African Journal of Agricultural Research Vol. 3 (11), pp. 787-791, November, 2008
Available online at http://www.academicjournals.org/AJAR
ISSN 1991-637X © 2008 Academic Journals

Full Length Research Paper

Relationship between seed size and NaCl on germination, seed vigor and early seedling growth of sunflower (*Helianthus annuus* L.)

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Accepted 11 November, 2008

In recent years, the use of sunflower seeds sorted by seed size has been extensively increased in Turkey but little is known about the effects of seed size on germination, emergence, seedling growth and seed vigor in arid and semiarid region suffering from salinity problem. Two seed sizes of cv. Muson and Sirena of sunflower classified as small (6 - 15 mm for Muson and 7 - 16 mm for Sirena) and large (>15 mm for cv. Muson and >16 mm for cv. Sirena) were exposed to 0, 10 and 20 dS m^{-1} of NaCl. Germination percentage (%), mean germination time (day), root and shoot length (cm) and dry matter (%) were investigated. Seed vigor was tested by accelerated ageing (AA) test performed at 45°C and 100% relative humidity for 3 days. Results showed that higher water uptake and hull rate was obtained from large seeds. Small seeds germinated and grew more rapidly compared to large seeds of the same cultivars under NaCl stress. NaCl caused lower root and shoot length but higher mean germination time and dry matter. Therefore, viability after accelerated ageing was lower in small seeds than large seeds. Emergence percentage did not change by seed size, but cotyledon length was shorter in small seeds. It was concluded that although large seeds produced vigorous germination and seedling growth yet small seeds could also be used for successful sunflower production in salt affected areas.

Key words: Germination, *Helianthus annuus*, seed size, NaCl, seed vigor

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crops in Turkey. It is also extensively cultivated for snacks in confectionary industry. It is widely cultivated in arid and semiarid regions of Turkey because of its well adaptation to low moisture availability. Under these conditions, salinity is a major constraint affecting cultivation since rainfall is insufficient to leach salt from root zone and evaporation tends to exceed rainfall (Pessarakli, 1999; Kaya et al., 2003). Approximately 2 - 2.5 million ha area in Turkey has suffered from salinity problem (Kaya et al., 2003).

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. Opening of all florets on a single head is usually completed in 10 – 15 days. The development of seeds in each whorl of the inflorescence occurs under varying environmental conditions causing variability in seed size and quality (Weiss, 1983; Munshi et al., 2003). Therefore, seed size decreases from periphery to the center of the head. Robinson (1974) found that large seeds produced more vigorous seedlings compared to small seeds while superiority of crops produced from large seeds was not determined on seed yield, hectoliter weight and oil

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percentage. Karadogan et al. (1998) and Munshi et al. (2003) reported that seed number, a thousand seed weight, crude protein and oil content were decreased from periphery to the center. On the other hand, Saranga et al. (1998) emphasized that seed vigor was negatively correlated with embryo mass but large seeds germinated and emerged later than small seeds.

Seed companies have started grading sunflower seeds with the same price. In general, farmers tend to sow large seeds with hesitation towards small seeds as they doubt germination behavior and uniform stand establishment capability of small seeds. This research focuses on determining the differences in seed size for germination, seedling growth and seed vigor. Also, the study aimed to expose the performance of different sized seeds of two sunflower cultivars under saline conditions.

MATERIALS AND METHODS

The seeds classified as small (6 - 15 mm for Muson and 7 - 16 mm for Sirena) and large (larger than 15 mm for Muson and 16 mm for Sirena) were obtained from May Seed Company, Bursa-Turkey. The one hundred seed weight, water uptake and hull rate of two cultivars are shown in Table 1.

NaCl concentrations at electrical conductivities of 10 and 20 dS m^{-1} were adjusted before start of the experiment by using WTW 3.15i conductivity meter (Germany). Distilled water served as a control (0 dS m^{-1}).

