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Evaluation of seed size and NaCl stress on germination and early seedling growth of sunflower (*Helianthus annuus* L.)

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Abstract

Sunflower is well adapted plant to tolerate moderately saline soil conditions**.** In order to investigate this circumstance, an experiment was conducted as a factorial with three factors arranged in completely randomized design with 4 replications and 50 seeds per replicate. Three cultivars (Azrgol, Eurofler and Record) made first factor, the second was made of seed size (small and large) and different NaCl solution levels (0.0, 10 and 15 ds.m^{-1}) made the third one. Germination percentage (%), mean germination time (day), root and shoot length (cm) and seedling dry weight (g) were investigated. In general, small size seeds germinated rapidly compared to large size ones of the same cultivars under salt stress. Also, NaCl caused lower root and shoot length but higher mean germination time. Interaction between seed size × cultivar × NaCl levels had different response to germination percentage. Small size seeds of Azargol and Record showed the highest germination percentage values at all NaCl concentration, while the maximum germination percentage was obtained in large size seeds of Eurofler cultivar under all levels of salt stresses. In conclusion, small size seeds can also be used for sunflower production in saline soils to achieve better stand establishment.

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Introduction

Sunflower (*Helianthus annuus* L.) is becoming an increasingly important source of edible vegetable oil throughout the world because of its high polyunsaturated fatty acid content and no cholesterol. Sunflower is appropriately classified as moderately tolerant to salinity. Each unit increase in salinity above 4.8 ds m^{-1} reduced yield by 5.0% (Francois, 1996). Yield reduction was attributed primarily to a reduction in seeds per head. Oil concentration in the seed was relatively unaffected by increased soil salinity up to 10.2 dS m^{-1} (Francois, 1996). Sunflower appears to be well adapted for growth under moderately saline soil conditions. The concept of developing salt-tolerant plants, even to the degree that they can be grown with seawater, captures the imagination of both the scientific and the public sector. There is a wide range of salt tolerance in vascular plants. There is currently a need to develop new direction and cohesive impetus in the area of salt-tolerant crops development. Strategies concerning selection and breeding for plant salt tolerance (Shannon, 1997). Sunflower is cultivated about 17000 ha area in Iran. Rapid and uniform field emergence is essential to achieve high yield with good quality and quantity in annual crops (Yari *et al.*, 2010). The effect of seed size on germination and seedling emergence of different crop species has been the subject of numerous published studies. However, results from these studies revealed more variety among species (Sadeghi *et al.*, 2011). Seed size is an important seed quality characteristic affected by variety, environment and management practices (Robinson, 1978). However, it is commonly variable depending on variation within population, even in a plant. Irregular flowering on the head is responsible for obtaining different sized seeds in the plant (Kaya and Day, 2012). However, their effects are modified by combination of factors including rainfall, temperature and number of seeds per pod (head) (Smithson *et al.*, 1985). Karadogan *et al.* (1998) and Munshi *et al.* (2003) reported that seed number, thousand seed weight, crude protein and oil content were decreased from periphery to the center. Saranga *et al.* (1998) emphasized that seed vigor was negatively correlated with embryo mass but large seed germinated and emerged later than small seeds. This study aimed to expose the superiority of different sized seeds of three sunflower cultivars under saline conditions.

Materials and methods

The Experiment was conducted in the laboratory of the Seed and Plant Certification and Registration Research Institute, Karaj, Iran in 2013. The seeds of three sunflower cultivars (Azargol, Record and Eurofler) were obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran. They were classified as small and large size seeds by passing through a series of sieves. One hundred seed weight of these three cultivars is shown in Table 1. NaCl concentrations at electrical conductivities of 10 and 15 dSm^{-1} were adjusted before the start of the experiment by using a conductivity meter (model ALF90SER-NA31245385).

Distilled water served as a control (0 dSm^{-1}). Three replication of 50 seeds for each cultivar were germinated between 3 rolled filter papers with 10 ml of respective test solutions. The papers were replaced every 2 day to prevent the accumulation of salts. Each rolled paper was put into a sealed plastic bag. Seeds were allowed to germinate at 25±1˚C under normal light for 10 days (ISTA, 2003). Seeds were considered germinated when radicle protruded for 2mm. Germination progress was measured at 24h intervals for 10 days. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980) with the following formula:

$$\text{MGT(day)} = \frac{\sum TiNi}{S}$$

Where N is the number of newly germinated seeds on each day and t is days of counting, S is the total number of germinated seeds.

Also final germination percentage, root length, shoot length and seedling dry weight were recorded after 10 days.

The experiment was conducted as a factorial with three factors arranged in completely randomized design with 4 replications and 50 seeds per replicate. Three cultivars (Azrgol, Eurofler and Record) made first factor, the second was made of seed size (small and large) and different NaCl solution levels (0.0, 10 and 15 ds.m^{-1}) made the third one.

Experimental data were analyzed using SAS (Statistical Software, SAS institute, 2002) and treatment means were compared using Duncan's multiple range test at 5% level of probability.

Results

Main effects of seed size and NaCl levels on germination percentage (GP), mean germination time (MGT), root length (RL), shoot length (SL), dry weight (DW) of the three cultivars were shown in Table 2.

Higher germination percentage was recorded in Record with small size seeds. Increasing NaCl level resulted decrease in germination percentage. Germination was severely limited at the highest NaCl level (15 ds.m^{-1}) which was reported 32.5%.

A three-way interaction was evaluated for mean germination time, germination percentage, seedling dry weight, root length and shoot length (Table 2). Mean germination time increased with an increase in NaCl level and seed size in all cultivars. The small size seeds of Eurofler showed the minimum time to germinate on all NaCl stresses.

