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Response of Bread Wheat Genotypes to Immature Embryo Culture, Callus Induction and Drought Stress

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Abstract: In order to evaluate the response of twenty genotypes of bread wheat (*Triticum aestivum* L.) to callus induction and *in vitro* drought stress. The immature embryos of wheat were used in a Completely Randomized Design (CRD) with six replications for callus induction and a 20×2 factorial experiment based on CRD design with three replications was carried out for response of genotypes to *in vitro* drought stress at the Agricultural College of Razi University, Kermanshah, Iran during 2010-2011. Significant differences were observed among the genotypes for Callus Growth Rate (CGR), Relative Fresh Weight Growth (RFWG), Relative Growth Rate (RGR) and Percentage of Callus Induction (PCI) indicating the presence of genetic variability, different responses of bread wheat genotypes to callus induction and possible selection of callus induction at *in vitro* level using immature embryos. Mean comparison of the traits measured in callus induction showed that genotypes 1 and 6 had the highest PCI (100%). Analysis of variance for CGR, RFWG and RGR, Relative Water Content (RWC), Percent of Callus Chlorosis (PCC) and Proline Content (PC) exhibited significant differences among the genotypes for all the characters in the stress condition (15% PEG). Screening drought tolerant genotypes and *in vitro* indicators of drought tolerance using mean rank, standard deviation of ranks and biplot analysis, discriminated genotypes (6), (19) and (1) as the most drought tolerant.

Keywords: Biplot analysis, embryo culture, *in vitro* indicators of drought tolerance, screening techniques

INTRODUCTION

Cereal crops belonging to Graminae family producing large edible grains which provide about one-half of man's food calories and a major portion of his nutrient requirements (Jain, 2001). High adaptation of bread wheat (*Triticum aestivum* L.) as well as its diverse consumption in the human nutrition lead to be presented as the most important cereal in the word, especially in the developing countries and it can provides 20% food resources of the world people (Farzi *et al*., 2010). Global warming and concomitant increase in drought effected areas limit plant production is also restricted by drought exposed areas and this loss lead to considerable economic and social problems because of its great importance on human nutrition (Ilker *et al*., 2011). Water deficit the main environmental constraint limiting cereal yield worldwide and particularly within the Mediterranean basin, a problem likely to become even worse in the future. Cereal plants respond to drought through morphological, physiological and metabolic modifications occurring in all organs and therefore traits associated with improved performance under water limited conditions or improved survival to extremely low water availability are diverse (Slafer *et al*., 2005). One possible way to ensure future food needs of an increasing world population involves the better use of water through the development of crop varieties which need less water and are more tolerant to drought (Shao *et al*., 2006; El-Shafey *et al*., 2009; Mafakheri *et al*., 2010). Development of cultivars with high yield is the main goal in water limited environments but success has been modest due to the varying nature of drought and the complexity of genetic control of plant responses (Mirbahar *et al*., 2009). Since yield is a complex trait and is strongly influenced by the environment, severe losses can be caused by drought, a stress common in most arid and semi arid areas. Accordingly, drought tolerance is one of the main components of yield stability and its improvement is a major challenge to geneticists and breeders (Eid, 2009). These efforts have been focused mostly on exploiting high yield potential and genotype selection for morphological, physiological and agronomic traits indicative of drought tolerance in field conditions (Dhanda *et al*., 2004).

Breeding for drought tolerance by selecting solely for grain yield is difficult because the heritability of yield under drought conditions is low, due to small genotypic variance or due to the large variances in the genotype-environment interaction (Ludlow and Muchow, 1990; Koszegi *et al*., 1996). Improvement of the wheat plant itself gives a long-term avenue for raising its yield in the

