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European Journal of Experimental Biology, 2012, 2 (4):984-994

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Association between *in vitro* and *in vivo* predictors of drought tolerance in the landraces of bread wheat (*Triticum aestivum* L.)

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ABSTRACT

In order to identify drought tolerant genotypes of bread wheat landraces (Triticum aestivum L.) two experiments were carried out in the Agricultural College, Razi university, Kermunshah, Iran, during 2009-2011. Experiment 1 was conducted in the field in a randomized complete block design with three replications under two different rainfed and irrigated conditions. Experiment 2 was carried out in the in vitro conditions using mature embryo cultures in a completely randomized design (CRD) with six replications for callus induction and a 20 × 2 factorial experiment with three replications for response of genotypes to in vitro drought stress. The results of analysis of variance for grain yield under irrigated (Yp) and rainfed (Ys) conditions exhibited the presence of a considerable genotypic variation among the genotypes (P < 0.01) indicating the possibility of discriminating drought tolerant landraces in the field conditions. Based on drought tolerance index (STI) genotypes No. 2, 5, 10, 15 and 18 identified as drought tolerant. Statical analysis also revealed highly significant differences between the genotypes for percentage of callus induction (PCI), 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. Genotypes were also different for in vitro indicators of drought tolerance such as: in vitro tolerance index (INTOL), callus growth index (CGI), percentage relative tolerance (Rt%) and percentage reduction (R%). To determine the most desirable drought tolerant genotypes according to all indices, mean rank and standard deviation of ranks of all in vitro and in vivo drought tolerance criteria were calculated and based on these two criteria the most desirable drought tolerant genotypes were identified as genotypes no. 2 (WC–4530), 10 (WC – 47399) and 18 (WC – 4931). Correlation analysis between in vivo and in vitro characteristics of drought tolerance also gave the same results.

Key words: Iranian landraces of bread wheat, embryo culture, in vivo and in vitro indices of drought tolerance**.**

INTRODUCTION

Cereal crops belonging to *Graminae* family produce large edible grains which provide about one-half of man's food calories and a major portion of his nutrient requirements. Wheat (*Triticum aestivum* L.) is foremost among cereals and indeed among all crops, as direct source of food for human environmental limitations of crop productivity throughout the world [1]. About two thirds of the world populations live on wheat grain [2].

The maximum potential of agricultural crops is seldom attained because of limitations on morphological and physiological processes imposed by stressse [4].

Drought is one of the most important and earliest studied abiotic stresses and one of the major limiting environmental factors for plant development and, hence, plant mass production. Plant defense against water deficit is a complex endeavour that the plant undertakes to protect itself [3, 33]

Crops exposed to this stressful environment are observed initially to have reduced growth rates. If water stress is more severe the response is manifested visually in a number of specific and recognizable symptoms [5]

In the absence of an understanding of the special mechanisms of tolerance the quantification of drought tolerance should be based on the grain yield in both stress and non-stress environments that can lead to selection of high yield genotypes under stress condition since, the response of selection under non-stress condition is maximal and heritability of the yield under these conditions is high [6, 7, 33]

In order to identify drought-tolerant genotypes in the field, several selection criteria have been proposed based on grain yield under stressed and non-stressed conditions. These indices are either based on drought tolerance or on the susceptibility of genotypes [8]. Fernandez [9] defined a new stress tolerance index (STI) and divided the manifestation of plants into the four groups of (1) – genotypes that express uniform superiority in non-irrigated and irrigated conditions (group A), (2) - genotypes which perform favorably only in non-stress conditions (group B), (3) - genotypes which yield relatively higher only in stress conditions (group C) and (4) - genotypes which perform poorly in non-irrigated and irrigated conditions (group D). Therefore, as Fernandez stated, the best index for stress tolerance selection is one that can be able to separate group A from others.

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 [10, 11, 12]

In addition to the classical method of breeding, modern technologies such as biotechnology and genetic engineering have been developed in support of the classical breeding method in research on plant tolerance to drought [3].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 [13]. 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 [14].

Mature wheat embryos have a high frequency of callus induction [15].Wheat-breeding programs have been struggling to improve the drought tolerance using a conventional approach of trailing breeding lines under drought field conditions. However, several attempts have been made to obtain drought tolerant varieties using tissue culture techniques to select the adapted genotypes of wheat varieties to water stress [16] for direct gene transformation and generation of genetic variable plants [17].