The large size seeds of Eurofler cultivar in general displayed the maximum MGT under 15ds.m^{-1} NaCl stress conditions. Interaction between seed size × cultivar × NaCl levels showed different reaction for germination percentage. Small size seeds of Azargol and Record stated the highest GP values at all NaCl concentration, while the maximum GP were obtained from large size seeds in Eurofler cultivar under all levels of salt stresses.

Germination percentage decreased in all cultivars by increasing NaCl concentration. Also, the maximum seedling dry weight was determined with 0.05g at 10dSm^{-1} in small size seeds of Azargol cultivar. Increasing NaCl concentration caused remarkable decrease in seedling dry weight compared to control.
Increased NaCl levels resulted in the decrease in root length of the cultivars (Table 2). Interaction between seed size and cultivars showed different response to root length. The longest root was obtained from Azargol with large seeds. Root length in Record with small size seeds was longer than with large size seeds.

Shoot length was severely influenced by salt stress compared to root length. Shoot length of cultivars decreased remarkably by increasing NaCl concentration compared to control. The longest shoot length was detected in the control with 8.08 cm for Eurofler cultivar. Clear difference for shoot length occurred among cultivars at NaCl level of 15ds.m^{-1}.

Table 1. One hundred seed weight (g).

Cultivars	Seed sizes	One hundred seed weight (g)
Azargol	small	3.50
	large	6.02
Record	small	4.30
	large	6.00
Eurofler	small	3.12
	large	5.44

Table 2. Main effects and interactions of seed size and NaCl levels on germination percentage (GP, %), mean germination time (MGT, d), root and shoot length (RL, SL, cm) and dry seedling weight (DW, g) of sunflower cultivars.

	GP	MGT	RL	SL	DW
Cultivar					
Azargol	46.22 c	2.25 b	10.26a	4.86a	0.03a
Record	74.44a	2.35b	9.00b	4.68 a	0.03a
Eurofler	67.38b	2.76 a	8.01b	4.7a	0.023b

	GP	MGT	RL	SL	DW
Seed size					
small	66.55 a	2.35 b	9.21 a	4.84 a	0.028a
large	58.81 b	2.55 a	8.97 a	4.64 a	0.027a
NaCl					
0	89.94 a	1.76c	14.20 a	7.69 a	0.037 a
10	65.61b	2.46 b	10.64 b	4.75 b	0.035 a
15	32.5 c	3.13 a	2.35 c	1.79 c	0.012 b

Table 3. Summary of anova.

S.O.V.	df	Shoot dry matter weight	total dry matter weight	MGT	GP%	SL	RL
Seed size	1	0.000046ns	0.00002ns	0.52**	808.9**	0.544ns	0.75ns
cultivar	2	0.00033**	0.00037**	1.29**	3882.9**	0.176ns	23.02**
NaCl	2	0.0025**	0.0035**	8.45**	14964.9**	156.35**	673.6**
Cultivar*seed size	2	0.00029**	0.00045**	0.90**	1212.01**	0.565ns	7.73*
NaCl*seed size	2	0.000023ns	0.000024ns	0.11ns	292.7*	0.082ns	7.26ns
NaCl*cultivar	4	0.000038n4s	0.0001*	0.2191**	343.01**	2.4**	0.54ns
NaCl*cultivar*seed size	4	0.00019**	0.00026**	0.10*	235.6*	0.996ns	4.622ns
Error	36	0.000015	0.000032	0.038	64.79	0.491	2.32
Total	53						
C.V.		17.96	10.1	7.9	12.84	14.76	16.77

Discussion

The primary action of osmotic inhibition is retardation of water uptake, which crucial for germination (Kahan, 1960). Seed size affected the water uptake and subsequent growth parameters of the investigated cultivars. The effect of salt stress was more remarkable on large seeds of the cultivars compared to small seeds. GP was significantly affected by NaCl, seed size and cultivar. Small size seeds of Azargol and Record showed the highest GP values at all NaCl concentration, while the maximum GP was obtained from large size seeds in Eurofler cultivar under all levels of salt stresses. Also, mean germination time of small seeds was lower than large seeds.

The lower mean germination time in control (0.0 ds/m^2) and the reduced mean germination time in small size seeds compared to large size seeds of the cultivars under salt stress could be explained by rapidly water uptake in small size seeds by early achievement of necessary moisture content required for germination. Salinity through enhancement of osmotic pressure leads to reduction of water absorption and disturbance in metabolic and physiological processes, so it causes delay in germination following by enhancing seed germination duration. Kaya and Day (2012) found NaCl adversely affected the time to germination and early seedling growth of sunflower. Also, the results are in line with the findings of Kaya *et al.* (2006) and Kaya and Day (2012) in sunflower, who observed that NaCl was responsible for delayed germination.

Different concentration of NaCl may have created an osmotic barrier resulting of corresponding water uptake, which is in agreement with Nieman (1965), Yasseen *et al.* (1987) and Kaya *et al.* (2008), emphasized that NaCl inhibits growth by reducing both cell division and cell enlargement.

Shoot length was severely influenced by salt stress compared to root. Each increase in NaCl concentration caused remarkable decrease in shoot length for the three cultivars compared to control. The results are in line with the findings of Farhoudi and Motamedi (2010) who observed that salt stress decreased shoot and root length but increased mean germination time in both large and small seeds in safflower. The findings showed that small size seeds produced the longest roots. It is assumed that small size seeds absorbed water more rapidly than the large size seeds, which resulted in fast root growth.

Conclusion

Small size seeds germinated and grew more rapidly under NaCl stress, showing that they could be preferred for the use in saline soils to achieve uniform stand establishment and higher levels of crop yield. Also, this study shows that NaCl had greater inhibitory effects on early seedling growth.

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