Pak. J. Bot., 41(2): 647-654, 2009.

SUNFLOWER (*HELIANTHUS ANNUUS* L.) RESPONSE TO DROUGHT STRESS AT GERMINATION AND SEEDLING GROWTH STAGES

SHAMIM AHMAD[1], RASHID AHMAD[1], MUHAMMAD YASIN ASHRAF[2*], M. ASHRAF[3] AND EJAZ AHMAD WARAICH[1]

[1]*Department of Crop Physiology, University of Agriculture Faisalabad, Pakistan*
[2*]*Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan*
[3]*Department of Botany, University of Agriculture Faisalabad, Pakistan.*

Abstract

Response of six sunflower hybrids/ breeding lines viz., G-101, SF-187, Hysun-33, Hysun-38, 64-A-93 and S-278 to drought stress imposed at germination and seedling growth stages was investigated in a laboratory experiment (25±3°C). Five water stress levels of zero (control), -0.35, -0.6, -1.33, and -1.62 MPa were developed using polyethyleneglycol-6000 (PEG-6000). Complete randomized design with three replications was used for this experiment. Germination stress tolerance index (GSI), plant height stress index (PHSI), root length stress index (RLSI) and dry matter stress index (DMSI) were used to evaluate the genotypic response to PEG-induced water stress. Plant height and dry matter stress tolerance indices for all sunflower hybrids decreased with increasing water stress. In contrast, an increase in RLSI was observed in all sunflower hybrids. Sunflower hybrids G-101 and 64-A-93 performed better and were classified as drought tolerant. The variation among hybrids for DMSI was found to be a reliable indicator of drought tolerance in sunflower.

Introduction

Adequate water and nutrient supply are important factors affecting optimal plant growth and successful crop production. Water stress is one of the severe limitations of crop growth especially in arid and semi-arid regions of the world as it has a vital role in plant growth and development at all growth stages. However, depending upon plant species, certain stages such as germination, seedling or flowering could be the most critical stages for water stress. Seed germination is first critical and the most sensitive stage in the life cycle of plants (Ashraf & Mehmood, 1990) and the seeds exposed to unfavorable environmental conditions like water stress may have to compromise the seedlings establishment (Albuquerque & Carvalho, 2003).

Sunflower being an oil seed crop is particularly susceptible to water shortage at germination stage. Sajjan *et al*., (1999) reported decrease in percent germination and biomass accumulation in sunflower with increasing osmotic stress in germinating media whereas mean germination time increased with increasing water deficit (El-Midaoui *et al*., 2001). Moreover, different genotypes of sunflower showed differential responses to all these stress treatments. Lenzi *et al*., (1995) reported that mutant seeds of sunflower showed a higher resistance to osmotic stress, i.e., germinating at more negative osmotic potentials. Germination of sunflower was inhibited in presence of polyethylene glycol -6000, at osmotic pressure lower than -5 bars (Smok *et al*., 1993). The adverse effects of water shortage on germination and seedling growth had also been well reported in different crops such as wheat (Dhanda *et al*., 2004), sugar beet (Sadeghian & Yavari, 2004), sorghum (Gill *et al*., 2002), and sunflower (Mohammad *et al*., 2002).

E-mail: niabmyashraf@gmail.com

Sunflower (*Helianthus annuus* L.) due to its significant share in vegetable oil production of Pakistan has emerged as an economically important crop of the country. But limited rainfall or shortage of water for irrigation during the growing season constraints its seed yield with significant reductions. Therefore, growing of drought tolerant cultivars will contribute to more stable sunflower production and the screening of the response of sunflower cultivars or breeding lines to drought stress can play a crucial role in breeding programmes. However, difficulties like uncontrolled climatic conditions, insufficient homogeneity of soil, large amount of plant material and time and labor consumption make field trials difficult for drought screening of genotypes. The present study was therefore, conducted with the objectives to determine the response of sunflower hybrids/breeding lines to drought stress at germination and seedling stages under controlled conditions and to evaluate germination and seedling growth as screening criteria for drought tolerance in sunflower.

