International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.9, pp 318-333, 2015

In vitro Selection for Drought Tolerance in Wheat (*Triticum aestivum* L.).

Hend M. Mandour[1]*, S.S.A.Soliman[2], M.S. Abd El-Hady[1], A.A.Mahmoud[2], and Hoda.M.H.El-Naggar[1]

[1]Botany Dept., Agric., Biol., Division, NRC, Dokki, Giza, Egypt.
[2]Genetics Dept., Fac., Agric., Zagazig Univ., Egypt.

Abstract: This study aims to detection the response of five wheat genotypes and their 10 F1 hybrids for embryo culture, evaluating the genetic response of the induced callus for drought tolerance and plant regeneration and determining the gene action and genetic control of three important criteria, i.e. callus induction frequencey, callus primary fresh weight and plant regeneration frequencey. The results revealed that there were highly significant differences ($P<0.01$) among the genotypes for callus induction, drought stress and plant regeneration criteria, indicating the presence of genetic variation, different responses of wheat genotypes under study to callus induction and *in vitro* drought stress, possible selection for callus induction and drought tolerance criteria at *in vitro* level using mature embryos.M2 medium gave the highest percentage of callus induction, variety Gemmeiza-10 (p3) showed maximum values of callus induction frequencey (CIF%) and callus relative fresh weight growth (CRFWG),while Misr-1(p4) and Giza-168 (p1) showed the best performance towards callus primary fresh weight (CPFW) and callus relative growth rate (RGR) ,respectively. Regarding drought stress and plant regeneration criteria, variety, Giza-168 (p1) gave the best performance towards all characters except INTOL and (CGI), where Gemmeiza-10 (p3) and Sids-13 (p2) were the best towards these criteria. In contrast variety Misr-1 (p4) gave the least performance towards all characters. Fluctuation behavior of F_1 was observed for almost crosses .Diallel analysis revealed that genotype Line-24 (P_5) had most dominant genes for callus induction frequency and plant regeneration frequency, while Giza-168(P_1) had most dominant genes for callus primary fresh weight. Misr-1(P_4), Line-24 (p_5) and Giza-168(P_1) had most recessive genes for callus induction frequency, callus primary fresh and plant regeneration frequency.

Key words: Genetic analysis, In vitro selection, Embryo culture, Wheat, Drought, Diallel analysis.

Introduction

Wheat (*Triticum aestivum* L.) belongs to grass family poaceau .It is self-pollinated annual plant and is the most widely grown cereal crop [1].Wheat is not only a main crop for more than one third of the world population [2], but also the most abundant sources of energy and nourishment for mankind [3], as well as an important staple food crop, dominant grain of world commerce.

Egypt, as a developing country has less share in global wheat production. Its consumption is increasing

day by day due to ever increasing population**.** In Egypt, wheat is the most important cereal crop covers 3.4 million feds (about 48% of cultivated area) with total annual production 9.5 million tons (1.3% of total production) [4]. In the same time, Egypt imported 7.7 million tons or about 81% percent of production to face the Egyptian local consumption of wheat [5]. Recently there is an increase in Egyptian wheat production but not sufficient to meet the demands of the Egyptian population [6]**.**

Abiotic stress is a major limiting factor in agricultural crop production in many countries and the main abiotic stresses of economic importance include drought [7]. Drought is the commonest, most serious threat and significant constraints to agricultural production, seriously affecting crop growth and yield quantity and quality [8]**;** [9]**;** [10]**;** [11] and [12].

Improvement of productivity under drought stress conditions is one of the most important breeding objectives in wheat. Improved yields of wheat depend on many factors, among which one of the most important factors is tolerance to environmental stress, particularly to water stress.

Drought tolerance is now considered by both breeders and molecular biologists to be a valid breeding target. Breeding for drought tolerance by selecting for grain yield only is difficult because the heritability of yield under drought conditions is low, or due to small genotypic variance, the large variances in the genotype-environment interaction and our poor understanding of the physiological basis of yield in water-limited conditions [13]**;** [14] and [15].Thus under stressful environments ,yield is not always the most suitable or easiest selection trait. Therefore, we need to deploy the biotechnological tools for addressing the critical problems of crop improvement for sustainable agriculture.

Tissue culture creates a wide range of genetic variation in plant species, which can be combined in plant breeding programs. In addition by *in vitro* selection, mutants with useful agronomic traits, such as disease resistance, salt or water stress tolerance can be obtained in a short duration [16]**;** [17] and [18].

Embryos are the most frequently used explants for the initiation of wheat tissue culture for either callus culture or direct DNA delivery techniques [19]**;** [20] and [21].Both mature and immature embryos have been used extensively in tissue culture protocols, but mature embryos were found to be a better choice in comparison to immature embryos [22].

Plants tolerant to drought stresses can be acquired by applying selective agents such as Polyethylene glycol (PEG) in the culture media. Poly ethylene glycol (PEG) has been used for many years to stimulate water stress in Plants [23]**;** [24] and [25].It can also used to stimulate water stress without the risk of being taken up by the plants [24]**.**Cells tolerant to selective agent are selected and subsequently regenerated into plantlets due to their 'totipotency'. The selected somaclones for desired characters may be genetically stable and helpful for crop improvement [26]**.**

Therefore, the present investigation aims to : (i) detection the response of five wheat genotypes and their 10 F1 hybrids for callus induction using embryo culture technique , (ii) screening the obtained callus for drought tolerance using different concentrations of PEG , (iii) evaluating the genetic response of the tolerant calli to plant regeneration and (iv) determining the gene action and genetic control of three important criteria ,i.e.callus induction frequencey, callus primary fresh weight and plant regeneration frequencey.

Materials and Methods

The present investigation was carried out at the Experimental Farm and Tissue Culture Lab of Genetics department, Faculty of Agricultural, Zagazig University, Egypt, during the period 2012-2015. Fifteen wheat genotypes (*Triticum aestivum* L.) were used in this investigation, i.e.5 parents namely, Giza-168 (P1), Sids-13 (P2), Gemmeiza-10 (P3), Misr-1 (P4), Line-24 (P5) and their F_1 hybrids that introduced to this experiment from diallel crosses without reciprocal. The origin and pedigree of the five cultivars are presented in (Table1)**.**

In 2012 winter season, the five wheat genotypes were sown in two successive sowing dates with two weeks interval in order to synchronize the flowering time for crossing purposes. Hand emasculation and pollination were performed so as to produce enough grains of all possible F_1 cross combinations of five parents, i.e.full diallel without reciprocal.

Table 1. Pedigree, breeding history and origin of the five parental genotypes used in this investigation.