The drought stress could be induced in the plant cell cultures by adding different compounds to the nutrient medium such as, polyethylene glycol (PEG) which stimulates water stress by acting as osmotic agent which reduce the potential of the medium in where the cell are growing [18]

PEG of high molecular weight is a non-penetrating inert osmoticum lowering the water potential of nutrient solutions without being taken up or being phytotoxic [19].The culturing of an embryo isolated from the seed and ovules of higher plants in special medium is defined as embryo culture. Through embryo culture, either the plant develops directly from the embryo, or first callus formation is stimulated and then shoots and roots occur (indirect organogenesis), so a lot of plants are obtained from just one embryo.

The most important aspect of embryo culture is determining a culture medium that will provide the regular growth of embryos that were cultured in different sizes. Nutrients required by embryos vary depending on embryo age. In short, while mature embryos can develop in a simple medium, embryos in the early development stage demand more complex medium [20].The objectives of the present investigations were to: (i) screen land races of bread wheat genotypes for drought tolerance under *in vivo* and *in vitro* conditions (ii) evaluate the ability of genotypes to induce callus using mature embryo culture and (iii) assess the correlation beween *in vitro* and *in vivo* predictors of drought tolerance.

MATERIALS AND METHODS

In vivo experiment

Twenty landraces of bread wheat (*Triticum aestivum* L.) listed in Table 1 were provided from Seed and Plant Improvement Institute of Karaj, Iran. They were assessed in a randomized complete block design with three replications under two irrigated and rainfed conditions during 2010-2011 growing season in the experimental field of the College of Agriculture, Razi University, Kermanshah, Iran (47° 20′ N, 34° 20′ E and 1351 m above sea level).

Mean precipitation in 2010–2011 was 509.50 mm.. The soil of experimental field was clay loam with pH7.1. Sowing was done by hand in plots with three rows 2 m in length and 20 cm apart. The seeding rate was 400 seeds per m^2 for all plots. At the rainfed experiment, water stress was imposed after anthesis. Non-stressed plots were irrigated three times after anthesis, while stressed plots received no water. At harvest time, yield potential (Yp) and stress yield (Ys) were measured from 3 rows 1 m in length.

Stress tolerance index (STI) was calculated using the following formula [9].stress tolerance index =

$$STI = \frac{Y_S \times Y_P}{\overline{Y}_P^{\,2}}$$

where Yp and Ys are the yield of a given genotype in irrigated and rainfed conditions respectively, and $\overline{Y}_p$ is the mean yield for all genotypes in irrigated condition.

***In vitro* experiment**

In order to evaluate the response of the same genotypes of bread wheat (*Triticum aestivum* L.) (Table 1) to callus induction and *in vitro* drought stress, an experiment was carried out as 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 conducted for response of genotypes to *in vitro* drought stress.

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:

Percentage of callus induction (PCI): PCI was evaluated 4 weeks (suitable for sub-culturing) after embryo culture in Petri dishes as [21]: (number of seeds producing callus)/(number of seeds plated in Petri dishes).

Relative fresh weight growth (RFWG): RFWG = $[(W_2-W_1)]/W_1$ [22]

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): RGR = $[LnW_2-LnW_1]/GP$ [23]

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): 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 [24]:

$CGR_{1=}d_4/4$, $CGR_2 = d_8/4$, $CGR_3=d_{12}/4$, $CGR_4 =d_{16}/4$

$CGR = (CGR_1+ CGR_2 + CGR_3 + CGR_4) / 4$

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: diameter of callus = DC =√length×width

Percentage of callus chlorosis (PCC): PCC was determined visually as percentage of necrotic callus, 16 days after moving callus to the PEG containing medium [21].

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 the following formula [25]:

RWC= [(FW-DW)/DW]×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 [26]:

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

where RGR = relative growth rate and was measured by the formula of Birsin and Ozgen [23].

Callus growth index (CGI): or increasing value of callus fresh weight was calculated as: $CGI= (W_1-W_0)/W_0$ [27]: 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 [27]:

Rt % = [(value under stress)/ (value under non- stress)] × 100

Reduction percentage (R%): R% was calculated for the two stress (15%) and non-stress level (0) using the following formula [27]:
(value under 15% stress level - value at 0% stress level).

