Assessment of safflower genotypes for individual and combined effects of drought and salinity stress at early seedling growth stages

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Abstract: Safflower is a highly valued oilseed crop. Safflower oil is a source of monounsaturated and polyunsaturated fatty acids which are important for a plurality of industries including the edible vegetable oil industry. Safflower is highly resistant to salinity and drought stress, which impede seed germination. Two safflower cultivars and 4 lines were screened against nine levels of osmotic stress treatments (using NaCl and PEG 6000, control, NaCl–0.2, NaCl–0.4, PEG 6000–0.2, PEG 6000–0.4, NaCl–0.2, NaCl–0.4, PEG 6000–0.2, NaCl–0.4, NaCl–0.2 + NaCl–0.4 MPa) for 8 days at seed germination stage. The osmotic stress was observed to induce a negative effect on the percentage of seed germination, shoot length, root length, root/shoot length ratio, fresh and dry weights of seedlings as well as on germination, and seedling vigor index. Lines and varieties significantly differed for respective and combined effects of stress treatments at early seedling growth stages. These results demonstrated that the respective effects of PEG 6000 and NaCl on safflower genotypes seedlings were less detrimental than the combined effects. Despite safflower varieties and lines were observed to be affected differently by individual and combined stresses, cv. Dinçer, lines G–8, and G–6 showed a tolerance response to the studied conditions. Significant positive correlations were identified between shoot length and germination percentage (r = 0.91*), and root length (r = 0.98**). A significant correlation was observed between seedling dry weight and root length (r = 0.94**), shoot length (r = 0.91*), and seedling fresh weight (r = 0.97**). A significant correlation was observed between seedling fresh weight and root length (r = 0.90*), and shoot length (r = 0.87*). Cv. Dinçer, lines G–8, and G–6 were determined to be significantly tolerant against salinity, drought, and combined stresses for the studied traits using principal component analysis. According to these findings, effects individually produced by salinity and drought on seedlings of safflower are less damaging to plant development when compared to the combined effects of said two stresses. This study emphasized the importance of monitoring the genotypes of safflower for salt and drought stress tolerances in breeding programs.

Key words: NaCl, PEG 6000, screening, germination, Carthamus tinctorius

1. Introduction

Safflower (Carthamus tinctorius L.) of the family Asteraceae, adapted to hot and drought-hit environments, is a self-pollinated annual multipurpose vegetable oilseed and dye crop. It is also grown for obtaining pharmaceutically important compounds the world over (Ali et al., 2020; Biradar et al., 2022; Hosseinizadeh–Bandbafha et al., 2022). About 690,000 t of safflower seeds are produced in an area of 840,835 ha worldwide. Turkey produced 21,883 t of safflower seeds on about 15,860 ha of land in 2019 (FAO, 2020).

Recurrent or unexpected droughts stemming from global warming have severe negative effects on the quantity and quality of crop yield as well as on all aspects of human life both in Turkey and in many countries around the world (Birpinar and Tugac, 2022; Kumar et al., 2022). Salinization along with drought affects approximately 1 billion ha (or about 7% of the earth’s land surface) of the world’s soils. Nearly one-third of the irrigated land (equal to about 70 Mha) is salt-affected and the extent of affected land grows continuously at the rate of approximately 1.0–2.0 Mha/year (Tian et al., 2020; Hopmans et al., 2021). Estimations of the Turkish State Meteorological Service suggest that, based on IPCC scenarios, the number of consecutive dry days is going to increase along with an increase of 2–3°C in average temperatures and precipitation is going to suffer a significant decrease until the year 2100 (Birpinar and Tugac, 2022). Drought and salinization, negatively affecting both plant growth and development, are major problems currently posing a challenge for most countries around the world (Goharrizi et al., 2021; Ashry et al., 2022; Kumar et al., 2022).

