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GT biplot Analysis of Genetic Diversity in Bread Wheat Using *In Vitro* Indicators of Drought Tolerance

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ABSTRACT: Mature embryos callus of 20 wheat genotypes were used in a factorial experiment based on completely randomized design (CRD) for screening drought tolerant genotypes. The results of analysis of variance for callus characteristics exhibited highly significant differences between the genotypes for callus growth rate (CGR), relative fresh weight growth (RFWG), relative growth rate (RGR), relative water content (RWC), percentage of callus chlorosis (PCC) and proline content (PC) indicating high genotypic variation and possible selection of drought tolerant genotypes at in vitro level. The genotype-by-trait (GT) biplot captured 94% of the total variation. Polygon view of 20 wheat genotypes with 9 callus characteristics indicated that the vertex genotype G6 (WC - 4640) had the highest values for RFWG, RGR, RWC,INTOL, PC, CGI and relative tolerance (RT%). Significant correlations among most of traits suggested that% RTcan be recommended as a suitable selection criterion for screening drought tolerant genotypes.

Keywords: *Triticum aestivum* L., traits correlation, genotype by traits (GT) biplot, in vitro indicators of drought tolerance

INTRODUCTION

Drought is a major abiotic stress which causes important agricultural losses, mainly in arid and semiarid areas. Drought stress causes moisture depletion in soil and water deficit with a decrease of water potential in plant tissues. The similarities of the effects induced by the stress in the plant cultured in vitro and in vivo conditions suggest that the in vitro system can be used as an alternative to field evaluations for studying the general effect of water-stress on plant growth and development. During past years, in vitro selection for cells exhibiting increased tolerance to water or drought stress has been reported (Barakat and Abdel-Latif, 1995; Errabii *et al.,* 2006; Mohamed *et al.,* 2000). In vitro selection can considerably shorten the time for the selection of desirable traits under selection pressure with minimal environmental interaction, and can complement field selection (Jain, 2001).

Tissue culture technique has been effectively utilized to induce tolerance which includes the use of some selective agents that permit the preferential survival and growth of desired phenotypes (Purohit *et al.,*1998). But in most cases, PEG has been used to stimulate water stress in plants.

PEG of high molecular weight is a non-penetrating inert osmotic cum lowering the water potential of nutrient solutions without being taken up or being phytotoxic (Hassan *et al.,* 2004). Osmotic adjustment through the accumulation of cellular solutes, such as proline has been suggested as one of the possible means of overcoming osmotic stress caused by loss of water (Gerdakaneh *et al.,* 2010, Shankhadhar *et al.,* 2000). Proline accumulation in higher plants is a characteristic physiological response to osmotic stress. Its degradation can provide carbon, nitrogen and energy source after stress (Hare *et al.,* 1999).

The GGE biplot methodology was developed originally for analysing multi-environment trial data. However, it can also be equally used for all types of 2-way data that assume an entry × tester structure (Yan, 2001, Yan and Kang, 2003). The genotype-by trait (GT) biplot analysis, proposed by Yan and Rajcan (Yan and Rajcan, 2002) is another powerful statistical tool for studying relationships among traits, evaluating cultivars based on multiple traits and for identifying those that are superior in certain traits. The genotypes can be generalized as entries, and the multiple traits as testers (Rubio *et al.,* 2004). The GT analysis allows visual display of the genetic correlation among traits (Yan and Rajcan, 2002).

It also provides information on the usefulness of cultivars for production as well as information that helps detect less important (redundant) traits, and identify those that are appropriate for indirect selection for a target trait. Up to now such investigation on biplot analysis of trait is not available on callus related traits.

The objectives of this research were to (i) evaluate callus related traits performance of wheat genotypes under stress conditions (ii) determine the interrelationship among wheat traits using GT biplot procedure (iii) Compare among genotypes on the basis of multiple traits.

MATERIALS AND METHODS

Twenty genotypes of bread wheat (*Triticum aestivum* L.) listed in Table 1 were provided from Seed and Plant Improvement Institute of Karaj, Iran. In order to evaluate the response of the mature embryos callus of wheat genotypes to in vitro drought stress, an experiment was carried out in a factorial experiment based on CRD design with three replications at the Agricultural College of Razi University, Kermanshah, Iran during 2010-2011.

