Journal on New Biological Reports
JNBR 4(1) 33 – 40 (2015)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

In vitro evaluation of durum wheat genotypes for drought tolerance

Maryam Razmjoo[1*], Reza Mohammadi[2], Lia Shooshtari[1]

[1]Department of Plant Breeding, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran
[2]Dryland Agricultural Research Institute, Kermanshah, Iran

***Corresponding author:** m.razmjoo67@gmail.com

| **Received**: 14 January 2015 | **Accepted**: 21February 2015 |

ABSTRACT

Durum wheat (*Triticum turgidum* var. *durum*) is considered as an important food crop. The most important challenge in production of most of the crops including durum wheat is drought stress. To evaluate drought tolerance of 25 durum wheat genotypes using polyethylene glycol (PEG) 6000, a factorial experiment based on completely randomized design with three replications was carried out in the tissue culture laboratory of islamic Azad University, Kermanshah, Iran in 2010-11. The results of ANOVA indicated significant differences between genotypes for traits *Diameter of callus* (DC) and *Callus Growth Rate* (CGR) under non-stress condition and for the traits *relative growth rate* (RGR), *Relative fresh weight growth* (RFWG), based on fresh weight, *Callus Growth Rate* (CGR), callus water content (CWC), *Relative Water Content* (RWC), fresh weight and dry weight is a significant difference (P<0.01) under drought stress. Cluster analysis based on the studied traits, classified the genotypes into four groups. The principal component analysis (PCA) and biplot technique classified the breeding lines 4, 11, 3 and 2 in same group as drought tolerant genotypes. The results verified a remarkable variation for callus induction ability in genetic materials under drought stress condition that can be used in durum wheat breeding program.

Key Words: Durum wheat, PEG, mature embryos, PCA, biplot analysis

INTRODUCTION

Improved yields of wheat depend on many factors, among which one of the most important is tolerance to environmental stress, particularly to water stress. Indeed, in durum wheat (*Triticum turgidum* var. *durum*), drought is a major non-biotic stress that causes remarkable yield loss. In the Mediterranean region, this loss ranges from 10 to 80% depending on the year (Nachit et al., 1998). Drought is one of the most common environmental limitations that cause significant reduction of growth, development and yield on present cultivated lands, together with major problem in the cultivation of crops on arid and semiarid areas (Jain, 2001). Using classic breeding techniques in traditional breeding programs for tolerance to environmental stress was responsible for creating the majority of commercial varieties, but their applications are sometimes limited (Purohit et al., 1998). Resent progress in genetic manipulation of plant cells has opened new possibilities in crop improvement. Callus culture is used as an *in vitro* technique for biochemical and physiological studies in response to stress at the cellular level

(Liu et al., 2006). 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. 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 environment 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).

The main objectives were to evaluate the response of durum wheat genotypes to drought stress in vitro and to compare the ability of durum genotypes to induce callus immature embryo culture under drought stress condition.

MATERIAL AND METHODS

To evaluate drought tolerance of 24 durum wheat genotypes along with an old bread wheat cultivar (Sardari) using polyethylene glycol (PEG) a factorial experiment based on completely randomized design (CRD) with three replication was carried out in the tissue culture laboratory of Islamic Azad University, Kermanshah, Iran in 2010-2011.