Entry	Name	pedigree	Drought tolerance	Origin
1	**Giza 168 (P1)**	MRL/BUC/SER CM93046-8M-0Y-0M-2Y-0B	Tolerant	Egypt
2	**Sids 13 (P2)**	KAUZ "S"//TSI/SNB"S". ICW94-0375-4AP-2AP-030AP -0APS-3AP-0APS-050AP-0AP-0SD	Tolerant	Egypt
3	**Gemmeiza 10 (P3)**	MAYA74"S"/0N//160-147/3/BB/GLL/4/CHAT"S"/5/ CROW"S". GM5820- 3GM-1GM-2GM-0GM.	Mod. Tolerant	Egypt
4	**Misr 1 (P4)**	OASIS/SKAUZ//4*BCN/3/2*PASTOR.CCMSSOY O1881T-050M-030Y-O3OM-030WGY-33M-0Y-0S	Sensitive	Egypt
5	**Line 24 (P5)**	THB/KEA/PF85455/4/RIVADENEIRA/5	Sensitive	Mexcio

Experimental procedures

This part was classified into 3 experiments on the basis of the objective .The first experiment aimed to determine the optimum hormone balance for callus induction from wheat mature embryos by using three different media compositions and also to determine the genetic response of the fifteen wheat genotypes .i.e., 5 parents and their 10 F_1 hybrids to callus induction, the second experiment aimed to evaluate the response of the obtained calli for drought tolerance by using selective media (M2 medim+ different concentrations of PEG) and the third experiment aimed to determine the genetic response of the tolerant calli to plant regeneration. As well as determination of some genetic parameters.The experimental work was conducted as follow:

(i) Callus induction

Grain surface sterilization and imbibition

Mature wheat grains were surface-sterilized with 70 % (vol/vol) ethanol for one minute and then rinsed with sterile distilled water for five times. After that, grains were treated with 30 % (vol/vol) NaOCl for twenty minutes, followed by five times washing with sterile distilled water. Grains were imbibed in sterile distilled water over night at room temperature. After imbibition, grains were again sterilized with 70 % (vol/vol) ethanol for one minute and five times washed with sterile distilled water [27].

Isolation of mature embryos from wheat grains

Mature embryos were aseptically removed from sterilized grains using blade and forceps. The instruments were sterilized at 250˚C in oven. Mature embryo were isolated and cultured with scutellum in contact with the medium to start initiation of callus formation.

For callus induction, the effect of three callus induction media was compared.

Culture media used for callus induction were:

1. MS+2mg/l 2,4D.
2. MS+2mg/l 2,4D+300mg/l casein hydrolysate.
3. MS+2mg/l 2,4D+4mg/l Silver nitrate.

Aseptically mature embryos were transfered to glass jars containing 40 ml of solidified basal MS medium [28] supplemented with 30 g/L sucrose and was adjusted to PH 5.8, solidified with 2.5g/L phytagel before autoclaving at 121˚C and 1.2 kg/cm² for 20 min .All the operations and inoculation were performed under aseptic conditions in a laminar airflow cabinet. Mature embryos in contact with medium were incubated for 2 weeks dark incubation at 25 ˚C. After establishment, calli were sub-cultured on the same callus induction medium at 2 weeks intervals until enough callus material was obtained to initiate the drought stress stage.

Criteria measured in callus induction stage

After 14 days of embryo culture, callus primary fresh weights (CPFW) and callus induction frequency (CIF%) were measured and after 28 days of embryo culture callus growth rate (CGR), callus relative fresh weight growth (CRFWG)**,** callus relative growth rate (CRGR) were measured.

Callus Induction Frequencey % (CIF %):

CIF was evaluated 2 weeks after embryo culture as: (number of embryos produced callus)/(number of embryos plated in Petri dishes). [20].

Callus Primary Fresh Weight (CPFW):

CPFW was evaluated by measuring fresh weight of callus 14 days after callus induction [29].

Data obtained for callus induction frequencey and callus primary fresh weight on M2 media were analyzed with diallel analysis and their genetic components variation were estimated.

Relative Fresh Weight Growth (RFWG):

RFWG = $[(W_2-W_1)]/W_1$ [28] where W1 and W2 are the initial weight of callus before and after four weeks, respectively.

Callus Relative Growth Rate (CRGR):

RGR = $[L_nW_2-L_nW_1]$ /GP [30], where W_1 and W_2 are the initial and final weight of callus and GP is the growth period, respectively.

(ii) *In vitro* experiment of drought tolerance

After four weeks of incubation on callus multiplication media, the obtained calli were separately subcultured on MS medium supplemented with different concentrations of polyethylene glycol (0, 5, 10, 15 and 20%) for the evaluation of drought tolerance. Before transferring to drought medium, fresh weight of calli were measured and after 14 days of transferring onto PEG-medium .RFWG, RGR, callus growth index (CGI), relative tolerance (RT%) and *in vitro* tolerance (IT) were calculated as follows:

In this stage CGR, RFWG and RGR were calculated the same as in callus induction stage

***In vitro* tolerance (IT):** was calculated as:

IT= RGR treatment / RGR control, [31]

Callus growth index (CGI) or increasing value of callus fresh weight was calculated as:

$$\mathbf{CGI} = \frac{RFWGstress - \mathbf{RFWGcontrol}}{\mathbf{2}}$$

Where, RFWG stress= $(W_1-W_0)/W_0$,
RFWG control= $(W_1-W_0)/W_0$, [32],W_0 is the weight of callus before treatment and W1 the final weight of callus after14 days of treatment and control for RFWG stress and RFWGcontrol, respectively.

Percetage of relative tolerance (Rt%): Rt% was calculated as:

Rt% = [a/b] × 100, [33].
Where a = fresh weight under stress after 14 days and b = fresh weight after 14 days under control

(iii) Plant Regeneration

The obtained calli were shifted to regeneration medium with 30 g/l sucrose, 2.5 g/l phytagel and plant growth regulators (2 mg/l TDZ) .The calli were incubated at 25±2°C temperature with 16 h light and 8 h dark photoperiod. The regeneration medium was refreshed every 15-21 days. Plant regeneration frequencey was calculated as follows:

(PRF%) = (number of regenerated calli / total number of calli) x 100.

Statistical Analysis:

1. All data were further analysed with ANOVA.
2. Means and their least significant differences for each studied character were calculated.
3. Diallel F_1 analysis was applied to estimate the genetic parameters for (CIF%) ,(CPFW) and (PRF%) as described by [33] and [34].The data were also subjected to Wr-Vr graph analysis following [35].The illustration of [36] was adopted to estimate the components of genetic variance and heritability in broad and narrow sense.

