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Study of Drought Tolerance with Cell Membrane Stability Testing and Relation with the Drought Tolerance Indices in Genotypes of Wheat (*Triticum aestivum* L.)

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Abstract: In order to investigate the genetic diversity of bread wheat landraces of Northwest of Iran to drought stress resistance, thirty genotypes selected from the collection of "Research Institute on Breeding and producing Seed and Seedling" along with six controls were studied in a complete randomized block design with three replications in drought stress and normal irrigation conditions in greenhouse. Cell-membrane stability of genotypes in induced stress (PEG6000 20% and 30%) and drought tolerance indices were calculated on the basis of grain yield. There was a significant difference in cell-membrane stability under inductive stress among genotypes which indicate genetic diversity among genotypes and make it possible to select drought tolerance cultivars and perform genetic studies and breeding programs. The significant correlation of cell membrane stability in both stress conditions with grain yield and also drought tolerance indices indicated that the test could be used as an easy and fast method to screen genotypes in the preliminary breeding stages. Mean comparison genotypes through the factors of cell-membrane stability and drought tolerance indices showed genotypes number 11198 and 11200 and Pishtaz cultivar were selected as the most resistant genotypes to be used in breeding programs for drought stress. Classifying the results of the cluster analysis identified the best three genotypes which confirmed the results of the other methods.

Key words: PEG6000 · Drought stress · Landraces · Cell membrane stability

INTRODUCTION

One of the important challenges facing crop physiologists and agronomists is understanding and overcoming the major abiotic stresses in agriculture which reduces crop productivity and yield. One of these stresses particularly predominant in arid and semi-arid regions is dryness stress, which decreases plant growth and development and also crop yield [1]. Drought, one of the environmental stresses, is the most significant factor that restricts plant growth and crop productivity in the majority of agricultural fields of the world [2]. Thus, drought indices which provide a measure of drought based on yield loss under drought conditions in comparison to normal conditions have been used for screening drought-tolerant genotypes [3]. Drought stress damages the plasma membrane, so that cell content percolates to the outside. Magnitude of this damage can be determined via ionic secretion measurement [4]. Water deficit causes changes in almost all the cell processes, which affect plant growth and development [5]. The primary site of damage under stress conditions is the plasma lemma. The membrane integrity is altered for the stress; a consequence of this is the increase of the cell permeability which is accompanied by electrolyte leakage from the cell [6]. Ashraf *et al.* [7] have suggested that development drought tolerant varieties can be a useful approach to increase crop production and yield under water stress conditions. As such the release of drought- tolerant genotypes, including desirable traits associated with water limitation, has become an established applied method for developing cultivars under dry conditions [8]. These modifications occur mainly in drought sensitive plants and lead to a loss of semi permeable properties of the cell membrane, which is the main reason of metabolic damages developed in water

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stress plants. Therefore the integrity and stability of cell membrane in water deficit conditions can be considered a possible adaptive value indicative of stress resistance. Cell membrane stability may be determined through estimation of the extent of cell membrane damage in desiccated of leaf fragment *in vitro* whit a polyethylene glycol solution (PEG) and subsequent measurement of electrolyte leakage into aqueous medium [5]. Polyethylene glycols or PEGs are a group of neutral osmotically active polymers with a certain molecular weight. PEG6000 (the number signifying molecular mass) is most frequently used in plant water deficit studies to induce dehydration by decreasing the water potential of the nutrient solution [9]. Electrolyte leakage tests have been widely used to assess the level of plant tolerance to various stresses. These tests determine the degree of cell membrane damage caused by stress based on electrolyte leakage from the cells. The technique is relatively simple, repeatable and rapid and requires inexpensive equipment, can be used on plant material from a variety of cultural systems and it is suitable for the analysis of large numbers of samples [10]. A consequence of the altered membrane integrity is the increase of the cell permeability which is accompanied by electrolyte leakage from the cell. An important strategy for the development of drought resistance in plants is the maintenance of cell membrane integrity after the imposition of water stress [11].

Saneoka *et al.* [12] and Azizi-e-Chakherchaman *et al.* [4] in Lentil studied the relationship between plasma membrane stability (obtained from EC measurement) and grain yield in stress and non stress conditions. They reported that plasma membrane stability in genotypes under stress was significantly lower than genotypes under non stress conditions. The Cell Membrane Stability has been exclusively used as selection criterion for different abiotic stresses including drought and high temperature in wheat [13, 14], rice [15], cotton [16] and sorghum [17]. Several associations were established between CMS and different agronomic traits by including *in vitro* with polyethylene glycol (PEG-6000) [18].