Germination of the seed begins with the intake of water under appropriate germination conditions only.
This is worsened in the presence of salinity and drought that damage and prevent the uptake of water by the seeds in the soil. Injurious effects of these stresses (salinity and drought) on the growth of plants decrease the osmotic potential of the medium, and ion toxicity, resulting in a deficiency in the availability of many nutrients (Alasvandyari et al., 2017; Shaki et al., 2019). Therefore, there is a need for breeding improved varieties that will be tolerant of drought and salinity and exhibit improved growth and development, preventing economic losses to the crop with the ability to grow on fallow, abandoned, and marginal lands. (Arslan and Culpan, 2018).

The negative effects of individual PEG 6000 and NaCl on different crops have been reported previously (Magangana et al., 2021; Kayacetin, 2021; Wu et al., 2021; Habib et al., 2022; Pamuta et al., 2022). A rather limited number of earlier studies conducted on crops other than safflower, such as barley, tomato, wheat, and sweet sorghum, have focused on the combined effects of PEG 6000 and NaCl on these crops (Aliakbari et al., 2021; Ors et al., 2021; Ahmed and Gomaa, 2022; Wang et al., 2022). Despite the studies conducted earlier, data available regarding the response of the safflower to combined salinity and drought stress still remains insufficient. Even though it has been observed that various species respond to salinity and drought stresses, stress thresholds may differ depending on genotypes.

Safflower, having adapted to survive in regions where availability of water is poor, is capable of developing morphological and genetic alterations, conditioned to metabolic and biochemical adjustments. Metabolic changes, however, may impose a limit on plant growth based on the intensity of water stress (Dos Santos et al., 2022), with a variable reduction in seed germination, depression in seedling growth, germination, and seedling responses that could vary depending on the safflower genotypes. Therefore, selecting the desired and tolerant genotypes against salinity and drought stress is desired in safflower breeding to develop new varieties. The available data on the response of safflower to the combined effects of PEG 6000 and NaCl stress remains inadequate, and there is a need for basic information on the cultivation and breeding thereof.

This study aimed to identify the effects of safflower genotypes for individual and combined effects of PEG 6000 and NaCl stress imposed at early seed germination stages, determine correlation coefficients among traits of safflower genotypes under stress treatments, and interpret the interrelationship of treatments × safflower genotypes interaction by principal component analysis.

2. Materials and methods

This study was conducted at the Oilseed Crop Unit, Central Research Institute for Field Crops Yenimahalle, Ankara, Turkey. Seeds of 6 genotypes [2 cultivars (Dinçer and Remzibey), 4 pure lines (G–308, G–6, G–8, and G–135)] were obtained from the institution mentioned above (Table 1). Sixty seeds of each genotype, divided into an equal number of 3 replications, were used in each treatment using glass Petri dishes (100 × 10 mm). They were treated with 10 mL of NaCl \(0\) and PEG 6000 \(0\) (control), NaCl \(-0.2\), NaCl \(-0.4\), PEG 6000 \(-0.2\), PEG 6000 \(-0.4\), PEG 6000 \(-0.2\)+NaCl \(-0.2\), PEG 6000 \(-0.2\)+NaCl \(-0.4\), PEG 6000 \(-0.4\)+NaCl \(-0.2\), PEG 6000 \(-0.4\)+NaCl \(-0.4\), PEG 6000 \(-0.4\)+NaCl \(-0.4\) MPa) every 2 days to regulate their desired water potentials for all genotypes as suggested by (Kayacetin and Khawar, 2021). They were treated with 10 mL of NaCl \(0\) and PEG 6000 \(0\) (control), NaCl \(-0.2\), NaCl \(-0.4\), PEG 6000 \(-0.2\), PEG 6000 \(-0.4\), PEG 6000 \(-0.2\)+NaCl \(-0.2\), PEG 6000 \(-0.2\)+NaCl \(-0.4\), PEG 6000 \(-0.4\)+NaCl \(-0.2\), PEG 6000 \(-0.4\)+NaCl \(-0.4\), PEG 6000 \(-0.4\)+NaCl \(-0.4\) MPa) every 2 days to regulate their desired water potentials for all genotypes as suggested by (Kayacetin and Khawar, 2021). The seeds were allowed to germinate at 22 ± 1 °C (Kayacetin, 2021) in the dark for 8 days. Germination percentage was measured and the filter papers were replaced every 2 days in new Petri dishes to prevent accumulation of respective PEG 6000 and NaCl solutions. The seeds were considered germinated when ~2mm radicle showed emergence.