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field. Thus, under stressful environments, yield *per se* is not always the most suitable or easiest selection trait and an approach based on the evaluation and incorporation of physiological traits into a potentially high-yielding genotype may improve its adaptability and thus its response to environmental variability (Steven *et al*., 1990; Blum, 2005). Much attention is shifted towards crop improvement programs. On of such biotechnological techniques is the plant tissue culture. Tissue culture techniques are becoming increasingly popular as an alternative means of plant vegetative propagation, mass production of chemicals, and genetic engineering (Shah *et al*., 2009). Resent progress in genetic manipulation of plant cells has opened new possibilities in crop improvement. Callus culture are used as an *in vitro* technique for biochemical and physiological studies in response to stress at the cellular level (Liu *et al*., 2006). Many researchers have used the *in vitro* culture of cells on media supplemented with PEG to study the mechanisms of drought tolerance and to utilize the somaclonal variation, as a source of variability to improve the drought tolerance (El-Shafey *et al*., 2009). Various osmotic agents have been employed in appropriate nutrient media to screen germplasm *in vitro* for drought tolerance. Although specific *in vitro* methods vary with plant types being screened, researchers have been able to control the drought environmental more precisely using *in vitro* or artificial selection techniques (Maruyama *et al*., 2008; He *et al*., 2009; Srinivasan *et al*., 2010). Polyethylen Glycol (PEG) of high molecular weights, have long been used to stimulate water stress in plants (Ruf *et al*., 1967; Kaufmann and Eckard, 1971; Corchete and Guerra, 1986). PEG of high molecular weight is a non - penetrating inert osmoticum lowering the water potential of nutrient solutions without being taken up or being phytotxic (Lawlor, 1970).

Osmotic solutions of NaCl, mannitol/sorbitol and Polyethylene Glycol (PEG) have been used as *in vitro* stress factors for selecting salt- and drought-tolerant genotypes in screening procedures for seed germination of wheat (Almansouri *et al*., 2001), sunflower (Punia and Jain, 2002) and potato (Gopal and Iwama, 2007).
The objectives of the present investigations were to:

- Screen bread wheat genotypes for drought tolerance under *in vitro* condition
- Evaluate the ability of genotypes to induce callus using immature embryo culture
- Screening *in vitro* indicators of drought tolerance

MATERIALS AND METHODS

In order to evaluate the response of twenty genotypes of bread wheat (*Triticum aestivum* L.) namely:

- WC - 5047
- WC - 4530
- WC - 4780
- WC - 4566
- WC - 47360
- WC - 4640
- WC - 47456
- WC - 47628
- WC - 47367
- WC - 47399
- WC - 47636
- WC - 4584
- WC - 46697 - 11
- WC - 4823
- Pishtaz
- WC- 47341
- WC - 47379
- WC - 4931
- WC - 47381
- WC - 5053

kindly provided from Seed and Plant Improvement Institute of Karaj, Iran to callus induction and *in vitro* drought stress. A Completely Randomized Design (CRD) with six replications was used for callus induction and a 20×2 factorial experiment based on CRD design with three replications was carried out for response of genotypes to *in vitro* drought stress at the Agricultural College of Razi University, Kermanshah, Iran during 2010-2011.

The genotypes were exposed to different concentrations of PEG 6000 (Merck, Germany) (0 as control and 15%) for 14 days. The growing morphogenic calli derived from immature embryos were also exposed to Murashige and Skooge (1962) medium containing different concentrations of PEG (0 and 15%). Spikes were harvested from main tillers 14 days post-anthesis. Spikes rinsed twice with water then were surface-sterilized in 70% (v/v) ethanol for 1 min, rinsed twice with sterile distilled water, incubated further in commercial bleach (5% sodium hypochlorite) for 10 min and rinsed several times in sterile distilled water. All the operations and inoculation were performed under strict aseptic conditions in a laminar airflow cabinet. Immature embryos were aseptically dissected from the seeds and placed scutellum up on MS medium supplemented with 30 g/L sucrose and was adjusted to PH 5.7, solidified with 8g/L agar and 2.5 mg/L 2,4-dichlorophenoxy acetic acid (2,4-D)(Merck, Germany). The medium was autoclaved at 121ºC for 20 min and incubated at 25ºC for 28 days in growth chamber and in the darkness. Callus was maintained by sub-culturing every 21-28 days on the same MS medium. In drought stress conditions the cultures were kept in an incubator without any light. The following callus characteristics were measured under stress conditions:

Percentage of Callus Induction (PCI): PCI was evaluated 4 weeks (suitable for sub-culturing) after embryo culture in Petri dishes as: (Arzani and Mirodjagh, 1999) (number of seeds producing callus)/(number of seeds plated in Petri dishes).

