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The Effects of Different Levels of Osmotic Stress on Germination and Seedling Growth in Promising Durum Wheat Genotypes

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ABSTRACT

A study was carried out to compare the appreciable differences between various durum wheat genotypes during the germination and seedling stages. Twenty promising spring durum wheat genotypes were tested under four (0.0, -0.3, -0.6 3 and -0.9 MPa) osmotic stresses conditions laid out in factorial experiments based on a completely randomized design (CRD) with three replications. Osmotic potentials were produced using different concentrations of polyethylene glycol (PEG 6000) at 20°C. Results revealed that all the recorded traits were significantly (P<0.01) affected by osmotic stress treatments. There were remarkable decreases in germination percentage, coleoptile, root and shoot length, shoot dry weight, seedling dry weight with increasing osmotic stress. However the germination rate showed a slight increase under low osmotic stress condition. The highest germination percentage was 93.5%, observed in D1 (control) treatment whilst the lowest (42%) was seen in the D4 (-0.9 MPa) treatment. Furthermore, root dry weight, root to shoot length ratio and root to shoot dry weight ratio also increased under low osmotic stress, although severe osmotic stress caused a sizeable decrease in their values. The genotypic effects on germination percentage and rate were highly significant (P<0.01), whilst its effects on coleoptile, root length and root dry weight were significant (P<0.05). Genotype four (G4) exhibited the highest germination percentage (84.17%) whilst G17 showed the lowest value (62.92%). Correlation studies amongst the different traits showed that the highest correlation coefficient was between seedling dry weight and shoot dry weight (r = 0.96), whilst the lowest value was between germination rate and root to shoot dry weight (r = - 0.13).

Keywords: coleoptile, PEG, root, shoot, tolerance

INTRODUCTION

Drought is a major abiotic stress in many agricultural lands throughout the world. Large parts of agricultural regions are subjected to mild or severe abiotic stresses. In these areas, water deficit decreases crop productivity more than any other environmental stresses. It is also particularly serious in arid and semi-arid regions. With regard to this the commercial production and human consumption of durum wheat (*Triticum turgidum* L. var. *durum*), the second most important *Triticum* species, next to common wheat, is gravely affected in arid and semi-arid regions of the world where it serves a staple food. It is well known that durum wheat has the hardest kernel of all wheat and is used to make semolina .Semolina with strong gluten properties makes pasta products with superior cooking characteristics as can be found in macaroni, spaghetti, and other pasta products (Dai and Li 2004; Casati and Walbot 2004; Canadian Food Inspection Agency 2006).

Drought stress is an accepted basic factor affecting the different growth and development stages in a plant and many investigations on the response of plants to drought has helped to overcome the negative effects of water stress on plants. Seed germination is considered to be the most critical growth stage under water stress conditions, as it is a requirement for the success of the stand establishment of crop plants. Furthermore, seed germination in bread and durum wheat is a serious problem with regard to final plant productivity (Begg and Turner 1976). Several recent studies have reported that increasing osmotic stress decreases significantly the germination percentage and rate, coleoptile length, root and shoot length, dry weight of root and shoot, in bread wheat varieties (Ashraf *et al*. 1992; Jaradat and Dawayri 1981; Dhanda *et al*. 2004; Ghodsi 2004; Rauf *et al*. 2007; Yagmur and Kaydan 2008). In addition to this, varietals and genotypic differences in drought tolerance of the wheat have also been studied and reported (Steiner *et al*. 1990). The results of Radhouane (2007) showed that longer root length is a useful trait and adaptive response under water stress to access deeper water in the soil.

The objectives of this study were to determine the osmotic stress and genotype effects on different traits at the germination and seedling stages in promising durum wheat genotypes. In addition to this, identification of the significant correlations amongst studied traits in related to stress tolerance were also computed during germination and seedling period.

MATERIALS AND METHODS

Laboratory studies were conducted at the Institute of Biological Sciences, University of Malaya. It was laid out in factorial experiments based on a completely randomized design (CRD) with three replications and two factors. The first factor was osmotic stress at four levels, i.e. 0.0, -0.3, -0.6 3 and -0.9 MPa. The second factor was 20 promising spring drum wheat genotypes. The seeds of the various durum wheat genotypes (**Table 1**) were obtained from the elite durum yield trial (EDYT, 2006-2007) done at the Seed and Plant Improvement Institute, Iran.