Table 1: Genotypes name and codes.

Genotype	Code	Genotype	Code
WC - 5047	G1	WC - 47636	G11
WC - 4530	G2	WC - 4584	G12
WC - 4780	G3	WC - 46697 - 11	G13
WC - 4566	G4	WC - 4823	G14
WC - 47360	G5	Pishtaz	G15
WC - 4640	G6	WC- 47341	G16
WC - 47456	G7	WC - 47379	G17
WC - 47628	G8	WC - 4931	G18
WC - 47367	G9	WC - 47381	G19
WC - 47399	G10	WC - 5053	G20

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 mature embryos were also exposed to Murashige and Skoog (MS) medium containing different concentrations of PEG (0 and 15%). Mature seeds were surface- sterilized in 70% (v/v) ethanol for 5 min, rinsed twice with sterile distilled water, incubated further in commercial bleach (5% sodium hypochlorite) for 20 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. The surface-sterilized seeds were incubated at 33°C for 2 h in sterile distilled water for imbibition to occur. The mature embryos were easily separated from the endosperm in imbibed 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 121°C for 20 min and incubated at 25°C 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:

Relative Fresh Weight Growth (RFWG) (Chen *et al.*, 2006)

$$RFWG = \frac{(W_2 - W_1)}{W_1}$$

where W_1 is the weight of callus before treatment and W_2 the final weight of callus after two weeks of treatment., respectively.

Relative Growth Rate (RGR) (Birsin and Ozgen, 2004)

$$RGR = \frac{(LnW_2 - LnW_1)}{GP}$$

where W_1 is the weight of callus before treatment and W_2 the final weight of callus after two weeks of treatment and GP is the growth period, respectively. The time interval between two consecutive measurements was 16 days.

Callus Growth Rate (CGR) (Compton, 1994)

CGR (mm/day) of cultured embryos on stress medium were measured at 4, 8, 12 and 16 days after transferring of calli to the medium. CGR was calculated using the following formulas:

$$CGR_1 = \frac{d_4}{4},\ CGR_2 = \frac{d_8}{4},\ CGR_3 = \frac{d_{12}}{4},\ CGR_4 = \frac{d_{16}}{4}$$

$$CGR = \frac{CGR_1 + CGR_2 + CGR_3 + CGR_4}{4}$$

where d_4, d_8, d_{12}, d_{16}, were diameter of callus in days 4, 8, 12 and 16, respectively.
Diameter of callus was calculated as:

$$DC = \sqrt{Length \times width}$$

Percentage of Callus Chlorosis (PCC) (Arzani and Mirodjagh,1999)
PCC was determined visually as percentage of necrotic callus, 16 days after moving callus to the PEG containing medium.

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

$$RWC = \frac{(FW - DW)}{DW} \times 100$$

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

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

$$INTOL = \frac{RGR_{Treatment}}{RGR_{Control}}$$

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

Callus growth index (CGI)
CGI or increasing value of callus fresh weight was calculated as: (Abdelsamad *et al.*, 2007)

$$CGI = \frac{(W1 - W0)}{W0}$$

where W0 is the weight of callus before treatment and W1 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.

Proline content (PC)
Extraction and estimation of free proline content were done according to the procedure described by: (Errabii *et al.*, 2006).

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

$$RT(\%) = \frac{(Value\ under\ stress)}{(Value\ under\ \text{non - } stress)} \times 100$$

Statistical analysis
The genotype trait (GT) biplot method outlined in Yan and Rajcan (2002) was used to display the genotype trait data in a biplot. The biplots, as described by Yan and Rajcan (2002), were constructed by plotting the first principal component (PC1) scores of the genotypes and the traits against their respective scores for the second Principal component (PC2) that resulted from singular-value decomposition (SVD) of trait-standardized data in each environment.

