Comparative Effects of NaCl and Polyethylene Glycol on Germination and Early Seedling Growth of Safflower

Saeed Saedipour

Department of Agronomy, Faculty of Agriculture, IAU, Shushtar Branch, Iran

Abstract

Seeds of KW13 cultivar of safflower (Carthamus tinctorius L.) were tested for salt and drought tolerance at germination and early seedling growth in NaCl and PEG-6000 solutions of different osmotic potentials (0, -0.2, -0.4, -0.8, and -1.0 MPa). Daily and final germination, as well as germination and seedling growth were recorded under controlled conditions. Results showed that germination and germination rate were delayed by both solutions, with differences between solutions. PEG-6000 had a lesser effect in terms of germination rate, mean germination time and the final germination and seedling growth than did sodium chloride. This conclusively proves that the adverse effect of sodium chloride on germination, emergence and early seedling growth was due to the specific ion rather than the osmotic effect. Seedling growth was reduced by both stresses, but PEG-6000 usually caused less damage than NaCl to safflower seedlings, suggesting that NaCl and PEG acted through different mechanisms.

Keywords: Carthamus tinctorius, drought, germination percentage, germination rate, seedling growth.

Introduction

Safflower (Carthamus tinctorius L.) is an important oilseed crop with 35-40% oil content. It has been used as a source of edible oil and dying since ancient times. More than 90% of the Iranian domestic need for oil is imported. Safflower, one of the native and valuable oil seeds in Iran, is tolerant to drought and salinity. It has a well-defined tap root that generally penetrates up to a depth of 2-3 m. This deep-rooting characteristic allows the plant to absorb moisture and nutrients from different volumes of soil (Weiss, 1983). According to a soil map from FAO-UNESCO, approximately 18-23 million h of arable land in Iran suffers from salinity problems. The salinity
problem is common in arid and semi-arid regions where rainfall is insufficient to leach salts out of the root zone. These areas often have high evaporation rates, which can encourage an increase in salt concentration at the soil surface (Demir et al., 2003). Under saline conditions, it is important to maintain and/or improve soil water availability to crops (Van Hoorn and Van Alpen, 1990). This can be accomplished through several strategies such as leaching salts from the soil profile, maintaining high soil water content in the root zone, selecting more salt tolerant plants and improving cropping systems (Meiri, 1984). Salt and water stresses are responsible for both inhibition or delayed seed germination and seedling establishment (Mayer and Poljakoff-Mayber 1975, Bewley and Black 1994). Under these stress conditions there is a decrease in water uptake both during imbibitions and seedling establishment, and in the case of salt stress, this can be followed by excessive uptake of ions (Prisco and O’Leary 1970). The decrease in both rate and percentage of germination and seedling emergence as a result of the increase in salt concentration in seed environment has been shown by several authors (Chartzoulakis and Klapaki 2000; Murillo-Amador and Troyo-Díez 2000; Murillo-Amador et al. 2001). It has also been shown that the inhibition of radicle emergence is mainly because of a decrease in water potential gradient between the external environment and the seeds (Eneas Filho et al. 1995). The effect of salinity on seedling establishment is more conspicuous than on seed germination (Khajeh-Hosseini et al., 2003). Salinity inhibition of embryo-axis growth during seedling establishment is the result of both delayed reserve mobilization (Gomes Filho et al. 1983; Prisco 1987) and membrane disturbance caused by salinity, and evidenced by increased leakage of materials from the embryo-axis (Prisco 1987). The accumulation of soluble salts in soils leads to an increase in osmotic pressure of the soil solution, which may limit the absorption of water by the seeds or by the plant roots. Salt damage to plants is attributed to the reduction in water availability, toxicity or specific ions, and nutritional imbalance caused by such ions. Polyethylene glycol (PEG) compounds have been used to simulate water stress effects in plants. Several works have studied the effects of NaCl and PEG on growth and ion absorption in rice (Bal and Chattopadhyay 1985), bean and cowpea (Vasquez Tello et al. 1990), bean (Costa-Franca et al. 2000) and tomato (Alian et al. 2000) in order to better understand plant response to salt stress. The present study with electrolyte (NaCl) as well as non electrolyte (PEG-6000) was attempted in order to study their effects on safflower to find out whether germination and early seedling growth are inhibited by salt toxicity or osmotic effect.

