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# SEED VIGOR AND ION TOXICITY IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) SEEDLINGS PRODUCED BY VARIOUS SEED SIZES UNDER NACL STRESS

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*Abstract* - Safflower is an important oilseed crop and is largely grown for edible oil production in low moisture or salt-affected soils of Turkey. The objective of the study was to find out the quality of seeds with different sized seeds, and the effects of seed size (3, 4 and 5 mm) and NaCl stresses at electrical conductivities of 5, 10, 20 and 30 dS $m^{-1}$ on the germination and early seedling growth of the safflower cultivar Dinçer. Our results show that heavier one-thousand-seed weight (70.5 g) and higher hull percentage (52.9 %) were obtained from large seeds while the oil content and water uptake of small seeds was higher than that of medium or large seeds. Germination percentage, germination time, root length, shoot length, and seedling fresh and dry weight showed size-dependent responses of the seeds to salt stress. In general, medium-sized (4 mm) seeds germinated more rapidly compared to small and large seeds against all levels of salt stress; however, the highest germination percentage and most vigorous seedling growth was recorded from large seeds after 10 days incubation. The inhibitory effect of NaCl levels was exhibited a drastic effect on seedling growth. Increased NaCl resulted in an increase in the $Na^+$ and $Cl^-$ content of the seedlings produced by all seed sizes, while the $K^+$ content was not changed. However, the seedlings from small seeds had the highest $Na^+$ and $Cl^-$ content of all measured NaCl levels. It was concluded that large seeds produced vigorous seedling growth due to a lower ion accumulation under NaCl stress. Consequently, large seeds could be used for successful stand establishment in contrast to their slow germination performance if the cultivation of safflower on saline soils is required.

*Key words:* Germination, seedling growth, salinity, ion analysis, safflower

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## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop with 25-30% oil content. It has been used as a source of edible oil and dying since ancient times (Weiss, 1983). During the last few years, the cultivation of safflower in arid and semiarid regions of Turkey has been expanding because it is well adapted to low-moisture soils (Bayramin and Kaya, 2009). Under these conditions, salinity is a major constraint affecting cultivation since rainfall is insufficient to leach the salt from the root zone and evaporation tends to exceed rainfall (Pessarakli, 1999; Kaya et al., 2003). Safflower is classified as a moderately salt-tolerant crop while varietal differences in salt tolerance have been detected by several researchers (Yermanos et al., 1964; Mass, 1986; Bassil and Kaffka, 2002; Kaya et al., 2004; Siddiqi et al., 2007).

Seed size is an important seed quality characteristically affected by variety, the environment and management practices (Robinson, 1978; Weiss, 1983). However, it is variable, depending on the variation within a population, even in a plant. Safflower has a regular flowering order, beginning on the main stem head and continuing on the primary, secondary and

tertiary heads (Baydar and Ulger, 1998). Yermanos et al. (1964) indicated that seed weight changed according to its position on the head in a plant and decreased from the primary to tertiary heads. Nevertheless, regular flowering, which can last approximately one month, is responsible for the different sized seeds in the plant, suggesting that the development of seeds in a head occurs under varying environmental conditions causing variability in seed size and quality (Weiss, 1983; Munshi et al., 2003). El-Saeed (1966) reported that the highest oil content of 37% was obtained from small seeds and this adverse relationship between seed size and oil percentage depended on an increase in proportion of the hull in large seeds. Siddiqi et al. (2007) found that salinity depressed seed germination of safflower lines but there was a clear difference among safflower lines for germination under NaCl stress. Farhoudi and Motamedi (2010) proposed that small seeds germinated faster and grew higher under saline conditions and that they could be preferred for use in saline soils to achieve better stands. Abud et al. (2010) observed no physiological quality and vigor of safflower seedlings according to seed size. Kaya et al. (2003) reported that the spiny variety of safflower was more tolerant to NaCl compared to spineless varieties and that seedling growth was inhibited by 5.1 dS $m^{-1}$ of NaCl.

In general, farmers prefer to use large or mixed seeds instead of small seeds as they doubt the germination behavior and uniform stand establishment capability of small seeds. There are conflicting reports on the effects of seed size on safflower seed quality, germination and seedling vigor. Consequently, this research was to determine seed quality, germination performance and seedling growth of safflower seeds graded by seed size and subject to different NaCl stresses.