RESULTS AND DISCUSSION

A. Analysis of variance and mean comparisons
Analysis of variance for Relative Fresh Weight Growth (RFWG), Relative Growth Rate (RGR),Callus Growth Rate (CGR), Percent of Callus Chlorosis (PCC), Relative Water Content (RWC), and Proline Content (PC) indicated highly significant differences ($p<0.01$) among the genotypes for all the characters in the stress condition (15%) (Table 2). The analysis of variance also showed significant differences among levels of (0, 15%) PEG concentration for traits RFWG, RGR CGR, PCC and RWC, and genotype × drought interaction for RGR, CGR, PCC and RWC. The result obtained from comparison of means revealed that the highest amounts of RFWG, RGR and PC belonged to genotypes G6 and G19. The highest amount of CGR was attributed to genotypes G19, G16 and G11, respectively. The highest RWC belonged to genotypes G6, G8 and G5, respectively. Also, genotypes G17 had higher PCC while genotypes G1 exhibited lower PCC in the stress condition (Table 3). Abdelsamad *et al* (2007) reported that significant differences of genotypic 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.

B. In vitro indicators of drought tolerance
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 genotypesG6 and G19 exhibited the highest INTOL (Table 3). Callus growth index (CGI) exhibited remarkable differences among the genotypes in the means of increasing value of selected calli. GenotypesG6 and G19 showed the highest callus increasing value. The highest amount of relative tolerance (% RT) in the induced drought stress condition was attributed to genotypesG6, G1 and G19, respectively, while the lowest amount of RT% belonged to genotypesG14, G5 and G13, respectively.

Table 2: Analysis of variance for mature embryos callus *characteristics* under stress condition.

S. O. V	df	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

Table 3: Comparing Genotypes for 9 mature embryos callus characteristics under stress condition.

Genotypes	CGR	RFWG	RGR	RWC	PCC	PC	INTOL	CGI	% RT
G1	1.27	0.3364	0.01590	83.20	16.14	5.02	0.5665	0.1857	90.92
G2	1.52	0.2888	0.01390	80.98	22.44	3.80	0.0028	0.0424	66.03
G3	1.33	0.4986	0.02170	82.89	21.30	4.55	0.2840	0.0459	59.77
G4	1.13	-0.0029	-0.00150	82.09	32.01	2.59	-1.2800	-0.1926	67.69
G5	1.08	0.1029	0.00175	85.95	33.03	3.24	-0.8394	-0.2904	51.84
G6	1.55	0.5000	0.02370	88.77	21.71	6.35	0.8809	0.5061	98.70
G7	1.58	-0.1278	-0.01170	71.34	46.01	2.06	-6.8750	-0.3148	64.00
G8	1.59	0.1886	0.01030	86.22	21.69	4.20	0.3464	0.0401	81.83
G9	1.37	0.4384	0.02120	83.82	19.99	4.11	0.3198	0.1417	68.46
G10	1.45	0.3435	0.01630	82.91	27.56	3.35	0.0900	0.0423	65.73
G11	1.60	0.1790	0.00850	84.15	31.88	3.29	-0.0502	0.0113	74.09
G12	1.34	-0.0086	-0.00220	82.48	40.86	1.51	-1.3700	-0.2194	64.29
G13	1.48	0.2375	0.00950	81.64	30.26	2.59	-0.3003	-0.0042	54.55
G14	1.74	0.4706	0.01720	75.12	32.61	2.82	-0.2013	-0.0346	47.37
G15	1.18	0.2373	0.01090	80.26	31.96	3.99	-0.0641	-0.0529	64.62
G16	1.61	0.2363	0.00960	82.74	27.45	4.31	-0.0252	-0.1476	70.69
G17	1.25	-0.0980	-0.00930	74.20	48.00	1.80	-3.5600	-0.3537	58.48
G18	1.01	0.1326	0.00720	83.51	28.75	4.29	-0.0649	-0.2345	59.64
G19	1.73	0.4853	0.02280	83.62	21.72	7.54	0.6618	0.2613	83.51
G20	1.22	0.3046	0.01500	83.39	25.02	3.07	0.1278	0.0592	66.93

C. Polygon view of the GT biplot

The polygon view of a GT biplot is the best way to visualize the interaction patterns between genotypes and traits (Yanand Rajcan, 2002). Fig. 1 is a GT biplot with a polygon view that presents the data of 20 wheat genotypes with 9 callus characteristics under stress conditions.