Relative Fresh Weight Growth (RFWG): RFWG = $[(W_2-W_1)]/W_1$ (Chen *et al*., 2006) where W_1 and W_2 are the initial weight of callus before and after four weeks, respectively.

Relative Growth Rate (RGR): RGR = $[LnW_2-LnW_1]/GP$ (Birsin and Ozgen, 2004) where W_1 and W_2 are the initial and final weight of callus and GP is the growth period, respectively. The time interval between two consecutive measurements was 21 days.

Callus Growth Rate (CGR): CGR (mm/day) of cultured embryos on MS medium were measured at 7, 14, 21 and 28 days, respectively after transferring calli to medium. CGR was calculated using the following formulas (Compton, 1994):

$CGR_1 = d_7/7$, $CGR_2 = d_{14}/7$, $CGR_3 = d_{21}/7$, $CGR_4 = d_{28}/7$
$CGR = (CGR_1 + CGR_2 + CGR_3 + CGR_4) / 4$

where d_7, d_{14}, d_{21}, d_{28}, respectively were diameter of callus in days 7, 14, 21 and 28, respectively. Diameter of callus was calculated as:

Diameter of callus = DC =√length×width

Percentage of Callus Chlorosis (PCC): PCN was determined visually as percentage of necrotic callus, 16 days after moving callus to the PEG containing medium.

Relative Water Content (RWC): callus samples of known fresh weight were dried in an oven set at 70−C for 24 h and RWC was calculated by following formula (Errabi *et al.*, 2006):

$$RWC = [(FW-DW)/DW]\times 100$$

where, FW and DW are the callus fresh and dry weights, respectively.

In Vitro Tolerance (INTOL): INTOL was calculated according to the following formula (Al-Khayri and Al-Bahrany, 2004):

$$INTOL = RGR_{treatment} / RGR_{control}$$

where, RGR = relative growth rate and was measured by the formula of Birsin and Ozgen (2004).

Callus Growth Index (CGI): or increasing value of callus fresh weight was calculated as: $CGI = (W_{1}-W_0)/W_0$ (Abdelsamad *et al*., 2007):

where, W_0 is the weight of callus before treatment and W_1 the final weight of callus after two weeks of treatment. Callus growth index was calculated for two levels of PEG (0 and 15%) and the average of two levels was used for calculation.

Relative tolerance (Rt%): percentage of Rt% was calculated for each genotype using the following formula (Abdelsamad *et al*., 2007):

$$Rt\ \% = [(\text{value under stress})/ (\text{value under non-stress})] \times 100$$

Reduction percentage (R%): R% was calculated for the two stress (15%) and non-stress level (0) using the following formula (Abdelsamad *et al*., 2007):
(value under 15% stress level n- value at 0% stress level).

Proline Content (PC): Extraction and estimation of free praline content were done according to the procedure described by Errabii *et al*. (2007).

Statistical analysis: Analysis of variance, mean comparison using Duncan,s Multiple Range Test (DMRT), correlation analysis between mean of the characters measured and principal component analysis (PCA), based on the rank correlation matrix were performed by MSTAT-C, SPSS ver. 16 and STATISTICA ver. 8. Standard Deviation of Ranks (SDR) was measured as:

$$S_i^2 = \frac{\sum_{j=1}^{m} (R_{ij} - \overline{R}_{i.})^2}{l - 1}$$

where, R_{ij} is the rank of *in vitro* drought tolerance indicator and $\overline{R}_{i.}$ is the mean rank across all *in vitro* drought tolerance indicators for the ith genotypes and $SDR = (S^2_i)^{0.5}$ (Arzani and Mirodjagh, 1999).

