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Effect of osmotic stress on germination and growth of Iranian wheat cultivars

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Abstract

In order to evaluate the response of wheat cultivars to drought stress, ten wheat cultivars, in the germination and seedling growth stages, were studies based on morphological traits. Germination test was done in a randomized complete block design with four replications. Treatments consisted of ten cultivars of wheat and five osmotic potential levels (0, -2.5, -5, -7.5 and -10 times, respectively). Stress levels and cultivars were respectively considered as the main and secondary factors. In this study, stress was imposed on plants at the reproductive phase. The results showed that as osmotic potential increased in the germination tests, measured traits decreased, and this reduction was maximum in the potential changes from -5 to -7.5 in most traits. These traits were not affected by moderate levels of stress. Also Varamin, Pavan and 68DH had the highest number of rootlet and rootlet length among the studied cultivars and thus it can be stated that this Fig. have a greater ability to resist the drought.

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Introduction

Common wheat (*Triticum aestivum*) grows in a wide range of climatic conditions of the world and in fact, this plant is one of the most adaptable crop varieties and forms the main food crop for humans (Nouri *et al.* 2011). Drought is the major factor of limiting agricultural production which prevents the plants from achieving the maximum yields (Naseri *et al.* 2010; Kadam *et al.* 2017). Like many other physiological and morphological characteristics, resistance or susceptibility to drought stress in wheat germination stage can have highly variable numbers of populations (Nakhforoosh *et al.* 2016). Therefore these studies provide the understanding of the physiological and genetic mechanisms of drought stress susceptibility and resistance (Jajarmi 2009).

Germination is one of the critical stages in the life cycle of plants, so that at this stage, durability, stability and final yield performance is guaranteed (Khayatnezhad *et al.* 2010). Due to the soil heterogeneity and impossibility of providing environmental factors in field conditions such as drought stress, conducting experimental researches on stress issues under laboratory conditions has been noticed recently. Investigating the reaction of genotypes to the osmoticom solutions such as polyethylene glycol is among these methods. Studies have shown that the percentage of seed germination in the polyethylene glycol 6000 solution was approximately equal to the percentage of seed germination in the soil with the same potential Aziz *et al.* (2008) Ghanifathi *et al.* (2011), in their study showed that the effects of different levels of osmotic potential on the most traits, particularly germination percentage, were significant (Aziz *et al.* 2008; Ghanifathi *et al.* 2011).

The genotype interactions at varying levels of osmotic potential for seedling length, ratio root length to shoot length, number of rootlets, rootlet length and shoot dry weight, were also significant. Qayyum *et al.* (2011) studied the seeds of a number of wheat cultivars at five levels of osmotic stress and reported that as osmotic stress increased, germination percentage, germination time mean and coleoptile length decreased (Qayyum *et al.* 2011).

Jajarmi (2009) used polyethylene glycol 6000 at four levels to investigate the effect of drought stress in three wheat cultivars. The results showed that as the osmotic potential decreased, germination percentage significantly decreased (Jajarmi, 2009). However, aims of the current study is following as; to evaluate the response of ten bread wheat cultivars to drought stress both in the germination and seedling growth stages.

Material and methods

Plant material

The present study was conducted on the seeds of ten bread wheat cultivars (68 DH, Sorkheh, Roshan, Gohar, Zagros, Varamin, Sardari, Karkheh, Chamran, Pavan), which they were papered by seed and plant improvement institute, Karaj, Iran.

Seed Germination and Stress Application

Seed surface sterilization was done under sterile conditions using 70 percent ethanol for 10min and then a solution of sodium hypochlorite 2.5 percent for 15min. After three times being washed with sterile distilled water, seeds were separately dried on several layers of sterilized filter paper. Sterilized seeds were then placed under a laminar hood in 9cm Petri dishes on filter papers. During the germination, the osmotic stress was applied in five levels (zero with distilled water, -2.5, -5, -7.5, -10 times) by using polyethylene glycol 6000. The mentioned cultures were kept in the growth chamber at a temperature of 1 ± 25, and 16-hr light and 8 hour dark period, for 10 days (Ghanifathi *et al.* 2011). This experiment was done in Laboratory of Biotechnology, Faculty of Agriculture, University of Ilam.

