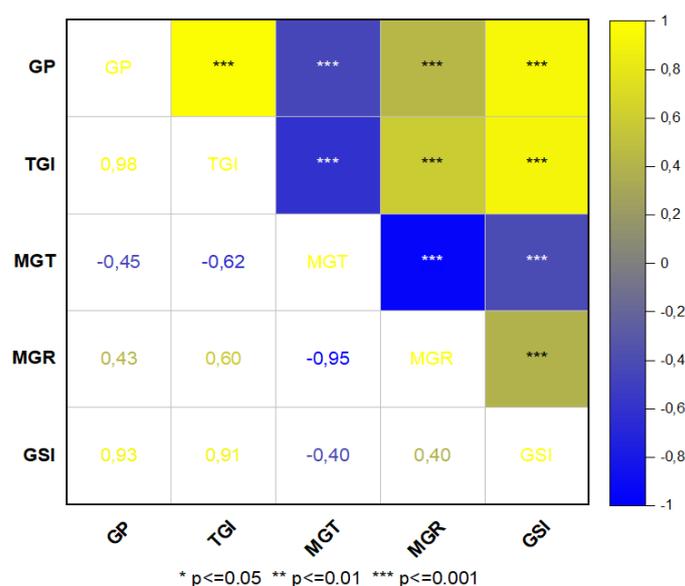
<sup>ns</sup> not significant, \*\* Significant at  $p \leq 0.01$  level

further reduces crop yields. Safflower and many other crop species are adversely affected by such conditions [14-17]. Different treatments were tested to alleviate these environmental conditions, such as chitosan application to seeds [18], seed priming [19] and foliar applications to plants [20]. Another way to increase crop productivity is to find suitable genotypes resistant to abiotic stress factors through screening [21, 22] and to incorporate necessary genes to elite lines through breeding [23].

In the present study, diverse safflower cultivars were screened for their germination capacities under 5 different salt concentrations. Different seed parameters were calculated using germination data. Seed germination parameters could be used to identify salinity tolerance of different genotypes at germination stage [24]. All tested cultivars had more than 50% germination up to 120 mM salt concentration (Table 3). However, increasing salt concentration to 180 and 240 mM differentiated

**Table 3.** The mean values of germination parameters in safflower cultivars under salt stress

Genotype	NaCl concentration (mM)	Germination percentage	Timson's germination index	Mean Germination Time	Mean germination rate	Germination stress index
Gila	0 (Control)	85.8 a-d	845.83 a-e	2.15 j-p	0.47 f-m	-
	60	77.5 d-i	737.50 e-n	2.47 e-l	0.41 i-p	0.90 e
	120	67.5 i-n	639.17 k-s	2.53 d-k	0.40 j-p	0.79 k-o
	180	60.8 l-o	574.17 o-u	2.58 c-j	0.39 k-p	0.71 s
	240	52.5 o-s	473.33 uv	2.98 b-e	0.34 m-p	0.61 t
UC-1	0 (Control)	79.2 c-h	831.67 a-f	1.49 t-y	0.67 a-c	-
	60	77.5 d-i	810.00 a-i	1.55 s-y	0.66 a-c	0.98 b
	120	66.7 j-n	691.67 g-p	1.62 p-y	0.62 a-e	0.84 g-i
	180	60.0 m-p	601.67 n-u	1.99 l-u	0.52 e-k	0.76 o-r
	240	49.2 r-s	488.33 t-v	2.06 j-s	0.49 f-l	0.62 t
Royal	0 (Control)	87.5 ab	924.17 ab	1.43 vy	0.70 a	-
	60	85.8 a-d	895.83 a-c	1.56 r-y	0.65 a-d	0.98 b
	120	84.2 a-e	855.00 a-e	1.84 n-y	0.55 c-h	0.96 b-d
	180	75.8 e-j	763.33 c-l	1.94 m-v	0.52 e-j	0.87 fg
	240	71.7 g-l	660.00 j-r	2.79 b-g	0.36 l-p	0.82 h-k
US-10	0 (Control)	86.7 a-c	920.00 ab	1.37 y	0.73 a	-
	60	73.3 f-k	773.33 c-k	1.45 vy	0.69 ab	0.85 gh
	120	70.8 h-m	745.83 d-m	1.47 u-y	0.69 ab	0.82 h-l
	180	67.5 i-n	686.67 h-p	1.83 n-y	0.56 c-g	0.78 m-p
	240	66.7 j-n	676.67 i-r	1.85 n-y	0.55 c-h	0.77 n-p
Leed	0 (Control)	89.2 a	944.17 a	1.41 vy	0.71 a	-
	60	70.0 h-n	703.33 f-o	2.02 k-t	0.55 c-h	0.79 l-o
	120	50.0 p-s	486.67 uv	2.27 g-o	0.45 f-n	0.56 u
	180	31.7 t	306.67 y	2.32 f-o	0.43 g-o	0.36 v
	240	28.3 t	262.50 y	2.77 b-h	0.36 l-p	0.32 y
AC Sunset	0 (Control)	84.2 a-e	820.83 a-h	2.24 h-o	0.45 f-n	-
	60	82.5 a-f	790.83 b-j	2.39 f-m	0.42 h-o	0.98 b
	120	81.7 a-f	746.67 d-m	2.79 b-g	0.36 l-p	0.97 bc
	180	68.3 i-n	627.50 l-s	2.84 b-f	0.36 l-p	0.81 i-l
	240	61.7 l-o	518.33 s-v	3.62 a	0.28 p	0.73 rs
Lesaf 414	0 (Control)	85.0 a-d	896.67 a-c	1.46 u-y	0.71 a	-
	60	84.2 a-e	884.17 a-c	1.48 u-y	0.70 a	0.99 a
	120	80.8 b-g	825.83 a-g	1.78 o-y	0.57 b-f	0.95 b-d
	180	70.8 h-m	698.33 f-o	2.15 j-p	0.47 f-m	0.83 h-j
	240	44.2 s	431.67 v	2.22 i-o	0.45 f-n	0.52 u
Quiriego 88	0 (Control)	86.7 a-c	880.83 a-d	1.83 n-y	0.55 c-h	-
	60	80.8 a-g	816.67 a-h	1.89 m-y	0.53 d-i	0.93 d
	120	70.0 h-n	700.83 f-o	1.99 l-u	0.50 e-k	0.81 j-m
	180	65.0 k-n	617.50 m-t	2.50 e-l	0.40 i-p	0.75 pr
	240	60.8 l-o	560.00 p-v	2.80 b-g	0.36 l-p	0.70 s
San Jose 89	0 (Control)	82.5 a-f	817.50 a-h	2.09 j-r	0.48 f-l	-
	60	73.3 f-k	720.00 e-n	2.18 i-o	0.46 f-n	0.89 ef
	120	67.5 i-n	651.67 k-s	2.36 f-n	0.43 h-o	0.82 h-l
	180	65.8 j-n	624.17 m-s	2.52 d-l	0.40 i-p	0.80 k-n
	240	59.2 n-r	550.00 r-v	2.71 c-i	0.37 l-p	0.72 s
FO2	0 (Control)	73.3 f-k	728.33 e-n	2.07 j-s	0.49 f-l	-
	60	71.7 g-l	702.50 f-o	2.21 i-o	0.46 f-n	0.98 b
	120	69.2 h-n	620.83 m-s	3.03 b-d	0.33 n-p	0.94 cd
	180	48.3 s	431.67 v	3.08 bc	0.33 n-p	0.66 t
	240	15.0 u	130.83 z	3.26 ab	0.31 op	0.20 z