Proline content (PC): Extraction and estimation of free proline content were done according to the procedure described by Errabii et al. [25].

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 genotype and $SDR= (S^2_i)^{0.5}$.
Rank sum (RS) = Rank mean ($\overline{R}$) + Standard deviation of rank (SDR).

RESULTS AND DISCUSSION

***In vivo* experiment**
The results of analysis of variance for grain yield under irrigated (Yp) and rainfed (Ys) conditions indicated the presence of a considerable genotypic variation among the genotypes under rainfed and irrigated ($P < 0.01$) conditions (**Table 2**).

STI showed that genotypes 18, 15, 5, 2 and 10 were the most, whereas genotypes 17 and 6 the least relative tolerant genotypes (**Table 6**).

Fernandez [9], divided the manifestation of plants into the four groups of (1) – genotypes that express uniform superiority in non-irrigated and irrigated conditions (group A), (2) - genotypes which perform favorably only in nonstress conditions (group B), (3) - genotypes which yield relatively higher only in stress conditions (group C) and (4) - genotypes which perform poorly in non-irrigated and irrigated conditions (group D). A three-dimensional representation of Ys, Yp and STI is shown in Figure 1. The area of the 3D plot was divided into 4 regions, A, B, C and D [22]. Genotypes 2, 5, 10, 15 and 18 were placed in a region of the plot which had the highest STI, Ys and Yp (**Fig. 1**).

Correlation analysis (Table 5) showed that STI was positively correlated with Ys and Yp. These results implied that STI was able to identify genotypes with high grain yield under both rainfed and irrigated conditions and to differentiate drought-tolerant from drought-sensitive genotypes. The observed relationships between Yp and Ys with STI are in consistent with those reported by Fernandez [9] for mungbean, Sio Se Marde et al. [28] for bread wheat and Mohammadi et al. [29] for wheat.

***In vitro* experiment**

Callus induction

Analysis of variance (**Table 3**) revealed significant differences between genotypes for PCI, CGR, RFWG, RGR, RWC, PCC and PC indicting different resposes of genotypes to callus induction characteristics.

Mean comparison using Duncan's multiple range test (DMRT) (**Table 4**) revealed that genotypes influenced callus induction frequency so that the range of PCI was between 73.33-100; genotypes 5, 6, 8, 11, 16 and 18 exhibited 100% callus induction while genotypes 17 and 9 had the least PCI. These results confirmed that callus induction is genotype dependant. Ozgen et al. [15] in winter wheat, Arzani and Mirodjagh [21] in durum wheat, Grigoryeva and Shletser [30] in durum and bread wheat, and Shah et al. [13] in bread wheat also reported that callus induction is genotype dependent.

Table 1. Genotypes name and codes.

Genotype	Code	Genotype	Code
WC – 5047	1	WC – 47636	11
WC – 4530	2	WC – 4584	12
WC - 4780	3	WC – 46697 – 11	13
WC – 4566	4	WC – 4823	14
WC – 47360	5	Pishtaz	15
WC – 4640	6	WC– 47341	16
WC – 47456	7	WC – 47619	17
WC - 47628	8	WC – 4931	18
WC – 47367	9	WC – 47381	19
WC – 47399	10	WC - 5053	20

Table 2. Analysis of variance for grain yield under rainfed and irrigated conditions.

		Mean squares	
		grain yield	
S.O.V.	df	rainfed	irrigated
Replication	2	3650.52*	6951.14**
Genotype	19	22584.23**	21851.37**
Error	38	1064.62	1049.19
C. V. %		11.43	8.06

** and **: Significant at 1% and 5% level of probability respectively; S.O.V: Source of variation, d.f: Degree of freedom.*

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

	Mean squares						
S.O.V	PCI	CGR	RFWG	RGR	RWC	PCC	PC
Genotype(G)	0.010^{**}	0.121^{**}	0.071^{**}	0.004^{**}	140.131^{**}	0.055^{**}	0.684^{**}
Drought(D)	-	1.126^{**}	0.421^{**}	0.014^{**}	3190.971^{**}	5.607^{**}	11.102^{**}
D×G	-	0.010^{ns}	0.018^{ns}	0.002^{ns}	81.198^{**}	0.013^{ns}	0.242^{**}
Error	0.002	0.011	0.011	0.001	3.811	0.015	0.004
CV%	2.33	7.92	9.77	3.37	2.29	8.87	3.87

*Ns; **: Non-significant and significant at 1% level of probability,respectively.*

Table 4. Mean comparison of *in vitro* and *in vivo* indicators of drought tolerance in wheat.