Seeds of 25 genotypes of durum wheat (Table 1) water-soaked for 24 hours and sterilize with 70% (v/v) ethanol for 30 second, rinsed twice with sterile distilled water, incubated further in commercial bleach (2.5% sodium hypochlorite) for 12 min and rinsed several times in sterile distilled water to remove all the effects of sodium hypochlorite. All the operations and inoculation were performed under strict aseptic conditions in laminar airflow cabinet. Mature embryos were aseptically dissected from the seeds and placed scutellum up on MS medium to Murashige and Skooge (1962) supplemented with 30 g/L sucrose and was adjusted to PH 5.8, solidified with 7g/L agar and 2mg/L 2, 4-dichlorophenoxy acetic acid. The medium was autoclaved at 121ºC for 20 min and Petri dishes containing culture medium supplemented with 30 g/L sucrose, 7 g/L agar, and with concentrations 2 mg/L of 2, 4-D for callus induction were used.Petri dishes were sealed using Parafilm and placed in growth chamber in darkness. The temperature was maintained at 25±1ºC. Plates were changed after 28 days to refresh the media and regularly checked for contamination. For the determination of callus growth rate, 4 week old calli were transferred to a sterile Petri dish and their weight was measured in aseptic conditions. Afterwards, callus pieces were re-transferred to callus culture medium. The genotypes were exposed to different concentrations of PEG 6000 (Merck, Germany) for 15 days, and to build a culture medium containing PEG in the study of diffusion method (Diffusion-based method) was used. In this method of preparation and sterile agar medium containing 7 g/L, and the culture was distributed in containers. The growing morphogenic calli derived from mature embryos were also exposed medium containing different concentrations of PEG (0, - 4 and -8), (Table 2). The dishes containing solid medium under sterile conditions, the volume of liquid medium containing PEG was added after 24 hours of PEG molecules spread on agar medium. And thus reduce the potential water concentration in both the solid and liquid equilibrium is reached. After this time the culture supernatant was removed and the solid medium containing PEG was used. Callus was transferred to medium containing PEG for 15 days in this environment inside the growth chamber and was kept in the dark. 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: (number of seeds producing callus)/ (number of seeds plated in Petri dishes), (Arzani and Mirodjagh, 1999).

Relative Fresh Weight Growth (RFWG)

RFWG = [(W2-W1)]/W1 where W1 and W2 are the initial weight of callus before and after four weeks, respectively (Chen et al., 2006).

Relative Growth Rate (RGR)

RGR = [LnW2-LnW1]/GP where W1 and W2 are the initial and final weight of callus and GP is the growth period, respectively. The time interval between two consecutive measurements was 15 days (Birsin and Ozgen, 2004).

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):

CGR1 = d7/7, CGR2 = d14 /7, CGR3 = d21/7, CGR4 = d28/7

CGR = (CGR1+ CGR2 + CGR3 + CGR4) / 4
Where d7, d14, d21, d28, 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

Relative Water Content (RWC): callus samples of known fresh weight were dried in an oven set at 700C for 24h and RWC was calculated by following formula (Errabi et al., 2006):
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 (Al-Khayri and Al- Bahrany, 2004):
INTOL = RGRtreatment / RGRcontrol
where, RGR = relative growth rate and was measured by the formula of Birsin and Ozgen (2004).

Relative tolerance (Rt%): percentage of Rt was calculated for each genotype using the following formula (Abdelsamad, 2007):
Rt % = [(value under stress)/ (value under non-stress)] × 100

Callus water content (CWC %) = (callus fresh weight (CFW) - callus dry weight (CDW)/ CFW.CDW)

Statistical Analysis: Analysis was carried out using MSTAT. C and SPSS var. 18. Mean comparisons were conducted using Duncan (α= %5 and %1).

Table 1. Genotypes name and codes

Code	Genotype	Code	Genotype
1	IDYN-88-4	14	IDYN-88-28
2	IDYN-88-5	15	IDYN-88-31
3	IDYN-88-6	16	IDYN-88-35
4	IDYN-88-7	17	IDYN-88-37
5	IDYN-88-8	18	IDYN-88-43
6	IDYN-88-11	19	IDYN-88-44
7	IDYN-88-14	20	IDYN-88-46
8	IDYN-88-15	21	IDYN-88-47
9	IDYN-88-17	22	Saji (check)
10	IDYN-88-19	23	Zardak (durum landrace)
11	IDYN-88-20	24	Gerdish (durum landrace)
12	IDYN-88-23	25	Sardari (bread wheat landrace)
13	IDYN-88-26		

Table 2.Concentrations used in the drought

Stress level		solution	PEG (g)
0	MS FULL+ 2,4-D	-	-
- 4	MS FULL+ 2,4-D	200 Ml	35.67g
-8	MS FULL+ 2,4-D	200Ml	52.39g

RESULTS AND DISCUSSION

ANOVA and mean comparison

Highly significant differences (*P*<0.01) were observed among the genotypes for CGR, DC and PCI, respectively indicating the presence of genetic variability, different responses of genotypes to callus induction and possible selection of callus induction in durum wheat genotypes using mature embryos of durum wheat (Table 3; and Fig. 1).
Mean comparison for the genotype based on each studied traits in callus induction showed that genotypes No. 9, 10 and 11 had the highest PCI (100%). The highest DC and CGR belonged to genotypes No. 20 and 7, while the lowest CGR and DC was attributed to genotypes 21 and 1, respectively. The lowest PCI was attributed to genotypes 14, 15, 16, 17, 18, 19, 20, 21 and 23 respectively (Table 5). The results of callus induction traits revealed that culture response was greatly influenced by the wheat genotypes and also emphasized a marked 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). 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.