Four replicates 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 days to prevent accumulation of salts. In order to prevent evaporation, each rolled paper was put into a sealed plastic bag. Seeds were allowed to germinate at 25 ± 1°C in the dark for 10 days (ISTA, 2003). A seed was considered germinated when the emerging radicle elongated to 2 mm. Germination percentage was recorded every 24 h for 10 days. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980). Ten grams seeds from each seed size and cultivar were placed in Petri dishes containing distilled water to determine water uptake of seeds necessary for germination. The water uptake was expressed as percentage increase in moisture content on fresh weight basis. To determine emergence percentage and cotyledon length, four replications of 50 seeds from each cultivar and seed size were sown in pots filled with sterilized sand to a depth of 3 cm. The pots were irrigated with tap water at the rate of 350 ml per pot once every two days. After 10 day incubation, seedling emergence was counted and cotyledon length was measured from randomly selected ten seedlings.

Accelerated ageing (AA) test

The accelerated ageing test (AA) was performed by using ageing temperature and time combination of 45°C for 3 days in a dark growth chamber. After ageing, 50 seeds per replicate were germinated in 3 layered filter papers at 25 ± 1°C in dark growth chamber for ten days.

The experiment made use of three factorial arranged in a completely randomized design with 4 replications and 50 seeds per replicate. The experimental data were analyzed by ANOVA and the differences were compared by Least Significant Difference (LSD) test ($P<0.05$). Data given in percentages were subjected to arcsine transformation before statistical analysis. For all investigated parameters, analysis of variance was performed using the MSTAT-C computer software program (Michigan State University).

RESULTS

Variation in one thousand seed weight, water uptake and hull rate of the investigated sunflower cultivars was shown in Table 1. As expected, one thousand seed weights of the cultivars and seed sizes were significantly different. Both large and small seeds of cv. Sirena were heavier than that of cv. Muson. Higher water uptake and hull rate was obtained from large seeds of both cultivars.

Main effects of seed size and NaCl levels on germination percentage (GP), mean germination time (MGT), root length (RL), shoot length (SL) and dry matter (DM) of the both cultivars were shown in Table 2. Higher germination percentage was obtained from cv. Muson and large seeds. Increasing NaCl resulted in decrease in germination percentage. Germination was severely limited at the highest NaCl level (20 dS m^{-1}) where germination was 80.9%.

A three-way interaction was determined for mean germination time, root length, shoot length and dry matter (Table 2). Mean germination time increased with an increase in NaCl and seed size in each cultivar. The small seeds of Muson and Sirena had the minimum time to germinate under all NaCl stresses. On the other hand, the large seeds took the maximum time to germinate under all levels of salt stresses. Clear difference between seed sizes for mean germination time occurred at NaCl level of 20 dS m^{-1}.

Increased NaCl levels resulted in decrease in root length of the cultivars (Table 3). However, the decrease was very dramatic in large seeds of the cultivars. The small seeds of respective cultivars gave the longest roots at both control and all levels of NaCl. Except for control, at all levels of NaCl no significant difference was observed for root length of seed sizes.

Shoot length showed a progressively decrease with increasing NaCl and seed size. The longest shoot length was detected in the control with 12.98 cm for large seeds of Sirena (Table 4). But, the superiority of the large seeds disappeared when NaCl concentration increased. At NaCl levels of 20 dS m^{-1}, no significant difference in shoot length was observed.

Compared to control, each increase in NaCl concentration and seed size caused remarkable decrease in seedling dry matter for the cultivars. The maximum seedling dry matter was determined with 27.91% at 20 dS m^{-1} (Table 4). Apparent superiority of small seeds for dry matter was observed in cv. Sirena while significant difference was not detected in cv. Muson.

The seed vigor of the cultivars in relation to seed size was tested by accelerated ageing test. Accelerated ageing test showed large seeds of both cultivars were superior to small seeds (Table 5). GP after accelerated ageing was 66.5 and 35.0% for large seeds of cv. Muson

Table 1. One thousand seed weight (g), hull rate (%) and water uptake (%) of cultivars in relation to seed sizes.