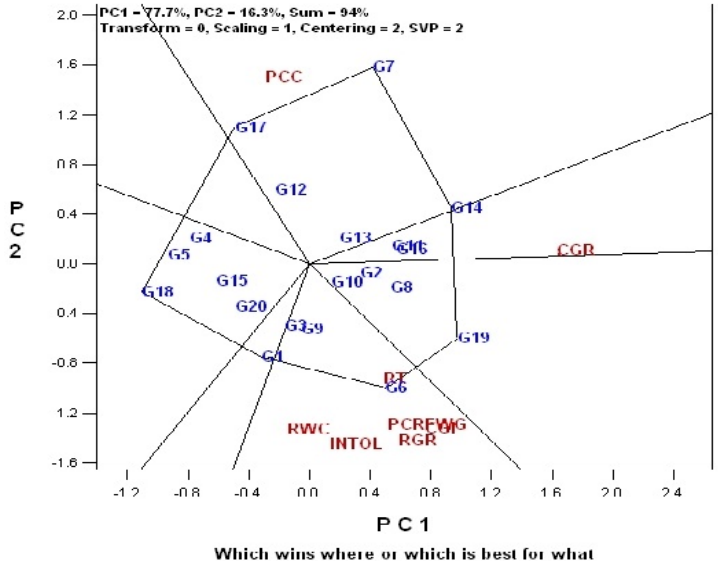

Fig. 1. Polygon view of the GT biplot to show which genotypes performed better in which callus characteristics.

The GT biplot explained 94% (PC1 = 77.7% and PC2 = 16.3 %) of the total variation of the standardized data (Fig. 1). This relatively high percentage variation reflects the accuracy of interrelationships among the measured characters/traits. The vertex genotypes were G7, G14, G19, G6, G1, and G18 and the characteristics fell into the sectors of G7, G14 and G6.Genotype G7 (WC - 47456) had the highest value for PCC; whereas G14 (WC - 4823) had the highest value for CGR. G6 (WC - 4640) had the highest values for RFWG, RGR, RWC, INTOL, PC, CGI, and RT%.

D. Vector view of the GT biplot

GT biplot can be used to visualize the relation among traits which facilitates identification of traits that can be used in indirect selection for a target character (Yan and Tinker, 2005). This biplot can be visualized from two perspectives. First, it shows the associations among the traits across 20 genotypes. Second, it shows the trait profiles of the genotypes, particularly those that are placed farther away from the biplot origin (Yan and Fregeau-Reid, 2008).

% RT was highly correlated with RFWG, RGR, RWC,INTOL, PC, and CGI and negatively correlated with PCC. Therefore, high positive correlations among the most traits suggest that one (i.e., % RT) of these traits should suffice as a selection criterion (Fig. 2).

Genotypes G6, G1 and G19 had the largest PC2 negative scores, respectively and were placed very close to RT, RFWG, CGI, PC, RGR, INTOL and RWC. Genotypes G7 and G17 had the largest PC2 positive scores and were placed very close to PCC. Genotypes G14 and G19 also had the largest PC1 positive scores and were placed very close to CGR (Fig. 2).

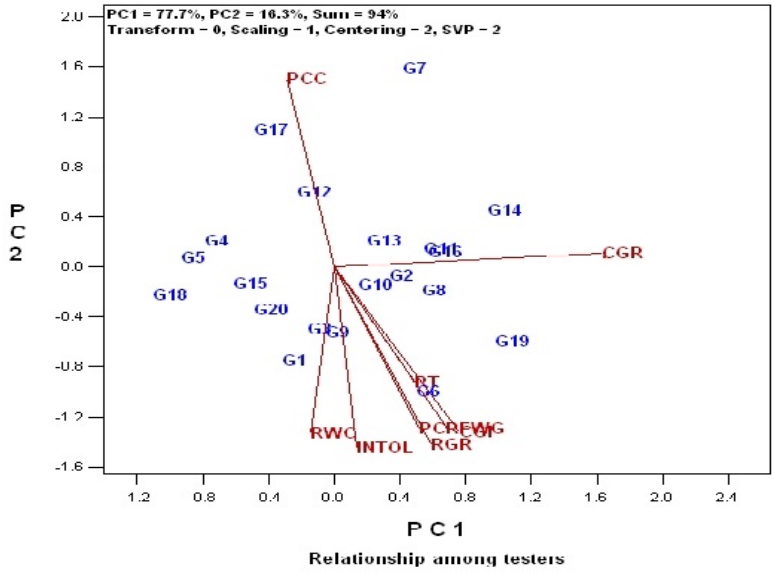

Fig. 2. Vector view of the GT biplot to show the interrelationship among all measured callus characteristics.