Effect of drought stress on the characters

variance for callus growth rate (CGR), relative fresh weight growth (RFWG), relative growth rate (RGR), relative water content (RWC), callus water content (CWC), FW and DW indicated highly significant differences (P<0.01) among the genotypes for all the characters in the stress condition (Table 4 and Fig. 1). El-Aref, (2002) reported a significant difference between maize genotypes for the same traits. The stress × genotype (G × S) interaction was significant for CGR and FW indicated highly significant differences among the genotypes for all the characters in the stress condition. The result obtained from comparison of means showed that the highest CGR, RFWG, RGR, RWC, FWG, DWG and CWC, respectively, belonged to genotypes 9, 6, 2, 2, 1, 1, and 6. The lowest CGR, RFWG, RGR, RWC, FWG, DWG and CWC respectively, was attributed to genotypes 13, 2, 20, 21, 15, 6 and 2 (Table 5). Ozgen et al, (1996) in winter wheat, Arzani and Mirodjagh (1999) in durum wheat, Grigoryeva and Shletser (2006) in durum and bread wheat, also reported that callus induction is genotype dependent.

Cluster analysis of *in vitro* characteristics

Cluster analysis of genotypes (UPGMA) based on RFWG, CGR, RGR, RWC, FWG, DWG and CWC and subsequent discriminant analysis for confirming the number of clusters, classified the

25 genotypes into four different clusters under stress condition. The first group consisted of majority of genotypes (12, 15, 20, 18, 14, 19, 21, 16, 17, 13, 11, 4, 9, 5, 6, 10, 8, 7) which classified with the check genotypes (22, 23, 24 and 25) in same group, while the genotypes 2, 3 and 1 separately each one classified in single clusters as second, third and fourth clusters (Fig. 2).

Table3-Analysis of variance for callus induction traits in durum wheat under non-stress condition

	Mean square			
S.O.V	DF	DC	PCI	CGR
Genotype	24	0.011**	39.66^{ns}	1.200**
Error	50	0.005	33.25	0.580
Total	74	-	-	-
CV%		19.12	5.35	5.86

Table 4 - Mean comparison for the genotypes based on the studied traits under stress and non-stress conditions

		Mean square						
S.O.V	DF	CGR	RFWG	RGR	RWC	DWG	FWG	CWC
Genotype(G)	24	0.007**	0.149**	0.006**	705.71**	0.155**	0.259**	692.54**
Stress(S)	2	0.002*	0.050*	0.001*	143.14^{ns}	0.016^{ns}	0.104^{ns}	117.61^{ns}
G × S	48	0.002**	0.014^{ns}	0.001^{ns}	61.89^{ns}	0.011^{ns}	0.130*	66.69^{ns}
Error	150	0.001	0.012	0.001	62.40	0.010	0.094	73.25
Total	224	-	-	-	-	-	-	-
CV%		23.81	12.69	11.73	23.71	17.18	20.48	21.30

Table5- Mean comparison for the genotypes based on the studied traits under stress and non-stress conditions