Materials and Methods

Experiment was carried out at Stress Physiology Laboratory, Salinity and Environmental Management Division, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. Six sunflower (*Helianthus annuus* L.) hybrids viz., G-101, SF-187, Hysun-33, Hysun-38, 64-A-93 and S-278 that are widely grown in the region were tested against drought stress at germination and seedling stages under laboratory conditions (25±3°C). Polyethylene glycol with a molecular weight of 6000 (PEG-6000) was used as a drought stimulator and five water stress levels of zero (control), -0.35, -0.6, -1.33, and -1.62 MPa were developed by dissolving 5, 10, 15, and 20 g of PEG per 100 mL distilled water. Seeds were surface sterilized with 10% Sodium hypochlorite solution for five minutes and then washed three times with distilled water. Ten seeds of each sunflower hybrid were planted in each Petri plate containing filter papers. The experiment was laid out in a completely randomized design with 3 replicates for each experimental unit.

Ten m*L* of designated treatment solution was applied daily in each Petri plate after washing out the previous solution. Number of seeds germinated was counted daily and data were recorded for 14 days. A seed was considered germinated when both plumule and radicle had emerged to 5 mm. Fresh and dry weights and root and shoot length were recorded after 14 days of the start of the experiment. Plant dry weights were recorded after drying at 70°C to a constant weight. From these measurements the Promptness index (PI), germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) were calculated using the following formulae given by Ashraf *et al*., (2006).

i. P.I = $nd_2(1.00) + nd_4(0.75) + nd_6(0.5) + nd_8(0.25)$ where n is the number of seeds germinated at day d
ii. G.S.I. (%) = [P.I of stressed seeds / P.I control seeds] x 100
iii. PHSI = (Plant height of stressed plant / Plant height of control plants) x 100
iv. RLSI = (Root length stressed plant / Root length of control plants) x 100
v. DMSI = (Dry matter of stressed plant / Dry matter of control plants) x 100

The data so collected was analyzed statistically using analysis of variance technique and the STATISTICA Computer Program was used for this purpose.

Results

Polyethylene glycol induced water stress significantly reduced the GSI (Table 1). The minimum GSI values (82.20, 70.29, 23.88, 72.28, 86.34 and 48.54%) were recorded at the highest 20% PEG (-1.62 MPa) which was maximum (89.34, 82.17, 50.00, 95.06, 96.58 and 87.14%) under 5% PEG (-0.35 MPa) in sunflower hybrids, Hysun-33, Hysun-38, S-278, 64-A-93, G-101, SF-187, respectively (Fig. 1). The trend was almost similar under 10 % (-0.60 MPa) and 15% PEG concentrations (-1.33 MPa).

Sunflower hybrids also differed significantly for this variable (Table 1). Sunflower hybrid G-101 had the highest germination stress tolerance indices (96.58, 95.74, 94.86 and 86.34%) under all (5, 10, 15 and 20%) PEG concentrations, while sunflower hybrid S-278 showed minimum (50.00, 50.00, 38.33 and 23.88%) germination stress tolerance indices (Fig. 1) under all PEG concentrations. Sunflower hybrid 64-A-93 was statistically at par with G-101 and ranked second with respect to GSI. Sunflower hybrid Hysun-33, Hysun-38 and SF-187 maintained the GSI between 70-90% and were ranked third with respect to GSI at all PEG concentrations. On the basis of GSI values sunflower hybrids can be divided into three groups, G101 and 64A93 being the best performing hybrids under water stress conditions, Hysun-33, Hysun-38 and SF-187 the hybrids having medium tolerance while S-278 the sensitive one to water stress.