RESULTS AND DISCUSSION

Callus induction: Highly significant differences ($p<0.01$) were observed among the genotypes for CGR, RFWG, RGR and PCI, respectively indicating the presence of genetic variability, different responses of genotypes to callus induction and possible selection of callus induction in bread wheat using immature embryos of wheat (Table 1). Immature embryo culture supplement with 2, 4-D gave good callus growth (Shan *et al*., 2000; El-Sherbeny *et al*., 2001). Immature embryos of 1.0-1.5 mm,14 d after anthesis were cultured on MS medium supplemented with 1.5 or 2.0 mg/L 2, 4-D and found that 90-100% of these embryos formed callus (Arun *et al*.,

Table 1: Analysis of variance for callus induction traits in bread wheat

		MS			
S.O.V	df	CGR	RFWG	RGR	PCI
Genotypes	19	0.003**	0.826**	0.009**	0.029**
Error	100	0.001	0.058	0.001	0.002
CV%		3.11	14.60	12.11	2.59

**: Significant at 1% level of probability

Table 2: Mean comparison for callus traits using DMRT*

Genotype	CGR	RFWG	RGR	PCI
1	0.1964abc	0.9124h	0.0309g	100.00a
2	0.262abc	1.5021fgh	0.0395efg	80.00bcde
3	0.2069abc	1.2092gh	0.0416defg	83.33bcde
4	0.183ab	3.1033abc	0.0663ab	75.00de
5	0.1998abc	3.1919abc	0.0687ab	76.60bcde
6	0.2246abc	1.3232fgh	0.0386efg	100.00a
7	0.2194abc	2.4822cde	0.0546bcde	50.00g
8	0.2383abc	1.9224defg	0.0495bcdef	88.33ab
9	0.3084d	4.2225a	0.0763a	88.33ab
10	0.2023abc	2.065def	0.053bcde	85.00bcd
11	0.2284abc	2.0867def	0.0523bcde	80.00bcde
12	0.2198abc	0.9387efg	0.0469cdefg	61.66e
13	0.2454bc	2.7803bcd	0.0627abc	76.66cde
14	0.2550c	3.898ab	0.0747a	78.33bcde
15	0.1965abc	1.8204defg	0.0492bcdef	78.33bcde
16	0.2447bc	2.4247cde	0.0582abcd	81.66bcde
17	0.2489b	c3.2705abc	0.0687ab	63.33f
18	0.1754a	0.4671i	0.0179h	83.33bcde
19	0.2422abc	0.9216h	0.0296g	93.33a
20	0.2295abc	1.0268h	0.0332fg	86.60bc

*: Common letters indicate no significant difference

1994). Arzani *et al*. (1999) reported that there were significant differences among cultivars for potential of regeneration from immature embryo and the Fresh Weight Growth of callus (FWG) distinguished cultivars more than callus induction frequency did for callus induction evaluation. Solid MS medium was optimum for immature embryo culture (Al-khayri and Al-Bahrany, 2000; Delport *et al*., 2000; Mendoza and Kaeppler, 2002) of wheat supplement with different combinations of plant growth regulators.

Mean comparison of traits in callus induction: Mean comparison of the traits measured in callus induction showed that genotypes, 1 and 6 had the highest PCI (100%). The highest amount of CGR, RFWG and RGR belonged to genotype no.9. While the lowest amount of CGR, RFWG and RGR was attributed to genotypes no.18, 1 and 18, respectively (Table 2). The results of callus induction traits (Table 2) obviously revealed that culture response was greatly influenced by the wheat genotypes and also emphasized a profound effect of genotypes on callus induction capacity, which is in agreement with reports of callus induction in durum wheat (Ozgen *et al*., 1996; Bommineni and Jauhar, 1996) and in bread wheat (Hess and Carman, 1998). The importance of genotype in determining the *in vitro* response of wheat tissues has been recognized and the efficiency of callus induction, callus growth rate and plant regeneration frequency have all been reported to be genotype dependent (Yadav *et al*., 2000; Yadava and Chawla, 2001; Schween and Schwnkel, 2003). Birsin and Ozgen (2004) reported that the genotype effects on callusing ability from *triticale* mature embryo cultures. Shah *et al.* (2009) exhibited significant differences between and among wheat cultivars for callus induction response and the callus induction was found to be genotype-dependent. In general, callus induction used as on efficient character for assessment of culture responses from mature embryo in wheat genotypes. The callus fresh weight is provided a more concise quantitative character for the development rate of callus.