The genotypes were measured and evaluated for germination percentage (GP), germination index (GI), seedling vigor index (SVI), root length (RL), shoot length (SL), root/shoot length percentage (R/S), and seedling fresh weight (SFW) in all replicates following Khodarahmpour et al. (2013). Seedling dry weight (SDW) was measured by drying the samples at 70 °C for 48 h in an oven (Böhm, 2002).

Table 1. List of safflower genotypes used in this study.

<table>
<thead>
<tr>
<th>No</th>
<th>Genotype</th>
<th>Origin</th>
<th>Phenotype</th>
<th>Registration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>flower color</td>
<td>spininess</td>
</tr>
<tr>
<td>1</td>
<td>Remzibey</td>
<td>Turkey</td>
<td>yellow</td>
<td>spiny</td>
</tr>
<tr>
<td>2</td>
<td>Dinçer</td>
<td>Turkey</td>
<td>orange</td>
<td>spineless</td>
</tr>
<tr>
<td>3</td>
<td>G–6</td>
<td>India</td>
<td>orange</td>
<td>spineless</td>
</tr>
<tr>
<td>4</td>
<td>G–308</td>
<td>Pakistan</td>
<td>yellow</td>
<td>spineless</td>
</tr>
<tr>
<td>5</td>
<td>G–135</td>
<td>USA, Arizona</td>
<td>white</td>
<td>spiny</td>
</tr>
<tr>
<td>6</td>
<td>G–8</td>
<td>Afghanistan</td>
<td>yellow</td>
<td>spiny</td>
</tr>
</tbody>
</table>
Seedling Vigor Index (SVI – %) = (Average radicle length + Average plumule length) × Germination percentage

Statistical analysis: A completely randomized two factorial experimental design with three replications was used. The main factor was safflower genotypes and the stress levels were the subfactors. Analysis of variance (ANOVA) was computed for seed germination and growth parameters. The comparison of the treatment means was performed by LSD Test (p < 0.05) using Mstat–C computer software. Correlation analyses were performed among the germination and seedling growth characters of safflower genotypes under individual and combined stress treatments PEG 6000 and NaCl genotypes, and the correlations were calculated using correlation coefficient (p < 0.05; p < 0.01). Principal component analysis was utilized in order to determine the effect of treatments × safflower genotypes in terms of studied traits.

3. Results and discussion
The individual and combined effects of PEG 6000 and NaCl stress on safflower’s early seedling growth stages were statistically significant (p < 0.05). Increasing concentrations of PEG 6000 and NaCl in the growth medium induced a marked reduction in final germination percentage, root length, shoot length, seedling fresh and dry weight, germination index, and seedling vigor index in vegetative growth of the safflower genotypes. The plants treated with a combination of both stresses, induced higher reduction in the tested attributes could be distinguished. Stress treatment, genotypes, and interaction between stress treatment and genotypes had a significantly negative effect on all tested attributes compared to the control treatment (Figure 1).