E. Comparison of traitsprofile of two specific genotypes

Traits profile of two genotypes can be easily compared on the GT biplot (Fig. 3). To compare two genotypes, here genotypes G6 (the lowest RT%) and G14 (the highest RT%) in Fig. 3, first connect their markers with a straight line; then draw a perpendicular line that passes through the biplot origin. This perpendicular divides traits into two groups; each of these two genotypes had larger values for a number of the traits. For instance, G6, had higher values than the G14 for RFWG, RGR, RWC, INTOL, PC, and CGI; in contrast, G14, had higher values than the G6 for CGR and PCC.

F. Comparing genotypic performance based on %RT

Fig. 4 is a graphic comparison of the relative performance of all genotypes based on RT%. The perpendicular line separates genotypes that performed below average from those performing above average for RT%. This figure indicates that genotypes G6 (WC - 4640) and G19 (WC - 47381) had the highest RT% and genotypes G7 (WC - 47456), G17 (WC - 47379) and G1 (WC - 5047) had the lowest RT%. The order of the genotypes for RT% was G6>G19>G1>G9>G8>G3>G10>G2>G16>G11>G20> G14>G13>G15>G18>G5>G4>G12>G17>G7.

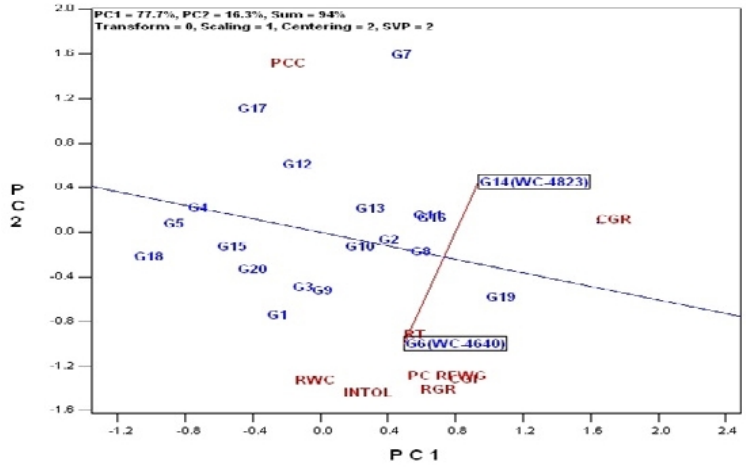

Fig. 3. The GT biplot for comparison of callus characteristics profiles of two specific genotypes.

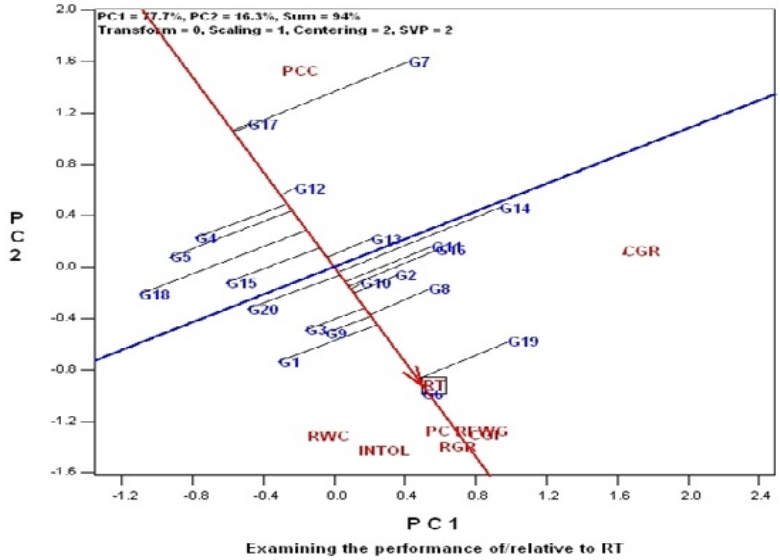

Fig. 4. The GT biplot to compare genotypic performance based on RT%.

G. Comparing performance of genotypes with check variety

Released wheat variety (i.e. "Pishtaz") was considered as check variety and it was compared with other breeding lines (Fig. 5). The concentric rings allow comparing the lines with Pishtaz. G20, G4 and G5 were closer to the central concentric ring, indicating these wheat breeding lines were similar to Pishtaz for these callus traits. G19 followed by G14 and G7 were appeared the farthest from the Pishtaz that means they were different from the standard variety for these studied traits.