Effect of drought stress on the characters: Analysis of variance for Callus Growth Rate (CGR), Relative Fresh Weight Growth (RFWG), Relative Growth Rate (RGR), Relative Water Content (RWC), Percent of Callus Chlorosis (PCC) and Proline Content (PC) indicated highly significant differences ($p<0.01$) among the genotypes for all the characters in the stress condition (15%) (Table 3). The analysis of variance also showed significant differences among levels of (0, 15%) PEG concentration for traits CGR, RFWG, RGR, RWC, PCC and genotype×drought interaction for CGR, RGR, RWC and PCC, respectively. The result obtained from comparison of means revealed that the highest amount of CGR, RFWG, RGR, RWC and PC, respectively belonged to genotypes no.14, 6, 6, 6 and 19, respectively. While the lowest amount of CGR, RFWG, RGR, RWC and PC, respectively was attributed to genotypes no. 5, 7, 7, 7 and 12, respectively (Table 4). The highest PCC and the lowest PCC were related to genotypes 17 and 1, respectively. The results indicated that CGR, RFWG, RGR and RWC decreased in the stress condition (%15 PEG level) as compared with non-stress condition (0%

Table 3: Analysis of variance for mature embryos callus characters under stress condition

		MS					
S.O.V	d.f	CGR	RFWG	RGR	RWC	PCC	PC
Genotype(G)	19	0.011**	0.010**	0.001**	0.005**	0.115**	0.830**
Drought(D)	1	0.012**	0.227**	0.016*	0.293**	2.095**	1.021^{ns}
D×G	19	0.002**	0.007^{ns}	0.0002**	0.005**	0.027**	0.483^{ns}
Error	80	0.001	0.004	0.0002	0.002	0.005	0.329
CV%		3.18	6.86	5.42	2.07	5.15	2.32

Ns, *,**: Non-significant, significant at 0.05 and significant at 0.01 level of probability, respectively.

Table 4: Mean comparison of the traits measured in stress condition (p<0.01)*

Genotype	CGR	RFWG	RGR	RWC	PCC	PC
1	1.27bcd	0.3364a	0.0159abc	83.20d	16.14g	5.02ab
2	1.52a	0.2888a	0.0139abc	80.98abc	22.44f	3.80cd
3	1.33abc	0.4986a	0.0217ab	82.89ab	21.30f	4.55bcd
4	1.13ef	-0.0029abc	-0.0015cde	82.09ab	32.01cde	2.59cd
5	1.08fg	0.1029abc	0.00175 bcde	85.95a	33.03bcd	3.24cd
6	1.55a	0.5000a	0.0237a	88.77a	21.71g	6.35a
7	1.58abc	-0.1278c	-0.0117e	71.34ab	46.01a	2.06bcd
8	1.59a	0.1886a	0.0103abc	86.22a	21.69ef	4.20bcd
9	1.37abc	0.4384a	0.0212ab	83.82a	19.99f	4.11bcd
10	1.45ab	0.3435a	0.0163abc	82.91ab	27.56bcd	3.35bcd
11	1.60a	0.1790a	0.0085abcd	84.15a	31.88bc	3.29bcd
12	1.34abc	-0.0086abc	-0.0022cde	82.48ab	40.86a	1.51abc
13	1.48a	0.2375a	0.0095abcd	81.64abc	30.26bcd	2.59cd
14	1.74abc	0.4706abc	0.0172abc	75.12cd	32.61b	2.82cd
15	1.18de	0.2373a	0.0109abc	80.26abc	31.96bc	3.99cd
16	1.61a	0.2363ab	0.0096abcd	82.74ab	27.45bcd	4.31bcd
17	1.25cd	-0.098bc	-0.0093de	74.20bcd	48.00a	1.80bcd
18	1.0g	0.1326a	0.0072abcd	83.51a	28.75bcd	4.29bcd
19	1.73abc	0.4853a	0.0228a	83.62a	21.72g	7.54abc
20	1.22de	0.3046a	0.0150abc	83.39a	25.02de	3.07d

*: Common letters indicate no significant difference

Table 5: Mean comparison of *in vitro* indicators of drought tolerance under stress (15% PEG) and non-stress (0 % PEG) using immature embryo culture (p<0.01)

Drought	CGR	RFWG	RGR	RWC	PCC	PC
0	1.46a	0.4577a	0.0220a	90.63a	19.76a	3.61a
15	1.35b	0.0166b	-0.0019b	73.09b	36.82b	5.58b

*: Common letters indicate no significant difference

PEG. Level). PC and PCC were increased in %15 PEG level as compared with 0% PEG level (Table 5).