Germination percentage, germination index, and seedling vigor index: Effect of different treatments on germination percentage, germination index, and seedling vigor index of safflower genotypes, as affected by PEG 6000 and NaCl treatments genotypes, is shown in Table 2; Figures 2, 3 and 4. The maximum germination percentage (96.67%) for the control treatment (no stress) was observed in Dincer. The minimum germination percentage value, as noted in G–135 under PEG 6000\( _{0.4} \)+NaCl\( _{0.4} \) MPa treatment, was 48.33%, whereas the maximum germination index (106.00%) and seedling vigor index (1322.92%) were determined for G–135 under NaCl\( _{0.4} \) MPa and Dincer under control treatment. The minimum germination index (55.36%) and seedling vigor index (126.17%) were noted on G–135 and G–135 under PEG 6000\( _{0.4} \)+NaCl\( _{0.4} \) MPa treatments, respectively. The maximum vigor index (1236%) was noted on Dincer under control treatment, while the minimum vigor index (126%) was detected on G–135 under PEG 6000\( _{0.4} \)+NaCl\( _{0.4} \) MPa treatment. Abido and Zsomkib (2018) also observed that water potential influenced the germination percentage of six safflower genotypes. Germination percentage showed a significant reduction in eight rice genotypes due to varying increased levels of PEG–induced water stress (Shereen et al., 2019). The water stress at early seedling growth stages induced delay, decrease, or complete suppression in germination (Kayacetin, 2021). Variations in seed germination of the genotypes affected by genetic differences could be utilized to select safflower genotypes and cultivars against undesired saline and drought conditions (Golkar and Taghizadeh, 2018; Davari et al., 2022). Consequently, the results of the current study exhibited that individual (NaCl and PEG 6000) and combined stress treatments influenced the seed germination negatively compared to the control treatment, in the selected genotypes at the initial stages of the seedling growth.

Shoot length, root length, and root/shoot length percentage: The differences among stress treatment, on genotypes and their interaction for root length, shoot length and root/shoot length percentage showed significant differences (Table 2; Figures 5, 6, and 7.). The maximum root length and shoot length values of Dincer under control treatment were 5.96 cm and 7.72 cm, respectively. The minimum root length and shoot length values of G–135 under PEG 6000\( _{0.4} \)+NaCl\( _{0.4} \) MPa were 1.02 cm and 1.60 cm, respectively, while the minimum root/shoot length percentage value of G–135 under PEG 6000\( _{0.4} \)+NaCl\( _{0.4} \) MPa was 0.64%. The maximum root/ shoot length percentage value for G–8 under PEG 6000\( _{0.4} \) MPa treatment was 1.13%. PEG 6000\( _{0.4} \)+NaCl\( _{0.4} \) MPa treatment led to the maximum decrease of root and shoot length of the genotypes, which was the most remarkable for the G–135 genotype. High genetic differences were noted in shoot length. Earlier studies have shown that root-related traits were affected significantly by water deficit (Zullo et al., 2020; Qayyum et al., 2021). Similar to these studies Kayacetin (2021) has also noted a significant influence of individual PEG–NaCl induced water stress in Brassica species. Rapid and early root elongation indicate increased drought resistance (Dayob et al., 2021). Shoot and root length along with root/shoot length percentage are reduced as water potential is improved (Table 2). Rana et al. (2017) noted a decline in shoot and root length, which acts as a barrier to the multiplication of root cells and shoots elongation, during utilization of seed reserves. As compared to plant shoots, the physiological changes in roots show less sensitivity to water deficiency (Ahmad 1979). GP, GI, and SVI parameters were also measured as given below (Abdul–Baki and Anderson, 1973):

\[
\text{Germination Percentage (GP – %) } = \frac{\text{Total number of germinated grains} \times 100}{\text{Total number of observed grains}}
\]

\[
\text{Germination Index (GI – %) } = \frac{\text{Germination percentage in each treatment} \times 100}{\text{Germination percentage in the control}}
\]
et al., 2017). These varying responses of genotypes to treatments, showing differential sensitivity depending on genetic sensitivity to water deficiency, have significance for the plant breeders; and help to select drought-tolerant genotypes at the early seedling stage without involving extensive field trials (Meher et al., 2017; Rana et al., 2017; Zuffo et al., 2020). Several plants react to both individual stresses by raising the root/shoot length percentage of assimilation used for growth. This improves root/shoot length percentage along with soil water volume available to the treated plants (Ahmad et al., 2017).