Osmotic potentials (-0.3, -0.6, and -0.9 MPa) were produced using different concentrations of polyethylene glycol 6000 (PEG) at 20°C according to the method of Michel and Kaufmann (1973). Consequently the amount of PEG used for each concentration (-0.3, -0.6, and -0.9 MPa) were 143.18, 213.64 and 267.98 g/l distilled water, respectively. The seeds were germinated using the paper method, on 9 cm diameter Petri dishes on the top of the filter

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Table 1 List of durum wheat genotypes used in study.

Genotype	Pedigree	Genotype	Pedigree
G1	ARIA	G11	GREEN_2/HIMAN_12
G2	PISHTAZ	G12	HUI/YAV79//RASCON_37
G3	STOT//ALTAR 84/ALD	G13	LIRO_3/LOTAIL_6
G4	RASCON_39/TILO_1	G14	MUSK_1//ACO89/FNFOOT_2
G5	E90040/MFOWL_13//LOTAIL_6	G15	CADO/BOOMER_33
G6	BRAK_2/AJAIA_2//SOLGA_8	G16	PLATA_3//CREX/ALLA/3/LOTAIL_6
G7	HAI-OU_17/GREEN_38	G17	GARAVITO_3/RASCON_37//GREEN_8
G8	SN TURK MI83-84 375/NIGRIS_5//TANTLO_1	G18	BOOMER_18/KITTI_1//LUND_4
G9	RAFI97/RASCON_37//BEJAH_7	G19	CPAN.6018/2*RAJ1555//2*PORRON_4
G10	RASCON_37/BEJAH_7	G20	HYDRANASSA30/SILVER_5//SILVER_3/RISSA

papers. Twenty healthy and equal-sized seeds of each genotype were selected and then sterilized with 5% sodium hypochlorite solution for three seconds. The seeds were germinated in covered sterilized Petri dishes containing germination paper moistened with 8 ml of the different solutions of PEG-6000. The Petri dishes were kept in an incubator for 8 days at 20 ± 0.5°C (Rehman *et al*. 1996; Ghodsi 2004).

Data were recorded daily for 8 days. For germination purposes, only those seeds that presented approximately 2 mm of root length were considered to have germinated and were used for germination percentage and rate calculations (Sapra *et al*. 1999; Afzal *et al*. 2004). The numbers of seeds germinated were counted daily and the germination percentage and rate were estimated. Mean germination time (MGT) was calculated to assess the germination rate (GR) according to Ellis and Roberts (1980) and Sapra *et al*. (1999). To determine the coleoptile length, 12 hours of light was given after the fourth day, to the seedlings in the incubator. At the end of the eighth day, 5 seedlings were randomly selected and the coleoptile, root, shoot and also seedling length measured. Additionally, root, shoot and seedling dry weight were measured after drying samples at 76°C for 48 hours in an oven.

For all the investigated parameters, analysis of variance was statistically performed according to factorial experiments using EXCEL, MSTAT-C and SPSS software packages. Significant differences among the mean values were compared using the Duncan's Multiple Range Test with at least P<0.05. Simple correlation coefficient among different characteristics was also calculated at germination and seedling growth stage under osmotic stress conditions.

RESULTS AND DISCUSSION

Germination percentage and rate

The results (**Table 2**) show that the germination percentage and rate were highly significant (P<0.01) affected by osmotic stress and genotype treatments. In addition to this the interaction effects of osmotic stress × genotype was highly significant for the germination percentage, whereas it was non significant for the germination rate.

There was a decrease in germination percentage with increasing osmotic stress. The highest value was observed was 93.5% in D1 (distilled water control) and the lowest (42%) in the D4 (-0.9 MPa) treatment. However with regard to germination rate, there was a slight increase in D2 (-0.3 MPa) compared to D1 (**Fig. 2**). This is probably in response to low osmotic stress exhibited by some genotypes, such as G4 (**Table 2**). Overall, the results observed was similar to that shown in the germination percentage experiments (**Fig. 1**), that is a decreasing water potential (-0.3 to -0.9 MPa) by PEG caused a decrease in germination rate. Similar reductions in the germination percentage and rate affected by increasing osmotic stress have been reported by other workers in bread wheat (Ghodsi 2004; Rauf *et al*. 2007), durum wheat (Jaradat and Dawayri 1981) and in triticale (Nazeri 2005; Kaydan and Yagmur 2008). The probable explanation for these observations is that there was a decrease in water uptake ability by the seeds during germination and the seedling stage and this subsequently decreased the germination

Table 2 Analysis of variance (ANOVA) for the different characteristics of durum wheat genotypes during germination and the seedling stages.