Genotype code	Yield, g/m^2 (rainfed)	Yield, g/m^2 (irrigated)	PCI	CGR	RFWG	RGR	RWC	PCC	PC
1	267.79 cd	377.71 de	93.33 ab	1.287 bcd	0.7749 a	0.0350 ab	87.03 abc	19.58 de	2.7579 fgh
2	413.84 ab	507.12 ab	81.67 bc	1.4 abcd	1.234 abcd	0.0476 a	88.07 a	19.12 cde	6.9338 a
3	242.38 cde	370.39 def	93.33 ab	1.218 d	-0.0542 efgh	-0.0042 ab	87.31 abc	32.52 abcde	2.6494 hi
4	228.25 cde	301.39 gh	91.67 ab	1.009 e	-0.0971 fgh	-0.0072 ab	87.5 abc	39.88 ab	1.5388 j
5	410.82 ab	516.40 ab	100 a	1.048 e	-0.0644 defgh	0.0014 ab	85.54 bc	20 e	3.6442 d
6	199.27 ef	279.75 gh	100 a	1.251 bcd	-0.0644 efgh	-0.0054 ab	85.59 abc	30.07 bcde	4.1039 cd
7	286.43 c	388.17 de	91.67 ab	1.277 bcd	0.6874 abc	0.0303 ab	85.44 c	24.33 bcde	3.9001 cd
8	248.29 cde	317.46 fg	100 a	1.513 a	-0.1133 gh	-0.0634 c	87.15 abc	33.85 abc	4.4346 c
9	254.33 cde	372.61 def	75 c	1.404 abcd	0.0400 cdefgh	0.0059 ab	85.31 c	30.79 bcde	2.6421 hi
10	383.88 b	472.81 bc	95 ab	1.367 abcd	0.601 ab	0.0289 ab	86.96 abc	26.32 bcde	4.7034 b
11	266.85 cd	400.34 d	100 a	1.396 abcd	0.0758 bcdefgh	0.0005 ab	86.91 abc	28.33 bcde	2.9985 ef
12	230.31 cde	429.76 cd	93.33 ab	1.426 abc	0.1507 abcdefg	0.0087 ab	86.88 abc	31.82 abcd	2.8142 ghi
13	251.76 cde	401.62 d	95 ab	1.551 a	0.5321 abcde	0.023 ab	86.13 abc	31.67 abcd	2.8031 fghi
14	259.01 cde	391.74 de	93.33 ab	1.377 abcd	0.1653 abcdefg	0.0074 ab	85.37 c	26.93 bcde	2.5685 hi
15	435.24 ab	560.58 a	96.67 ab	1.543 a	0.307 abcdef	0.0142 ab	85.33 c	26.67 bcde	3.2152 e
16	227.71 cde	404.84 d	100 a	1.379 abcd	0.3737 abcdef	0.0176 ab	86.95 abc	24.4 bcde	4.1334 cd
17	150.29 f	250.78 h	73.33 c	1.385 abcd	-0.2229 h	-0.0196 bc	69.58 e	49.47 a	1.4681 j
18	464.29 a	547.87 a	100 a	1.439 ab	0.557 abcd	0.0247 ab	88.97 ab	25.21 bcde	2.3937 i
19	267.41 cd	406.95 d	95 ab	1.318 bcd	0.4007 abcde	0.0192 ab	88.01 abc	28.75 bcde	2.8918 efg
20	219.42 de	337.35 efg	95 ab	1.247 cd	-0.0303 fgh	-0.0067 ab	73.69 d	31.79 abcde	1.3230 i

Note: Means followed by the same letter(s) in each column are not significantly different.

Table 5. Association between *in vivo* and *in vitro* indicators of drought tolerance in wheat.

