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Evaluation of osmotic stress tolerance in wheat genotypes and solidarity of assessed traits using in vitro mature embryos culture

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Abstract: ***In order to evaluate the response of wheat cultivars to drought stress, ten cultured wheat cultivars, in a mature embryo stage were studies based on morphological traits. The study was performed in a randomized complete block design with eight replications in the mature embryo stage. The osmotic stress by polyethylene glycol (6000PEG) Treatments, 0, -2.5, -5, -7.5, -10 were applied in liquid medium on in vitro matured embryos. The results showed that the tolerance of genotypes to applied levels of drought stress was different. The effect of drought stress on assessed traits was significant. Genotypes responded differently at different levels of applied drought stress in terms of these characteristics including number of rootlets, rootlet length, shootlet length, callus volume, regeneration percentage, callus dry weight, seedling fresh weight, seedling dry weight, callus induction percentage, and callus fresh weight and it refers to the fundamental differences between the genotypes studied. The results can be used as indicators for the selection of drought tolerant genotypes. 68DH and Pavan were identified as tolerant cultivars among these genotypes.***

Keywords: Osmotic stress, mature embryos culture, PEG, wheat**.**

1. Introduction

Drought stress is the most significant factor restricting crops production including wheat, *Triticum aestivum*, in Iran and around the world (Khayatnezhad et al., 2010). Wheat is an important cereal in the world. As world population increases and water resources for crop production decreases, the development of drought resistant cultivars is an important goal. Compared to other environmental stresses, water scarcity extremely limits crop growth and production (Tutan and Baser, 2004). Development of drought resistant crops using traditional methods is very time consuming and sometimes impossible. So today, more useful methods including tissue culture are used for produce drought resistant plants and other unfavorable factors (Sinaki, 2007). Using non-biological stress factors in the selection of in vitro culture of plants has had favorable outcomes (Moayedi, 2010). Plant embryo culture is widely used as a good way to produce callus. The advantage of using embryos in wheat breeding is rapid regeneration and also overcoming the natural barriers in the crossing ability between different races and cultivars of wheat (Kintzies, 1996). (Ozgen et al., 1998) examined the efficient callus induction and plant regeneration from mature and immature embryos of winter wheat genotypes and reported that mature embryos had a high frequency of callus induction and plant regeneration capacity and so they can be used as the source of efficient explants in wheat tissue culture. (Jajarmi, 2009) studied effects of drought stress on seven wheat cultivars on six levels of water treatment. The results showed significant differences among cultivars and the levels of drought stress. Shootlet length was more sensitive to drought stress among others. (Joshi et al., 2011), in a study on drought tolerance using mature embryos, showed that as PEG concentration increased, callus volume decreased and also total content of proline significantly increased. (Galovic et al., 2005) studies effects of drought stress in several winter wheat genotypes. They transferred them to MS medium containing 5% PEG after callus production. Significant statistic differences were observed among genotypes in their response to the induced stress. The callus growth under stress conditions significantly reduced the callus fresh weight in all genotypes. (Abdelghany et al., 2002) examined the growth and regeneration of callus from mature embryos of wheat varieties under different concentrations and compared related traits with their growth and performance in field conditions. Significant interactions were observed between PEG variety and concentration for the ability to survive and regeneration of callus.

2. Materials and methods

This experiment was conducted in the Biotechnology laboratory at the University of tabriz in the spring of 2011 on ten wheat cultivars (68DH, Sorkhe, Roshan, Gohar, Zagros, Varamin, Sardari, Karkhe, Chamran, Pavan) on mature embryos stage. To conduct this experiment, and to prepare explants, the seeds of different wheat genotypes were soaked in water for 16 hours (Tutan and Baser, 2004). For the superficial sterilization of seeds, first 70% ethanol for 30 seconds and then sodium hypochlorite 2.5% for 15 min were used. Samples were washed five times with distilled water to clean residual sodium hypochlorite on seeds (Borles and Sunkar, 2005). After