Landraces are important genetic resources for improvement of crops in dry areas, since they have accumulated adaptation to harsh environment over long time. Collection and characterization of various agronomic and physiological traits of landraces are primary steps in plant breeding programs. This method can be used for a large number of samples and may be applicable to rapid evaluation of drought resistance in large number of genotypes. The objectives were to study the effectiveness and reliability of physiological techniques such as electrolyte leakage tests for screening wheat genotypes under levels of osmotic stress and the relation of this trait with the drought tolerance indices and facilitate its introduction within cereal farming system prevailing under dry areas of Iran.

MATERIAL AND METHOD

Experiments were undertaken on thirty wheat landraces selected from the collection of "Research Institute on Breeding and producing Seed and Seedling Iran" along with six controls (Pishtaz, Alvand, Azar2, Fankang, Konia2002 and Gork79) was evaluated under irrigated and drought stress conditions. Based on randomized complete block design with three replications, the experiment was carried out in the greenhouse agricultural research station of Islamic Azad University, Ardabil branch, Iran (Northwest of Iran), during the 2008 and 2009 cropping year.

To do the experiment, the pot which had 20cm diameter and 30cm height were selected and they contained 10kg soil. Each pot had been filled with cultivated soil, sand and manure with a ratio of 1:1:1 and four seeds had been planted in 3cm depth with equal spaces. In three leaves phase, in order vernallization, the pots were moved out of the greenhouse from 21 December until 30 January for 40 days. After this period, the pots were moved to the greenhouse once again. All the pots were watered in three days period to reach the irrigation capacity. In flowering phase, drought stress was exerted through every day watering control pots and not watering stress pots until they reached to 80% soil moist evacuation via weight.

Optimization of the Electrolyte Leakage Measurement for the Estimation of Cell Membrane Stability in Wheat: To measure cell membrane stability, the genotypes were planted on base of complete randomized block design in the greenhouse with three replications. Ten seeds were planted in every pot; each of these pots was 25×15×15 big. In flowering phase, the same size leaves which were as old as each other were selected and picked up.

Five leaves per genotype were collected, immediately weighed and cut into segments (cut in 1 cm segments), segments originating from the same leaf were put into 20 ml of deionised water in a test tube and washed slowly using a rotary shaker (100 rpm) at room temperature to remove solutes from both leaf surfaces and damaged cells due to cutting and then exposed either to 0% (control) or to 20% and 30% PEG 6000 for 15h in the dark. Electrolyte

leakage was then measured before (ECi) and after (ECf) 4 h of rehydration and ultimately after autoclaving (ECt). Cell membrane damages were expressed as an index of damage calculated as Id = [(Rs – Rc) / (1-Rc)] * 100, where Rs and Rc represent (ECf-ECi) / (ECt-ECi) for control or PEG-treated tissues, respectively [19].

Drought tolerance indices were calculated by using the following equations:

MP= (Ypi + Ysi) / 2 Rosielle and Hamblin [20];
HARM= 2(Ypi×Ysi)/ (Ypi×Ysi) Jafari *et al.* [21];
STi= (Ypi×Ysi) Yp2 Fernandez [22];
Tol= (Ypi- Ysi) Roseille and Hamblin [20].

Where in these equations, Ysi and Ypi are stressed optimal (potential) yields of a given genotype, respectively. Ys and Yp are average yields of all genotypes under stress and optimal conditions, respectively.

The analysis of variance (ANOVA) for each character was performed following the Duncan's new multiple range test [23], to test the significance difference between means. For evaluated relation between traits used in Pearson correlation. The data were statistically analyzed by Mstat-c and Spss software's.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) showed that there is a significant difference in possibility level of 1% between the genotypes from cell membrane stability point of view in both inductive stress levels (20% and 30% PEG) (Table 1). The results of analysis according to factorial experiment. On the base of complete randomized block design showed that there is a significant difference in 1% possibility level between (20% and 30%) inductive stress (Table 2). The interaction between genotype and condition was also significant in this experiment. In 20% inductive stress, the amount of damage was different from 12.65% to 8.36%. And 29 genotypes had stable membrane cell with f common letter. Fewer than 30% inductive stress, the amount of damage was different from 19.55% to 44.9%, that 30 genotypes which had f common letter considered as one group and had identical membrane stability (Table 3).