## MATERIALS AND METHODS

This research was conducted at the Seed Physiology and Technology Laboratory of the Central Research Institute for Field Crops in Ankara, Turkey, in 2010. Fresh seed samples from plants harvested in 2010 in Ankara, which is a semi-arid region of Turkey, were used as material. By passing the seeds of the safflower cultivar Dinçer through a series of sieves, the seeds were separated into small (3 mm), medium (4 mm) and large (5 mm) sizes. The 1000-seed weight, hull percentage, water uptake, oil, protein and moisture content of the graded seeds were determined. NaCl concentrations at electrical conductivities of 5, 10, 20 and 30 dS $m^{-1}$ were adjusted before the start of the experiment by using a WTW 3.14i conductivity meter (Germany). Distilled water served as a control (0 dS $m^{-1}$).

Four replicates of 50 seeds for each seed size and NaCl level were germinated between three rolled filter papers with 20 ml of respective test solutions. The papers were replaced every 2 days to prevent the accumulation of salts. Each rolled paper was placed in a sealed plastic bag to prevent evaporation. Seeds were allowed to germinate at 25±1°C in the dark for 10 days. A seed was considered germinated when the emerging radicle grew to 2 mm. Germination percentage was recorded every 24 h for 10 days. Mean germination time (MGT) was calculated for the speed of germination according to ISTA (2003). Root and shoot lengths and seedling fresh and dry weights were measured on the $10^{th}$ day. Dry weight was measured after drying samples at 70°C for 48 h in an oven. Ten grams of seeds from each seed size were placed in Petri dishes containing distilled water to determine the water uptake of the seeds necessary for germination. The water uptake was measured as the fresh weight percentage increase in seed weight.

In order to evaluate the toxic effect of $Na^+$ and $Cl^-$ accumulation, all seedlings from each replication were sampled for mineral analysis. The samples were weighed and separately dried at 70°C for 48 h for mineral analysis ($Na^+$, $K^+$ and $Cl^-$ analysis). Chloride contents were determined with a titrimetric method (Johnson and Ulrich, 1959) and sodium and potassium analyses were performed using a flame photometer (Kacar and Inal, 2008).

The experiment was designed with two factors (3x5) arranged in a completely randomized design

with four replications. The first factor was seed size (3, 4 and 5 mm) and the second was NaCl stress (0, 5, 10, 20 and 30 dS $m^{-1}$). Data given in percentages were subjected to arcsine transformation before statistical analysis. Analysis of variance (ANOVA) for all investigated parameters was performed by using the MSTAT-C computer software, version 2.10 (Michigan State University, USA). Significant differences among means were compared by Duncan Multiple Range test ($p < 0.05$).

## RESULTS

The one-thousand-seed weight, hull percentage, water uptake, oil and protein content of safflower was significantly affected by seed size ($P<0.05$), while moisture content was not changed (Table 1). Increased seed size resulted in an apparent increase in the 1000-seed weight, as expected. The large seeds had significantly higher hull content than the small and medium seeds. The small, medium and large seeds of Dinçer took up 31.7%, 27.6% and 27.5% water before germination, respectively. This showed that seed size greater than 3 mm was not accompanied by a corresponding decrease in the uptake of water to germinate the seeds. However, the oil content of the small, medium and large seeds was 28.4%, 25.3% and 23.0%, respectively, declining with increasing seed size. The moisture content of the sorted seeds did not vary significantly.

The comparative effects of seed size and NaCl levels on germination percentage, mean germination time (MGT), root length, shoot length, seedling fresh and dry weights are shown in Table 2. A higher germination percentage was obtained from the medium seeds with 97.8%, followed by large and small seeds, respectively. Increasing NaCl resulted in a decrease in the germination percentage, though the difference was not significant.

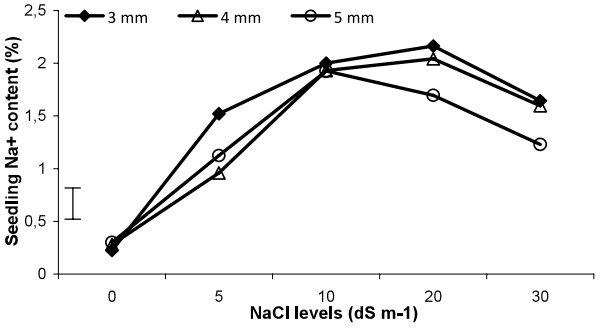



**Fig. 1.** Changes in seedling $Na^+$ content in relation to seed size and NaCl levels. Bar shows LSD value for seed size x NaCl interaction.