**Figure 1.** Relationships and correlation between germination parameters generated by Heat map using mean values of studied traits in safflower genotypes under salt. Color scale displays the intensity of normalized mean values of different parameters. (GP, Germination percentage; TGI, Timson's germination index; MGT, Mean germination time; MGR, Mean germination rate; GSI, Germination stress index)

cultivars based on their germination capacities. Leed (28.3%), FO2 (15%), Lesaf 414 (44.2%) and UC-1 (49.2%) had lower than 50% germination at 240 mM salt concentration. The other safflower cultivars all had higher than 50% germination percentages.

Timson's germination index uses daily cumulative germination percentage and the higher value of Timson's index correlates to speedy germination. Consequently, control seeds, which have not been exposed to salt stress, had higher values for Timson's index, hence speedier germination as observed by mean germination times as well. As germination percentage decreased and mean germination time increased with higher salt concentrations, Timson's germination index, speed of germination, decreased. The lowest values for Timson's germination index was found for FO2 and Leed, which had the lowest germination percentages and high mean germination times at 240 mM salt concentrations.

Mean germination time is another germination parameter used to calculate relative germination speed. It is used to compare effects of different treatments on speed of germination but it does not always correlate with time to germinate to reach a specific germination percentage [25]. Increased salt concentrations caused an increase in mean germination times of cultivars. However, mean germination times of FO2 (3.26) and Leed (2.77) was lower than AC Sunset (3.62) at 240 mM salt concentration, whose germination percentage was higher than FO2 and Leed. Mean germination time did not correspond to germination percentages at 240 mM salt concentration may have overestimated speed of germination in FO2 and Leed. Similarly, San Jose 89 had high mean germination time at 0 mM but

mean germination time increase for 240 mM was 0.62, lower than Royal's mean germination time.

Mean germination rate is based on mean germination time and they have inverse relationship to each other. As mean germination time is higher, mean germination rate gets lower. Consequently, higher mean germination rates were observed in control seeds, but mean germination rate values did not correspond to germination percentages. Both San Jose 89 and Lesaf 414 had similar germination percentages for their controls, their mean germination rates were very different (Table 3) due to differences in mean germination times, and negative correlation (-0.95) observed between the two parameters (Table 3).

Germination stress index was also calculated to determine effects of salt stress on germination capacity of seeds. Leed showed 29% decrease in germination stress index from 60 mM to 120 mM salt concentration. The highest decreases after Leed at the same salt concentrations was observed in Quiriego 88 and Gila by 13% and 12%; respectively. At 180 and 240 mM salt concentrations, both Leed and FO2 had the lowest germination stress index values compared to other genotypes. Based on germination stress index, Leed was more susceptible than FO2 and Royal was the most tolerant cultivar to salt stress at germination stage.

Based on germination indices, it could be concluded that the germination percentage, Timson's germination index and germination stress index were the best indices for the assessment of the germination capacity compared to mean germination time and mean germination rate. These two indices

might be affected by the seed quality and seed features, such as imbibition time. These indices might reflect differences related to those characters rather than the differences in germination capacity under stress conditions. Based on these indices, Leed and FO2 were sensitive and Royal and US-10 were resistant to salt stress at the germination stage.

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### Declaration of Ethical Code

*In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.*

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