Results and Discussion

Callus induction

Effect of interaction between hormone balance and genotypes on callus induction .

Callus induction and growth from mature embryos was visible in all media and in all genotypes after 6-10 days in the dark and lasted up to four weeks. The induced calli were soft, white and friable.

The analysis of variance revealed the presence of highly significant differences ($P<0.01$) among the genotypes for (CIF%), (CPFW) , (CRFWG) and (CRGR), indicating the presence of genetic variation, different responses of genotypes to callus induction and possible selection of callus induction in bread wheat using mature embryos (Table 2). The differences between the three media were significant except in (CIF%) indicating that this criterion is genotype dependent. The media × genotype (M × G) interaction was significant in CIF, CPFW and CRFWG except for CRGR displaying different responses of characters to media compositions, while CRGR was stable and independent of media compositions. Similar findings were found by [37].Genotype effects on callusing ability from wheat mature embryo cultures were reported in bread wheat by [38] and [39].

Table 2. Analysis of variance for callus induction criteria using mature embryos of wheat genotypes under study.

SOV		M.S of the studied characters			
	df	**CIF**	**CPFW**	**CRGR**	**CRFWG**
Replication	2	726.8**	160.8	0.00012	0.00145
Genotypes	14	20738**	3086.5**	0.00043**	0.034**
Media	2	47.32	5671.7**	0.0027**	5.124**
GxM	28	556**	1028.8**	0.000026	0.04**
Error	88	175.3	263.8	0.0000188	0.006

**,Significant at 0.01

Mean values of callus induction criteria.

Mean values of the three media compositions (Table 3) indicated that the best media was M2 which gave the highest callus induction frequency (85.5%) followed by M1 (85%) and M3 (81.6%).

Mean values of (CIF%), (CPFW) , (CRFWG) and (CRGR) are present in (Table 4). Gemmeiza-10 (p3) possessed higher values for (CIF) and (CRFWG), while Misr1 (p4) and Giza-168 (p1) possessed higher value for,(CPFW) and (CRGR) respectively. In contrast, Genotype Line-24 (p5) showed lower values for (CIF%) ,(CPFW) and (CRFWG),while genotype Misr-1 (p4) showed lower values for (CRGR) .Fluctuation behavior of F_1 was observed for almost crosses of the four criteria. Hybrid Gemmeiza-10xMisr-1 (p3xp4) possessed higher values for (CPFW) and (CRFWG), while Misr1xLine-24 (p4xp5) and Giza-168xSids-13 (p1xp2) possessed higher value for,(CIF%) and (CRGR) respectively. In contrast, Hybrid Giza-168xLine-24 (p1xp5) showed lower values for (CIF%) ,(CPFW) and (CRFWG),while Hybrid Sids-13xMisr-1 (p2xp4) showed the lowest values for (CRGR). Various amounts of CIF%, CPFW, CRFWG and CRGR in different genotypes exhibited that the characters measured were genotype dependent .Similar results were found by [39]**;** [40]**;** [41]**;** [42]**;** [43] and [44] who exhibited significant differences between wheat cultivars for callus induction response and revealed that callus induction was genotype-dependent. In general, callus induction used as an efficient character for assessment of culture responses from mature embryo in wheat genotypes.

Table 3.Mean performance of the five parental genotypes and their 10 F1 hybrids on M1, M2 and M3 media to Callus induction

Genotypes	M1	M2	M3	Mean
P1 (Giza-168)	80	90	70	80
P2 (Sids-13)	91.7	94	91.7	92.5
P3 (Gemmeiza-10)	100	100	100	100
P4 (Misr-1)	68.4	84	93.4	81.9
P5 (Line-24)	75	34	34	47.7
P1×P2	75	70	80	75
P1×P3	70	80	100	83.4
P1×P4	100	80	100	93.4
P1×P5	50	67	75	64.1
P2×P3	90	82	90	87.3
P2×P4	91.7	100	38.4	91.7
P2×P5	91.7	100	75	88.9
P3×P4	100	100	75	91.7
P3×P5	100	100	56.7	85.6
P4×P5	91.7	100	100	97.2
Mean	85	85.5	81.6	
LSD 0.05	48.7	22.3	27.3	
LSD 0.01	68.03	30.96	37.8	

Table 4. Mean performance of wheat genotypes under study to callus induction criteria over all media .

Genotypes	CIF(%)	CPFW(mg)	CRFWG	CRGR
P1	80	44	0.038	0.62
P2	92.5	94.5	0.04	0.57
P3	100	92.2	0.043	0.34
P4	81.9	110	0.041	-0.037
P5	47.7	40	0.037	0.11
P1×P2	75	75.6	0.042	0.87
P1×P3	83.4	84.5	0.038	0.64
P1×P4	93.4	82.5	0.042	0.37
P1×P5	64.1	65.7	0.033	0.46
P2×P3	87.3	82.2	0.04	0.5
P2×P4	91.7	111	0.04	-0.06
P2×P5	88.9	80	0.04	0.38
P3×P4	91.7	102.2	0.047	0.17
P3×P5	85.6	72.2	0.042	0.22
P4×P5	97.2	97.8	0.034	0.02
LSD 0.05	23.2	28.5	0.0075	0.14
LSD 0.01	32.2	39.6	0.01	0.19

Drought tolerance

Analysis of variance (Table 5) revealed highly significant differences ($P < 0.01$) between genotypes for CGI, CRGR, RT%,CRFWG and INTOL and different drought levels indicating the presence of genetic variation, different responses of genotypes to different drought intensities and *in vitro* selection of drought-tolerant genotypes. The stress × genotype (G × S) interaction was significant for CGI, RT%, RGR, and INTOL except for CCRFWG displaying different responses of characters to different levels of drought (PEG), while CRGR was stable and independent of different drought levels. Similar findings were reported by [45] and [46]**.** [18] reported a significance difference between Maize genotypes for the same characteristics.

Table 5. Analysis of variance of evaluated traits on mature embryo calli under drought stress conditions.