Genotypes	Non-stress			stress						
	CGR	PCI	DC	CGR	RFWG	RGR	RWC	DWG	FWG	CWC
1	12.81 ad	96.7a	0.74d	0.077abc	0.567 cd	0.065 b	94.08b	0.95a	2.163a	721.6r
2	14.00a	96.7a	1.36 ab	0.066abc	0.499d	0.068 a	95.99 a	0.89a	1.646ab	615.0r
3	13.20 ad	96.7a	0.79 d	0.074 abc	0.621bcd	0.063 a	93.77 a	0.49b	1.186bc	648.3q
4	12.90 ad	96.7a	1.12 bd	0.065 abc	0.830ab	0.060 a	93.15 a	0.17c	0.988bc	1287i
5	12.97 ad	96.7a	1.02bd	0.11 abc	0.935a	0.063 a	93.76 a	0.080c	1.290bc	1193l
6	13.05 ad	96.7a	1.05 bd	0.13ab	0.952a	0.066 a	94.50 a	0.060c	1.293bc	1901a
7	14/14 a	96.7a	1.01bd	0.10 abc	0.943a	0.065 a	94.38 a	0.066c	1.243bc	1551c
8	14.28a	96.7a	1.12 bd	0.10 abc	0.946a	0.066a	94.63 a	0.069c	1.354bc	1436e
9	12.38 bd	100a	0.90 cd	0.14ab	0.929a	0.059 a	92.89 a	0.066c	0.976bc	1441e
10	13.40ac	100a	1.28 ac	0.088 abc	0.938a	0.066 a	94.62 a	0.065c	1.099bc	1542c
11	13.20 ad	100a	0.95 bd	0.074abc	0.888a	0.061 a	93.52a	0.17c	1.247bc	1177l
12	12.15cd	96.7a	1.55 a	0.12 abc	0.926a	0.058 a	92.59 a	0.067c	0.928bc	1482d
13	13.15 ad	93.33a	1.06 bd	0.090 abc	0.882a	0.059 a	92.82 a	0.17c	1.130bc	1230k
14	12.80ad	90a	1.24 ac	0.080 abc	0.920a	0.060 a	93.04 a	0.077c	0.990bc	1312h
15	13.80 ab	90a	1.02 bd	0.051 c	0.926a	0.058 a	92.62 a	0.061c	0.845c	1582b
16	12.25 cd	90a	0.96 bd	0.051 c	0.941a	0.064 a	94.18 a	0.074c	1.350bc	1413f
17	13.32 ad	90a	0.89 cd	0.084 abc	0.879a	0.062 a	93.62 a	0.13c	1.010bc	1414f
18	13.26ad	90a	1.14 bd	0.080 abc	0.913a	0.055 a	91.33 a	0.072c	0.863bc	1352g
19	13.30 ad	90a	1.13 bd	0.71 abc	0.918a	0.056 a	91.81 a	0.097c	1.211bc	969.3o
20	12.18 cd	90a	1.37 ab	0.059 bc	0.910a	0.053 a	90.16 a	0.087c	0.889bc	1260j
21	11.85 d	90a	0.94 bd	0.080abc	0.921a	0.057 a	91.10 a	0.077c	0.996bc	1251j
22	12.90 ad	93.33a	1.17ad	0.072 abc	0.922a	0.058 a	92.25 a	0.095c	1.244bc	1124m
23	13.00ad	90a	1.11 bd	0.11 abc	0.919a	0.056a	91.91 a	0.073c	0.908bc	1360g
24	12.45 bd	93.33a	1.06 bd	0.074 abc	0.852ab	0.057 a	92.33 a	0.16c	1.048bc	1102n
25	12.34 bd	93.33a	1.01bd	0.14 a	0.776abc	0.058 a	92.57 a	0.31c	1.209bc	772p
LSD	1.24	8.25	0.11	0.067	0.23	0.051	0.051	0.21	0.64	18.23

*Means in same column followed by common letters are not significant at 5% level of probability