There was a significant difference among the genotypes from yield amount point of view in stress and non stress conditions. The results of average comparison (Table 4) showed that 13, 12, 33, 15 and 10 genotypes have the most yield in stress condition and 33, 3, 10, 15 and 11 genotypes have the most yield in non- stress condition. However there is no significant difference between these genotypes with the genotypes of common letters. The results of calculating and drought tolerance indices showed that 15, 10, 27 and 30 genotypes and Pishtaz were the most tolerant genotypes from MP, STI and HARM indices point of views to drought stress (Table 4).

Ahmadizadeh [24] studied the genotypes of durum wheat in two conditions: drought stress and normal irrigation. He announced that considering the significant correlation of quantitative indices of MP, GMP, STI and

Table 1: Result of analysis of variance for studied traits

		Means Square			
S.O.V	d.f	Ys	Y_P	I_d20	I_d30
Replication	2	1.22^{NS}	0.72^{NS}	70.65^{NS}	75.78^{NS}
Genotype	35	4.47^{**}	4.15^{**}	113.71^{**}	136.13^{**}
Error	70	0.46	0.89	28.87	48.45

** and Ns, significant at 1% level of probability and non-significant, respectively.

Table 2: Analysis of variance, according to Factorial experiment for cell

S.O.V	d.f	Means Square
Replication	2	134.73*
Condition	1	5852.92**
Genotype	35	173.40**
Condition × Genotype	35	76.43**
Error	142	38.28

** and * significant 1% and 5% level of probability, respectively.

Table 3: Drought tolerance indices means and comparison means cell membrane stability, Ys and Yp for studied wheat genotypes