SOV	M.S of the studied characters					
	df	**CGI**	**RFWG**	**RGR**	**INTOL**	**RT%**
Replication	2	0.02	0.0045	0.0038	0.0065	9.45
Genotypes(A)	14	**0.019****	**0.45** **	**0.0022****	**0.87****	8334.7**
PEG level(B)	4	**3.42****	**14.5** **	**0.065****	**30****	176694.4**
AxB	56	**0.025****	**0.0071**	**0.00014****	**0.2****	4777.6**
Error	148	0.0027	0.012	**0.000067**	0.06	306

****Significant at 0.01 .**

Mean values of *in vitro* drought tolerance criteria

Mean values of (CGI), (RFWG), (RGR) , (INTOL) and (RT%) are present in (Table 6) . Giza-168 (p1) possessed higher value for (RFWG), (RGR) and (RT), while Sids-13 (p2) and Gemmeiza-10 (p3) possessed higher value for, (CGI) and (INTOL) ,respectively. In contrast, variety Misr-1 showed a lower values for the five studied criteria. Fluctuation behavior of F_1 was observed for almost crosses of five criteria. Hybrid Giza-168xSids-13 (p1xp2) showed the higher values for the five studied criteria. In contrast the least values were attributed to hybrids Sids-13xMisr-1 (p2xp4) and Misr-1xLine-24 (p4xp5).

Table 6. Mean performance of wheat genotypes under study to drought stress criteria

Genotypes	CGI	RFWG	RGR	INTOL	RT%
P1	-0.28	0.37	0.056	0.43	25
P2	-0.13	0.34	0.046	0.28	34
P3	-0.32	0.2	0.0076	0.46	21
P4	-0.38	-0.02	0.0032	-0.26	-52.5
P5	-0.32	0.07	0.0037	-0.13	6.6
P1×P2	-0.21	0.52	0.086	0.53	70
P1×P3	-0.27	0.38	0.06	0.36	37.1
P1×P4	-0.28	0.22	0.0033	0.2	17.6
P1×P5	-0.3	0.27	0.0037	0.28	31
P2×P3	-0.34	0.3	0.04	0.24	20.3
P2×P4	-0.27	-0.036	-0.0032	-0.033	15.4
P2×P5	-0.35	0.23	0.0032	0.19	23.3
P3×P4	-0.39	0.13	-0.06	-0.07	15.7
P3×P5	-0.38	0.1	0.0004	0.026	19.5
P4×P5	-0.4	0.036	-0.23	-0.24	13.4
LSD 0.05	0.027	0.19	**0.014**	0.429	30.6
LSD 0.01	0.0.038	0.26	**0.019**	0.59	42.5

Various amount of RFWG, CGI, CRGR, IT and RT% in different genotypes exhibited that the measured criteria were genotype dependent. Similar results were found by [42] who reported that, response in tissue culture such as callus intiation is genotype dependent. Values of CGI,RFWG, RGR, INTOL and RT were decreased with increasing drought stress level .Likewise**,** [18] reported that these criteria reduced while studying somaclones achieved from Maize calli under drought stress with PEG. [47] and [48] reported that increasing the levels of PEG(0-30%) reduced CRGR, PCWC and INTOL in date palm (*Phoenix dactylifera* L.). [49] and [50] also reported the same results in bread and durum wheat and [51] in soybean, respectively, which is in consistent with the results of this experiment.

Plant regeneration

Effect of interaction between PEG concentrations and genotypes on plant regeneration.

The analysis of variance over four drought stress levels (0,5,10 and 15%) for genotypes under study to plant regeneration revealed the presence of highly significant differences among the genotypes for plant

regeneration frequency indicating the presence of genetic difference between them (Table 7). Similar findings were found by [20] who reported that there were significant differences between wheat cultivars for their regeneration potential from immature embryo.

Table 7. Analysis of variance for plant regeneration frequency using mature embryos of wheat genotypes under study

SOV	df	Mean squares
Replication	**2**	**1.25**
Genotypes(A)	**14**	**134.4****
PEG level(B)	**4**	**2084.4****
AxB	**56**	**15.8****
Error	**148**	**6.6**

**,*Significant at 0.01

Mean values of plant regeneration frequencey (PRF %) are present in (Table 8). Regeneration was obtained in all genotypes under the first three PEG concentrations, and in most genotypes under 15% PEG, but was completely absent under 20% PEG .

Table 8. Mean performance of wheat genotypes under study to plant regeneration under drought stress conditions

Genotypes	0	5	10	15
P1	61.7	50	43.4	33
P2	53.4	43.4	33	25
P3	50	28.4	23.4	15
P4	40	20	4	0
P5	45	25	22.4	10
P1×P2	60	48.5	42	33
P1×P3	55	28.4	34.7	20
P1×P4	51.7	25	20	7
P1×P5	50	26.7	21.7	20
P2×P3	55	42	30	17
P2×P4	48	35	25	0
P2×P5	48.03	37	34	15
P3×P4	45	27	20	0
P3×P5	50	32	25	0
P4×P5	42	24	19	5
LSD 0.05	7.1	7.4	5.6	5.4
LSD 0.01	10.1	10.3	7.8	7.5

Genotype Giza-168 (p1) possessed higher value for (PRF %) under all PEG concentrations. In contrast, Misr-1 (p4) possessed lower values for the same criterion under all PEG concentrations. Fluctuation behavior of F_1 was observed for almost crosses. Hybrid Giza-168xSids-13 (p1xp2) possessed the higher values of (PRF %) under all PEG concentrations. In contrast, hybrid Misr-1 xLine-24 (p4xp5) possessed lower values for (PRF%) under control, 5% and 10% PEG, while under 15% PEG ,hybrids Sids-13xMisr-1 (p2xp4),Gemmeiza-10xMisr-1 (p3xp4) and Gemeiza-10xLine-24 (p3xp5) possessed lower values for (PRF%). [38] and [52] investigated that the percentage of callus induction and plant regeneration in tissue culture of wheat were usually affected by the explants source, effect of genotype and effect of medium composition.

Diallel analysis

Highly significant differences of considerable amount of genotypic variability for all characters under study were found (Table 9). In fact, the development of any plant breeding program is dependent upon the existence of genetic variation, the efficiency of selection and expression of heterosis and largely dependent upon the magnitude of genetic variation present in plant population, [53] and [54].

Table 9. Analysis of variance, t^2 values, regression coefficient and their test of significant for the studied characters.

SOV	df	M.S of the studied characters		
		CIF	CPFW	PRF(%)
Replication	2	860.7**	59.85**	11.7
Treatment	14	1253.4**	2467.6**	112.2**
Error	28	162.2	238.6	15.2
t2		0.0038	0.142	0.006
b		0.989	1.12	0.927
S.E(b)		0.16	0.257	0.26
H0:b=0		6.18**	4.35**	3.56**
H1:b=1		0.07	0.46	0.28

**, Significant at 0.01

Mean values and gene action of callus induction frequency, callus primary fresh weight-on M2 media- and plant regeneration frequencey on control media

Mean values of (CIF%), (CPFW) -on M2 media- and (PRF%) on control media are present in (Table 10) . Gemmeiza-10 (p3) , Misr-1(p4) and Giza-168 (p1) possessed higher values for (CIF%), (CPFW) and (PRF%), respectively. In contrast, genotypes, Line-24 (p5) and Misr-1(p4) showed lower values for the studied criteria. Fluctuation behavior of F_1 was observed for almost crosses.