	CGR	RFWG	RGR	RWC	INTOL	PCC	PC	CGI	Rt%	R%	Ys	Yp	STI
CGR	1												
RFWG	0.252	1											
RGR	0.205	0.944**	1										
RWC	0.019	0.452*	0.457*	1									
INTOL	0.072	0.497*	0.483*	0.311	1								
PCC	0.000	-0.712**	-0.679**	-0.614**	-0.812**	1							
PC	0.122	0.604**	0.522*	0.504*	0.365	-0.576**	1						
CGI	0.293	0.962**	0.933**	0.497*	0.526*	-0.696**	0.560*	1					
Rt%	-0.275	-0.479*	-0.478*	0.206	0.096	0.000	-0.232	-0.406	1				
R%	0.204	0.247	0.266	-0.029	0.323	-0.352	0.195	0.291	-0.233	1			
Ys	0.129	0.571**	0.544*	0.426*	0.550*	-0.661**	0.435	0.632**	0.050	0.050	1		
Yp	0.243	0.588**	0.578**	0.475*	0.666**	-0.695**	0.383	0.654**	0.135	0.100	0.924**	1	
STI	0.172	0.546*	0.525*	0.412	0.560*	-0.628**	0.394	0.607**	0.079	0.051	0.990**	0.951**	1

Table 6. Ranks, mean ranks, standard deviation of ranks and rank sum of *in vitro* and *in vivo* indices of callus induction and drought tolerance in wheat.

Genotype no.	Y_S (g/m^2)	R	Y_P (g/m^2)	R	STI	R	CGR	R	RFWG	R	RGR	R	RWC	R
1	267.79	7	377.71	13	0.62	10	1.2865	14	0.7749	2	0.035	2	87.0484	6
2	413.84	3	507.12	4	1.30	4	1.3995	7	1.2338	1	0.0476	1	89.0746	1
3	242.38	14	370.39	15	0.56	15	1.2182	18	-0.0542	16	-0.0042	14	86.4752	9
4	228.25	16	301.39	18	0.43	18	1.0092	20	-0.0971	18	-0.0072	17	88.0172	3
5	410.82	4	516.4	3	1.31	3	1.0477	19	0.0994	13	0.0014	13	85.6081	13
6	199.27	19	279.75	19	0.35	19	1.2512	16	-0.0644	17	-0.0054	15	85.2214	17
7	286.43	6	388.17	12	0.69	6	1.2768	15	0.6874	3	0.0303	3	85.8047	12
8	248.29	13	317.46	17	0.49	16	1.5133	3	-0.1133	19	-0.0121	18	87.2462	4
9	254.33	11	372.61	14	0.59	13	1.4042	6	0.1323	12	0.0059	12	85.4568	15
10	383.88	5	472.81	5	1.12	5	1.3671	12	0.6010	4	0.0288	4	86.4727	10
11	266.85	9	400.34	10	0.66	8	1.3957	8	0.0758	14	-0.0235	20	86.7087	8
12	230.31	15	429.76	6	0.60	12	1.4259	5	0.1506	11	0.0084	10	86.4262	11
13	251.76	12	401.62	9	0.61	11	1.5507	1	0.5328	6	0.0229	6	85.1046	18
14	259.01	10	391.74	11	0.63	9	1.3768	11	0.1652	10	0.0074	11	85.4973	14
15	435.24	2	560.58	1	1.51	2	1.5427	2	0.3069	9	0.0142	9	85.2286	16
16	227.71	17	404.84	8	0.57	14	1.3791	10	0.3737	8	0.0176	8	87.0933	5
17	150.29	20	250.78	20	0.23	20	1.3854	9	-0.2229	20	-0.0196	19	69.5837	20
18	464.29	1	547.87	2	1.58	1	1.4388	4	0.5569	5	0.0246	5	88.9182	2
19	267.41	8	406.95	7	0.67	7	1.3175	13	0.4007	7	0.0192	7	86.7771	7
20	219.42	18	337.35	16	0.46	17	1.2472	17	-0.0303	15	-0.0067	16	73.7484	19

Table 6 continued.