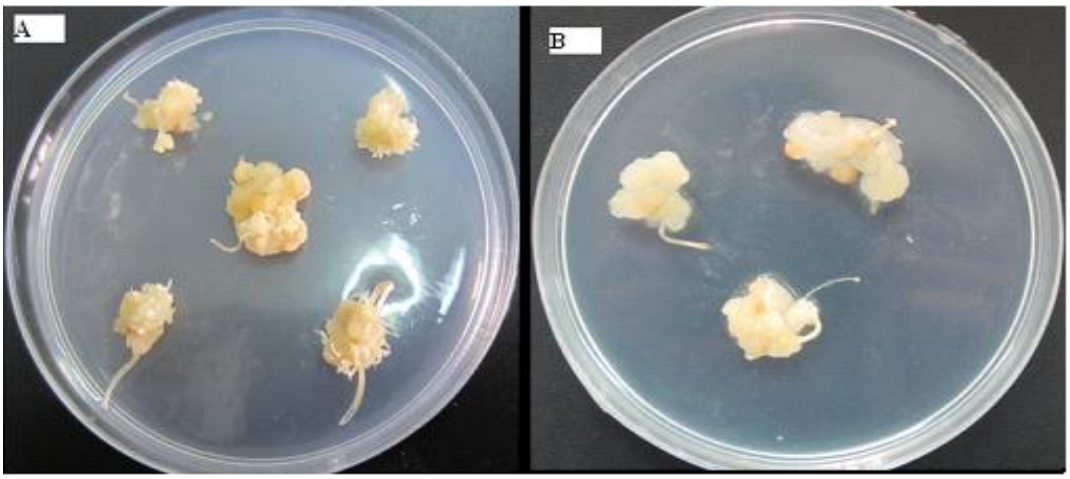

Fig. 1. (A) Calluses placed in medium with 2, 4-D and non-stress hormone, **(B)** calluses under drought stress environment.

Cluster analysis of the genotypes under non-stress condition classified the genotypes in four groups (Fig. 3). First group included genotypes 21, 16, 25, 24, 22, 13, 4, 6, 5, 17, 15, 14, 23, 19 and 18 in same group, while the second group comprised the genotypes 11, 9, 3 and 1 and the third group consisted of genotypes 10, 2, 8 and 7, and the fourth group included genotypes 12 and 20.

The characteristics of groups from cluster analysis under stress and non-stress conditions is presented in Table 6. Based on the results of the non-stress condition in the first PCI induction rate was higher than the other groups. In the second group relative CGR rate was higher than the other and each class in the fourth DC superior to the other groups. The results showed that the mean DWG of callus in drought conditions in the first group was superior to the other groups, RFWG and RWC traits in the second group and RGR, CGR and CWC traits in the third group were superior to the other groups. The FWG of callus in group fourth was higher than the other groups. The third group of drought stress condition and according to the most genotypes in this group could be introduced as a larger group. Moreover, according to the genotypes with other genotypes in the control group, the group could be considered as resistant to drought .

Correlation analysis

A positive significant correlation between RFWG with RGR, RWC, CWC and RGR and a negative significant correlation with FWG, DWG and INTOL were observed (Table 7). INTOL showed negative correlated (P<0.05) with RT% and CWC, RWG, RGR. Wheat is notorious for its ability to induce callus, which is a major hindrance in direct gene transfer and consequently for genetic improvement programs. In order to provide a successful platform for gene transfer, good quantity and quality callus is important (Suleman et al., 2001). In the present study, all the genotypes produced callus cultures with medium to relatively high quality (showing the first visible indication of embryogenic callus that was milky white to yellow in color and compactness in surface morphology) and could be used in future research (Rai et al., 2011).

To better understand the relationships, similarities and dissimilarities among the *in vitro* indicators of drought tolerance, a 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 PCA1 and PCA2 (Fig. 4). The PCA1 and PCA2 axes accounted 60.93% 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). PCI, DC and INTOL we refer to group 1= G1 indices which introduce genotypes no. 4, 11, 3, 2 as drought tolerant. Traits RWC, RGR, CWC and RFWG in a single group (G2) is suitable genotypes 5, 15, 10, 7, 8, 6, 9, 14, 16, 12, 23, 20, 17, 21 and 13 and FWG, DWG and RT% in a single group (G3) is suitable genotypes 1, 19 and 25. Indices in G1 were positively correlated (an acute angle), the same conclusion was obtained for the G2 indices, while G1 was negatively correlated with G3 indices (an obtuse angle independence (right angle) and negative correlations (obtuse angle were observed between G1 with G2 and G2 with G3 *in vitro* indices, respectively).

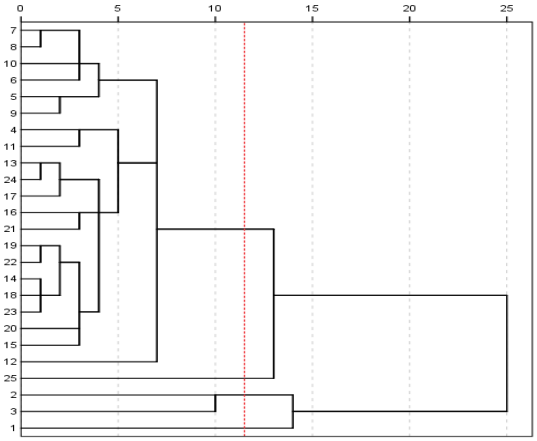

Fig.2. Dendrogram resulting from cluster analysis and discriminant function analysis of 25 genotypes based on measured characteristics of callus from mature embryos in stress condition.