Estimation of heterosis over mid and better parent

Data presented in (Table 11) showed heterosis values over mid and better parent for the three studied characters. Concerning mid-parent heterosis, hybrid Misr-1xLine-24 (p4xp5) possessed the highest heterosis effects for callus induction frequencey* and callus primary fresh weight* ,while hybrid Giza-168xLine-24 (p1xp5) possessed the highest heterosis effects for plant regeneration frequencey** (*and** for positive and negative heterosis respectively). Concerning better parent heterosis, hybrid Giza-168xLine-24(p1xp5) possessed the highest heterosis effects for callus induction frequencey* and plant regeneration frequencey**, while hybrids Sids-13xGemmeiza-10 (p2xp3) possessed the highest heterosis effects for callus primary fresh weight** (*and** for positive and negative heterosis ,respectively).

Table 10. Mean values of Callus induction frequency (%), Callus fresh weight (mg) under M2 medium and Plant regeneration frequency (%) under control medium

Genotypes	Callus induction frequency %	Callus fresh weight (mg)	Plant regeneration frequency %
P1	50	**76.7**	61.7
P2	**93.3**	**103.3**	53.3
P3	**100**	**120**	50
P4	**83.3**	**143.3**	40
P5	**33.3**	**26.7**	45
P1×P2	**70**	**80**	60
P1×P3	**80**	**80**	55
P1×P4	80	**86.7**	**50**
P1×P5	100	**56.7**	51.7
P2×P3	**81.7**	**83.3**	55
P2×P4	**100**	**130**	48.3
P2×P5	**100**	**90**	48.3
P3×P4	**100**	**110**	45
P3×P5	**100**	**83.3**	50
P4×P5	**100**	**126.7**	41.7
LSD 0.05	**23.2**	**37.2**	**7.1**
LSD 0.01	**32.95**	**52.9**	**10.1**

Table (11): Heterosis values over mid-parent (MP%) and better parent (BP%) for 10 F_1 bread wheat hybrids for callus induction frequency,callus primary fresh weight (mg) under M2 medium and plant regeneration frequency under control medium.

plant regeneration frequency %		Callus primary fresh weight (mg)		Callus induction frequency (%)		Crosses
(BP%)	MP%)(	(BP%)	MP%)(	(BP%)	MP%)(	
-2.8	**4.4**	-22.6	-10.9	-24.97	-2.3	P1xp2
-10.9**	-1.5	-33.3	-18.5	-20	6.7	P1xp3
-16.2**	**1.7**	-39.5*	-21	-3.96	**19.94**	P1xp4
-19**	-6.3*	-25.7	10.1	**43.3**	59.95**	P1xp5
2.4	-1.7	-44.1*	-25.4	-18.3	-15.5	P2xp3
-9.4*	**3.5**	-9.3	**5.4**	7.2	**13.3**	P2xp4
-9.4*	-1.7	-12.9	38.5*	7.2	57.97**	P2xp5
-10*	**0**	-23.2	-16.5	0	**9.1**	P3xp4
0	5.3	-41.9*	**31.5**	0	49.9**	P3xp5
-7.3*	-1.9	-11.6	49.1**	20.1	71.5**	P4xp5
±3.18	±2.75	±16.7	±14.47	±20.34	±14.38	S.E

*,**Significant at 0.05 and 0.01,respectively .

The estimates of genetic components of variation and their proportions are presented in (Tables 12-13). The magnitude and high significance values of D and H_1 estimates indicate the importance of both additive and dominance of all studied criteria except (PRF%) which show an additive effect only. However, the relative magnitudes of both types of gene action varied from trait to trait (Table13). h_2 estimates indicate the heterozygous loci in the F_1 hybrids. All criteria possessed significantly of heterozygous loci, except callus fresh weight. The results showed the positive F values for callus induction frequencey and callus fresh weight, indicating that dominant genes are more frequent than recessive ones among parental genotypes. These results are in agreement with those reported by [55] in (*Brassica napus*) .

Table 12:Estimates of genetic components of variation in F_1 of diallel crosses for the studied callus induction frequency, callus fresh weight under M2 medium and plant regeneration frequency under control medium

Componets	Callus induction frequency %	Callus fresh weight (mg)	Plant regeneration frequency %
D±S.E(D)	**825.3** ± 70**	**1929.8** ±9.94**	**63.5**± 2.6**
F±S.E(F)	**744.6*±176.1**	**1008.7**± 24.8**	**-5.16± 4.8**
H1±S.E(H1)	***±189.71030.5**	**695.3**± 26.8**	**2.14± 5.2**
H2±S.E(H2)	**115.8±172.8**	**-122.6*± 24.4**	**0.36± 4.7**
h^2±S.E(h^2)	**6506.8**± 116.5**	**37.3±16.7**	**-12.8*±3.18**
E±S.E(E)	**11.5±28.7**	**75.6** 4.4**	**5**±0.8**

* and ** significant at 0.05 and at 0.01 probability levels respectively ; D, Additive effects; H1, overall dominance effect; H2, reflection of the proportion of genes with positive and negative effects; F, covariance of additive and dominance effects; h^2, dominance effects of all loci in heterozygous phase and E, environmental variance

Mean degree of dominance $(H_1/D)^{1/2}$ indicated the presence of over dominance for callus induction frequency ,while it is partial dominance for callus fresh weight and plant regeneration frequencey (Table 14). Estimates of proportion of positive and negative genes ($H_2/4H_1$) in parents ranged from 0.028for callus induction frequency to 0.044 for callus fresh weight. As this ratio is far from 0.25, hence positive and negative alleles are un symmetrically distributed in these characters. By comparing Wr +Vr values for each array with

the mean of the common parent, i.e. comparing (Wri +Vri) with Yri we can see the direction of dominance Table (14). If the correlation coefficient (r) between them is negative it means that parents containing most increasing genes have the lowest values of Wri +Vri and thus contain most dominant genes, and correlation will be positive if the case is reversed. Therefore, the results revealed that the direction of dominance was toward callus induction frequencey and callus fresh weight as a dominant and plant regeneration frequencey as a recessive.

High heritability estimates in broad-sense h^2 (bs) were obtained for the all studied characters indicated the little effects of environmental conditions for these traits. On the other hand, narrow-sense heritability was also high for the three studied characters suggesting that early generation selection for these characters should be effective.