***In vitro* indicators of drought tolerance:** Callus Growth Index (CGI) displayed remarkable differences among the genotypes in the means of increasing value of selected calli. Genotype no.6 showed the highest callus increasing value (Table 6). The highest amount of relative tolerance (Rt%) in the induced drought stress condition was attributed to genotype no.6 (Table 6), while the lowest amount of reduction percentage (R%) from 0.0 to 15% PEG belonged to genotype no.6 and the highest amount of R% was shown by genotype no.14 (Table 6). The amount of callus growth was expressed as *in vitro* tolerance (INTOL) to eliminate inherent differences associated with the Relative Growth Rate (RGR) of the genotypes in response to induced drought stress by PEG. Based on INTOL genotype no. 6 exhibited the highest INTOL (Table 6). With regard to callus (resulted from immature embryos) increasing value, percentage of relative tolerance (Rt%), the amount of reduction percentage (R%) and INTOL genotype no. 6 was selected as the most drought tolerant at *in vitro* condition (Table 6).

The increasing value of proline concentration during stress condition has been suggested as an osmoticum, a desiccation-protectant, a sink for nitrogen and reducing power during stress or a source of nitrogen and reducing power during recovery from stress (Steven *et al*., 1990). Abdel-Ghany *et al*. (2004) expressed that there were highly significant interactions between cultivars for manitol concentration for callus survival and regeneration ability from immature embryos of wheat. Hamdy and Aref (2002) examined the immature embryo culture of maize for improving drought tolerance in January 25, cultivars and reported that analysis of variance revealed highly significant differences between the tested genotypes as well as between the different levels of drought (PEG concentration) for all studied characters. Early works of Singh *et al*. (1972) displayed a significant positive correlation between drough resistance and proline accumulation in barley. Since then, a number of workers have reported enhanced accumulation of proline content in different plants (Szegletes *et al*., 2000; Chandrasekar *et al*., 2000; Deora *et al*., 2001). Al-khayri and Al-Bahrany (2000) examined the response of palm (*Phoenix dactylifera* L.) calli to water stress. Callus growth, water content and proline accumulation were assessed. They showed that increasing water stress caused a progressive reduction in growth as expressed in callus fresh mass, relative growth rate and index of tolerance. Abdulaziz and Al-Bahrany (2002) studied the callus to varing degree of Polyethylene Glycol (PEG)-induced water stress.They studied callus growth, water content and prolin accumulation. Their results indicated that increasing water stress induced by increasing concentration of PEG caused a progressive reduction in callus fresh weight. Significant reduction in callus weight was recorded in response to 50g/L PEG. increasing with a progressive reduction in callus water content, which caused increase in proline accumulation reaching

Table 6: (I): Ranks (R), ranks mean ($\overline{R}$) and standard deviation of ranks (SDR) of *in vitro* indicators of drought tolerance using immature embryo culture