Similarly Zouzou et al, (2008) in cotton observed that callus percentage was positively correlated with dry weight of callus. Callus growth rate exhibited no significant correlation with callus relative growth rate. A significant correlation was not found among drought tolerance indices (INTOL and RT%) with callus relative growth, callus relative growth rate and callus growth rate. Similar results were reported by Arzani et al, (1999). In contrast, Birsin et al, (2004) reported that negative correlation coefficient was observed among percentage of callus induction with callus weight and culture efficacy are negatively, also between regeneration percentage and number of regenerated plants.

In conclusion, the findings indicated that high variation in callus induction ability in durum wheat genotypes. The genotypes significantly responded well to *in vitro* culture based on the studied traits. The results verified the importance of durum wheat breeding lines in *in vitro* selection program for drought stress.

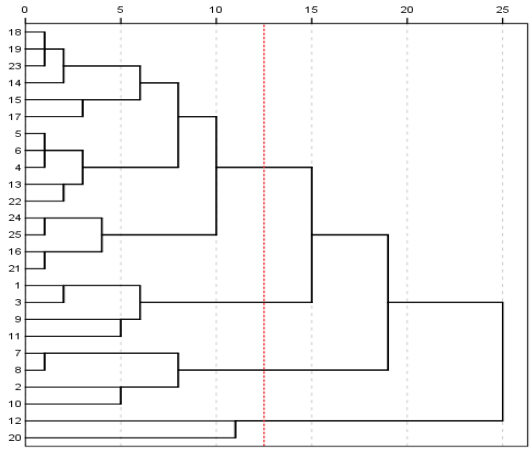

Fig.3.Dendrogram resulting from cluster analysis and discriminate function analysis of 25 genotypes based on measured characteristics of callus from mature embryos under non-stress conditions.

Table 6. Characteristics of the group from cluster analysis in tissue culture under stress and non-stress conditions

Group	Stress						Non-stress			
	CGR	RFWG	RGR	RWC	DWG	FWG	CWC	PCI	DC	CGR
I	0.13	0.85	0.058	92.58	0.18	1.06	1127	98.33	0.85	12.89
II	0.07	0.56	0.065	94.88	0.69	1.41	631.6	97.49	1.11	13.05
III	0.11	0.70	0.054	92.93	0.095	1.09	1344	92.22	1.06	12.88
IV	0.077	0.56	0.065	94.08	0.95	2.16	721.6	93.33	1.46	12.16

Table7. Correlation coefficients between the studied traits under stress and non-stress conditions

	Stress							Non-stress				
	CGR	RFWG	RGR	RWC	DW	FW	CWC	RT%	INTOL	DC	PCI	CGR1
CGR	1											
RFWG	0. 218	1										
RGR	0. 253	0.977**	1									
RWC	0.305	0.836**	0.930**	1								
DW	-0.191	-0.968**	-0.950**	-0.796**	1							
FW	-0.049	-0.624**	-0.559**	-0.331	0.771**	1						
CWC	0.254	0.909**	0.942**	0.893**	-0.916**	-0.674**	1					
RT%	0.257	-0.047	-0.091	-0.142	0.036	-0.037	-0.086	1				
INTOL	-0.347	-0.551**	-0.476*	-0.355	0.423*	0.151	-0.404*	-0.445*	1			
DC	-0.096	-0.146	-0.040	0.110	0.144	0.215	-0.024	-0.265	0.268	1		
PCI	0.376	-0.221	-0.120	0.047	0.253	0.364	-0.103	-0.117	0.337	0.222	1	
CGR1	0.005	0.155	0.135	0.052	-0.214	-0.325	0.172	-0.114	-0.054	-0.004	-0.044	1