Analysis of variance for the data for PHSI revealed highly significant differences among different PEG concentration (Table 1). Plant height stress tolerance index was decreased with the increase in PEG concentrations and was minimum (50.41, 35.53, 0.00, 56.67, 68.52 and 39.53%) at the lowest osmotic potential (-1.62 MPa), maintained with the application of 20%. The maximum PHSI values of 97.74, 90.12, 85.45, 93.15, 96.98 and 91.50%, were recorded under 5% PEG concentration (-0.35 MPa) in sunflower hybrids Hysun-33, Hysun-38, S-278, 64-A-93, G-101, SF-187 respectively (Fig. 2). Almost similar trend was observed under 15 (-1.33 MPa) and 20% PEG (-1.62 MPa) concentrations with respect to decrease in PHSI with the increase in PEG concentrations.

Different sunflower hybrids were significantly different for PHSI (Table 1). Sunflower hybrid, G-101 maintained the highest PHSI (68.52%) at 20% PEG (-1.62MPa) treatment followed by 64-A-93 (56.67%) while S-278 failed to germinate at this stress level. Hysun-33 maintained the highest PHSI (88.43%) followed by sunflower hybrid G-101 (81.93%) while the minimum PHSI were recorded in S-278 at 15% PEG concentration. Under 10% PEG concentration G-101 and Hysun-33 were statistically similar with each other and maintained the highest value for PHSI, 94.45 and 94.37% respectively. The minimum PHSI was recorded in S-278 while 64-A-93, SF-187 and Hysun-38 maintained 81-86% PHSI under 10% PEG concentration. The trend was almost same for 5% PEG treatment for all the 6 sunflower hybrids. At 5% PEG, G101 showed the maximum PHSI which was lowest in S-278. Hysun-33, 64-A-93 and SF-187 maintained the PHSI between 91-98% (Fig. 2). The interaction among treatments was also statistically significant (Table 1).

The effect of different PEG concentrations on RLSI was statistically highly significant for root length stress tolerance index in all the sunflower hybrids (Table 1). Root length stress tolerance index increased with increase in PEG concentration. The highest values for root length stress tolerance index (240.42, 145.68, 0.00, 211.14, 285.12 and 155.89%) was recorded at 20% PEG treatment while the minimum values of 104.50, 107.11, 59.72, 107.69, 104.61 and 101.68% were observed under 5% PEG in all sunflower hybrids studied (Fig. 3). Almost similar response was observed under 15 (-1.33 MPa) and 20% PEG (-1.62 MPa) concentrations with respect to increase in RLSI with the increase in PEG concentrations.

Table 1. Mean square values from analysis of variance tables for germination stress tolerance index (GSI), plant height stress tolerance index (PHSI), root length stress tolerance index (RLSI) and dry matter stress tolerance index (DMSI) of six sunflower hybrids under different PEG-6000 concentrations.

	d.f.	GSI	PHSI	RLSI	DMSI
PEG Concentration	3	1416.263**	9065.454**	19491.34**	3916.741**
Hybrids (H)	5	4284.461**	3061.685**	40742.40**	929.668**
PEG Conc. X H	15	105.416**	303.017**	4348.067**	21.854**
Error	48	4.836	6.770	10.471	0.967

** = Significant at 0.01 probability level

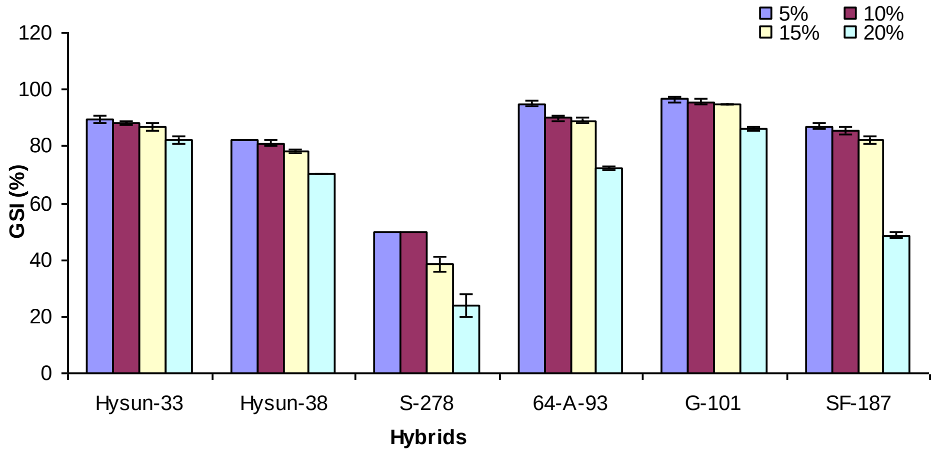

Fig. 1. Effect of different PEG concentrations on germination stress tolerance index (GSI) of six sunflower hybrids.