Genotype no	CGR	R	RFWG	R	RGR	R	RWC	R	INTOL	R	PCC	R
1	1.27	14	0.3364	7	0.01590	7	83.20	9	0.5665	3	16.14	1
2	1.52	8	0.2888	2	0.01390	9	80.98	16	0.0028	9	22.44	7
3	1.33	13	0.4986	9	0.02170	3	82.89	11	0.2840	6	21.30	3
4	1.13	18	-0.0029	17	-0.00150	17	82.09	14	-1.2800	18	32.01	15
5	1.08	19	0.1029	16	0.00175	16	85.95	3	-0.8394	17	33.03	17
6	1.55	7	0.5000	1	0.02370	1	88.77	1	0.8809	1	21.71	5
7	1.58	6	-0.1278	20	-0.01170	20	71.34	20	-6.8750	20	46.01	19
8	1.59	5	0.1886	13	0.01030	11	86.22	2	0.3464	4	21.69	4
9	1.37	11	0.4384	5	0.02120	4	83.82	5	0.3198	5	19.99	2
10	1.45	10	0.3435	6	0.01630	6	82.91	10	0.0900	8	27.56	10
11	1.60	4	0.1790	14	0.00850	14	84.15	4	-0.0502	11	31.88	13
12	1.34	12	-0.0086	18	-0.00220	18	82.48	13	-1.3700	19	40.86	18
13	1.48	9	0.2375	10	0.00950	13	81.64	15	-0.3003	15	30.26	12
14	1.74	1	0.4706	4	0.01720	5	75.12	18	-0.2013	14	32.61	16
15	1.18	17	0.2373	11	0.01090	10	80.26	17	-0.0641	12	31.96	14
16	1.61	3	0.2363	12	0.00960	12	82.74	12	-0.0252	10	27.45	9
17	1.25	15	-0.0980	19	-0.00930	19	74.20	19	-3.5600	16	48.00	20
18	1.01	20	0.1326	15	0.00720	15	83.51	7	-0.0649	13	28.75	11
19	1.73	2	0.4853	3	0.02280	2	83.62	6	0.6618	2	21.72	6
20	1.22	16	0.3046	8	0.01500	8	83.39	8	0.1278	7	2502	8

Table 6: continued

Genotype no	PC	R	CGI	R	Rt%	R	R%	R	Sum	$\overline{R}$	SDR
1	5.02	3	0.1857	3	90.92	2	0.93	2	51.00	5.10	4.09
2	3.80	10	0.0424	7	66.03	10	5.94	16	101.00	10.10	3.28
3	4.55	4	0.0459	6	59.77	15	4.82	10	73.00	7.30	4.62
4	2.59	17	-0.1926	15	67.69	8	3.34	5	144.00	14.40	4.42
5	3.24	13	-0.2904	18	51.84	19	8.46	18	156.00	15.60	4.76
6	6.35	2	0.5061	1	98.70	1	0.21	1	21.00	2.10	2.13
7	2.06	18	-0.3148	19	64.00	14	6.13	17	173.00	17.30	4.39
8	4.20	7	0.0401	9	81.83	4	3.44	7	66.00	6.60	3.50
9	4.11	8	0.1417	4	68.46	7	4.86	11	62.00	6.20	3.01
10	3.35	11	0.0423	8	65.73	11	5.90	14	94.00	9.40	2.40
11	3.29	12	0.0113	10	74.09	5	5.91	15	102.00	1.20	4.31
12	1.51	20	-0.2194	16	64.29	13	4.76	9	156.00	15.60	3.62
13	2.59	16	-0.0042	11	54.55	18	9.83	19	138.00	13.80	3.35
14	2.82	15	-0.0346	12	47.37	20	14.56	20	125.00	12.50	6.86
15	3.99	9	-0.0529	13	64.62	12	3.23	4	119.00	11.90	3.84
16	4.31	5	-0.1476	14	70.69	6	5.05	12	95.00	9.50	3.65
17	1.80	19	-0.3537	20	58.48	17	5.38	13	177.00	17.70	2.35
18	4.29	6	-0.2345	17	59.64	16	1.59	3	123.00	12.30	5.43
19	7.54	1	0.2613	2	83.51	3	3.41	6	33.00	3.30	1.94
20	3.07	14	0.0592	5	66.93	9	4..06	8	91.00	9.10	3.31

significant increase over the control. Abdelsamad *et al*. (2007) declared that significant differences of genetic responses were observed for the four wheat genotypes at 10 and 20% PEG for callus induction, callus fresh weight, growth index, relative water content and relative tolerance percentage. El-Shafey *et al*. (2009) indicated that osmotic stress due to PEG application highly significantly decreased the fresh weight of the non irradiated rice calli as well as irradiated once in response to 10 and 15 % PEG, as compared with the control.