disinfection under a laminar hood, seed embryos were transferred to dishes containing MS liquid medium with 0.1 mg kinetin and 2 mg 2,4-D and treatments, 0, -2.5, -5, -7.5, -10 times osmotic stress by polyethylene glycol (6000PEG) onto the paper bridge. Five embryos were placed in each dish and after closing the Petri dishes with parafilm, they were placed in a growth chamber with a temperature of 1 ± 25 ° C and 16 hours light and 8 hours of darkness for 35 days. Size of calluses was determined by Hooker and Niberz scale. Number of rootlet, rootlet length, shootlet length, callus volume, regeneration percentage, callus dry weight, seedling dry weight, seedling fresh weight, callus induction percentage, and callus fresh weight were evaluated. This study was performed in randomized complete block design with eight replications. Genotype and various levels of polyethylene glycol were experimental factors in this study. Before performing statistical computations, the data were tested for normality using Minitab software. The test of homogeneity of variances was performed on the data. Analysis of variance, specification of correlation coefficients and comparison of mean values were performed using SAS software and based on Duncan test at the 5% level. Excel software was used to draw shapes.

3. Results

The results of analysis of assessed traits variance on in vitro mature embryos culture indicates that the effects of genotype and also effects of drought stress levels on the number of roots, root length, coleoptile length, shootlet length, callus volume, callus fresh weight, callus induction percentage, and regeneration percentage were obtained significant at 1% probability level**.** The cultivar × stress interactions for all traits were significant at 1% probability level and it indicates that the cultivars vary at different levels of osmotic stress in terms of the ability to produce the above traits (Table 1).

Comparing the mean values of assessed traits in Table 2 using Duncan's multiple range test, and considering the significant effect of genotype on studied traits in mature embryos, it is completely obvious that 68DH and Zagros cultivars, having the highest number of rootles and root length, are significantly different from other cultivars. Paven cultivar had the highest amount of callus volume and callus fresh weight. 68DH, Sorkhe and Pavn had the highest percentage of callus induction. 68DH and Zagros cultivars, however, showed a high percentage of regeneration among other cultivars (Table 2).

With significant effect of osmotic stress levels, Duncan's mean comparison test was performed, by which it is determined that the maximum values were observed in the number of roots on the second level of osmotic stress (bar -2.5) with mean value of 1.01, and length of rootles with a mean value of 0.375 cm showed significant difference with the other levels on the same level of stress. Coleoptile length, shootlet length, callus volume,callus fresh weight, respectively, with the mean values of 0.907 cm and 1.278cm and 1.38 and 0.0016g, showed significant difference with other levels. Callus induction percentage on the first two levels, and regeneration percentage on the first three levels did not differ significantly and it shows that effects of drought stress levels was the same on these traits (Table 3).

The significant interaction of cultivar × stress indicates that cultivars vary in different levels of osmotic in terms of the ability to produce rootlets. Most cultivars can produce more rootlets on smooth levels of osmotic stress significantly increased which shows an adaptation to stress conditions (Figure 1). Rootlet length of the cultivars decreased in various levels as Polyethylene Glycol concentration increased (Figure 2). Also as osmotic potential increased, Coleoptile length decreased in all cultivars (Figure 3). According to Figure 4, the maximum reduction was observed in the bar -2.5 which indicates intolerance of higher levels of stress in embryonic stage for shootlet length. Also as osmotic potential decreased, the amount of callus volume was increased. The highest amount of callus volume was observed on (bar 0) level for 68DH and Pavan (Figure 5). Figure 6, the highest amount of fresh weight was observed in 68DH, Chamran and Pavan. As osmotic potential increased, callus fresh weight decreased. Figure 7 indicates that as osmotic potential increases, callus induction percentage decreases. Also as osmotic potential increases, the rate of regeneration decreases. The maximum reduction in regeneration was observed in Pavan and 68DH on -5bar level, and in Chamran, Roshan and Zagros on bar -7.5 level (Figure 8).

4. Discussion

The results of this experiment was compatible with the results obtained by (Joshi et al., 2011; Galovic et al., 2005). It can be concluded that a part of mechanisms of drought tolerance in wheat due to multiple mechanisms, such as the loss of old leaves and osmotic potential acts at the cellular level.

According to the results of Pearson correlation coefficients table of the assessed traits of mature embryos (Table 4), it was found that the number of rootlet correlated with the rootlet length, and callus volume. It is also shown that rootlet length and callus volume have a positive and significant correlation at the 1% probability Coleoptile length had a significant positive correlation with shootlet length, callus volume, and callus fresh weight level. Shootlet length also had a significant positive correlation with callus volume and callus fresh weight at the 1% probability level. Callus volume was positively correlated with number of rootlets, rootlet length, coleoptile length, and shootlet length at the 1% probability level. Callus fresh weight showed a high correlation with coleoptile length, shootlet length, and callus volume.

