

Study of drought tolerance of bread wheat (*Triticum aestivum* L.) genotypes in seedling stage

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Abstract. Seed germination is one of the main stages of growth which is a basic requirement for determining optimum plant density in field. This character is affected by many biotic and abiotic factors including ability of genotypes to tolerate water stress imposed by environmental factors. The aim of this research was first, to investigate the reaction of twenty bread wheat genotypes to determine the parameters and suitable water stress at seed germination and, second, to determine the parameters and appropriate water potential for screening the tolerant genotypes. Drought levels imposed a significant reduction on all measurement characters. The analysis of variance showed that genotype \times stress interaction notified significant differences ($P < 0.1$) for all the studied traits. Therefore, the studied traits indicated the necessary diversity in the stress effects. Subcategorizing genotypes into levels 0, -0.04 and -0.8 MPa showed that only genotype No. 12 presented no significant difference by rootlet length and dry rootlet weight traits. Therefore, the germination percent and the vigor index are suitable for selection of the drought tolerant genotypes. By correlation analysis promptness Index (PI), germination percent to 5 day (GP5), germination stress index (GPF) traits and vigor Index (VI) indicated significant positive correlation with germination stress index (GSI). The stress tolerance index (STI) as field criteria indicated significant and positive correlation with GSI. This shows that genotypes' selection based on high GSI value in laboratory conditions is suitable in field conditions based on stress tolerance index which is also much more recommendable. Based on the first two components, genotypes No. 5, 10, 12 and 13 in the vicinity of drought tolerance indices were identified as tolerant high yielding genotypes in both conditions. Three-dimensional plot based on STI and GSI showed that genotypes No. 5, 10, 12 and 13 with high yield in both conditions and relatively equivalent drought tolerance.

Key words: Bread wheat, germination, polyethylene glycol, drought stress.

Introduction

Plants show some adaptive strategies in response to various abiotic stresses such as salt, drought, cold and heat stress, which ultimately affect plant growth and yield (McCue & Hanson 1990). Interest in crop responses to environmental stresses has increased greatly, because of experiencing severe losses from heat, cold, drought and high consent rations of toxic mineral elements (Blum 2011).

Water deficit can be defined as the absence of adequate moisture necessary for normal plant growth and to complete the life cycle (Zhu 2002). Water stress is a problem in 45% of the world's geographical area and is a major limitation to the productivity of agricultural systems and food production worldwide (Boyer 1982).

However, in wheat, grain filling processes under stress conditions were partly limited by low grain water status as well as by the reduced assimilate supply to the grain (Ahmadi & Baker 2001). It seems that grain water status or sensitivity of grain growth to grain water status is not the only factor controlling grain growth. Genetic gain in yield was 0.6 and 0.4% per year under non-stress and stress conditions, respectively (Cox et al. 1988).

Drought stress reduced significantly the yield of some genotypes and some of them revealed tolerance to drought, which suggested the genetic variability for drought tolerance in this material (Talebi et al. 2009).

Evaluation of crop tolerance to environmental stresses during seed germination and seedling emergence is a main measure to choose them for cropping in different circumstances since common investigations in field conditions are

time consuming and influenced by many companion variables of soil, climate and agricultural practices, so fast and precise evaluation of crop response to stress would be achieved using an experiment in controlled environment conditions (Khalesro & Aghaalikhani 2006).

The capacity for osmotic adjustment was the main physiological attribute associated with wheat resistance under cyclic water stress which enabled plants to recover from water deficit (Izanloo et al. 2008). Seedling and early growth vigor in wheat and barley might be important for plant water status under drought stress since faster ground cover reduces soil surface evaporation and increases the amount of soil moisture available for transpiration (Rebetzke & Richards 1999).

Water stress at different growth stages causes various morpho-physiological alters in the plant to acclimatize under such conditions. For example, water stress at seedling stage might lead to higher dry root weights, longer roots, coleoptiles and higher root/shoot ratios which could be exploited as selection criteria for stress tolerance in crop plants at very early stages of growth (Takele 2000; Dhanda et al. 2004; Kashiwagi et al. 2004).

Polyethylene glycol (PEG) is a polymer produced in a range of molecular weights. Lagerwerff et al. (1961) were the first to indicate that PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in a relatively controlled manner. It was assumed that PEG of large molecular weight did not penetrate the plant and thus was an ideal osmotic for use in hydroponics culture. During the 1970s and 1980s PEG of higher molecular weight (4000–8000) was quite commonly used in

physiological experiments to induce drought stress in nutrient solution cultures. Several papers also reported theoretical or measured calibrations of PEG water potentials against molecular weight and concentration. Examples for PEG calibration can be found in Michel & Kaufmann (1973) and Money (1989).