A two-way (seed size x NaCl level) interaction was determined for the MGT, root length, shoot length, fresh and dry weight of the seedlings (Table 2). The MGT increased with each increase in NaCl stress and seed size. The medium seeds (4 mm) took the minimum time to germinate under all salt stress conditions. In contrast, the large seeds (5 mm) took the maximum time to germinate under all salt stress conditions.

Increased NaCl concentration decreased the root length; however, this decrease was more prominent with large seeds (5 mm). Significant and higher inhibition due to NaCl was very evident at 10 dS $m^{-1}$ for small, 30 dS $m^{-1}$ for medium and 20 dS $m^{-1}$ for large seeds.

**Table 1.** Seed characteristics of safflower cv. Dinçer with different seed sizes

| Seed size | 1000 seed weight (g) | Hull percentage (%) | Water uptake (%) | Oil content (%) | Protein content (%) | Moisture content (%) |
|---|---|---|---|---|---|---|
| Small | 37.1 | 48.6 | 31.7 | 28.4 | 17.2 | 8.52 |
| Medium | 50.1 | 50.7 | 27.6 | 25.3 | 16.6 | 8.73 |
| Large | 70.5 | 52.9 | 27.5 | 23.0 | 16.1 | 8.67 |
| *$LSD_{0.05}$* | *1.96* | *2.97* | *1.79* | *1.47* | *0.73* | *ns** |

*: ns shows non-significant at p<0.05 level.

**Table 2.** Germination and seedling growth of safflower cv. Dinçer affected by seed size and NaCl level.

| Seed size | NaCl (dS m$^{-1}$) | Germination percentage (%) | MGT (day) | Root length (cm) | Shoot length (cm) | Seedling fresh weight (mg plant$^{-1}$) | Seedling dry weight (mg plant$^{-1}$) |
|---|---|---|---|---|---|---|---|
| Small | 0 | 99.0 (85.9) | 1.52$^{f}$ | 6.40$^{ef}$ | 6.09$^{bc}$ | 589$^{c}$ | 36.1$^{g*}$ |
| | 5 | 98.0 (83.1) | 1.68$^{e}$ | 7.88$^{cd}$ | 6.07$^{bc}$ | 524$^{d}$ | 35.8$^{g}$ |
| | 10 | 93.0 (75.2) | 1.88$^{d}$ | 4.44$^{g}$ | 2.91$^{e}$ | 304$^{ef}$ | 37.3$^{g}$ |
| | 20 | 95.0 (77.4) | 2.09$^{c}$ | 4.42$^{g}$ | 2.11$^{f}$ | 253$^{f}$ | 37.3$^{g}$ |
| | 30 | 90.0 (72.6) | 2.68$^{a}$ | 1.89$^{h}$ | 0.76$^{g}$ | 137$^{g}$ | 36.5$^{g}$ |
| *Mean* | | *95.0 (78.8)$^{b}$* | *1.97* | *4.40* | *3.59* | *362* | *36.6* |
| Medium | 0 | 99.0 (85.9) | 1.43$^{f}$ | 6.62$^{def}$ | 5.88$^{cd}$ | 680$^{b}$ | 42.8$^{f}$ |
| | 5 | 99.5 (88.0) | 1.79$^{de}$ | 5.91$^{ef}$ | 5.95$^{bc}$ | 613$^{c}$ | 52.8$^{c}$ |
| | 10 | 98.5 (86.5) | 1.83$^{d}$ | 9.81$^{ab}$ | 5.44$^{d}$ | 533$^{d}$ | 47.0$^{de}$ |
| | 20 | 97.5 (82.4) | 2.04$^{c}$ | 6.95$^{def}$ | 2.05$^{f}$ | 304$^{ef}$ | 45.5$^{def}$ |
| | 30 | 94.5 (77.6) | 2.49$^{b}$ | 2.46$^{h}$ | 0.79$^{g}$ | 167$^{g}$ | 44.8$^{ef}$ |
| *Mean* | | *97.8 (84.1)$^{a}$* | *1.91* | *6.35* | *4.02* | *459* | *46.6* |
| Large | 0 | 98.0 (83.1) | 1.68$^{e}$ | 8.57$^{bc}$ | 6.45$^{b}$ | 778$^{a}$ | 49.0$^{d}$ |
| | 5 | 99.5 (88.0) | 1.85$^{d}$ | 7.12$^{de}$ | 6.93$^{a}$ | 698$^{b}$ | 58.5$^{a}$ |
| | 10 | 96.5 (80.5) | 1.89$^{d}$ | 10.59$^{a}$ | 6.12$^{bc}$ | 667$^{b}$ | 57.5$^{ab}$ |
| | 20 | 96.0 (79.5) | 2.04$^{c}$ | 5.68$^{f}$ | 1.95$^{f}$ | 309$^{e}$ | 57.8$^{ab}$ |
| | 30 | 94.0 (77.9) | 2.59$^{ab}$ | 1.51$^{h}$ | 0.60$^{g}$ | 164$^{g}$ | 54.5$^{bc}$ |
| *Mean* | | *96.8 (81.8)$^{ab}$* | *2.01* | *6.69* | *4.41* | *524* | *55.5* |

*: Different letters in each column show significant at p<0.05 level. The values in bracket show arcsin transformed values.