Callus induction percentage was the only trait that correlated positively and significantly with regeneration percentage at the 1% probability level. So it can be concluded that in the mature embryos culture experiment of different cultivars of wheat, the highest amount of rootlet number, rootlet length, coleoptile length, shootlet length, callus volume, and callus fresh weight was observed in 68DH and Pavan, and also the highest percentage of callus induction was obtained in 68DH and Pavan. The highest percentage of regeneration was observed in 68DH, Zagros and Pavan. Therefore it can be declared that 68DH and Pavan are recognized as tolerant cultivars to drought stress compared to other cultivars in this study. For a better assessment of osmosic stress, it is proposed that this research be conducted in field conditions and at different growth stages and also with the use of higher concentrations of PEG.

Table 1 ANOVA table the effect of genotype and osmotic stress levels in all traits

		Mean of Squares							
Source	D.F	Number of Radical	Length of Radical	Length of Coleoptile	Length of Plumule	Callus Volume	Callus wet weight	Percentage of Callus	Percentage of Regeneration
Replication	7	0.03**	0.065**	0.084*	0.196*	0.77*	0.000003 ns	0.857 *	1348.57**
Genotype	9	6.37**	0.79**	1.79**	2.837**	25.99**	0.00003**	10.5**	18988.9**
Stress	4	0.41**	1.253**	11.63**	23.44**	15.08**	0.00002**	64.6**	27057.5**
	36	0.026**	0.131**	1.109**	2.039**	5.75**	0.00001**	0.94**	1574.16**
Error	343	O.O1	0.024	0.039	0.093	0.33	0.000003	0.351	479.18

*, ** and ns significant at $p \leq 0.05$, $p \leq 0.01$ and non-significant, respectively

Table 2 - Comparison of evaluated traits mean of different wheat cultivars using Duncan's multiple range test

	Number of Radical	Length of Radical	Length of Coleoptile	Length of Plumule	Callus Volume	Callus wet weight	Percentage of Callus	Percentage of Regeneration
DH68	1.065^{a}	0.385^{a}	0.725^{a}	0.943^{a}	1.07^{b}	0.0022^{ab}	63.5^{a}	71^{a}
Sorkh	0.785^{b}	0.176^{b}	0.185^{de}	0.256^{c}	1.005^{b}	0.0015^{b}	60.5^{a}	45^{bc}
Roshan	0.34^{c}	0.096^{c}	0.152^{def}	0.191^{cd}	0.73^{c}	0.0015^{b}	43^{b}	35^{c}
Gohar	0.505^{c}	0.162^{bc}	0.096^{efg}	0.196^{b}	0.29^{ef}	0.00023^{d}	18^{c}	22^{d}
Zagros	1.245^{a}	0.437^{a}	0.323^{c}	0.487^{b}	0.495^{cde}	0.00033^{d}	37.5^{b}	65.5^{a}
Varamin	0^{d}	0^{d}	0.0335^{g}	0.0335^{e}	0.34^{ef}	0.00041^{d}	9^{d}	5.5^{e}
Sardari	0.08^{d}	0.016^{d}	0.70^{fg}	0.0925^{de}	0.09^{f}	0.00023^{d}	4^{d}	9.5^{e}
Karkhe	0.695^{b}	0.188^{b}	0.203^{d}	0.239^{c}	0.43^{de}	0.00065^{cd}	34.5^{bc}	36.5^{c}
Chamran	0.425^{c}	0.122^{bc}	0.330^{c}	0.425^{b}	0.63^{cd}	0.0013^{bc}	39.5^{bc}	36.5^{c}
Pavan	0.41^{c}	0.184^{b}	0.47^{b}	0.514^{b}	2.92^{a}	0.0025^{a}	69.5^{a}	53.5^{b}