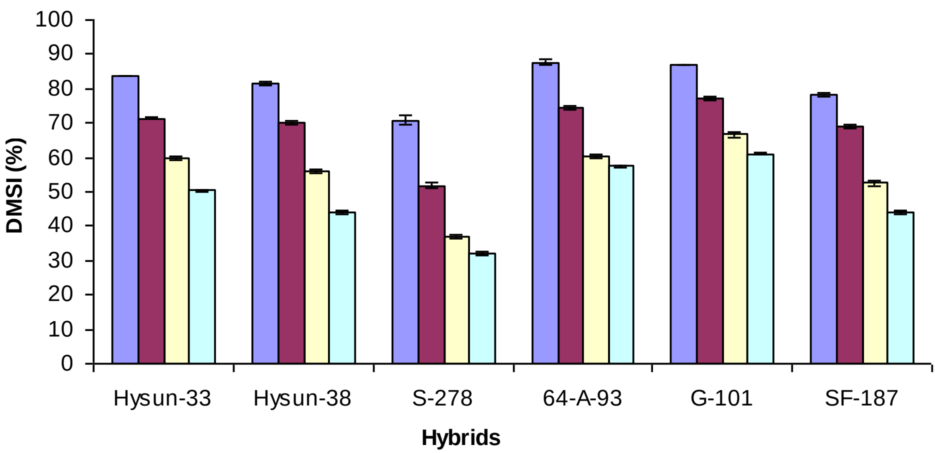

Fig. 2. Effect of different PEG concentrations on dry matter stress tolerance index (DMSI) of six sunflower hybrids.

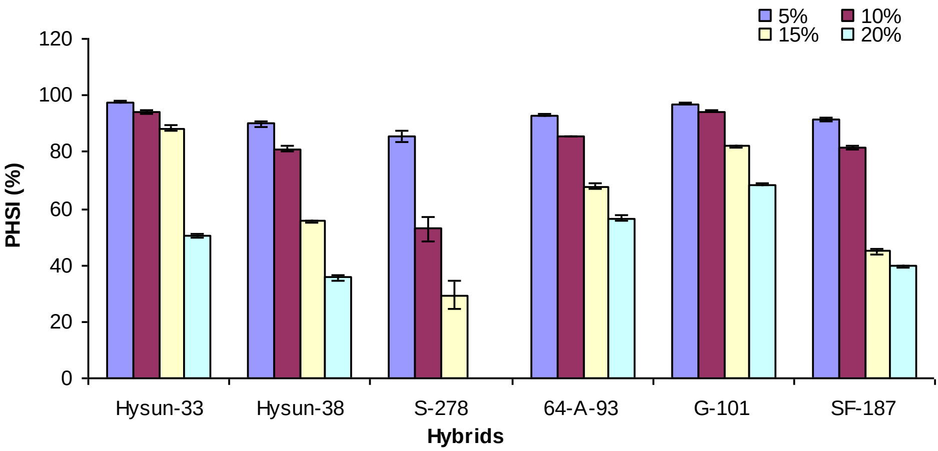

Fig. 3. Effect of different PEG concentrations on plant height stress tolerance index (PHSI) of six sunflower hybrids.