The purpose of this study was to investigate the ability of bread wheat genotypes to increase their germination potential by making the water uptake slower with the osmotic active compound polyethylene glycol 6000 (PEG 6000) during seed imbibition's, also evaluation water stress at seed germination and to determine parameters and an appropriate water potential for screening tolerant genotypes in laboratory conditions.

Materials and methods

Laboratory experiment

In order to study drought stress, an experiment was carried out in a completely randomized design with four replications and four level of PEG6000 (0, -0.4, -0.8 and -1.2 MP). The seeds of twenty bread wheat genotypes were obtained from International Maize and Wheat Improvement Center (CIMMYT), Mexico. The experiment was carried out in the laboratory Agriculture and Natural Resource Research Center of Kermanshah, Iran. The value PEG for the necessary potential, calculated according to Michel & Kaufmann (1973) formula, includes:

$$OP = (-1.18 \times 10^{-2}) \times C - (1.18 \times 10^{-4}) \times C^2 + (2.67 \times 10^{-4}) \times CT + (8.39 \times 10^{-7}) \times C^2T$$

where OP = the osmotic pressure; C=PEG concentration; T=Temperature.

Germination test for both controls and artificial agent treatments were analyzed performing ISTA standard germination test for 50 seeds in petri dishes (12 cm) at 22-24°C during 8 days (ISTA 2008). In order to study the diversity among different genotypes, traits were measured such as the number of germinated seeds, the maximum rootlet and shoot let length, the coleoptile length, number of secondary root, dry rootlet weight and dry shoot let weight.

In order to consider the diversity genotypes to water stress and evaluate the main criteria for segregating genotypes used the indices as below:

1. Promptness index (PI) (Bouslama & Schapaugh 1984):

$$PI = nd_2(1.0) + nd_4(0.8) + nd_6(0.6) + nd_8(0.4) + nd_{10}(0.2)$$

In this formula nd_2 , nd_4 , nd_6 , nd_8 and nd_{10} are the number of seeds germinated in the 2, 4, 6, 8 and 10 days, respectively.

2. Germination stress index (GSI) (Bouslama & Schapaugh 1984):

$$GSI = \left[\frac{PI, \text{Under stress condition}}{PI, \text{Under non-stress condition}} \right] \times 100$$

3. Vigor index (VI) (Abdul-Baki and Anderson 1970)

$$VI = \frac{(\text{Percent of germination} \times \text{Sum of the Rootlet and shootlet length})}{100}$$

4. Percent of germination:

$$\text{percent of germination} = \frac{\text{the sum of germination seeds to i day}}{\text{the sum of germination seeds}}$$

Statistical analysis

Data was analyzed using SAS 9.1 (SAS 2004) for analysis of variance and interpreting the comparison of its traits.

Results and Discussion

The analysis of variance showed that there was a significant difference between genotypes and the different levels of

drought stress (PEG) for measuring traits ($P < 0.1$) of germination test (Table 1). This related to significant interaction effect. Also genotype \times stress interaction showed significant differences for all the studied traits ($P < 0.1$). This result showed explanatory different reaction genotypes studied to stress difference levels. So the studied traits indicated necessary diversity in the affect stress and can be suitable criteria for selecting suitable genotypes and necessary tolerance to deficit water stress conditions for germination.

The results of interpreting comparison of measured traits genotype \times stress interactions carried out for germination test. The means comparison results for these traits showed that genotypes' response to varying osmotic potential of polyethylene glycol was different. The results slicing for genotypes (data no show) showed that by Promptness index genotype No. 16, germination percent final genotypes No. 1, 2, 6, 10, 11, 12, 13, 15, 18, dry rootlet weight genotypes No. 1, 3, 4, 5, 7, 8, 9, 11, 12, 14, 17, 18 and vigor seed index only genotype No. 12, on the levels 0 and -0.04 MPa were not significantly different. The results showed the stability of these genotypes on the levels 0 and -0.04 MPa by these traits and lose stability with increasing levels PEG and other genotypes with increase PEG value by these traits showed no stability (Table of mean comparison not shown).

By shoot length and dry shoot weight traits in different levels of PEG showed no stability and ratio to PEG value showed susceptibility.

Screening genotypes in the levels 0, -0.04 and -0.8 MPa indicated that only genotype No. 12 showed no significant difference by rootlet length and dry rootlet weight traits. These traits could be selected to screen bread wheat genotype for yield potential under water limited conditions. The relation between these traits with yield is positive. Dhanda et al. (2004), reported vigor index was a susceptible trait with regard to shootlet length, germination percent and rootlet length.

The seed germination percentage decreased with decreasing of levels of osmotic potential of PEG. This result correlated with the findings of Yamur and Kaydan (2008). The explanation of the reduction in germination percentage by effects of PEG may be that the decrease in water potential gradient between seeds and their surrounding media adversely affects seed germination (Dodd and Donovan 1999).