Materials and Methods
This experiment was carried out at the Plant Physiology Laboratory and Research Greenhouse of Horticultural Science Department of Shooshtar Islamic Azad University in Iran. Germination, and early seedling growth (7 days) of this cultivar was studied in two experiments using distilled water (control) and osmotic potentials (-0.20, -0.40, -0.80 and -1.00 MPa), which were prepared adding NaCl or polyethylene glycol (PEG MW 6000) to distilled water according to Van’t Hoff’s equation (Lang 1967) to have the same osmotic potential in both NaCl and PEG.
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Germination

Seeds of safflower were previously disinfected by immersion in a calcium hypochlorite solution, containing 5% active chlorine, for 5 min. The seeds were then washed three times with sterilized distilled water. Germination tests were carried out in sterilized Petri dishes [(150×15 mm)] covered at the bottom with a cotton layer] that had been autoclaved. Each dish was moistened with uniform amounts of desired osmotic solutions (NaCl or PEG-6000 solutions, osmotic potentials of 0, -0.20, -0.40, -0.80 and -1.00 MPa). Germination tests were carried out in a dark growth chamber (Percival, Model MB-60B, Controlled Environment, Percival Manufacturing Company, Boone, Iowa 50036, USA) at 25 °C ± 0.5 °C and 80% ± 1% of relative humidity. Three millilitres of the appropriate solutions were added daily to each dish. Seeds were considered germinated when the radicle was at least 2-mm long. Germination percentage was recorded every 24 h for 10 d. Mean germination time (MGT) was calculated for the rate of germination (Ellis and Roberts, 1980) with the following formula:

\[ MGT = \frac{\sum (fx)}{\sum f} \]

where \( f \) is the number of newly germinated seeds on each day and \( x \) is days of counting.

Germination index (GI) was calculated by the method of Wang et al (2004) with the formula as following:

\[ GI = \sum (G/T_i) \]

where \( G_i \) is the germination percentage at the \( i \)th day, and \( T_i \) is days of germination test. The number of germinated seeds was recorded daily (germination rate), and the final germination percentage was determined after 7 days. The experimental design was hierarchical with respect to two factors arranged in a completely randomized design with three replications of 20 seeds per replicate. The first factor had two levels (NaCl and PEG-6000) and the second one had five levels (0, -0.20, -0.40, -0.80 and -1.00 MPa). Using this design the data for the final germination percentage was analyzed, after arcsine transformation (Sokal and James Rohlf 1988), with two-way analyses of variance (ANOVAS). Germination rates, which were the sum of germinated counts per day, were not transformed before analysis. Data were analyzed using the Statistical Analysis System (SPSS.16).

Seedling growth

Five seedlings were chosen randomly and seedling growth was measured by dry and fresh weights of different parts of the seedling on the 7 day after germination. Dry weight was determined after drying each organ in a forced-air dryer at 80 °C for 48 h. The total height (hypocotyl + epicotyl) was measured with a digital caliper (General, no. 143, General Tools Manufacturing Co., Inc., New York, NY). The variables total dry and fresh weights, and the height were analysed in a two-way analysis using this design an analysis of variance (ANOVAS) was performed, and the F-test was applied to determine the values that were statistically different (Snedecor 1956). All statistical tests were carried out using the Statistical Analysis System (SPSS.16).
Results
Germination
The ANOVA for all investigated characters were significant at $P < 0.001$ (Table 1). The germination rate decreased with the decrease in osmotic potential in both NaCl and PEG solutions, but it declined considerably with decreasing water potential under NaCl, especially at -0.8 and -1 MPa, that non-seed germinated (Fig. A), it is evident that NaCl was toxic, because under the same water potential by PEG the germination percentage 51.67 and 43.33 were obtained respectively (Fig. A).