Table 13: The proportion of genetic components, most dominant and recessive genotypes and heritability in broad and narrow sense for studied characters.

Parameters	CIF %	CPFW (mg)	PRF%
$(H1/D)^{1/2}$	1.11	0.6	0.18
$H_2 / 4 H_1$	0.028	0.044	0.042
Dom/Res proportion	2.35	2.54	0.63
r	-0.8	-0.44	0.2
r^2	0.64	0.19	0.1
h^2 (bs)	0.978	0.95	0.875
h^2 (ns)	0.924	0.917	0.873

Graphical analysis of Wr/Vr graph for studied criteria

The F_1 graphic analysis for the three studied characters are shown in figs (1:3). An inspection of Wr-Vr graphs showed that regression line cuts the Wr axis below the point of origin indicating over dominance for callus induction frequencey, while it cuts above the point of origin dominance for callus fresh weight and plant regeneration frequencey indicating partial dominance. These observations supported the previous results obtained from the estimation of $(H1/D)^{1/2}$ ratios and confirmed the importance of dominance gene effects in the inheritance of these characters**.**

The order of the array points along the regression line showed that genotype Line-24 (P_5) had most dominant genes for callus induction frequencey and plant regeneration frequencey, while Giza-168(P_1) had most dominant genes for callus primary fresh weight. Misr-1(P_4), Line-24 (p_5) and Giza-168(P_1) had most recessive genes for callus induction frequencey ,callus primary fresh and plant regeneration frequencey respectively.

Table(14):Values of Vr,Wr,Wr + Vrand Yr for callus induction frequency, callus primary fresh weight and plant regeneration frequency in the F_1 wheat generation

Genotypes	callus induction frequency				callus primary fresh				plant regeneration frequency			
	Wr	Vr	Vr+Wr	Yr	Wr	Vr	Vr+Wr	Yr	Wr	Vr	Vr+Wr	Yr
Giza-168 (P_1)	**240.2**	**152.2**	**392.4**	**50**	**486.94**	**129.86**	**616.8**	**76.3**	**35.4**	25.9	**61.3**	61.7
Sids-13 (P_2)	0.95	**168.7**	**169.65**	**93.3**	**526.05**	**413.46**	**939.51**	**103.3**	**38.5**	**24.4**	**62.9**	**53.3**
Gemmeiza -10 (P_3)	**12.4**	**110.4**	**122.8**	**100**	**582.28**	**337.1**	**919.38**	**120**	**31.5**	**17.5**	**49**	**50**
Misr-1 (P_4)	**62.6**	**102.4**	**165**	**83.3**	**1207.9**	**792.89**	**2000.8**	**143.3**	**39.1**	**22.8**	**61.9**	**40**
Line-24 (P_5)	**828**	**889.5**	**1717.5**	**33.3**	**841.48**	**573.89**	**1415.4**	**26.7**	**25.7**	**12.95**	**38.65**	**45**
Total	**1144.15**	**1423.5**	**2567.7**	**359.9**	**3644.65**	**2247**	**5891.7**	**469.6**	**170.2**	**103.6**	**273.75**	**250**
Mean	**228.8**	**284.7**	**513.53**	**71.98**	**728.93**	**449.4**	**1178.4**	**93.92**	**34.04**	**20.7**	**54.75**	**50**

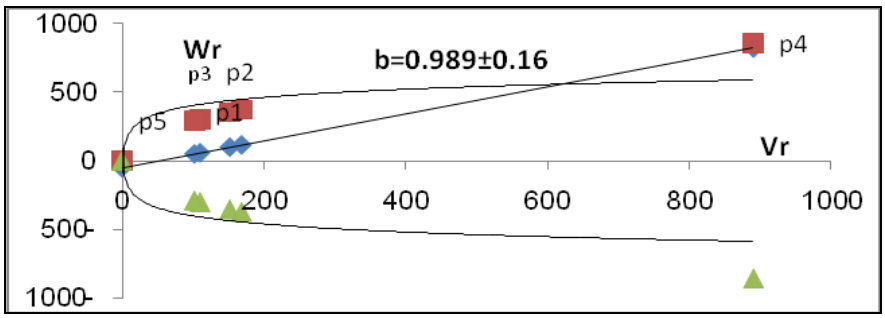

Fig.1:F_1 graphic analysis of callus induction frequencey under M2 medium.

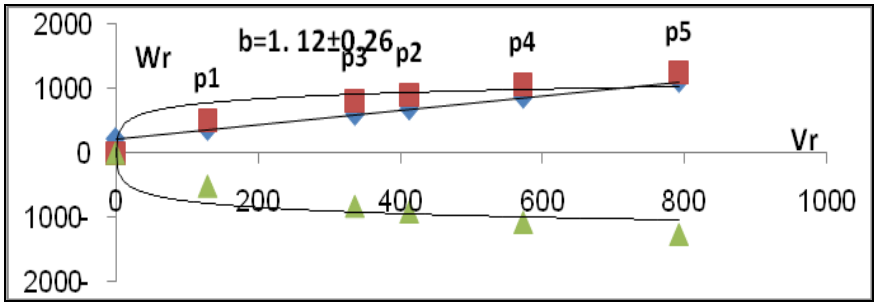

Fig.2:F_1 graphic analysis of callus primary fresh weight under M2 medium.

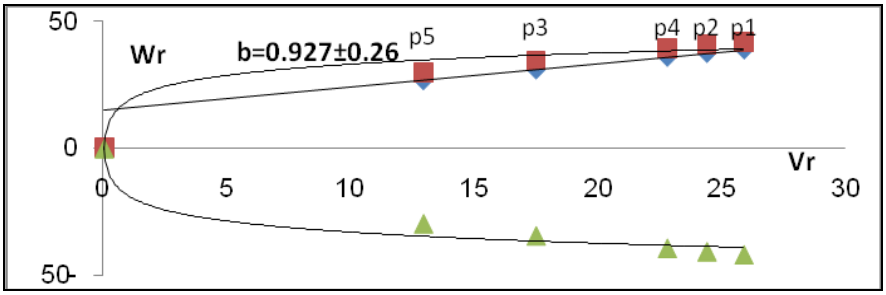

Fig. 3:F_1 graphic analysis plant regeneration frequencey (%) under control medium.