**Seedling dry weight and seedling fresh weight:** The maximum seedling fresh weight (20.21 mg) and seedling dry weight (2.89 mg) were the most remarkable for Dincër under control treatment, while the minimum seedling fresh weight (6.65 mg) and seedling dry weight (1.77

Figure 1. Effect of different treatments PEG 6000 and NaCl on seedling of safflower genotypes.
Table 2. Effect of different treatments on studied traits of safflower genotypes.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination percentage (%)</th>
<th>Germination index (%)</th>
<th>Seedling vigor index (%)</th>
<th>Root/shoot length percentage</th>
<th>Seedling fresh weight (mg)</th>
<th>Seedling dry weight (mg)</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 6000 (MPa)</td>
<td>NaCl (MPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>91.11a</td>
<td>100.00a</td>
<td>823.82a</td>
<td>0.81e</td>
<td>16.47a</td>
<td>2.52a</td>
<td>4.94a</td>
</tr>
<tr>
<td>N&lt;sub&gt;0.2&lt;/sub&gt;</td>
<td>90.28ab</td>
<td>99.11a</td>
<td>601.56b</td>
<td>0.83de</td>
<td>14.04b</td>
<td>2.35bc</td>
<td>3.62b</td>
<td>3.00b</td>
</tr>
<tr>
<td>N&lt;sub&gt;0.4&lt;/sub&gt;</td>
<td>87.50b</td>
<td>96.20b</td>
<td>568.40c</td>
<td>0.88cd</td>
<td>14.38b</td>
<td>2.42b</td>
<td>3.43c</td>
<td>3.03b</td>
</tr>
<tr>
<td>P&lt;sub&gt;0.2&lt;/sub&gt;</td>
<td>Control</td>
<td>80.56c</td>
<td>88.61c</td>
<td>469.46d</td>
<td>0.93bc</td>
<td>12.28c</td>
<td>2.28cd</td>
<td>3.02d</td>
</tr>
<tr>
<td>N&lt;sub&gt;0.2&lt;/sub&gt;</td>
<td>74.72d</td>
<td>82.07d</td>
<td>455.18c</td>
<td>0.88cd</td>
<td>14.38b</td>
<td>2.42b</td>
<td>3.43c</td>
<td>3.03b</td>
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<tr>
<td>N&lt;sub&gt;0.4&lt;/sub&gt;</td>
<td>71.11e</td>
<td>78.10e</td>
<td>362.14e</td>
<td>0.89c</td>
<td>10.78d</td>
<td>2.27de</td>
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<tr>
<td>P&lt;sub&gt;0.4&lt;/sub&gt;</td>
<td>Control</td>
<td>64.17f</td>
<td>70.43f</td>
<td>327.98f</td>
<td>0.95ab</td>
<td>9.88e</td>
<td>2.19ef</td>
<td>2.47d</td>
</tr>
<tr>
<td>N&lt;sub&gt;0.2&lt;/sub&gt;</td>
<td>56.67g</td>
<td>62.18g</td>
<td>254.85g</td>
<td>0.95ab</td>
<td>9.01f</td>
<td>2.14f</td>
<td>2.29g</td>
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<tr>
<td>N&lt;sub&gt;0.4&lt;/sub&gt;</td>
<td>52.50h</td>
<td>57.63h</td>
<td>213.28h</td>
<td>0.88cd</td>
<td>8.42g</td>
<td>2.01g</td>
<td>2.38d</td>
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<tr>
<td>Cultivars–genotypes</td>
<td>Remzibey</td>
<td>72.22c</td>
<td>77.38c</td>
<td>387.67d</td>
<td>0.91ab</td>
<td>10.19d</td>
<td>2.20c</td>
<td>2.74e</td>
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<tr>
<td>Dinçer</td>
<td>78.70a</td>
<td>81.42b</td>
<td>627.61a</td>
<td>0.88b</td>
<td>13.64a</td>
<td>2.43a</td>
<td>4.09a</td>
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<td>G–6</td>
<td>76.11b</td>
<td>81.55b</td>
<td>536.36b</td>
<td>0.95a</td>
<td>13.04b</td>
<td>2.37ab</td>
<td>3.46b</td>
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<tr>
<td>G–308</td>
<td>71.11c</td>
<td>80.50b</td>
<td>388.74d</td>
<td>0.89b</td>
<td>11.48c</td>
<td>2.32b</td>
<td>2.82d</td>
<td>2.51d</td>
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<tr>
<td>G–135</td>
<td>70.56c</td>
<td>84.67a</td>
<td>294.88e</td>
<td>0.83c</td>
<td>9.43e</td>
<td>2.04cd</td>
<td>2.22f</td>
<td>1.85e</td>
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<td>G–8</td>
<td>77.04ab</td>
<td>84.04a</td>
<td>482.52c</td>
<td>0.93a</td>
<td>12.78bc</td>
<td>2.34b</td>
<td>3.15c</td>
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<tr>
<td>Fvalue&lt;sub&gt;S&lt;/sub&gt;:</td>
<td>26.66</td>
<td>13.91*</td>
<td>339.14*</td>
<td>8.77*</td>
<td>98.32*</td>
<td>35.02*</td>
<td>724.44*</td>
<td>159.91*</td>
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<tr>
<td>Fvalue&lt;sub&gt;T&lt;/sub&gt;:</td>
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<td>336.31*</td>
<td>578.92*</td>
<td>10.10*</td>
<td>162.95*</td>
<td>27.76*</td>
<td>829.86*</td>
<td>105.7*</td>
</tr>
<tr>
<td>Fvalue&lt;sub&gt;S×T&lt;/sub&gt;:</td>
<td>2.66*</td>
<td>3.05*</td>
<td>31.64*</td>
<td>3.50*</td>
<td>8.34*</td>
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</tr>
<tr>
<td>CV (%):</td>
<td>4.64</td>
<td>4.48</td>
<td>7.46</td>
<td>8.62</td>
<td>7.48</td>
<td>5.43</td>
<td>4.06</td>
<td>9.09</td>
</tr>
</tbody>
</table>