Significant differences were observed between solutions, osmotic potentials and interactions with regard to germination rate (Fig. 4), whereas in the final germination percentage solutions and osmotic potentials and their interaction were found to be significant (Table 1), which indicates that solutions and osmotic potentials were different. Final germination percentage decreased and delayed as osmotic potential increased at both solution compare to control (Fig 2, 3), proves that the adverse effect of osmotic effect at lower water potential (-0.2 and -0.4 MPa) and specific ion in more osmotic potential (-0.8 and -1.0 MPa) on germination. Mean germination time was delayed by decreasing water potential. Salinity affected more adversely than did PEG (Fig 5). Decreasing water potential by NaCl and PEG caused a remarkable decrease in seedling fresh weight (Fig.6). Differences determined among the solution were significant. NaCl had more inhibitory than PEG at all osmotic potential and cause to decrease fresh weight of seedling (Fig.6). Seedling dry weight showed a trend similar to that of fresh weight, and, depending on the decline in seedling fresh weight, dry weight decreased with increasing NaCl and PEG (Fig.7), however salt stress had severe effect than drought in decreasing dry matter, where as even -0.2 MPa of NaCl solution was further inhibited (61%) than -0.4 MPa PEG solution (47%) compare to control treatments, and under higher osmotic potential by salinity no seeds were able to grow at -0.8 and -1 MPa (Fig. 7). Increasing NaCl and PEG resulted in decrease in root length, but as other measurements salinity cause to more decline than PEG, different solution of PEG had the same effect on radicle length and there were not found significant differences between them (Fig.8). Shoot length was severely influenced by salt and drought stress but inhibition was greater in NaCl (Fig.9). No shoot length was recorded at -0.8 and -1 MPa of NaCl. Salinity stress depressed the shoot growth rather than their root growth.

Discussion
In this study, the cultivar (KW13) of safflower was evaluated for its tolerance to an imposed saline and water stress in controlled conditions during germination and the early seedling growth stage. Results showed that, the cultivar display distinct responses to salinity and drought stress. In this sense, the genes coding for enzymes variability offers a valuable tool for studying mechanisms of salt and drought tolerance. One of these mechanisms depends on the capacity for osmotic adjustment, which allows growth to continue under saline conditions. This is basically true for water stress, although osmotic adjustment is not achieved in the same way under both stresses. Under salt stress, this process is accomplished by uptake and accumulation of
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inorganic ions, mainly Na and Cl. Under water stress, it is achieved by synthesis and accumulation of organic compatible solutes (Alian et al. 2000). However, in this study unfortunately, these ions and organic solutes were not measured.

NaCl and PEG adversely affected the germination and seedling growth of safflower but NaCl had a greater inhibitory effect than did PEG. Salinity (NaCl) may affect germination by facilitating the intake of toxic ions, which may change certain enzymatic or hormonal activities of the seed (Smith and Comb 1991). These physicochemical effects upon the seed seem to result in a slower and/or lower rate of germination or emergence. Both osmotic and toxic effects of salts have been implicated in inhibition of seed germination (Zhu JiaoJun 2006). Other studies with legumes such as Prisco and O’Leary (1970) where it was reported that germination of red kidney bean (Phaseolus vulgaris L.) was inhibited by osmotic effects of NaCl greater than -0.8 MPa osmotic potential, but both osmotic and toxic effects of NaCl inhibited germination at less than -1.2 MPa osmotic potential.

Both, NaCl and PEG inhibited absorption, but PEG to a greater extent, and this distinct is obvious from compare of initial slope after beginning of germination in germination accumulative curves (Fig 2&3), especially at moderate potential (-0.2 and -0.4 MPa), suggesting that ion uptake and osmotic adjustment occurred in seeds treated with the NaCl solutions, but the severe inhibition induced by NaCl happened in higher potential (-0.8 & -1 MPa) in result of toxic ions. Redman (1974) found NaCl to be toxic to germinating alfalfa seeds based on differences in recovery from germination in isosmotic NaCl and mannitol solutions. Redman (1974) also found differences among alfalfa cultivars in tolerance to both osmotic and toxic effects of NaCl. On the other hand.