Conclusion

In conclusion, genotype was one of the main significant factors for successful callus induction frequencey from mature embryos of wheat .Callus induction ranged from 47.7 to 100% in different genotypes, therefore these genotypes had different response to the PEG concentration, hence callus induction is genotype dependent and can be considered as an index for *in vitro* screening for drought tolerant plants. On the other hand, the genotypes with the lowest callus relative growth under drought stress medium could be categorized as low-tolerant to drought at the cellular levels. In addition, mean values of drought criteria at different stress levels indicated that increasing PEG concentrations up to 10% reduced the *in vitro* parameters but higher levels tend towards stability and growth was almost stopped. The reason for this may be reduction of osmotic potential of the environment. Thus, it is obvious that *in vitro* selection can be used as an effective tool to screen a large number of genotypes to water deficit. More investigations such as field and hydroponic conditions studies are needed to corroborate this thought. However, it is suggested to breeders not generally select for specific traits to improve yield under drought principally because drought is unpredictable from year to year and this also means that the physiological responses to drought are also complex and unpredictable. It could also concluded that MS media supplemented with 2mg/l 2,4D and 300 mg/l casein hydrolysate is optimum to callus induction. Screening drought tolerant genotypes *in vitro* discriminated genotypes Giza-168, Sids-13, and their hybrid as the most drought tolerant genotypes, while genotype Misr-1 as the most sensitive to drought.Genotype Line-24 (P_5) had most dominant genes for callus induction frequencey and plant regeneration frequencey, while Giza-168(P_1) had most dominant genes for callus primary fresh weight. Misr-1(P_4), Line-24 (p_5) and Giza-168(P_1) had most recessive genes for callus induction frequencey, callus primary fresh and plant regeneration frequencey.

References

1. Zhou,H;J.D., Berg ;S.E.Blank; C.A.Chay; G.Chen; S.R.Eskelsen; J.E.Fry; S.Hoi; T.Hu; P.J.Isakson; M.B.Lawton; S.G.Metz; C.B.Rempel; D.K.Ryerson; A.P.Sansone; A.L.Shook ;R.J.Starke; J.M.Tichota and S.A.Valenti (2003). Feild efficacy assessment of transgenic Roundup Ready wheat.Crop Science, 43 (3):1072-1075.
2. Charkazi, F.; S.S. Ramezanpour and H. Soltanloo (2010). Expression pattern of two sugar transporter genes (SuT4 and SuT5) under salt stress in wheat. Australian Journal of Crop Science 3, 194-198
3. Debasis ,P.and K.H. Paramjit (2003).Genetic transformation of Indian bread (T. aestivum) and pasta (T. durum) wheat by particle bombardment of mature embryo-derived calli. BioMedCentral Plant Biology 3, 5.
4. F.A.O.(2013).(http://www.faostat.fao.org/).
5. U.S.D.A.(2012).http://www.usda.gov/wps/portal/usda/usdahome?navid .
6. El-Maghraby, M.A. ; M.E. Moussa; N.S. Hana and H.A. Agrama (2005).Combining ability under drought stress relative to SSR diversity to common wheat. Euphytica, 141: 301-308.
7. Hu, Z.M.(2003).Salt tolerance transferred from wild triticeae species into wheat. ISB News Rep. (http://www.isb.vt.edu).
8. Yang, X. Q. S. Q. Zhang; Z. S. Liang and Y. Shan (2004). Effects of water stress on chlorophyll fluorescence parameters of different drought resistance waiter wheat cultivars seedlings. Acta Bot. Boreal. Occident. Sin., 24, 812-816.
9. Shi, Z. ; S. Q. Shi; W. F.Xiao and L. W. Qi (2008). Influence of dehydration on characteristics of chlorophyll fluorescence of detached leaves in Haloxylon ammodendron and Populus euphratica. *Forest Research*, 21(4), 566-570.
10. Shi, G. Y. ; W. X. Liao; L. F.Qin and L. L. Lu (2009) . PEG simulated water stress effects on physiological and biochemistry indexes of germination of Toona sinensis seeds. *Journal of Forestry Sci and Tech.*, 4, 142-145.
11. Aazami, M.A.; M. Torabi and E. Jalili (2010). *In vitro* response of promising tomato genotypes for tolerance to osmotic stress. Afr. J. Biotechnol., 9(26): 4014-4017.
12. Subhani,G.M. ;M. Hussain;J. Ahmad and J. Anwar (2011). Response of exotic wheat genotypes to drought stress . J. Agric. Res. 49(3): 293-305.
13. Ludlow, M.M. and R.C.Muchow (1990). A critical evaluation of traits for improving crop yields in water limited environments.Adv Agron., 43:107-153.
14. Koszegi, B.; E.Farshadfar, A. Vagujfalvi and J. Sutka (1996).Drought tolerance on wheat/rye disomic chromosome addition lines.Acta Agron Hung,44:121-126.
15. Passioura, J.B. (2002). Environmental biology and crop improvement . Funct. Plant Biol., 29: 537-546.
16. Mercado, J.A.; C.Sancho; B.Jimenez; U.Peran; A.Pliego and M. Quesada (2000). Assessment of *in vitro* growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato. Plant cell tissue and Organ Culture 62: 101-106.
17. Jain,S.M. (2001).Tissue culture-derived variation in crop improvement. Euphytica, 118:153–166.
18. El-Aref, H.M. (2002).Employment of maize immature embryo culture for improving drought tolerance. Proceeding of the 3rd Scientific Conference of Agriculture Sciences, Fac. of Agric., Assiut Univ., Assiut, Egypt, 20-22. October, pp. 463-477.
19. Chawla,H.S.andG.Wenzel (1987).Regeneration potential of callus from from wheat and barley. Archive Fur Zuchtungsforschung.Bockhorn, 8059,Grunbach,GermanFedral Republic, 17(6):337-343.
20. Arzani,A. and S.S. Miradjagh (1999).Response of durum wheat cultivars to immature embryo culture, callus induction and in vitro salt stress. Plant Cell, Tissue and Organ Culture, 58: 67-72.
21. Chawla, H. S. (2000).Introduction to plant biotechnology. Science puplishers, Inc.
22. Ozgen, M. ; M.Turet;S. Altmok and C.Sancak (1998).Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum L.*) genotype.Plant Cell Rep.,18: 331–335.
23. Kaufmann, M.R. and A.N. Eckard (1971). Evaluation of water stress control with PEG by analysis of guttation. *Plant Physiol.*, 47: 453–458.
24. Corchete, P. and H. Guerra. (1986). Effect of NaCl and PEG on solute content and glycosidase activities during germination of lentil seeds. Plant Cell Environ., 9: 589-593.
25. El-Shafey, N.M. ;A. H. Raifa;M. A. G. Mahmoud and O. El-Sheihy (2009). Pre-exposure to gamma rays alleviates the harmful effect of drought on the embryo-derived rice calli *Aust J Crop Sci*, 3(5):268–277.