Correlation among the traits may be the consequence of pleiotropic or the genetic linkage among the characters whereas correlation coefficients describe only the relationship between two traits (Yan & Rajcan 2002). Correlation coefficients of measured traits were displayed in Table 2. Traits of PI, GP5, GPF and VI indicated significant positive correlation with GSI. High germination stress index value is criteria for evaluation drought resistance, and the traits that correlate with GSI, are suitable for stress conditions and selection base on these traits is suitable for drought resistance.

Germination percent traits were associated to rootlet emersion and roots before the other member of the plant outside of the seed, as before other member under environment stress. So germination percent and the vigor index were suitable for selection of the drought tolerance genotypes. These results were accordance with the records reported by Zarei et al. (2007).

The traits of shoot let length (SL), coleoptile length

Table 1. Analysis of variance for the studied traits in bread wheat genotypes on the germination test.

S.V	d.f	PI	GP5	GPF	SL	Means of Square					
						Col.L	RL	NSR	DSW	DRW	VI
Genotype	19	14.83**	465.92**	258.88**	245.72**	65.83**	713.37**	1.32**	178.55**	358.30**	1317.21**
Stress	3	3628.99**	143384.26**	146206.43**	109754.34**	12972.25**	93810.57**	204.41**	68298.78**	64379.03**	399537.24**
Genotype×Stress	57	6.53**	185.97**	127.9**	132.31**	31.11*	431.32**	0.41**	128.89**	262.92**	763.36**
Error	260	0.66	47.7	25.73	22.74	4.47	44.92	0.06	21.65	37.05	114.48
CV%	-	9.02	12.26	7.85	12.83	12.03	15.45	11.84	13.88	16.65	14.64

PI: Promptness Index; GP5: Germination Percent to 5 day; GPF: Germination Percent Final;

SL: Shoot let Length; RL: Rootlet Length; Col.L: Coleoptile Length;

NSR: Number of Secondary Root; DSW: Dry Shoot let Weight;

DRW: Dry Rootlet Weight; VI: Vigor Index

*P<0.05, ** P<0.01 and ^{ns} non-significant

Table 2. Simple correlation coefficients of Pearson's between traits in laboratory conditions.

	PI	GP5	GPF	SL	Col.L	RL	NSR	DSW	DRW	VI	GSI
GP5	0.892**										
GPF	0.868**	0.893**									
SL	0.425*	0.321 ^{ns}	0.344 ^{ns}								
Col.L	0.255 ^{ns}	0.261 ^{ns}	0.217 ^{ns}	0.861**							
RL	0.355 ^{ns}	0.331 ^{ns}	0.369 ^{ns}	0.666**	0.638**						
NSR	0.281 ^{ns}	0.170 ^{ns}	0.176 ^{ns}	0.312 ^{ns}	0.329 ^{ns}	0.007 ^{ns}					
DSW	0.408*	0.317 ^{ns}	0.363 ^{ns}	0.752**	0.667**	0.309 ^{ns}	0.464*				
DRW	0.114 ^{ns}	0.075 ^{ns}	0.209 ^{ns}	0.352 ^{ns}	0.285 ^{ns}	0.279 ^{ns}	0.726**	0.536**			
VI	0.575**	0.518**	0.573**	0.829**	0.684**	0.921**	0.151 ^{ns}	0.507*	0.344 ^{ns}		
GSI	0.741**	0.723**	0.684**	0.322 ^{ns}	0.339 ^{ns}	0.286 ^{ns}	0.161 ^{ns}	0.173 ^{ns}	-0.099 ^{ns}	0.406*	
STI	0.069 ^{ns}	0.171 ^{ns}	0.186 ^{ns}	-0.068 ^{ns}	-0.004 ^{ns}	-0.116 ^{ns}	-0.297 ^{ns}	0.084 ^{ns}	-0.310 ^{ns}	-0.123 ^{ns}	0.384*

PI: Promptness Index; GP5: Germination Percent to 5 day; GPF: Germination Percent

Final; SL: Shoot let Length; RL: Rootlet Length; Col.L: Coleoptile Length;

NSR: Number of Secondary Root; DSW: Dry Shoot let Weight;

DRW: Dry Rootlet Weight; VI: Vigor Index; GSI: Germination Stress Index;

STI: Stress Tolerance Index

*P<0.05, ** P<0.01 and ^{ns} non-significant (1-tailed)

(Col.L), rootlet length (RL), number of secondary root (NSR) and dry shoot let weight (DSW) indicated positive correlation and dry rootlet weight (DRW) trait indicated negative correlation with GSI but not statistically significant. The findings were in accordance with Zarei et al. (2007).

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