There is no significant difference among means indicated with the same letter at p < 0.05 level.

Figure 2. Effect of individual and combined treatments × safflower genotypes on GP (%).
The safflower accessions or genotypes induced lengthy roots due to a limited supply of water, showing an adaptive reaction to increased water uptake. This decrease in water contents of safflower genotypes could be elaborated by a decrease in water-flow from the roots to the respective shoots. Consequently, highly tolerant genotypes are desired while selecting the response of the genotypes, and reactions to both salt and drought stress for screening. The safflower accessions or genotypes inducing lengthy roots due to a limited supply of water show an adaptive reaction to increased water uptake capacity. There is insufficient evidence to back that salt and drought tolerance in genotypes could exhibit advantageous growth utilizing denser root/shoot dry matter, and their longer lengths under water-induced stress. These results are in accordance with earlier observations made for wild mustard as reported by Kayacetin et al. (2018); for B. juncea, B. rapa, B. nigra and S. alba by Kayacetin (2021) as well as for safflower by Kayacetin and Khawar (2021); for soybean by Fu et al. (2022). Individual both NaCl and PEG 6000 treatments inhibited seed germination and seedling growth properties in the safflower genotypes used in this study. Wang et al. (2022) reported that the combined stress induced by PEG 6000 and NaCl, which produced negative

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**Figure 3.** Effect of individual and combined treatments × safflower genotypes on GI (%).