26. Rai, M. K.; R. K. Kalia; R. Singh; M. P.Gangola and A. K. Dhawan (2011).Developing stress tolerant plants through in vitro selection-An overview of the recent progress. Environmental and Experimental Botany, 71: 89-98.
27. Bi ,R.M. ;M.Kou;L.G.Chen;S.R. Mao and G.Wang (2007) .Plant regeneration through callus initiation from mature embryo of *Triticum*. Plant Breeding 126: 9-12.
28. Murashige, T.; F. Skoog (1962).A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473-479.
29. Chen, J.J. ; R.Q Yue; H..X. Xu and X.J Chen.(2006). Study on plant regeneration of wheat mature embryos under endosperm supported culture. *Agric Sci China*, 5(8):572–578.
30. [30] Birsin,M.A.and M. Ozgen. (2004). A comparison of callus induction and plant regeneration from different embryo explants of triticale (x *Triticosecale wittmack*) Cell. Mol. Biol. Lett. 9: 353-361.
31. Al-Khayri, J.M. and A. M. Al-Bahrany.(2004).Growth,water content and proline accumulation in drought-stressed callus of date palm. *Biol. Plant*, 48(1):105–108.
32. AbdEl-samad,A.;O.E.El-Sayed and H.F.Ibrahim.(2007).Development of drought tolerant double haploid wheat using biochemical genetic markers on in vitro culture, J Appl Sci Res, 3, 1589-1599.
33. Hayman, B.I. (1954a).The analysis of variance of diallel tables. Biometrics, 10:235-244.
34. Hayman, B. I. (1954a).The theory and analysis of diallel crosses.Genetics, 39:789-809.
35. Jinks, J.L. (1954).The analysis of continuous variations in a diallel cross of *Nicotiana rustica* varieties. Genetics, 39: 767-787.
36. Singh, R.K. and B.D. Chaudhary (1985).Biometrical method in quantitative genetic analysis, Kalyani Pub.
37. Farshadfar, E.; S.S. Esmaili1and V.Rasoli (2012). Chromosomal localization of the genes controlling callus induction and *in vitro* drought tolerance criteria in wheat-barley disomic addition lines using mature embryo culture. *Annals of Biol. Res., 3 (3):1334-1344*.
38. Fennell, S; N. Bohorova; M. van Ginkel; J. Crossa and D. Hoisington (1996). Plant regeneration from immature embryos of 48 elite CIMMYT bread wheats.*Theor. Appl. Genet*, 92: 163–169.
39. Hess, J.R. and J.G .Carman (1998). Embryogenic competence of immature wheat embryos: genotype, donor plant environment, and endogenous hormone
40. levels. Crop Sci. 38:249-253.
41. Yadav, M.K. ;N.K. Singh and G.K. Garg (2000).Development of lines of Indian wheat genotypes for efficient regeneration using mature embryos. In:Symposium on Biotechnology for sunstainable agriculture, 27-29 April 2000, G.B. Plant University of Ag & Tech, Pantnagar, India.
42. Yadava, R. and H.S. Chawla (2001). Interaction of genotype, growth regulators and amino acids on plantregeneration from different developmental states of influorescence in wheat. J. Genet Breed, 55: 261-297.
43. Schween, G. and H.G. Schwnkel (2003).Effect of genotype on callus induction, shoot regeneration and phenotypic stability of regenerated plants in the greenhouse of Primula spp. Plant Cell Tissue Organ Cult., 72: 53-61.
44. Zouzou, M. ; T.H. Kouakou;M. Kone; N.G. Amani and Y.J. Kouadio (2008). Effect of genotype, explants, growth regulators and sugars on callus induction in Cotton (*Gossypium hirsutum* L.). Australian J. Crop Sci. 2(1): 1-9.
45. Shah, M.M. ;Q. Khalid;U.W. Khan; S.A.H. Shah;S.H. Shah;A. Hassan and A. Pervez, 2009. Variation in genotypic responses and biochemical analysis of callus induction in cultivated wheat . Genet. Mol.Res., 8(3): 783-793
46. Elyasi,P. ;E.Farshadfar and M.Aghaee (2012). Response of bread wheat genotypes to Immature Embryo Culture, Callus induction and drought stress. Current Research journal of Biological Sciences 4(4): 372-380.
47. Farshadfar,E. ;B. Jamshidi and M. Chehri (2014). Assessment of immature embryo culture to select for drought tolerance in bread wheat . International Journal of Biosciences (IJB) ,4, 4: 194-203.
48. Biswas, J.; B.Chowdhury; A.Bhattacharya and A.Mandal
49. (2002). *In vitro* screening for increased drought tolerance in rice.*In Vitro* Cell Devision Biology of Plant 38, 525–530.
50. Al-Khayri, J.M. and A. M. Al-Bahrany.(2000).*In vitro* plant regeneration of Hassawi rice (*Oriza Sativa* L.)from mature embryo- derived calluss. Pakistan Journal of BiologicalSciences 3, 602-605.
51. Turhan, H. and I.Baser (2004). Callus induction from mature embryo of winter wheat (*Triticum aestivum* L.). Asian Journal of Plant Sciences 3, 17-19.

52. Lutts, S. ; M.Almansouri; J.M.Kinet (2004). Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Science 167, 9-18.
53. Sakthivelu, G.; M. K. Akitha Devi; P.Giridhar; T. Rajasekaran ;A.A. Ravishankar; T. Nedev and G. Kosturkova (2008).Drought –induced alternations in growth,osmotic potential and *in vitro* regeneration of soyabean cultivars. General Applied Plant Physiology 34,103-112 .
54. Filippov, M. ;D.Miroshnichenko;D. Vernikovskaya and S. Dolgov (2006). The effect of auxins, time exposure to auxin and genotypes on somatic embryogenesis from mature embryos of wheat. Plant Cell, Tissue and Organ Culture, 84:213-222.
55. Singh, P. and S.S. Narayanan (1993). Biometrical techniques in plant breeding. 1st ed. Kalayani Publishers, New Delhi, India.
56. Singh, R.K. and B.D. Chaudhary (1999). Biometrical methods in quantitative genetic analysis. Kalayani Publishers, Ludhiana, India.
57. Etedal,F., A .Khandan; A. Motalebi-Azar; Z. Hasanzadeh; A. Amiri Sadeghan ;A. Pezeshki; A. Pezeshki and S. Kazemiani (2011). Biometric-genetic analysis of *in vitro* callus proliferation in rapeseed (*Brassica napus*).African J. Agric. Res. 7(48): 6474-6478.

*****