Genotype no.	PCC	R	PC	R	CGI	R	Rt%	R	R%	R	INTOL	R	$\overline{R}$	SDR	RS
1	19.5833	2	2.7579	13	0.2701	6	48.6414	16	7.75	12	1.0716	2	8.07	5.18	13.25
2	19.119	1	6.9338	1	0.5355	1	41.5611	19	11.08	19	0.8386	3	5.00	6.46	11.46
3	32.52	17	2.6494	14	-0.0717	15	81.9639	3	2	3	-23.9629	18	13.15	5.04	18.19
4	39.875	19	1.5388	18	-0.1144	19	74.1275	6	2.15	4	-26.0431	19	15.00	6.19	21.19
5	20.00	3	3.6442	7	-0.0045	13	92.7325	1	3	5	19.5696	1	7.53	5.89	13.42
6	30.0702	12	4.1039	5	-0.0782	17	67.1083	9	8.29	14	-2.8074	14	14.84	4.16	19.00
7	24.3283	4	3.9002	6	0.2184	7	35.9843	20	1.67	2	0.7478	5	7.76	5.38	13.14
8	33.8533	18	4.4346	3	-0.1269	18	48.5309	17	6.15	11	-17.0975	17	13.38	6.13	19.51
9	30.7857	13	2.6421	15	0.0589	10	78.1181	4	8.83	17	-5.1248	16	12.15	3.76	15.91
10	26.3153	6	4.7034	2	0.3379	2	56.8453	13	4.84	9	0.6832	6	6.38	3.54	9.92
11	28.3333	10	2.9985	9	-0.0242	14	87.6387	2	3.7	6	-0.3607	13	10.07	4.42	14.49
12	31.82	16	2.8142	11	0.0032	12	63.866	10	4.32	8	0.2154	9	10.46	3.09	13.55
13	31.6666	14	2.8031	12	0.2777	5	59.7171	11	5.3	10	-0.0119	12	9.76	4.39	14.15
14	26.9333	9	2.5685	16	0.0352	11	76.3587	5	8.47	16	0.0407	11	11.07	2.95	14.02
15	26.6666	8	3.2152	8	0.1343	8	72.2899	7	8.15	13	0.385	8	7.15	4.46	11.61
16	26.4047	7	4.1334	4	0.0956	9	67.2232	8	8.4	15	0.4807	7	9.23	3.85	13.08
17	49.4666	20	1.4681	19	-0.2311	20	49.7627	15	0.5	1	-26.0952	20	17.15	5.80	22.95
18	25.1452	5	2.3937	17	0.2929	3	57.7282	12	4.22	7	0.7824	4	5.23	4.58	9.81
19	28.75	11	2.8918	10	0.2802	4	50.2958	14	9.82	18	0.1477	10	9.46	3.77	13.23
20	31.7914	15	1.323	20	-0.0747	16	48.2352	18	14.54	20	-3.287	15	17.07	1.80	18.87