H. Comparing performance of pishtaz variety for all callus characteristics

What is good with standard variety was shown in Fig. 6. The perpendicular divides traits into two groups. For instance, the Pishtaz, had high average values for characteristics i.e. RWC and INTOL than trait CGR.

I. Comparing genotype performance based on proline content (PC)

Genotypic profiles of each of traits can be easily compared on the GT biplot. For instance, PC, had average above values in genotypes G6, G9 and G1 than genotypes G7, G17 and G2. Proline Content of the genotypes was in the following order: G6 > G19 > G1 > G9 > G3 > G8 > G2 > G10 > G20 > G16 > G11 > G14 > G15 > G13 > G18 > G5 > G4 > G12 > G17 > G7 (Fig. 7). In recent years, tissue culture based in vitro selection has emerged as a feasible and cost-effective tool for developing stress-tolerant plants (Rai *et al.*, 2011).

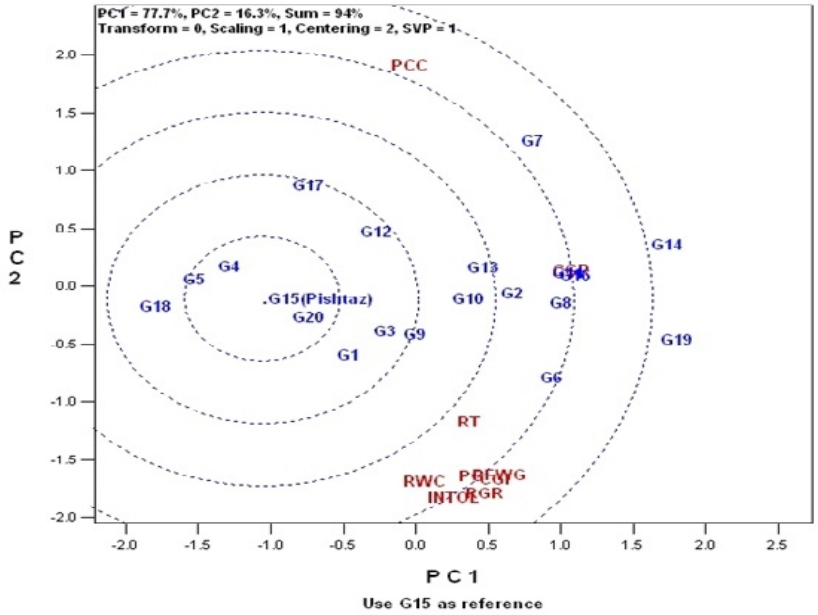

Fig. 5. The GT biplot to compare performance of genotypes with check variety (i.e. "Pishtaz").

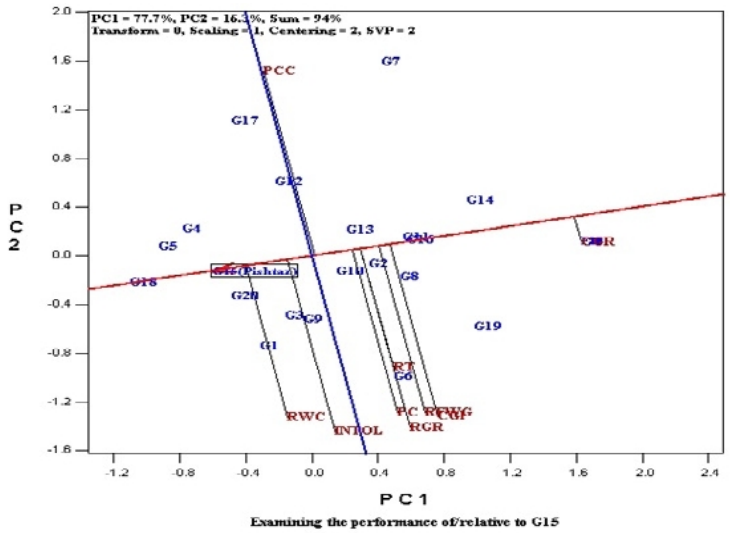

Fig. 6. The GT biplot to compare performance of Pishtaz variety at all callus characteristics.