Traits for Evaluation

In order to measure germination related traits (number of germinated seeds in each culture, germination percentage, and germination rate) the counting germinated seeds was done on a daily basis and at the end of the last day (after 8 days) other traits including the experimental unit, numbers of rootlet, rootlet length, coleoptile length, shoot length, ratio of root fresh weight to shoot fresh weight, and ratio of root dry weight to shoot dry weight were measured.

Germination percentage (GP) and germination rate (Rs) were calculated using the following procedure (Ghanifathi *et al.* 2011):

1) Germination percentage (GP):

100 × Ng / Nt = FGP

Ng = total number of germinated seeds;

Nt = total number of seeds evaluated

2) Germination rate (Rs): Si / Di 3 = Rs

Si = number of seeds germinated on day i;

Di = number of days to count n

Statistical Analysis

The experiment test was done in a randomized complete block design (RCBD) with four replications. The experimental factors included various cultivars and levels of polyethylene glycol (PEG). Before performing statistical analyzes, the data were tested for normality using Minitab software.

The homogeneity of variance test was also performed on the data.

Analysis of variance, calculation of correlation coefficients, and comparison of mean values were done using SAS software and based on Duncan's multiple range tests at 5 percent level of probability.

Results and discussion

ANOVA Results

According to the results of the ANOVA table (Table 1), the effect of genotype and osmotic stress levels in all traits were significant at 1 percent level of probability. This shows that there are genetic differences between cultivars used in this experiment.

Due to the significant interactions between osmotic stress levels and Fig. in 1 percent level of probability for all traits studied in this experiment, it can be concluded that the different cultivars in different osmotic potentials show different responses (Moayedi *et al.* 2010).

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

		Mean of Squares							
Source	D.F	Number of Rootlet	Rootlet Length	Coleoptile Length	Shoot Length	Germination Percentage	Germination rate	Ratio of root fresh weight to shoot fresh weight	Ratio of root dry weight to shoot dry weight
Genotype	9	21.146**	0.919**	0.0248**	0.115**	2.58**	0.496**	0.56**	0.487**
Stress	4	75.596**	3.821**	0.175**	5.255**	9.98**	1.134**	13.228**	9.627**
Genotype ×Stress	36	2.095**	0.107**	0.0025**	0.0468**	0.181**	0.024**	0.761**	0.593**
Error	150	0.459	0.0249	0.00057	0.0193	0.04	0.0037	0.194	0.238

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

Mean Comparisons

Considering the significant effect of genotype on the evaluated traits in this study, performing the mean comparison using Duncan's test (Table 2), it was observed that Varamin had the highest number of roots. 68DH showed a better reaction than other cultivars for rootlet length and coleoptile length. However, there were no significant differences between 68DH and Gohar in shoot length. It was also observed that 68DH also had better response for germination percentage and germination rate than other cultivars.

The highest amounts were allocated to Sardari in the ratio of root fresh weight to shoot fresh weight, and ratio of root dry weight to shoot dry weight. According to Table 2, no significant differences were observed between Sardari and Roshan in rootlet number, rootlet length, coleoptile length, shootlet length, and germination percentage and germination rate. Sorkheh has the lowest ratio of root fresh weight to shoot fresh weight. However, there were no differences between Sorkheh and Chamran in the ratio of root dry weight to shootlet dry weight.