Table 3 - Mean comparison of levels of osmotic stress on studied traits

	Control	-2.5 Bar	-5 Bar	-7.5 Bar	-10 Bar
Number of Radical	0.537^{c}	1.01^{a}	0.705^{b}	0.295^{d}	0.227^{d}
Length of Radical	0.164^{c}	0.357^{a}	0.237^{b}	0.075^{d}	0.051^{d}
Length of Coleoptile	0.907^{a}	0.297^{b}	0.073^{c}	0.009^{c}	0.007^{c}
Length of Plumule	1.278^{a}	0.32^{b}	0.073^{c}	0.009^{d}	0.007^{d}
Callus Volume	1.38^{a}	1.08^{b}	0.74^{c}	0.48^{d}	0.32^{d}
Callus wet weight	0.0016^{a}	0.0014^{ab}	0.0016^{b}	0.00^{ab}	0.0003^{c}
Percentage of Callus	44^{ab}	52^{a}	40.75^{b}	30^{c}	22.75^{d}
Percentage of Regeneration	53.5^{a}	53.25^{a}	47^{a}	20.25^{b}	16^{b}

Table 4 - Pearson correlation coefficients of evaluated traits

	X_{21}	X_{22}	X_{23}	X_{24}	X_{25}	X_{26}	X_{27}	X_{28}
(X_{21}) Number of Radical	1							
(X_{22}) Length of Radical	0.935**	1						
(X_{23}) Length of Coleoptile	0.233	0.299	1					
(X_{24}) Length of Plumule	0.184	0.246	0.99**	1				
(X_{25}) Callus Volume	0.659**	0.572**	0.669**	0.652**	1			
(X_{26}) Callus wet weight	0.344	0.325	0.847**	O.836**	0.845**	1		
(X_{27}) Percentage of Callus	-0.035	0.043	0.347	0.348	0.434	0.431	1	
(X_{28}) Percentage of Regeneration	-0.211	-0.06	0.255	0.257	0.172	0.297	0.79**	1

*, ** and ns significant at $p \leq 0.05$, $p \leq 0.01$ and non-significant, respectively