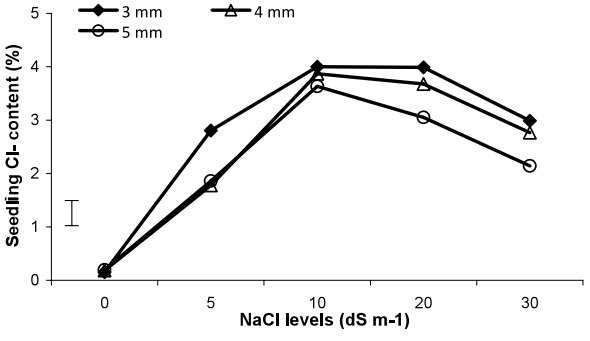



**Fig. 2.** Changes in seedling $Cl^-$ content in relation to seed size and NaCl levels. Bar shows LSD value for seed size x NaCl interaction.

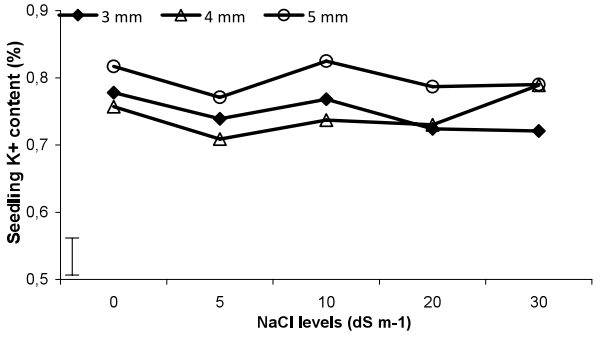



**Fig. 3.** Changes in seedling $K^+$ content in relation to seed size and NaCl levels. Bar shows LSD value for seed size x NaCl interaction.

Shoot length showed a progressive decrease with increasing NaCl. The longest shoot was detected in the control, with 6.45 cm for large seeds. But, the superiority of the large seeds disappeared when the NaCl concentration was increased. At NaCl levels of 20 and 30 dS m$^{-1}$, no significant difference in shoot length was observed.

Depending on the decrease in shoot and root length, seedling fresh weight gradually declined with the increasing NaCl levels. Higher seedling fresh weights were recorded for the large seeds exposed to NaCl stresses.

Compared to the control, each increase in NaCl concentration and seed size caused a slight increase

in seedling dry weight. The maximum seedling dry weight was determined from large seeds exposed to 5 dS $m^{-1}$ of NaCl with 58.5 mg $plant^{-1}$. Large seeds showed apparent superiority in seedling dry weight.

Mineral analysis of the seedling samples from the three seed sizes showed that increased levels of NaCl affected significantly the $Na^+$, $Cl^-$ and $K^+$ content of safflower seedlings (Fig 1, 2 and 3). As expected, the accumulation of $Na^+$ and $Cl^-$ increased with increasing NaCl levels up to 10 dS $m^{-1}$ and they dropped at 20 dS $m^{-1}$. Considering seed size, the seedlings from small seeds had a higher $Na^+$ content than the others. A similar trend was noted for $Cl^-$ in all seed sizes. A lower $Cl^-$ content was recorded for the large seeds. Contrarily, $K^+$ was higher in seedlings from large seeds and the maximum value was obtained from 10 dS $m^{-1}$. A linear increase or decrease in the $K^+$ content of safflower seedlings produced from different sized seeds was not observed.

## DISCUSSION

Seed weight and hull percentage were increased proportionally by increasing the seed size. Abud et al. (2010) found that an increased seed size leads to an increase in seed weight, while there were no changes in the physiological quality and vigor of safflower seedling under optimum conditions. However, our results showed that seed quality parameters such as seed weight, hull percentage, oil and protein content varied with seed size, while higher seedling vigor was obtained from large seeds under both optimum and NaCl conditions. The oil content declined as seed size increased and this relationship was attributed to the increase in the proportion of hull in the large seeds. El-Saeed (1966) stated that increased seed size resulted in a decrease in oil content depending on an increasing in the hull percentage.