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

	Number of Rootlet	Rootlet Length	Coleoptile Length	Shoot Length	Germination Percentage	Germination rate	Ratio of root fresh weight to shoot fresh weight	Ratio of root dry weight to shoot dry weight
DH68	2.91[b]	3.72[a]	1.96[a]	3.97[a]	73.2[a]	5.03[a]	0.547[bc]	0.545[bc]
Sorkhe	1.94[c]	1.58[d]	0.92[d]	2.87[bc]	27[d]	1.5[e]	0.468[c]	0.445[c]
Roshan	0.55[d]	0.37[e]	0.28[e]	2.22[c]	8[e]	0.37[g]	0.86[ab]	0.832[ab]
Gohar	2.69[b]	2.004[d]	1.29[c]	3.89[a]	37.2[c]	1.94[d]	0.82[ab]	0.767[abc]
Zagros	1.73[c]	1.46[d]	0.84[d]	3.84[ab]	23.6[d]	1.3[e]	0.657[bc]	0.683[abc]
Varamin	3.71[a]	2.53[b]	1.53[ab]	3.37[ab]	56.2[b]	3.54[b]	0.699[bc]	0.57[abc]
Sardari	0.6[d]	0.34[e]	0.44[e]	2.68[c]	10[e]	0.48[g]	1.023[a]	0.912[a]
Karkhe	1.78[c]	1.16[d]	0.84[d]	3.88[ab]	18.8[e]	1.01[f]	0.696[bc]	0.731[abc]
Chamran	1.71[c]	1.97[c]	0.65[d]	3.88[ab]	23.4[d]	1.19[ef]	0.619[bc]	0.465[c]
Pavan	3.02[b]	2.39[bc]	1.42[bc]	3.4[ab]	57.2[ab]	3.37[c]	0.551[b]	0.596[abc]

Means that have at least one common letter are not significantly different at 5% level.

Performing mean comparison test (Table 3), it was determined that for levels of osmotic stress, no significant difference was observed between control surfaces and -2.5 bar in rootlet number, rootlet length, coleoptile length, shootlet length, germination rate and germination percentage, and highest amount of these traits was observed in these surfaces.

The ratio of root fresh weight to shoot fresh weight, and root dry weight to shoot dry weight had the highest amounts in the control surface. It should be noted that as the osmotic potential increases, measured traits decrease, so that their least amount was observed on the fifth level of stress (-10bar). Similar to our results, several works also showed notable differences in the germination percentages and germination rate of wheat genotypes, which they were grown under osmotic stress (Dhanda *et al.* 2004; ÖZTÜRK *et al.* 2016). ÖZTÜRK *et al.* (2016) reported no germination in -10 bar osmotic level in the 64 certified and local bread wheat genotypes, which they were collected from Turky (ÖZTÜRK *et al.* 2016).

Unlike their work, the average germination percentage of our investigated genotypes was 4.2, showing that genetic source of bread wheat genotypes of Iran is relatively rich than Turky wheat genotypes, in terms of germination in osmotic stress.

Similar to our findings, Dhanda *et al.* (2004), and Baloch *et al.* (2012) observed notable decrease in the root lengths of wheat genotypes under osmotic stress (Dhanda *et al.* 2004; Baloch *et al.* 2012). Ahmadizadeh *et al.* (2011) reported 45.55% and 64.91% decreases in root lengths of wheat genotypes under −0.6 and −0.8 MPa treatments, respectively (Ahmadizadeh *et al.* 2011). In addition, ÖZTÜRK *et al.* (2016) and Nagel *et al.* (2014) observed measurable reduction in shoot length of investigated wheat genotypes.

This phenomenon also was observed in our results, so that there was significant reduction in terms of all investigated traits, including shoot length, coleoptile length, and rootlet length (Nagel *et al.* 2014; ÖZTÜRK *et al.* 2016).

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

Traits	Control	-2.5 Bar	-5 Bar	-7.5 Bar	-10 Bar
Number of Rootlet	3.195[a]	3.34[a]	2.513[b]	1.09[c]	0.193[d]
Rootlet Length	3.262[a]	2.79[a]	1.93[b]	0.833[c]	0.069[d]
Coleoptile Length	1.794[a]	1.71[a]	1.229[b]	0.335[c]	0.021[d]
Shoot Length	6.573[a]	5.92[a]	3.6[b]	0.873[c]	0.065[d]
Germination Percentage	3.265[a]	3.129[a]	2.289[b]	1.005[c]	0.183[d]
Germination rate	54.3[a]	50.8[a]	39[b]	19[c]	4.2[d]
Ratio of root fresh weight to shoot fresh weight	1.604[a]	0.853[b]	0.538[c]	0.352[c]	0.121[d]
Ratio of root dry weight to shoot dry weight	1.319[a]	0.983[b]	0.503[c]	0.364[c]	0.101[d]

Means that have at least one common letter are not significantly different at 5% level

Correlation Results

Based on the results of the calculation of simple correlation coefficients among evaluated traits in germination test (Table 4), a positive significant correlation at 1 percent level of probability was observed between the number of rootlet and all other evaluated traits in this study, except for the ratio of root dry weight to shoot dry weight. There was also a positive significant correlation at 1 percent level of probability between rootlet length and all other evaluated traits in this study, except for the ratio of root fresh weight to shoot fresh weight and the ratio of root dry weight to shoot dry weight. There was a positive significant correlation at 1 percent level of probability between coleoptile length and evaluated traits in germination test. However, the correlation between this trait and ratio of root fresh and dry weight to shoot fresh and dry weight was not significant.