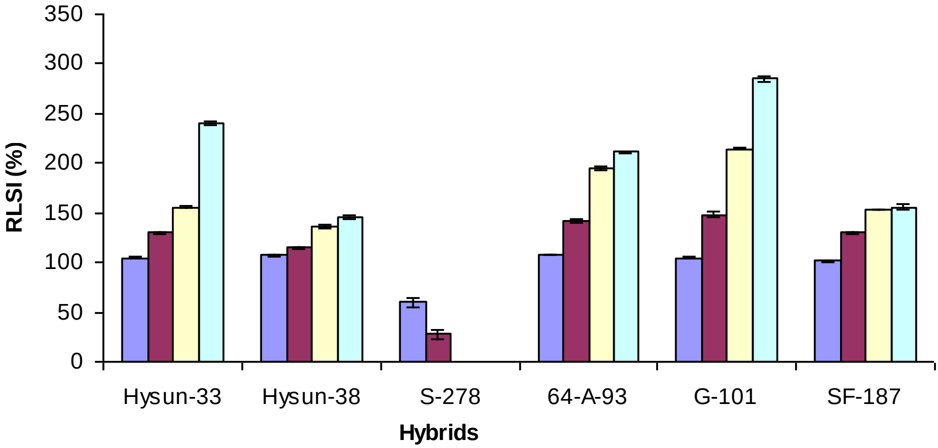

Fig. 4. Effect of different levels of PEG-induced water stress on root length stress tolerance index (RLSI) of six sunflower hybrids.

Sunflower hybrids were also statistically highly significant for this variable (Table 1). Sunflower hybrid, G-101 maintained the highest value of RLSI (148.32, 214.38 and 285.12%) under 10, 15 and 20% PEG concentration, respectively, while under 5% PEG concentration the maximum RLSI was recorded in hybrid 64-A-93 which was at par with Hysun-38. The sunflower hybrid S-278 failed to germinate under 15 and 20% PEG concentration but showed the minimum values of RLSI (59.72 and 28.24%) under 5 and 10% PEG concentrations, respectively. Sunflower hybrid 64-A-93 was at par with G-101 and was the second most successful hybrids with respect to RLSI under all the PEG concentrations other than 20%, Hysun-33 maintained the second highest (240.42%) RLSI. Hysun-33, Hysun-38 and SF-187 maintained the RLSI between 101-240% and occupied the third position in maintaining RLSI at all PEG concentration levels (5, 10, 15 and 20%). The interaction among the treatment was also highly significant for RLSI (Table 1).

Dry matter stress tolerance index was decreased significantly with the increase in PEG concentrations in sunflower hybrids (Table 1). The minimum DMSI vales were recorded at 20% PEG concentration (50.34, 44.07, 32.24, 57.36, 61.10 and 44.16%) where as the maximum values for DMSI were observed under 5% PEG application (83.66, 81.37, 70.86, 87.59, 87.03 and 78.34%) in all sunflower hybrids, respectively (Fig. 4). Almost the similar trend was observed under 15 (-1.33 MPa) and 20% PEG (-1.62 MPa) concentrations with respect to decrease in DMSI in all sunflower hybrids (Fig. 4).

Analysis of variance for the data for dry matter stress tolerance index revealed highly significant differences among different sunflower hybrids (Table 1). The sunflower hybrid, G-101 maintained the highest value for DMSI (87.03, 77.16, 66.67 and 61.10%), which was the minimumt in S-278 (70.86, 51.67, 37.10 and 32.24%) at 5, 10, 15 and 20% PEG concentration respectively (Fig. 4). The sunflower hybrid 64-A-93 maintained second highest values for DMSI (87.59, 74.41, 60.45 and 57.36%) at 5, 10, 15 and 20% respectively, which was at par with G-101 at 20% PEG treatment. Hysun-33, Hysun-38 and SF-187 maintained the DMSI between 78-84, 69-72, 52-60 and 44-50% respectively and occupied the third position for DMSI (Fig. 4). The interaction between sunflower hybrids and PEG concentrations was also statistically significant for DMSI.

Discussion

Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought simulator (Ashraf *et al.*, 1996; Turhan, 1997). In the present experiment PEG-6000 was used to create the osmotic stress, as most of the researchers (Smok *et al.*, 1993; Hu and Jones, 2004) utilized it for the development of water deficit environment in growth chamber studies.