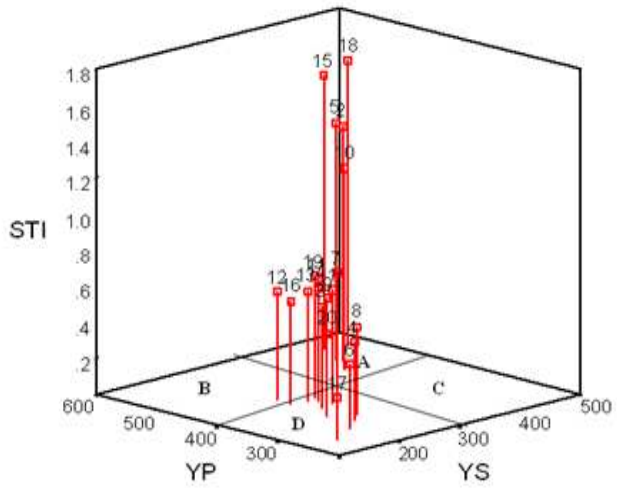

Fig.1. Three-dimensional plot between Yp, Ys and STI

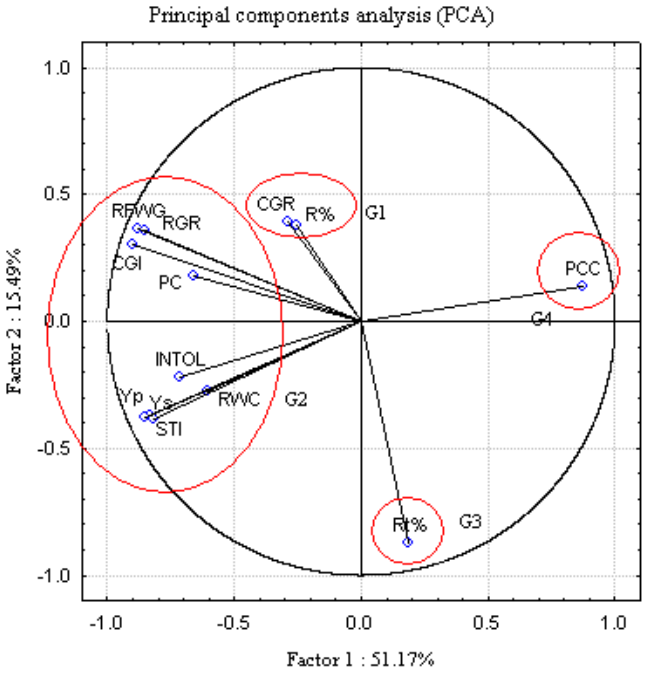

Fig.2. Biplot analysis of *in vitro* and *in vivo* indicators of drought tolerance

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% PEG) (**Table 3**). The analysis of variance also showed significant differences among levels of (0, 15%) PEG concentration and genotype × drought interaction for RWC and PC. The results obtained from comparison of means exhibited that the highest amount of CGR, RFWG, RGR, RWC, PC belonged to genotypes no.13, 2, 2, 2 and 2, respectively. While the lowest amount of CGR, RFWG, RGR, RWC an PC was attributed to genotypes no. 17 (**Table 4**). The highest PCC and the lowest PCC were related to genotypes 17 and 2, 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% PEG. Level). PC and PCC were increased in %15 PEG level as compared with 0% PEG level. Abdulaziz and Bahrany [31] studied the callus to varing degree of polyethylene glycol (PEG)-induced water stress. They studied callus growth, water content and proline accumulation. Their results revealed 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 significant increase over the control.

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 genotype no.5 exhibited the highest INTOL (**Table 6**). Callus growth index (CGI) exhibited remarkable differences among the genotypes in the means of increasing value of selected calli. Genotype no.2 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.5 (**Table 6**), while the lowest amount of reduction percentage (R%) from 0.0 to 15% PEG belonged to genotype no.5 and the highest amount of R% was shown by genotype no.8 (**Table 6**). With regard to callus (resulted from mature embryos) increasing value, percentage of relative tolerance (Rt%), the amount of reduction percentage (R%) and INTOL genotypes no. 2 and 5 were selected as the most drought tolerant at *in vitro* condition (**Table 6**). Abdelsamad et al. [27] 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.

Screening *in vitro* and *in vivio* indicators of drought tolerance

The relationships among different indices are graphically displayed in a biplot of PCA1 and PCA2 (**Fig. 2**). The first and second components justified 66.66% of total variations among the genotypes. The PCA1 and PCA2 mainly distinguished 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 data set. Nevertheless, the angles are informative enough to allow a whole picture about the interrelationships among the drought indices [32]. CGR and R% we refer to group 1= G1. The PCs axes separated RFWG, RGR, CGI, PC, INTOL, RWC, Ys, Yp and STI in a single group (G2). Rt% and PCC were separated as groups (G3), (G4). The vector view of the biplot (**Fig. 2**) provides a summary of the interrelationships among the *in vitro* and *in vivo* indicators. The cosine of the angle between the vectors of two indices approximates the correlation between them. For example, G2 indices were positively correlated (an acute angle), while G2 was negatively correlated with G4 indices.

A significant correlation coefficient was found among stress tolerance index (STI) with relative fresh weight growth (RFWG), relative growth rate (RGR), tolerance index (INTOL), callus growth index (CGI) and negative correlation coefficient was found between stress tolerance index (STI) and proline content (PC) (**Table 5**).

Screening drought tolerant genotypes

The estimates of *in vitro* and *in vivo* indicators of drought tolerance (**Table 7**) indicated that the identification of drought-tolerant genotypes based on a single criterion was contradictory. For example, according to INTOL, the desirable drought-tolerant genotype was WC – 47360 (5), WC – 5047 (1), while according to RFWG, RGR the desirable drought-tolerant genotype was WC – 4530 (2) and with stress tolerance index (STI), genotype WC – 4931(18) was the most drought tolerant. To determine the most desirable drought tolerant genotypes according to the all *in vitro* and *in vivo* indicators of drought tolerance, mean rank and standard deviation of ranks of all *in vitro* and *in vivo* 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 (18), (10), (2) and (15) 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), (4) and (8) as the most sensitive to drought.

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