Cultivars	Seed sizes	One thousand seed weight	Water uptake	Hull rate
Muson	Large	74.7 c	77.3 a	23.21 a*
	Small	61.3 d	63.4 c	17.94 c
Sirena	Large	91.0 a	75.2 a	21.96 a
	Small	82.7 b	72.0 b	20.24 b

*Means with the same letter(s) are not significantly different at P < 0.05 level.

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 matter (DM, %) of sunflower cultivars.

	GP	MGT	RL	SL	DM
Cultivar					
Muson	99.6 a	2.22 b	4.16 a	5.94	12.7 b[1]
Sirena	84.5 b	3.59 a	3.50 b	5.75	17.9 a
Seed size					
Large	93.2 a	2.96	4.09 a	5.76	15.7
Small	90.9 b	2.85	3.58 b	5.92	15.0
NaCl					
0	99.0 a	2.19 c	5.30 a	10.8 a	8.2 c
10	96.3 b	2.82 b	4.10 b	5.13 b	14.1 b
20	80.9 c	3.70 a	2.09 c	1.59 c	23.7 a
Summary of ANOVA					
Cultivar (C)	*	*	*	NS	*
Seed size (S)	*	NS	*	NS	NS
C x S	*	NS	*	NS	NS
NaCl (N)	*	*	*	*	*
C x N	*	*	*	*	*
S x N	*	*	*	NS	*
C x S x N	NS	*	*	*	*

[1]Means with the same letter(s) are not significantly different for each main effect within each column at P < 0.05 level. *: Significant at p<0.05 level; NS: Not significant.

Table 3. Interactive effects of NaCl levels and seed sizes on mean germination time (MGT) and root length (RL) of sunflower cultivars after 10 days incubation.

Cultivar	Seed size	MGT (day) NaCl levels (dS m^{-1})			RL (cm) NaCl levels (dS m^{-1})		
		Control	10	20	Control	10	20
Muson	Large	1.99	2.15	2.74	3.71	5.66	2.47
	Small	1.99	2.10	2.33	4.99	5.67	2.47
Sirena	Large	2.34	3.22	5.02	3.67	2.12	1.45
	Small	2.43	2.81	4.71	9.12	2.97	2.00
LSDint (P<0.05)			*0.29*			*1.02*	

and Sirena, respectively. The minimum viability after accelerated ageing was obtained from small seeds of cv. Sirena. Detrimental effects of accelerating ageing were found more deleterious for cv. Sirena because of lower viability. Emergence percentage of the cultivars was also significantly different from germination percentage. In addition, cotyledon length of the cultivars varied with seed size and large seeds produced longer cotyledon in

Table 4. Interactive effects of NaCl levels and seed sizes on shoot length (SL) and dry matter (DM) of sunflower cultivars after 10 days incubation.

Cultivar	Seed size	SL (cm)			DM (%)		
		NaCl levels (dS m^{-1})			NaCl levels (dS m^{-1})		
		Control	10	20	Control	10	20
Muson	Large	8.65	6.77	1.76	8.10	8.97	21.89
	Small	10.67	5.96	1.81	7.33	9.69	20.38
Sirena	Large	10.89	3.08	1.33	6.10	16.61	24.62
	Small	12.98	4.72	1.48	11.21	21.19	27.91
LSDint (P<0.05)			1.12			2.16	

Table 5. Germination percentage (GP, %), germination percentage after accelerated ageing (GP after AA, %), emergence percentage (EP, %) and cotyledon length (CL, cm) of the cultivars as affected by seed sizes.

Cultivar	Seed size	GP	GP after AA	EP	CL
Muson	Large	99.0 a	66.5 a	99.0 a	1.82 a*
	Small	99.5 a	45.0 b	99.0 a	1.73 b
Sirena	Large	99.5 a	35.0 c	98.8 a	1.87 a
	Small	97.5 b	10.0 d	96.3 b	1.68 b

*Means with the same letter(s) are not significantly different at $P < 0.05$

both cultivars.