The effect of NaCl stress on germination was more prominent in small seeds compared to medium and large. NaCl adversely affected the germination time of safflower in concordance with inhibition of germination percentage. Several researchers reported that the primary action of NaCl is retardation of water uptake by creating an osmotic potential which is crucial for germination (Almansouri et al., 2001; Khajeh-Hosseini et al., 2003; Okçu et al., 2005). Small seeds germinated slower than medium and large seeds under increased NaCl. The results of this study are in agreement with the observations of Ghorashy et al. (1972) and Patil and Bangal (1985). They observed that NaCl reduced the germination percentage and delayed the MGT. Farhoudi and Motamedi (2010) reported that salt stress increased the MGT in both large and small seeds. The lower MGT in the control (0 dS $m^{-1}$) and reduced MGT in small seeds compared to medium and large seeds under all levels of NaCl stress, may be due to more rapid water uptake; the different concentration of NaCl may have created an osmotic barrier resulting in the inhibition of corresponding water uptake. Okçu et al. (2005) emphasized that retardation of germination under NaCl stress was due to osmotic stress created by NaCl.

Root length was severely influenced by increasing NaCl stress; however, large seeds produced the longest roots. Similarly, El-Saeed (1966) observed that large seeds produced longer roots compared to medium and small seeds. Shoot growth was more adversely affected by hypocotyl injury due to different levels of NaCl than the root, especially at 10 dS $m^{-1}$ of NaCl for small seeds and 20 dS $m^{-1}$ for medium and large seeds. The results are in agreement with Charjan and Tarar (1992), who proposed that large seeds exhibited better performance than small seeds. However, Farhoudi and Motamedi (2010) found that small seeds germinated faster; consequently, shoot and seedling growth were higher compared to large seeds.

As a result of decreasing root and shoot lengths the seedling fresh weight decreased under increased NaCl concentrations in all seed sizes of safflower cv. Dinçer. Seedling dry weight floated with increasing NaCl levels, as seen in Table 2, while the effects of salinity varied with seed size. The dry weight of small seeds was not changed by NaCl, while medium and large seeds produced higher dry matter compared to

small seeds. This study further showed that seedling growth was greater in large and medium seeds compared to small seeds, and NaCl had greater inhibitory effects on early seedling growth than on germination.

Accumulation of the $Na^+$ ion caused changes in ion balances and the imbalance of mineral nutrients resulted in a reduction or an inhibition of plant growth (Mer et al., 2000). The $Na^+$ and $Cl^-$ contents of dry seedlings increased with increasing salinity levels and peaked at 20 dS $m^{-1}$. This means that $Na^+$ and $Cl^-$ accumulations are hazardous for seedling growth. The accumulation of $Na^+$ and $Cl^-$ in the seedlings from small seeds was higher than in the others. This may explain that the rapid germination and earlier seedling growth in small seeds leads to higher uptake of the ion from the media. On the other hand, large seeds had more seed reserves than small and medium seeds for producing seedlings. Soltani et al. (2002) elicited that the seedlings from large seeds of chickpea utilized seed reserves at a faster rate and that the large seeds have an advantage in providing energy more rapidly to growing seedlings. Our results are supported by the findings of Soltani et al. (2002). In the study, the $K^+$ concentration in safflower seedlings floated as NaCl increased. Any linear relationship between $K^+$ and NaCl level was not determined while the highest $K^+$ contents were measured in large seeds at all NaCl levels. However, Craig et al. (1990) and Rehman et al. (1996) reported that increasing NaCl concentrations resulted in increasing $K^+$ leakage from seeds. Broadly speaking, the main reason for the restricted seedling growth of the safflower was related to the accumulation of $Na^+$ and $Cl^-$ regardless of $K^+$; consequently, lower $Na^+$ and $Cl^-$ accumulation in the seedlings from large seeds at higher levels of NaCl resulted in more vigorous seedling growth.

In conclusion, the beneficial effects of large seeds under both saline and non-saline conditions were clearly observed in this study. Large seeds (5 mm) germinated properly and produced more vigorous seedlings under NaCl stress, showing that they had the advantage in salt-infected soils of achieving more vigorous plant growth. Accumulation of $Na^+$ and $Cl^-$ was also harmful for seedling growth while the least influenced seedlings were large seeds due to a lower ion accumulation. Furthermore, the seeds should be graded for seed size if any further study is conducted on the salinity effects on safflower during germination.

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