No.	Code	I_d20	I_d30	YP	YS	MP*	TOL*	SSI*	STI*	HARM*
1	12075	19.54^{c-f}	25.20^{b-f}	7^{abc}	3.87^{a-d}	5.43	3.13	0.98	0.84	4.98
2	10532	16.48^{def}	25.20^{b-f}	5.4^{c-f}	3.80^{a-d}	4.60	1.60	0.65	0.64	4.46
3	12196	36.80^{a}	35.49^{a-f}	8.37^{ab}	2.33^{cd}	5.35	6.03	1.59	0.60	3.65
4	11479	16.16^{def}	41.68^{abc}	6.77^{abc}	2.63^{bcd}	4.70	4.13	1.34	0.55	3.79
5	11028	27.57^{a-e}	44.90^{a}	4.83^{c-f}	2.33^{cd}	3.58	2.50	1.14	0.35	3.15
6	11486	20.47^{c-f}	31.31^{a-f}	4.77^{c-f}	2.97^{bcd}	3.87	1.80	0.83	0.44	3.66
7	11076	13.33^{ef}	34.39^{a-f}	5.07^{cde}	3.90^{a-d}	4.48	1.17	0.51	0.61	4.41
8	12193	22.35^{c-f}	22.87^{def}	5.37^{c-f}	2.90^{bcd}	4.13	2.47	1.01	0.48	3.77
9	12194	21.96^{c-f}	30.80^{a-f}	4.93^{c-f}	3.47^{bcd}	4.20	1.47	0.65	0.53	4.07
10	11200	23.19^{b-f}	19.55^{f}	8.50^{a}	4.03^{a-d}	6.27	4.47	1.16	1.06	5.47
11	11072	12.65^{f}	21.92^{ef}	7.23^{abc}	3.97^{a-d}	5.60	3.27	0.99	0.89	5.12
12	11063	15.89 ^{def}	23.07^{def}	5.93^{cde}	4.43^{abc}	5.18	1.50	0.56	0.81	5.07
13	11020	14.20^{ef}	22.20^{ef}	5.17^{c-f}	4.43^{abc}	4.80	0.73	0.31	0.71	4.77
14	11037	36.13^{ab}	31.02^{a-f}	3.17^{f}	2.27^{d}	2.72	0.90	0.63	0.22	2.64
15	11198	14.71^{ef}	20.22^{f}	8.27^{a}	5.67^{a}	2.71	2.90	0.47	1.05	6.82
16	11035	16.36^{def}	39.26^{a-e}	5.23^{c-f}	3.33^{bcd}	4.28	1.90	0.80	0.54	4.07
17	10335	27.10^{a-e}	37.66^{a-f}	6.07^{b-e}	2.37^{cd}	4.22	3.70	1.34	0.44	3.41
18	10429	21.79^{c-f}	37.70^{a-f}	5.40^{c-f}	2.70^{bcd}	4.05	2.70	1.10	0.45	3.60
19	11039	20.31^{c-f}	31.68^{a-f}	6.33^{a-e}	2.50^{bcd}	4.42	3.83	1.33	0.49	3.58
20	12076	23.36^{a-f}	42.55^{ab}	5.33^{c-f}	2.60^{bcd}	3.97	2.73	1.13	0.43	3.50
21	12078	27.17^{a-e}	36.96^{a-f}	6.37^{a-e}	2.87^{bcd}	4.62	3.50	1.21	0.56	3.95
22	11073	25.39^{a-f}	32.17^{a-f}	5.43^{c-f}	3.60^{bcd}	4.52	1.83	0.74	0.61	4.33
23	10523	31.27^{abc}	41.06^{a-d}	6.90^{abc}	3.03^{bcd}	4.97	3.87	1.23	0.65	4.21
24	11196	23.62^{a-f}	38.94^{a-e}	4.90^{c-f}	2.70^{bcd}	3.80	2.20	0.99	0.41	3.48
25	11074	23.15^{b-f}	32.44 $^{a-f}$	4.80^{c-f}	2.13^{d}	3.47	2.67	1.22	0.32	2.95
26	11201	18.45^{c-f}	26.77 $^{a-f}$	6.27^{a-e}	2.90^{bcd}	4.58	3.37	1.18	0.56	3.97
27	12195	20.83^{c-f}	28.80 $^{a-f}$	7.17^{abc}	3.90^{a-d}	5.53	3.27	1.00	0.86	5.05
28	11490	24.05^{a-f}	27.75 $^{a-f}$	7.20^{abc}	1.97^{d}	4.58	5.23	1.60	0.44	3.09
29	11021	13.82^{ef}	35.67 $^{a-f}$	4.10^{def}	2.90^{bcd}	3.50	1.20	0.64	0.37	3.40
30	11037	17.96^{c-f}	26.80 $^{a-f}$	5.63^{a-d}	3.83^{a-d}	5.18	2.70	0.90	0.77	4.82
31	FENKANG	19.54^{c-f}	30.70^{a-f}	3.90^{ef}	2.37^{cd}	2.63	2.53	1.43	0.16	2.02
32	ALVAND	13.49^{ef}	23.78^{c-f}	4.20^{def}	2.80^{abc}	3.50	1.40	0.73	0.36	3.36
33	PISHTAZ	16.51^{def}	31.60^{a-f}	7.27^{abc}	4.57^{ab}	5.92	2.70	0.81	1.20	5.60
34	AZAR2	30.09^{a-d}	34.36^{a-f}	6.40^{a-d}	2.27^{d}	4.33	4.13	1.42	0.45	3.35
35	GARAK 79	17.54^{c-f}	32.15^{a-f}	4.33^{c-f}	3.23^{bcd}	3.78	1.10	0.56	0.43	3.70
36	KONYA 2002	16.34^{def}	35.74^{a-f}	4.87^{c-f}	3.77^{a-d}	4.32	1.10	0.50	0.57	4.25

Values with the same superscript letters are non significantly different at $P < 0.05$.

* These indices have been calculated according to the mean of the dates and analysis variance has not been done on them.

Table 4: Correlation coefficients between studied traits and drought tolerance indices

	Id 20%	I_d30	Yp	Ys	MP	Tol	SSI	STI
I_d30	0.65**							
Yp	-0.09	-0.18						
Ys	-0.75**	-0.67**	0.29					
MP	-0.39*	-0.43**	0.89**	0.68**				
Tol	0.37*	0.22*	0.79**	-0.34*	0.43**			
SSI	0.52*	0.38*	0.47**	-0.64**	0.04	0.89**		
STI	-0.53**	-0.54**	0.74**	0.84**	0.95**	0.20	-0.19	
Harm	-0.61**	-0.59**	0.60**	0.91**	0.89**	0.003	-0.38*	0.97**