	Source of variation (Mean square)				
Characters	**Osmotic stress**	**Genotype effects**	**Interaction effects**	**Error**	**CV %**
Germination percentage (G %)	33592.4**	370.07**	324.01**	164.62	16.87
Germination rate (GR)	0.190 **	0.009 **	0.001 ns	0.004	20.05
Coleoptile length (mm)	97.143 **	0.188 *	0.169 *	0.107	13.64
Shoot length (cm)	1106.51**	1.494 ns	1.527ns	1.140	18.72
Shoot dry weight (mg)	1111.11**	3.137 ns	2.614 ns	2.496	18.13
Root dry weight (mg)	826.39**	4.017 *	2.533 ns	2.291	21.89
Seedling dry weight (mg)	36.68.23**	10.71 ns	7.826 ns	7.534	20.87
Root / Shoot length	171.77**	1.28 ns	2.531*	1.595	24.42
Root / Shoot dry weight	129.06**	2.77 ns	2.82 ns	2.104	22.78
Degrees of freedom (df)	3	19	57	160	-

* Significant difference at $P < 0.05$ ** Significant difference at $P < 0.01$

Table 3 Response of germination percentage (G %) and germination rate (GR%) to genotype effects.

Genotype	G%	GR%	Genotype	G%	GR%
G1	84.13 a	27 abc	G11	79.58 a	33 ab
G2	76.20 ab	30 abc	G12	81.67 a	26 bc
G3	73.33 ab	29 abc	G13	76.67 ab	31 abc
G4	84.17 a	35 a	G14	72.92 ab	28 abc
G5	73.33 ab	29 abc	G15	73.75 ab	31 abc
G6	81.67 a	34 ab	G16	69.58 ab	28 abc
G7	71.25 ab	29 abc	G17	62.92 b	28 abc
G8	73.75 ab	29 abc	G18	82.92 a	32 abc
G9	78.75 ab	30 abc	G19	71.25 ab	28 abc
G10	80.42 a	34 ab	G20	72.92 ab	29 abc
LSD value	13.66	6.7		13.66	6.7
Sx	3.704	1.8		3.704	1.8

Column sharing the same letters indicates non-significant differences at the 0.01 level percentage and rate in the water stressed seeds.

As shown in **Table 3** the various studied genotypes showed different responses for germination percentage and rate. Genotype four (G4) exhibited the highest germination percentage (84.17%) whilst G17 showed the lowest germination percentage (62.92%) amongst all the genotypes studied. In addition, the highest and lowest values for germination rate were also obtained from G4 (0.35) and G17 (0.25) genotypes, respectively.

Coleoptile, root and shoot length and root to shoot length ratio

Analysis of variance (**Table 2**) indicated that the effect of osmotic stress on coleoptile, root and shoot length and also root to shoot length ratio were highly significant (P<0.01). Moreover, genotype effect for coleoptile and root length was significant (p<0.05).

As can be seen in **Fig. 3** the highest coleoptile, root and shoot length were observed in the D1 treatment whilst the lowest growth were observed in the D4 osmotic stress level. However the influence of osmotic stress on decreasing shoot length was more than its effect on decreasing coleoptile and root length. It was observed that coleoptile, root and shoot length decreased with increasing osmotic stress, whereas root to shoot length ratio increased (**Fig. 4**). This agreed with the results reported by Jaradat and Dawayri (1981) on durum wheat and Ghodsi (2004) and Adda *et al.* (2005) working on bread wheat. Their findings showed that with increasing osmotic stress (-0.3 to -0.1.2 Mpa) coleoptile, shoot, and root length decreased in durum and bread wheat genotypes. Nazeri (2005) and Yagmur and Kaydan (2008) studying triticale reported similar results as well. They also indicated that shoot, root and coleoptile length decreased with the increasing concentrations of PEG 6000 in all studied triticale varieties, and the highest negative effects were obtained at the highest PEG 6000 concentration (-1.44 MPa) that resulted in no shoot growth in some genotypes and varieties. On the other hand, the root to shoot length and dry weight ratio significantly increased under osmotic stress of PEG 6000 in comparison to control solutions.