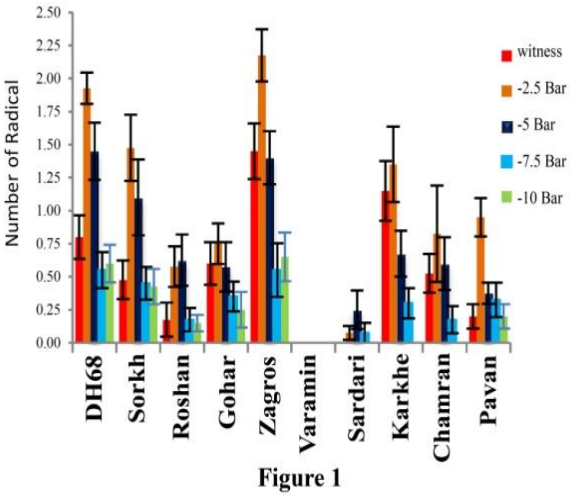


Figure 1

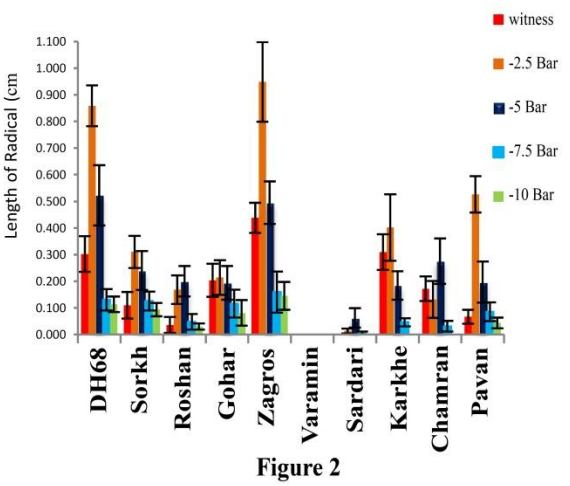


Figure 2

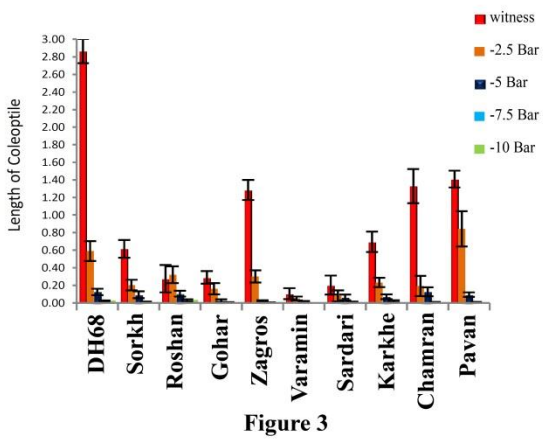


Figure 3

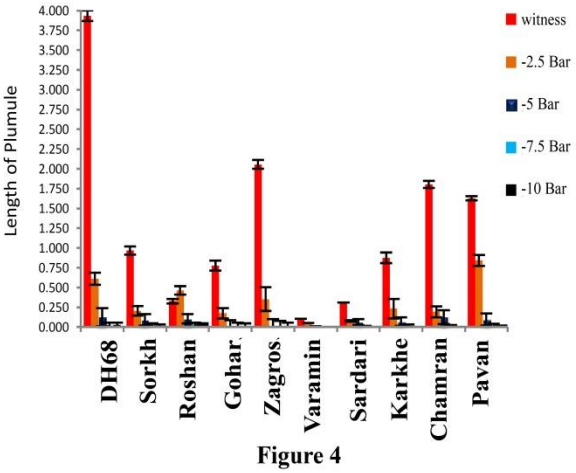
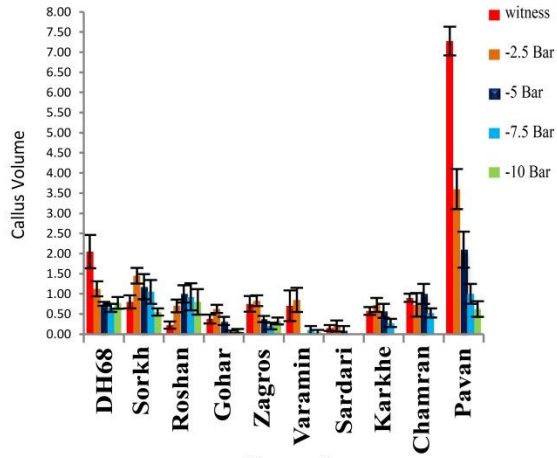


Figure 4



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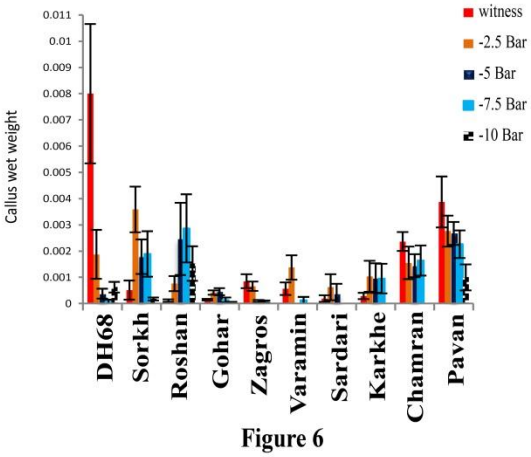

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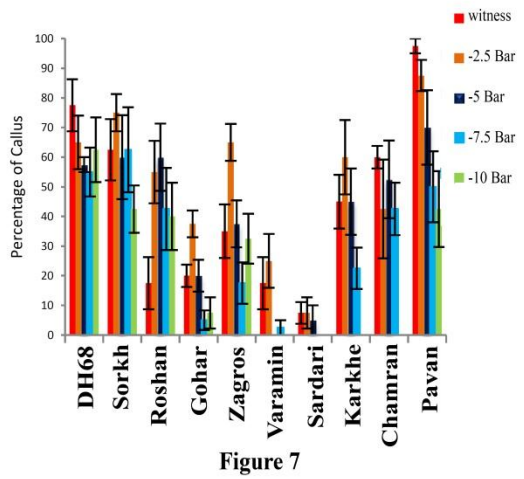

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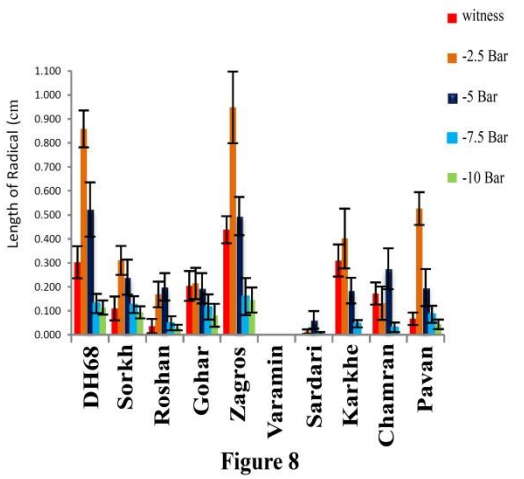

Figure 8

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