** and * significant 1% and 5% level of probability, respectively

Table 5: Yield mean in drought stress and normal irrigation, drought tolerance indices, index of damage 20% and 30% values of wheat genotypes of each cluster

Group		HARM	STI	SSI	Tol	MP	Ys	Yp	Id 20%	Id 30%
1		3.43	0.44	1.15	3.13	4.10	2.53	5.66	27.28	39.85
2	Mean	5.04	0.83	0.67	2.00	5.26	4.26	6.26	13.93	22.18
3		4.08	0.59	0.95	2.57	4.49	3.26	5.78	19.34	32.78

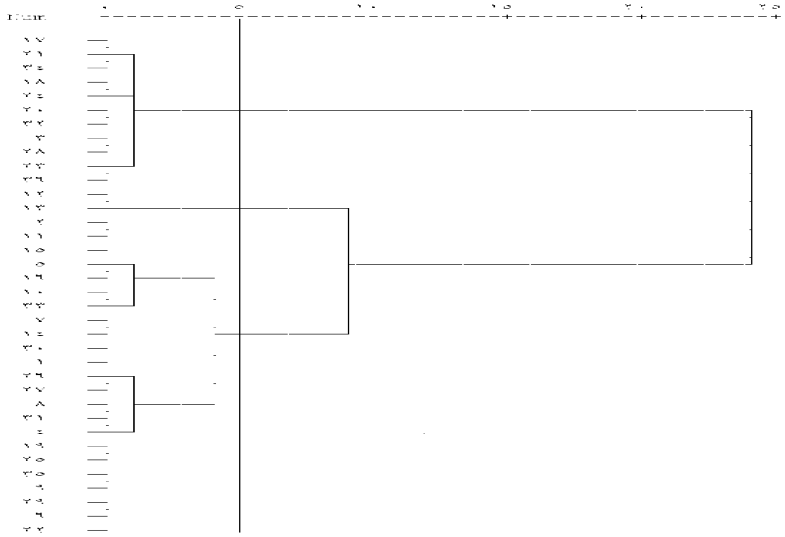

Fig. 1: Dendogram of cluster analysis of wheat genotypes classified according to Yp, Ys, drought tolerance indices, index of damage 20% and 30%.

TOL drought tolerance with the grain yield in both experimental conditions, these indices are suitable to determine the tolerant genotypes which agreement to the results of this experiment and from these indices point of view 14, 13, 8, 7 and 12 genotypes were introduced as tolerant genotypes. Selection on the base of TOL indices makes easy the selection of genotypes with high yields in stress and low yield in normal conditions [20]. The closer the amount of YP and YS, the less the sensitivity of that cultivar to drought will be and the smaller the amount of SSI to drought will be [25].

Correlation coefficients showed that there was a significant difference between the amounts of damage to cell membrane, fewer than 20% and 30% PEG and the amount of yield in drought stress conditions and negative significant correlation (Table 4). It can be inferred that the more the damage to the cell membrane, the less the amount of yield in drought stress condition has been. These results are also in agreement with findings of the study of Ahmadizadeh [24], Garcia del moral *et al*. [26] and Franca *et al*. [27]. There were significant relations between stability membrane and resistance at drought.

MP, STI and HARM indices had a positive and significant correlation (in 1% possibility level) with the yield in stress and non-stress conditions. On the other words, the genotypes with high yield can be identified indirectly in stress and non-stress conditions according to the above indices: TOL and SSI indices had negative correlation in stress conditions with the yield in non-stress condition which corresponds to the results of Golabadi *et al.* [28], also Similar reports were reported by Sio-se Mardaeh *et al*. [29] and Talebi *et al*. [3].

A significant correlation was observed between tolerance and sensitivity to stress indices. Cluster analysis divided the genotypes into three groups according to the grain yield in stress and non-stress conditions and drought tolerance indices. Average of traits of the clusters (Table 5) showed that the first cluster which had the genotypes with the least stable cell membrane under 20% and 30% PEG stress, yield in two conditions and MP, STI and HARM indices and the high TOL and SSI. The second cluster had the genotypes of resistant to drought with a high yield potential, so that the most cell membrane stability of yield in two conditions and STI, HARM and MP indices had the least amounts of TOL and SST indices. The third cluster was the middle amount of the first and the second clusters from drought tolerance and cell membrane stability indices and yield in two conditions point of views (Table 5).

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