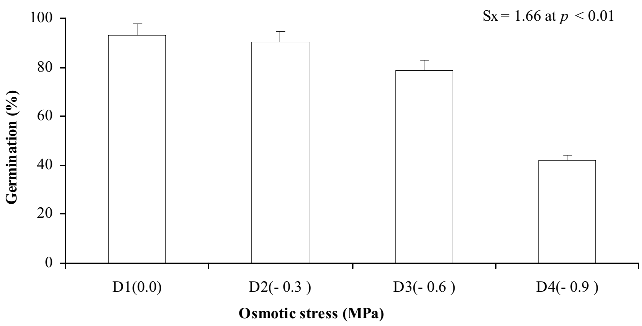

Fig. 1 The effect of osmotic stress on germination percentage.

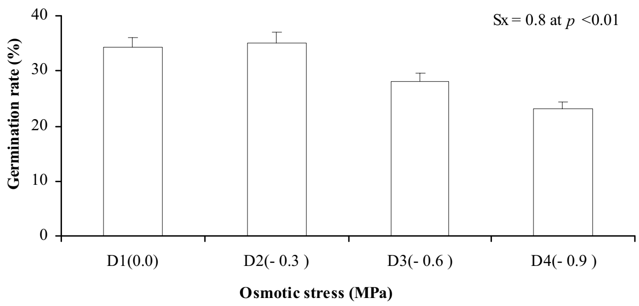

Fig. 2 The effect of osmotic stress on germination rate.

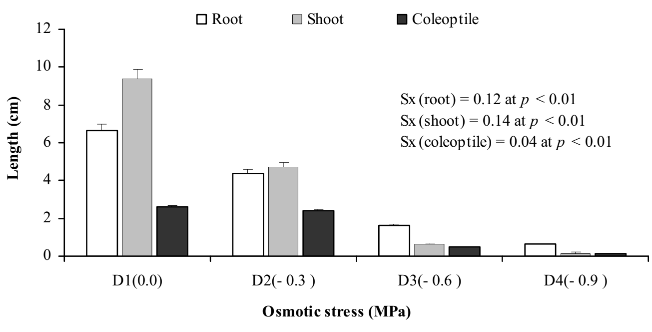

Fig. 3 The effect of osmotic stress on coleoptile, root and shoot length.

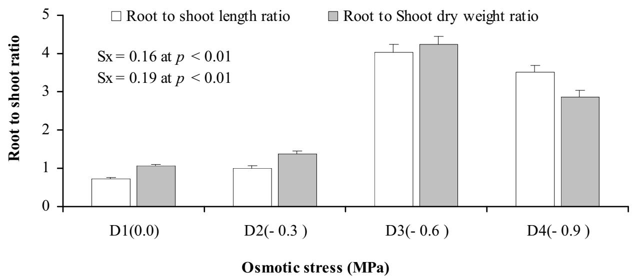

Fig. 4 The effect of osmotic stress on root to shoot length and dry weight ratio.

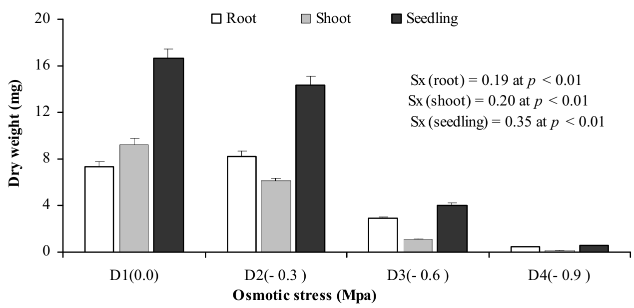

Fig. 5 The effect of osmotic stress on root, shoot and seedling dry weight.

According to Miralles *et al.* (2000) the reduction in growth affected by osmotic stress causes a decrease in cell size in plant roots and shoots. Dhanda *et al.* (2004) suggested that selection of the genotypes according to the root length and root to shoot length ratio under osmotic stress may be a useful method in predicting the drought tolerance of genotypes. The results showed that the highest and lowest coleoptile length was obtained in the G14 and G15 genotypes. In addition, the maximum values for root length was observed in G7, whilst the minimum values was observed in G8 genotypes.