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

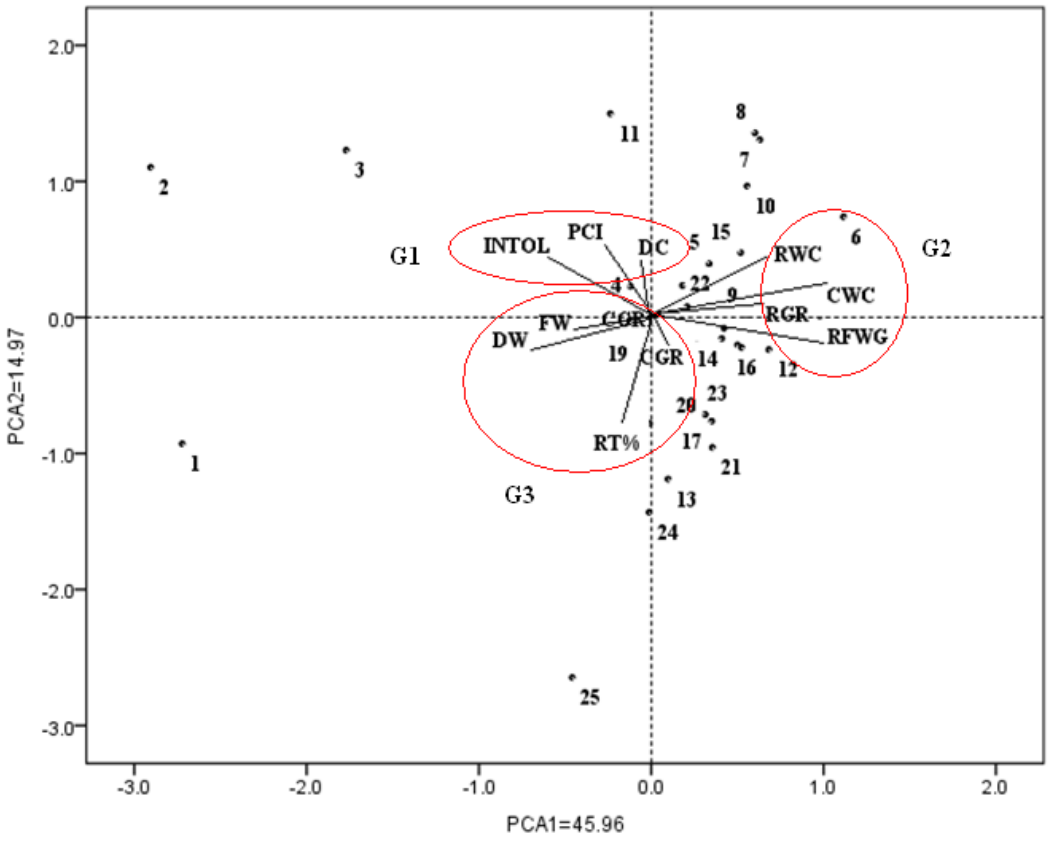

Figure4. Biplot analysis of in vitro indicators of drought tolerance using immature embryo culture and non-stress.

REFRENCES

Al-Khayri JM and Al-Bahrany A. 2004. Growth, water content and proline accumulation in drought stressed callus of date palm. Biol Plant 48(1): 105-108.

Abdelsamad A, El-Sayed OE, Ibrahim F. 2007. Development of drought tolerance haploid wheat using biochemical genetic markers on *in vitro* culture. J ApplSc Res 3(11): 1589-1599.

Arzani A and Shahram Mirodjagh S. 1999. Response of durum wheat cultivars to immature embryo culture, callus induction and *in vitro* salt stress. Pl Cell Tissue Organ Cul 58: 67-72.

Bommineni VR and Jauhar PP. 1996. Regeneration of plantlets through isolated scutellum culture of durum wheat. Pl Sc 116: 197-203.

Birsin MA and Ozgen M. 2004. Acomparison of callus induction and plant regeneration from different embryo explants of triticale (X *Triticosecale* Wittmack). Cellular and Molecular Biol Lett 9: 353-361.

Chen JJ, Yue RQ, Xu HX, Chen XJ. 2006. Study on plant regeneration of wheat mature embryos under endosperm supported culture. Agricul Sc China 5(8): 572-578.

Compton ME. 1994. Stutistical methods suitable for the analysis of plant tissue culture data. Plant Cell Tiss Org Cult 37: 217-242.

Corchete P and Guerra H. 1986. Effect of NaCL and PEG on solute content and glycosidase activities during germination of lentil seeds. Pl Cell Environ 9: 589-593.

Errabi T, Gandonou CB, Essalmani M, Abrini J, Idaomar M, Skali-Senhagi N. 2006. Growth, praline and ion accumulation in sugarcane callus caltures under drought-induced osmotic stress and its subsequent relief. Af J Biotech 5(16): 1488-1493.