The study regarding the effect of water stress created by PEG-6000 indicated that germination, plant height and dry matter stress tolerance indices decreased with the increase in PEG-6000 concentration while root length stress tolerance index increased. The variation among hybrids showed that germination stress tolerance index decreased with the increase in PEG-6000 concentration in all the hybrids. However G-101 performed better than others. Many reports indicated that GSI can be utilized as screening criteria for stress tolerance. However, many are of the view that germination criteria did not seem to reflect stress tolerance in different plants, but rather to indicate seed quality differences, nor did this procedure reflect the yield stability of genotypes (Ashraf *et al*., 1996). Several factors influence the seed such as, seed age, seed maturity (Shete *et al*., 1992), storage conditions (Elemery, 1991), seed biochemical composition (Reuzeau *et al*., 1992), genetic variability (Sajjan *et al*., 1999) and ecological conditions (Smok *et al*., 1993). The genotypes may respond different to these factors which could be reflected in their respective scores. In the present study, every effort was made to collect the seed of same age and size and provide identical environmental conditions to germinating achenes, it is felt that such precautions eliminated the effect of above mentioned factors on seed germination. So to avoid all the above mentioned factors hybrids of different well known seed producing companies had been used in the present experiment.

The germination test may be useful for identifying vigorous seed lots and genotypes capable of quickly establishing adequate population under low soil moisture conditions. But the genetic differences among various sunflower hybrids may be correlated to seedling growth which has been studied in the present investigation. In the present case hybrids of different seed companies were used to evaluate for drought tolerance. It is well established fact that certain parameters were genetically controlled like germination in the hybrid seed, so the differences in GSI are due to the osmotic stress created by PEG-6000 (Fig. 1). In present study the findings are very similar to the former case, in which germination decreased due to the increase in PEG-6000 concentration. Present study strongly support that GSI can be utilized to screen sunflower germplasm for drought tolerance.

The variation in performance of sunflower hybrids determined by the seedling growth as dry biomass, plant height and root length indicate that seedling growth is a reliable and efficient procedure for screening sunflower hybrids for moisture stress tolerance. Although plant height (Fig. 3) and dry matter (Fig. 2) decreased with the increase in water stress, created by PEG-6000 but different hybrids showed different performance under stress environment. Sunflower hybrid G-101 was at the top of the list in all the growth parameters recorded in the present study. The behavior of the entire hybrids was similar to that of G-101. Similar findings were recorded by Ashraf *et al*., (1996) for wheat. There are many reports which are in agreement with the present findings indicating that drought stress severely reduce the growth and biomass of the plant. But the varieties having genetic potential to maintain the higher growth under stress conditions are drought tolerant.

On the basis of the results of this study, sunflower hybrids can be classified into three groups, G-101 and 64-A-93 the best performing under drought conditions, Hysun-33, Hysun-38 and SF-187 the hybrids having medium tolerance to water stress and S278 the sensitive one. It is also concluded that variation among hybrids for DMSI was found to be a reliable indicator of drought tolerance in sunflower.

References

Albuquerque, F.M.C. de and N.M. de Carvalho. 2003. Effect of type of environmental stress on the emergence of sunflower (*Helianthus annuus* L.), soyabean (*Glycine max* (L.) Merril) and maize (*Zea mays* L.) seeds with different levels of vigor. *Seed Sci. Technol*., 31: 465-467.

Ashraf, M. and S. Mehmood. 1990. Response of four *Brassica* species to drought stress. *Environ. Expt. Bot.*, 30: 93-100.

Ashraf, M.Y. and S.S.M. Naqvi. 1995. Studies on water uptake, germination and seedling growth of wheat genotypes under PEG-6000 induced water stress. *Pak. J. Sci. Ind. Res.*, 38:130-133.

Ashraf, M.Y., K. Akhtar, F. Hussain and J. Iqbal. 2006. Screening of different accessions of three potential grass species from Cholistan desert for salt tolerance. *Pak. J. Bot.*, 38:1589-1597.

Ashraf, M.Y., M.H. Naqvi and A.H. Khan. 1996. Evaluation of four screening techniques for drought tolerance in wheat (*Triticum aestivum* L.). *Acta Agron. Hung*., 44: 213-220.