Root, shoot, seedling dry weight and root to shoot dry weight ratio

The results in **Table 2** show that the root, shoot, seedling dry weight and also root to shoot dry weight ratio were highly significant (P<0.01) affected by osmotic stress. In addition to this, the effect of genotype treatment was significant for root dry weight. In the present study the highest and lowest values for shoot dry weight were obtained from D1 and D4 treatments respectively. With increasing osmotic stress shoot dry weight showed a decrease whilst root dry weight increased under low osmotic stress conditions (D2). However under more severe osmotic stress conditions (D3 and D4) a sizeable decrease in root dry weight was seen. The maximum root dry weight was observed in D2 whilst the minimum was observed in D4. Seedling dry weight exhibited a similar trend to shoot dry weight and with increasing osmotic stress, seedling dry weight also decreased (**Fig. 5**). The responses of root, shoot and seedling dry weight to osmotic stress in this study were similar to the results obtained by Jaradat and Dawayri (1981), Gawronska and Grzelok (1993), Dhanda *et al.* (2004) and Yamur and Kaydan (2008).

Table 4 Correlation coefficient among different characteristics (G% :germination percentage, GR: germination rate, CL: coleoptile, RL: root length, SL: shoot length, RDW: root dry weight, SDW: shoot dry weight, SLDW: seedling dry weight) of various durum wheat genotypes in germination and seedling stage under osmotic stress condition

Traits	G%	GR	CL	SL	RL/SL	RDW	SDW	SLDW	RDW/SDW
G%	1	0.53**	0.68**	0.58**	-0.85	0.70**	0.60**	0.68**	0.57
GR		1	0.60**	0.54**	-0.22**	0.62**	0.58**	0.62**	-0.13**
CL			1	0.88**	-0.45**	0.89**	0.90**	0.93**	-0.20**
SL				1	-0.43**	0.77**	0.95**	0.90**	-0.25**
RL/SL					1	-0.36**	-0.47**	-0.42**	0.47**
RDW						1	0.84**	0.95**	-0.10
SDW							1	0.96**	-0.28**
SLDW								1	-0.21**
RDW/SDW									1

* Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level

Correlation studies among different traits

Correlation studies among the different traits showed that the highest correlation coefficient was between seedling dry weight and shoot dry weight and also between seedling dry weight and root dry weight (r = 0.96 and r = 0.95). The lowest correlation coefficient was seen between germination rate and root to shoot dry weight (r = - 0.13). In the present study the germination percentage and rate showed a positive and highly significant correlation with coleoptile length, root length, shoot length, root dry weight, shoot dry weight and also seedling dry weight. Root and shoot length also showed positive and highly significant correlation with coleoptile length, root dry weight, shoot dry weight and seedling dry weight. However they exhibited a negative and highly significant correlation with root to shoot length ratio and root to shoot dry weight ratio. Dhanda *et al*. (1995) showed that under drought conditions, genotypes with strong root growth are particularly important in tolerating drought. There was a negative and highly significant correlation between root to shoot length ratio with root dry weight, shoot dry weight and seedling dry weight. Root to shoot length ratio showed a positive and highly significant correlation with shoot to root dry weight ratio. The highest and lowest correlation coefficient for root to shoot length ratio was observed with germination percentage (r = -0.85) and germination rate (r = - 0.22). It can be seen that the effects of severe osmotic stress on decreasing shoot length was more than its effect over decreasing root length (**Table 4**).

CONCLUSIONS

Seed germination is considered to be the most critical growth stage under water stress conditions, as it is a requirement for the success of the stand establishment of crop plants. The response of germination traits in durum promising genotypes changes at the germination and seedling stage under control and water stress conditions. In the other word, the germination characteristics depend on genetic factors, and also osmotic stress conditions. The results of present study showed that germination percentage and rate, coleoptile, root and shoot length, shoot, root and seedling dry weight, root to shoot length and dry weight ratio were highly significant affected by osmotic stress treatments. In addition to this, In addition to this the difference in germination percentage and rate in the various durum wheat genotypes studied were highly significant whilst for coleoptile and root length and also root dry weight the differences were significant.

It was observed that germination percentage, coleoptile length, root length, shoot length, shoot and seedling dry weight decreased with increasing osmotic stress, whereas root to shoot length ratio and root to shoot dry weight ratio increased. Furthermore, germination rate and root dry weight increased from control (stilled water) to -0.3 Mpa treatment, while their values decreased with increasing the severity of the osmotic stress (-0.3 to -0.9 Mpa). Correlation studies among the different traits showed that the highest correlation was between seedling dry weight and shoot dry weight. On the other hand, the lowest was seen between germination rate and root to shoot dry weight.

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