**Figure 4.** Effect of individual and combined treatments × safflower genotypes on SVI (%).

mg) were the most remarkable for Remzibey and G–135, respectively under PEG 6000ₐ₀Nₐ₀NaClₐ₀, MPa (Table 2; Figures 8 and 9). Water contents of all genotypes showed a significant reduction comparing dry seedlings weights with those of fresh seedling weights which were highly visible in Remzibey. This decrease in water contents of safflower genotypes could be elaborated by a decrease in water-flow from the roots to the respective shoots. Consequently, highly tolerant genotypes are desired while selecting the response of the genotypes, and reactions to both salt and drought stress for screening. The safflower accessions or genotypes inducing lengthy roots due to a limited supply of water show an adaptive reaction to increased water uptake capacity. There is insufficient evidence to back that salt and drought tolerance in genotypes could exhibit advantageous growth utilizing denser root/shoot dry matter, and their longer lengths under water-induced stress. These results are in accordance with earlier observations made for wild mustard as reported by Kayacetin et al. (2018); for B. juncea, B. rapa, B. nigra and S. alba by Kayacetin (2021) as well as for safflower by Kayacetin and Khawar (2021); for soybean by Fu et al. (2022). Individual both NaCl and PEG 6000 treatments inhibited seed germination and seedling growth properties in the safflower genotypes used in this study. Wang et al. (2022) reported that the combined stress induced by PEG 6000 and NaCl, which produced negative
effects on sweet sorghum seedlings, was more severe compared to effects caused individually by drought or salinity stress. A significant decrease in germination and seedling growth was observed with PEG 6000 and NaCl at the osmotic potentials that indicated specific ionic effects and points that germination is solely controlled by the osmotic potential.

Correlation analysis: The correlation coefficients among germination and seedlings characters of safflower genotypes under individual and combined treatments of PEG 6000 and NaCl are shown in Table 3. They exhibited significant correlation coefficients between root length and germination percentage \( r = 0.91^{*} \). Shoot length exhibited positive correlation with germination percentage \( r = 0.91^{*} \) and root length \( r = 0.98^{**} \). Significant correlation coefficients were noted between seedling fresh weight and root length \( r = 0.90^{*} \), and shoot length \( r = 0.87^{*} \). Seedling dry weight showed a significant correlation with root length \( r = 0.94^{**} \), shoot length \( r = 0.91^{*} \), and seedling fresh weight \( r = 0.97^{**} \). Seedling vigor index
showed a positive and highly significant correlation with germination percentage ($r = 0.94^{**}$), root length ($r = 0.99^{**}$), shoot length ($r = 0.99^{**}$), seedling fresh weight ($r = 0.87^*$) and seedling dry weight ($r = 0.90^*$) also had a positive correlation. The rest of the components showed nonsignificant correlations among them showing that more than one gene controls their functions. Kayacetin (2022) determined that seedling dry weight showed a significant correlation with germination speed, root length, shoot length, and seedling fresh weight; with root length, and shoot length under individual drought and salinity stress. Khan et al. (2020) also determined that the highest correlations...
between SL and RL ($r = 0.83$), shoot length and root length could suggest that screening for these parameters is desired and could be beneficial in breeding studies. According to Hellal et al. (2018), correlation analysis revealed root/shoot length to be correlated parameters for barley genotypes under PEG 6000 stress. Ahmed et al. (2019) reported that while shoot length indicated a nonsignificant correlation, wheat root length, and fresh and dry weights showed positive and significant correlations among one another under the influence of stress, and that controlled conditions, though not extensively achievable with all the studied parameters, were long–lived. Since correlated response as an improvement in characters may introduce even further improvements in another character, a significant positive association between the parameters of germination and seedling growth may result in a rapid and high level of improvement during selection under both salinity and drought stress. These characters were also determined to be primary direct contributors to germination and seedling growth parameters (Maurya et al., 2019). The correlation analysis exhibited that root/shoot length percentage was the highly correlated parameter. The study of the latter or subsequent parameters could act as a beneficial indicator for the screening of potential tolerant genotypes.

**Table 3.** Correlation coefficients among germination and seedling growth characters of safflower genotypes under individual and combined stress treatments PEG 6000 and NaCl.