Dhanda, S.S., G.S. Sethi and R.K. Behl. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. *J. Agron. Crop Sci*., 190:1-6.

Elemery, M.I. 1991. Effect of storage conditions (T ºC and RH%) and packaging materials on germination percentage of onion and sunflower seeds. *Ann. Agric. Sci*., 29: 657-667.

El-Midaoui, M., A. Talouizte, M. Benbella, H. Serieys, Y. Griveau and A. Berville. 2001. Effect of osmotic pressure on germination of sunflower seeds (*Helianthus annuus* L.). *Helia,* 24: 129-134.

Gill, R.K., A.D. Sharma, P. Singh and S.S. Bhullar. 2002. Osmotic stress-induced changes in germination, growth and soluble sugar content of *Sorghum bicolor* (L.) Moench seeds. *Bulg. J. Plant Physiol*., 28:12-25.

Hu, F.D. and R.J. Jones. 2004. Effects of plant extracts of *Bothriochloa pertusa* and *Urochloa mosambicensis* on seed germination and seedling growth of *Stylosanthes hamata* cv. Verano and Stylosanthes scabra cv. Seca. *Aust. J. Agric. Res*., 48:1257-1264.

Iqbal, N. and M.Y. Ashraf. 2006. Does seed treatment with Glycinebetaine improve germination rate and seedling growth of sunflower (*Helianthus annuus* L.) under osmotic stress. *Pak. J. Bot*., 38: 1641-1648.

Lenzi, A., M. Fambrini, S. Barotti, C. Pugliesi and P. Vernieri. 1995. Seed germination and seedling growth in a wilty mutant of sunflower (*Helianthus annuus* L.): effect of absisic acid and osmotic potential. *Environ. Exp. Bot.*, 35:427-434.

Mohammad, M. El, M. Benbella and A. Talouizete. 2002. Effect of sodium chloride on sunflower (*Helianthus annuus* L.) seed germination. *Helia*, 37: 51-58.

Pelah, D., A. Altman and O. Shoseyov. 1997. Dourgh tolerance: a molecular perspective. In: *Horticulture Biotechnology*. *In vitro* Culture and Breeding. (Eds.): Altman & M. Ziv, *Acta. Hort*., 447: 439-445. ISHS.

Reuzeanu, C., D. Goffner and G. Cavalie. 1992. Relationship between protein composition and germination on capacity of sunflower seeds. *Seed Sci. Res*., 2: 223-230.

Sadeghian, S.Y. and N. Yavari. 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. *J. Agron. Crop Sci.*, 190:138-144.

Sajjan, A.S., V.P. Badanur and G.M. Sajjanar. 1999. Effect of external water potential on seed germination, seedling growth and vigor index in some genotypes of sunflower. In: *Proc. Symp. Recent Advances in Management of Arid Ecosystem*, (Eds.): S.A. Faroda, N.L. Joshi, S. Kathju and A. Kar. pp. 215-218.

Schutz, W. and P. Milberg. 1997. Seed germination in *Launaea arborescens*: a continuously flowering semi-desert shrub. *J. Arid Environ*., 36: 113-122.

Smok, M.A., M. Chojnowski, F. Corbineau and D. Come. 1993. Effects of osmotic treatments on sunflower seed germination in relation with temperature and oxygen. In: *Proc. 4th Intl. Workshop on seed: Basic and Applied Aspects of Seed Biology*, (Eds.): D. Come and F. Corbineau. Angers, France, pp. 1033-1038.

Turhan, H. 1997. Salinity studies in potato (*Solanum tuberosum* L.). Ph.D. Thesis, The University of Reading, UK. pp. 247.

Willenborb, C.J., R.H. Gulden, E.N. Jhonson and S.J. Shirtliffe. 2004. Germination characteristics of polymer-coated canola (*Brassica napus* L.) seeds subjected to moisture stress at different temperatures. *Agron. J.*, 96: 786-791.

(Received for publication 9 January 2009)