A positive significant correlation at 1 percent level of probability between shootlet length and all other evaluated traits in this study was reported. There was also a positive significant correlation at 1 percent level of probability between the ratio of root fresh weight to shoot fresh weight and shootlet length, and a positive significant correlation at 5 percent level of probability between the ratio of root dry weight to shoot dry weight and number of rootlet. According to Table 4, the ratio of root dry weight to shoot dry weight has a positive significant correlation with shootlet length and the ratio of root fresh weight to shoot fresh weight at 1 percent level of probability. A positive significant correlation at 1 percent level of probability was observed between generation percentage and all other evaluated traits in this study. However, there was no significant correlation between this trait and ratio of root fresh and dry weight to shoot fresh and dry weight.

Table 4. Pearson correlation coefficients of evaluated traits

	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8
(X_1) Number of Rootlet	1							
(X_2) Rootlet Length	0.925**	1						
(X_3) Coleoptile Length	0.908**	0.908**	1					
(X_4) Shoot Length	0.802**	0.755**	0.907**	1				
(X_5) Ratio of root fresh weight to shoot fresh weight	0.239**	0.127	0.375	0.604**	1			
(X_6) Ratio of root dry weight to shoot dry weight	0.253	0.134	0.393	0.6**	0.923**	1		
(X_7) Germination Percentage	0.910**	0.950**	0.876**	0.7**	0.150	0.211	1	
(X_8) Germination rate	0.863**	0.942**	0.876**	0.68**	0.105	0.172	0.982**	1

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

Given the significant effect of genotype on rootlet length, it can be stated that root length is an important trait for resistance to stress. All the cultivars with higher rootlet growth have greater ability of resistance to drought (Tahmasebi *et al.* 2016). According to the results of this experiment, 68DH had the highest rootlet length. Elongation rate of rootlet and shootlet is of great important. Seeds with suitable rootlet and shootlet growth in drought conditions can achieve better and faster establishment and guarantee a higher performance in appropriate environmental conditions (Leishman and Westoby 1994).

Considering the role of rootlet in plants water uptake, rootlet number and rootlet length are regarded as the most important traits. According to the results, the numbers of Varamin, Pavan and 68DH had the highest amount of rootlet number and root length. According to the results, Varamin, Pavan and 68DH had the highest number of root and root length. As a result, it can be stated that these cultivars have the ability to resist the drought. Coleoptile length is effective for the rapid rise of shootlet from the soil. The results of this experiment indicated that mild stress does not affect coleoptile length and as osmotic potential increases, coleoptile length decreased.

The reason for this could be an increase in PEG concentration and also osmotic pressure of the medium which leads to reduction of seeds uptake and prevention of seedlings natural performance (Yin *et al.* 2011). Coleoptile length is effective for the rapid rise of shootlet from the soil. In this experiment, 68DH, Varamin and Pavan had the longest coleoptile. The highest germination percentage and rate were observed in 68DH, Varamin and Pavan. In accordance to our results, Nagel *et al.* (2014) revealed significantly correlation between the coleoptile length and germination rate, plant height of wheat lines, which they were germinated under osmotic stress. They concluded that low correlations between seed germination parameters under the control and osmotic stress treatments indicate a different genetic background of investigated lines (Nagel *et al.* 2014).

Conclusion

According to the obtained results, it can be stated that 68DH, Varamin and Pavan are drought resistant at the germination stage. According to this experiment, it was found that moderate levels of stress do not evaluate the traits. Therefore, the reduction was observed in the measured traits as stress levels were increased. Maximum traits loss in this experiment was observed in the -5bar to -7.5 bar level. So the -7.5 bar is regarded as the best level to assess the drought resistance of cultivars used in this experiment.

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