Bal and Chattopadhyay (1985) found in Oryza sativa L. that at least in the germination and seedling growth of this species, a specific ion (NaCl) effect rather than osmotic effect (PEG) is the prime cause of salt injury. In the same way, Parmar and Moore (1968) working with PEG and NaCl found that below -0.8 MPa only osmotic effects operated and above that value both osmotic and toxic effects were evident. These results are totally consistent to the findings in this study.

The fresh and dry weights, root and shoot length of the seedlings 7 days after imbibitions show that growth was inhibited by both NaCl and PEG-6000. Apparently, the presence of NaCl or PEG in the germination medium reduces the uptake of water by the seedlings and inhibits the mobilization of the cotyledon reserves to the growing embryonic axis. These data are in agreement with others involving studies of germination in the presence of NaCl or nonionic osmotic solutions such as mannitol or polyethylene glycol (Gomes Filho and Sodek 1988; Jajarmi 2007). Differences between NaCl and PEG were significant for all investigated characters. Inhibited of NaCl was more than that of PEG.

Since the inhibition imposed by NaCl was more than that of PEG, it would appear that the toxic effect was the main factor related to growth inhibition especially above -0.4 MPa. It is evident that at isosmotic concentrations (NaCl) functioned as a stronger growth inhibitor, inducing more effective stress than PEG, given that toxic stress is more harmful to plants during the succulent seedling stage. One possible explanation for these findings is that the cell membrane reflection coefficient for NaCl is more
than for PEG, as a result, the seedlings subjected to salt stress should osmotically adjust faster and more efficiently in PEG than those grown in NaCl solutions.

In conclusion, this paper shows that (a) safflower exhibited variability in their drought and saline tolerance at the germination, and early growth stages; (b) contrary of other finding in other plants, NaCl was more harmful than PEG to seeds during germination and early seedling growth (c) this work is useful for plant breeders by proposing early tests for screening new or existing cultivars for their tolerance to salt or drought.

Table 1: Mean squares from analysis of variance of data for different parameters of Safflower.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>%</th>
<th>GR</th>
<th>MGT</th>
<th>Fr wt</th>
<th>Dry wt</th>
<th>Radicle length</th>
<th>Shoot length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>1</td>
<td>2707.5***</td>
<td>22.14***</td>
<td>51.20***</td>
<td>0.446***</td>
<td>0.033***</td>
<td>35.74***</td>
<td>51.74***</td>
</tr>
<tr>
<td>Osmotic Potential</td>
<td>4</td>
<td>5261.7***</td>
<td>42.79***</td>
<td>6.63***</td>
<td>0.773***</td>
<td>0.051***</td>
<td>24.15***</td>
<td>70.41***</td>
</tr>
<tr>
<td>S x O P</td>
<td>4</td>
<td>1036.7***</td>
<td>0.848***</td>
<td>19.45***</td>
<td>0.043***</td>
<td>0.003***</td>
<td>6.04***</td>
<td>7.62***</td>
</tr>
<tr>
<td>Error</td>
<td>20</td>
<td>11.7</td>
<td>0.01</td>
<td>0.049</td>
<td>0.005</td>
<td>0.001</td>
<td>0.054</td>
<td>0.259</td>
</tr>
</tbody>
</table>

G, GR, MGT, Fr wt and *** abbreviate of Germination, Germination Rate, Mean Germination Time, Fresh weight and significant at 0.001 respectively.

**Figure 1:** Germination rate of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.

**Figure 2:** Germination cumulative percent of safflower under decreasing external osmotic potentials created by NaCl solution. The bars represent the standard error of the mean.
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Figure 3: Germination cumulative percent of safflower under decreasing external osmotic potentials created by PEG-6000 solution. The bars represent the standard error of the mean.

Figure 4: Germination rate of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.

Figure 5: Mean germination time of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.
Figure 6: Fresh weight of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.

Figure 7: Dry weight of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.

Figure 8: Radicle length of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.
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Figure 9: Shoot length of safflower under decreasing external osmotic potentials created by NaCl and PEG-6000 solutions. The bars represent the standard error of the mean.

References