Screening in vitro indicators of drought tolerance: To better understand the relationships, similarities and dissimilarities among the *in vitro* indicators of drought tolerance, Principal Component Analysis (PCA), based on the rank correlation matrix was used. The main advantage of using PCA over cluster analysis is that each statistics can be assigned to one group only (Khodadadi *et al*., 2011). The relationships among different indices are graphically displayed in a biplot of PCA_1 and PCA_2 (Fig. 1). The PCA_1 and PCA_2 axes which justify 77.74% of total variation, mainly distinguish the indices in different groups. One interesting interpretation of biplot is that the cosine of the angle between the vectors of two indices approximates the correlation coefficient between them. The cosine of the angles does not precisely translate into correlation coefficients, since the biplot does not explain all of the variation in a dataset. Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the in vitro indices (Yan and Kang, 2003). Rt% and RWC we refer to group $1 = G_1$ indices which introduce genotype No. 6 as drought tolerant. The PCs axes separated PC, CGI and INTOL in a single group (G_2) that identify genotypes No. 19, 6 and

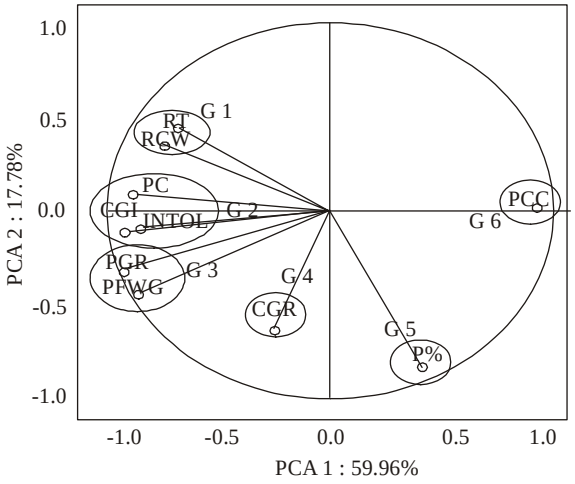

Fig. 1: Biplot analysis of in vitro indicators of drought tolerance using immature embryo culture

6 as the most drought tolerant. RFWG and RGR in a single group (G_3) that introduce genotype No. 6 as drought tolerant. CGR, R%, PCC were separated as groups 4 (G_4), 5(G_5), 6(G_6), respectively that distinguished genotypes 14, 6 and 1, respectively as drought tolerant ones. This procedure was also employed in chickpea (Zali *et al*., 2011) for clustering stability statistics and in durum wheat (Mohammadi *et al*., 2011a) for screening selection criteria of different climate and water regime conditions.

Screening drought tolerant genotypes: The estimates of *in vitro* indicators of drought tolerance (Table 6) indicated that the identification of drought-tolerant genotypes based on a single criterion was contradictory. For example, according to PCC, the desirable drought-tolerant genotype was (1), while according to RFWG, RGR, RWC, INTOL, R% and CGI, respectively the desirable drought-tolerant genotype was no. (6) and with regard to the indices CGR and PC genotypes no. (14) and (19) were the most drought tolerant, respectively.

Ranking method: To determine the most desirable drought tolerant genotype according to the all indices mean rank and standard deviation of ranks of all *in vitro* drought tolerance criteria were caculated and based on these two criteria the most desirable drought tolerant genotypes were identified.

In consideration to all indices, genotypes (6 = WC-4640), (19 = WC-47381) and (1 = WC-5047) showed the best mean rank and low standard deviation of ranks in stress condition, hence they were identified as the most drought tolerant genotypes, while genotypes (17 = WC-47379), (7 = WC-47456) and (5 = WC-47360), respectively as the most sensitive to drought, therefore they are recommended for crossing and genetic analysis of drought tolerance using diallel mating design or generation mean analysis and also for the QTLs (quantitative trait loci) mapping and marker assissted selection. Genotypes (15 = Pishtaz), (10 = WC-47399), (9 = WC-47367) and (3 = WC-4780) , respectively were distinguished as semi-tolerant genotypes. The same procedures have been used for screening quantitative indicators of drought tolerance in wheat (Mohammadi *et al*., 2011b), in maize (Farshadfar and Sutka, 2002) and in rye (Farshadfar *et al*., 2003).

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