DISCUSSION

Seed size affected the water uptake and subsequent growth parameters of the cultivars. The effect of NaCl stress was more deleterious on large seeds of the cultivars compared to small seeds. Also, mean germination time of small seeds was slightly lower than large seeds. Saranga et al. (1998) found higher mean germination time in large seeds compared to small seeds. On the other hand, NaCl adversely affected the time to germination and early seedling growth of sunflower, but any inhibitory effects of NaCl were more dramatic on seedling growth than germination. The results are in agreement with Kaya et al. (2006) in sunflower, Murillo-Amador et al. (2002) in cowpea and Okcu et al. (2005) in pea, who observed that NaCl was responsible for delayed germination. Delgado and Sanchez-Raya (2007) observed that seed germination declined by increasing NaCl. Mohammed et al. (2002) reported that germination percentage of sunflower considerably declined with increasing NaCl concentrations after 1 and 2 days incubation while the decline in germination reduced when incubation period was extended to 10 days. The lower mean germination time in control (0.0 dS/m) and reduced mean germination time in small seeds compared to large seeds of the cultivars under all levels of NaCl could be explained by more rapid water uptake in small seeds by early achievement of necessary moisture content required for germination. The results are in line with the findings of Kaya et al. (2006) in sunflower; Murillo-Amador et al. (2002) in cowpea, Khajeh-Hosseini et al. (2003) found that in soybean salinity influenced germination by decreasing water uptake. Small seeds with low hull rate absorbed water faster compared to large seeds and resulted in early germination. Saranga et al. (1998) emphasized that thicker and heavier pericarp of large seeds may explain the slower rate of germination relative to small seed. Also, Reuzeau et al. (1992) stated that lipid concentration was higher in small seeds with high germinability than in big seeds which were poor germinator.

Seedling growth was severely influenced by NaCl stress; however, small seeds produced the longest roots. It is assumed that small seeds absorbed water more rapidly compared to large seeds, which resulted in fast root growth. Our findings showed that NaCl had greater detrimental effects on seedling growth than on germination in line with Turhan and Ayaz, (2004), Muralidharudu et al. (1998) in sunflower and Kaya et al. (2003) in safflower. It is assumed that reducing cell division and plant metabolism induced by accumulation of Na ion caused changes in ion balances such as Na:Ca and K:Na in plant cell in agreement with Ashraf and O'leary (1997), Delgado and Sanchez-Raya (1999), Ashraf et al. (2003) in sunflower, therefore, the rate of seedling growth was not determined. This finding is in line with earlier observation made for sunflower by Saranga et al. (1998) who stated root length was diminished by increasing seed mass.

A significant increase in dry matter was observed under NaCl stress, indicating that increasing NaCl decreased tissue water content. Under stress conditions, lower tissue water content and higher dry matter was determined by Gill et al. (2003) in sorghum and Siddique et al. (2000) in wheat.

Heavier seeds resulted in longer cotyledon length under nonsaline conditions, indicating that large seeds produced more vigorous seedlings but no difference was determined for emergence percentage. Soltani et al. (2002) reported that advantage of large seeds was due to greater seed reserve utilization. Also, seed vigor test, accelerated ageing, exhibited that large seeds of the cultivars had the highest viability.

Conclusion

It was concluded that the use of large seeds produced more vigorous seedlings under non saline conditions while the superiority was used up under saline conditions. On the other hand, differences between large and small seeds in terms of germination, emergence and seedling growth were similar. In a previous research, Robinson (1974) demonstrated that no significant differences among seed size in terms of yield, hectoliter weight, oil content was determined in sunflower. Furthermore, the beneficial effects of small seeds under both saline and non-saline conditions were clearly observed in this study. Small seeds germinated and grew more rapidly under NaCl stress, showing that they could be preferred on salt-infected soils to achieve uniform stand establishment. To achieve a uniform plant density in case of saline conditions, farmers should not hesitate to sow small seeds of sunflower cultivars.

ACKNOWLEDGEMENT

The authors acknowledge the help from Prof. Dr. Sebahattin ÖZCAN, Turkey for practical guidance and helps for this study.

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