<table>
<thead>
<tr>
<th></th>
<th>GP</th>
<th>RL</th>
<th>SL</th>
<th>R/S</th>
<th>SFW</th>
<th>SDW</th>
<th>GI</th>
<th>SVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>1.00</td>
<td>0.91*</td>
<td>0.91*</td>
<td>0.49</td>
<td>0.78</td>
<td>0.80</td>
<td>0.15</td>
<td>0.94**</td>
</tr>
<tr>
<td>RL</td>
<td>1.00</td>
<td>0.98**</td>
<td>0.59</td>
<td>0.90*</td>
<td>0.94**</td>
<td>−0.13</td>
<td>0.99**</td>
<td></td>
</tr>
<tr>
<td>SL</td>
<td>1.00</td>
<td>0.44</td>
<td>0.87*</td>
<td>0.91*</td>
<td>−0.11</td>
<td>0.99**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R/S</td>
<td></td>
<td>1.00</td>
<td>0.54</td>
<td>0.63</td>
<td>−0.32</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFW</td>
<td></td>
<td></td>
<td>1.00</td>
<td>0.97**</td>
<td>0.03</td>
<td>0.87*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDW</td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>−0.18</td>
<td>0.90*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td>−0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SVI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**correlation is significant at p < 0.01; *correlation is significant at p < 0.05. GP, germination percentage; RL, root length; SL, shoot length; R/S, root/shoot length percentage; SFW, seedling fresh weight; SDW, seedling dry weight; GI, germination index; SVI, seedling vigor index
Principal component analysis: Principal component analysis was performed effectively through categorization of the genotypes under various environmental conditions, drought and salinity stress in particular. The interrelationship of treatments × safflower genotypes interaction by the principal component analysis for studied traits is shown graphically in Figure 10. The results exhibited that the first two components PCA1 (73.92%) and PCA 2 (15.12%) accounted for 99.04% of total variation with a positive correlation with all indices except GI. Genotypes that possessed high PC1 and low PC2 values are more stable under treatments. Cv. Dinçer, G–6, and G–8 were noted as the most stable and best genotypes (treatments). On the other hand, G–308 and cv. Remzibey were classified as unstable genotypes. G–135 was classified as the most unstable genotype. Stable genotypes under all treatments are important in plant breeding studies subjected to high-stress conditions. The study showed that the growth parameters of cv. Remzibey, G–308, and G–135 were severely influenced compared to cv. Dinçer, G–6, and G–8. PCA analysis was performed on mean data acquired from each genotype that was subjected to various NaCl and PEG stresses. Kaya et al. (2019) reported that cluster analysis revealed two main groups and 81.0% of data variability was represented by PCA1 (67.2%) and PCA2 (13.8%) respectively. Safflower varieties are decisively emphasized to be more tolerant to salinity in comparison to sunflower hybrids, with differences among genotypes.

4. Conclusion
Even though the adverse effects caused by PEG 6000 and NaCl stress on the growth factor of safflower are well known in the literature, combined effects produced by these treatments on safflower have yet to be the subject of extensive research. This study evaluated individual and combined effects of PEG 6000 and NaCl stress factors at the early stages of seedling growth.

This study highlighted the significance of screening safflower genotypes before sowing in drought and salt-affected areas. Salinity and drought stress reduced the plant growth of safflower seedlings.

Individual PEG 6000 and NaCl stress decreased seed germination percentage, shoot length, root length, root/shoot length percentage, seedling dry weight, seedling fresh weight, germination index, seedling vigor index when compared to their performance under the control treatment. Similarly, these parameters showed a greater reduction in all tested attributes under combined drought and salt stress and, the highest reduction in early seedling growth traits was recorded under PEG 6000_{0.4}+NaCl_{0.4} MPa at the osmotic potentials compared to the other treatment of the study.

Lines and varieties significantly differed for individual and combined effects of stress treatments at early seedling growth stages. Among the tested genotypes cv. Dinçer, lines G–8, and G–6 were more tolerant to drought, salt, and combined stress than other safflower cultivars and genotypes. In novel studies, these genotypes may be utilized as a genetic source in order to breed further genotypes that exhibit tolerance to salinity and drought stresses.

It is recommended for this experiment be repeated under field conditions. Additionally, further investigations on safflower’s response to stress factors could prove fruitful in establishing future field plans.

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References