In vitro selection makes possible to save the time required for developing disease resistant and abiotic stress tolerant lines of commercial crops and other plant species. However, In vitro selected variants should be finally field-tested to confirm the genetic stability of the selected trait (Jain, 2001).

The genotype-by-trait (GT) biplot allowed comparative evaluation of genotypes for multiple traits and helped identify genotypes that were desirable relative to several traits (Yan and Rajcan, 2002). The biplot provided good insight into the pattern of associations of the traits. In our study, the GT biplot captured 94% of the total variation.

This relatively high percentage variation reflects the accuracy of interrelationships among the measured traits. Application of GT biplot to this investigation on wheat genotypes shows visual interrelationships among the callus traits, which provides more information than the simple correlation coefficients that only describe the relationships between two traits. Traits correlation table (Table 4) revealed that RT% was positively and significantly associated with three traits PC, CGI, and RWC. Whereas GT biplot showed strong positive relationship between all of the measured traits, except CGR and PCC as indicated by the small acute angles between their vectors (Fig. 2).

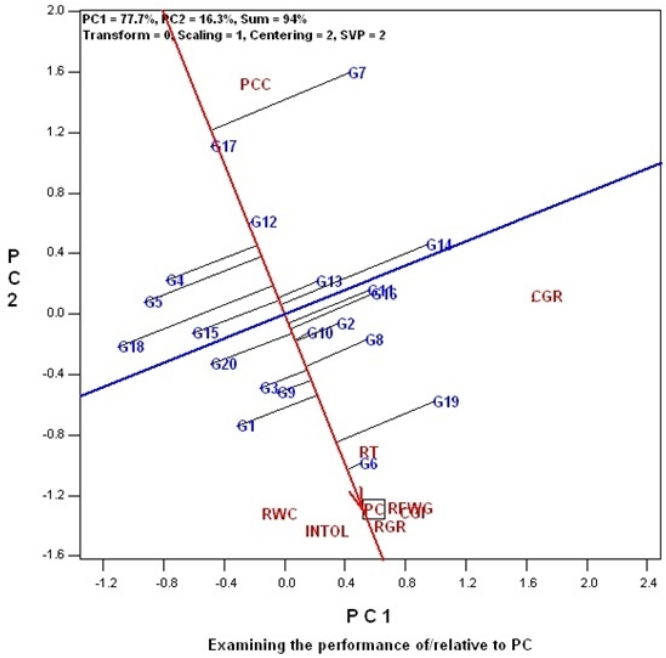

Fig. 7. The GT biplot to compare genotype performance based on proline content (PC).

Table 4: Pearson correlation coefficients between 8 mature embryos callus characteristics and percentage of relative tolerance (RT%) under stress condition.

	CGR	RFWG	RGR	RWC	INTOL	PCC	PC	CGI	%RT
CGR	1								
RFWG	0.364	1							
RGR	0.303	0.983 **	1						
RWC	-0.136	0.444	0.555*	1					
INTOL	0.033	0.764**	0.836**	0.778**	1				
PCC	-0.118	-0.803**	-0.871**	-0.695**	-0.823**	1			
PC	0.260	0.710**	0.745**	0.556*	0.601**	-0.748**	1		
CGI	0.407	0.835**	0.866**	0.562**	0.668**	-0.785**	0.760**	1	
% RT	0.262	0.308	0.388	0.517*	0.328	-0.532*	0.675**	0.713**	1

* and **: significant at the 5% and 1% probability levels, respectively.

Hence, the GT biplot effectively revealed that % RT is a good scope for selecting genotypes on a series of wheat callus traits. Thus, genotypes G6 (WC - 4640) and G19 (WC - 47381) were ranked as the top drought-tolerant genotypes, while genotypesG7 (WC - 47456), G17 (WC - 47379) and G1 (WC - 5047) were identified as the most sensitive to drought (Fig. 4).

Yan and Kang (2003) reported that within a sector, the genotype at the vertex of the polygon is the winner in all environments or traits falling in the sector. Thus, the polygon view of 20 wheat genotypes with 9 callus characteristics (Fig. 1) indicated that the vertex genotype G6 (WC - 4640) had the highest values for RFWG, RGR, RWC, INTOL, PC, CGI, and Relative tolerance (RT%).

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