El-Aref HM. 2002. In vitro selection of salt tolerant tomato plants and the changes in gene expression under salinity stress. J AgriculSc 33 (1): 23-46.

El-Shafey NM, Raifa AH, Mahmoud MAG, El-Sheihy O. 2009. Pre-exposure to gamma rays alleviates the harmful effect of drought on the embryo-derived rice calli. Aus J Crop Sc 3(5): 268-277.

Grigoryeva LP, Shletser IA. 2006. Screening wheat cultivars for morphogenesis ability in

immature embryo culture in vitro. Biologia 3(41):64–66.

He S, Han Y, Wang Y, Zhai H, Liu Q. 2009. In vitro selection and identification of sweet potato (*Ipomoea batatas*(L) Lam.) plants tolerant to NaCl. Plant Cell Tissue Organ: Cult 96: 69-74.

Jain SM. 2001. Tissue culture-derived variation in crop improvement. Euphytica, 118: 153-166.

Kaufmann MR and Eckard AN. 1971. Evaluation of water stress control with PEG by analysis of guttation. Pl Physiol 47: 453-458.

Khodadadi M, Fotokian MH, Miransari M. 2011. Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. Aus J Crop Sc 5(1): 17-24.

Lawlor DW. 1970. Absorption of PEG by plants and their effects on plant growth. New Phytologist 69: 501-513.

Liu T, Nada K, Handa C, Kitashiba H, Peny Wen X, Miny PX, Moriguchi T. 2006. Polyamine biosynthesis of apple callus under salt stress: Importance of arginine decarboxylase pathway in sress response. J Exp Bot 57: 2589-2599.

Maruyama H, Koyama R, Oi T, Yagi M, Takeda M, Kanechi M, Inagaki N, Uno Y. 2008. In vitro evaluation of osmotic stress tolerance using a novel root recovery assay. Plant Cell Tissue Organ Cult 95: 101-106.

Murashinge T Skooge F. 1962. Arevised medium for rapid growth and bioussays with tbacco tissue cultures. Pl Physiol 15: 473-497.

Nachit M, Picard E, Monneveux P, Labhilili M, Baum M, Rivoal R. 1998. An international durum wheat improvement programme for the Mediterranean basin. Cahiers-Agricultures 7: 510-515.

Ozgen MT, Ozcan S, Sancak C. 1996. Callus induction and plant regeneration from emmature and mature embryo of winter durum wheat genotypes. Pl Breed 15: 455-458.

Purohit M, Srivastava S, Srivastava PS, In: Srivastava (ed.) PS. 1998. Plant Tissue Culture and Molecular Biology: Application and Prospects. Narosa Publishing House, New Delhi, 554.

Rai MK, Kalia RK, Singh R, Gangola MP, Dhawan AK. 2011. Developing stress tolerant plants through in vitro selection-an overview of the recent progress.Environ Exp Bot 71(1): 89 - 98.

Ruf RH, Eckard ER, Gifford RD. 1967. Compounds of osmotic adjustment of plants to rapid changes in root medium osmotic pressure. Soil Sc104: 159-162.

Shah MM, Khalid Q, Khan UW, Shah SAH, Shah SH, Hassan A, Pervez A. 2009. Variation in genotypic responses and biochemical analysis of callus induction in cultivated wheat. Genetics and molecular research 8(3): 783-793.

Srinivasan T, Kumar KRR, Kirti PB. 2010. Establishment of efficient and rapid regeneration system for some diploid wild species of Arachis. Pl Cell Tissue Organ Cult 101: 303-309.

Suleman P, Al-Musallam A, Menzes CA. 2001. The effect of solute potential and water stress on black scorch caused by *Chalar aparadoxa* and *Chalara radicicola* on date palms. Pl Dis 85: 80–83.

Yan W, Kang MS. 2003. *Biplot* Analysis: A graphical Tool for Breeders, Geneticists and Agronomist, CRC Press, Boca Raton, Florida pp: 313.

Zouzou M, Kouakou TH, Koné M, Amani NG, Kouadio YJ. 2008. Effect of genotype, explants, growth regulators and sugars on callus induction in cotton (Gossypium hirsutum L.). Aus J Crop Sc 2(1): 1-9.