Egypt. J. Plant Breed. 8: 273-286 (2004)

REGENERATION FROM EGYPTIAN WHEAT CALLI SUBJECTED TO PEG-INDUCED STRESS CONDITIONS

A. M. M.Al-Naggar [1], Sawsan S. Youssef[2], A. E. I.Ragab [3]and M. R. Al-Bakry[3]

[1]Department of Agronomy and [2]Department of Genetics, Fac. of Agric. Cairo Univ.Giza.
[3]Plant Biotechnology Unit, Plant Res. Dept., Nuclear Res. Center, Atomic Energy Authority.

ABSTRACT

Immature embryo culture response, resistance of callus to different concentrations of PEG-induced water stress and plantlet regeneration were studied in some Egyptian wheat (Triticum aestivum L.) cultivars and their F_1 crosses. All genotypes induced callus with variation among them in the frequency of callus induction. Sids 1 cultivar and the F1's Sids 1 X Gemmeiza 5 and Sids 1 X Giza 168 showed the highest callus induction frequency (100.00%). The ability of callus for maintenance on MS medium supplemented with 1 mg/l 2,4-D was shown for all genotypes except for Giza 168 cultivar and Sakha 8 X Giza 168 cross. In general, callus fresh weight (CFW) and callus growth rate (CGR) gradually decreased with increasing PEG concentration in the culture medium. Results indicated that the most drought tolerant genotypes, based on their absolute values of CFW and CGR traits under both 15 and 20% PEG concentrations are the cross Sids 1 X Gemmeiza 5 followed by the cultivar Sids 1 and then the cross Sids 1 X Giza 164. The number of regenerated plants (RP) per 300 mg callus at 0, 15 and 20% PEG concentrations was 1.79, 1.38 and 0.50 for crosses, and 0.60, 0.06 and 0.06 for parents, respectively. Two parents and five F1's were able to regenerate plants from their calli. The highest RP (3) was recorded by Sids 1 X Gemmeiza 5 cross, while the lowest (0.44) was shown by Giza 164 cultivar.

Key words: ***Bread wheat, Triticum aestivum, Immature embryo, In vitro selection, Drought, PEG, Regeneration, Callus induction, Maintenance.***

INTRODUCTION

Tissue culture is utilized in a number of applications for wheat improvement. The regeneration of plants from callus is central to most of the strategies that have been proposed for the genetic manipulation of wheat using *in vitro* techniques. Plants regenerated from callus cultures could provide useful germplasm for breeding programs. However, selection at the callus level requires an effective system of initiating, maintaining, and subsequently regenerating plants from callus (Özgen *et al* 1996).

Tissue culture technologies provide novel approaches to improve crops for complicated traits such as drought tolerance. The idea in tissue culture methods is that cultivated cells are used as the selection units rather

than whole plants. The ability to regenerate plants from tolerant cells and to obtain enhanced tolerance at the plant level would be considered a great success. *In vitro* selection for cells exhibiting increased tolerance to water stress has beeen reported in tomato (Bressan *et al* 1981), chili pepper (Santoz-Diaz and Ochoa-Alejo 1994), maize (Dolgikh *et al* 1994), and wheat (Al-Naggar, 1989, Galiba *et al* 1989, Trivedi *et al* 1991, Barakat and Abdel-Latif 1995 a and b and El-Wafa 1999). The regeneration of drought tolerant plants has been achieved successfully in *Nicotiana plumbaginifolia* (Sumaryati *et al* 1992), maize (Dolgikh *et al* 1994) and wheat (Barakat and Abdel-Latif 1995 a , b). The objectives of this study were to screen a number of Egyptian wheat cultivars and their crosses for their ability of callus induction from immature embryos, callus resistance to PEG-induced water stress and the ability of selected calli for regeneration, in an attempt to obtain some drought tolerant plants.

MATERIALS AND METHODS

Egyptian wheat (*Triticum aestivum* L.) plants of 15 genotypes i.e. six cultivars (Sahel 1, Sids 1, Sakha 8, Gemmeiza 5, Giza 164 and Giza 168) and their nine F1 crosses (Sahel 1 x Gemmeiza 5, Sahel 1 x Giza 164, Sahel 1 x Giza 168, Sids 1 x Gemmeiza 5, Sids 1 x Giza 164, Sids 1 x Giza 168, Sakha 8 x Gemmeiza 5, Sakha 8 x Giza 164 and Sakha 8 x Giza 168) were used as the genetic material of the present study. Plants were grown under field conditions during the winter season 2001/2002 and properly watered and fertilized to maintain vigorous growth.

Immature embryos of the 15 wheat genotypes were used as explants for callus initiation and maintenance. According to Vasil and Vasil (1999) spikes were collected when immature embryo length was 0.8-1.5 mm, and wrapped in moist paper towels. Caryopses from the middle of each spike were removed, and surface-sterilized with 70% ethanol for five minutes, followed by washing with four changes of sterile distilled water. Aseptically, immature embryos (0.8-1.5 mm) were removed, and placed in Petri dishes on Murashige and Skoog's (1962) (MS) medium supplemented with 2 mg/l 2, 4-D with the scutellum exposed and the embryo axis in contact with the medium. Twenty-five embryos were cultured in each Petri dish. Culture dishes were sealed with Parafilm, and incubated in the dark at 27 °C to initiate proliferation of scutellar cells. After initiation, calli were maintained on the (MS) medium supplemented with 1.0 mg/l 2,4-D and subcultured at approximately 21-day intervals for 7 months (210 days). Callus induction frequency (CIF) was determined as follows:

CIF % = 100 (No. of embryos inducing callus / No. of cultured embryos)

Embryogenic calli of all wheat genotypes under study except Giza 168 and Sakha 8 X Giza 168 (i.e. for 5 parents and 8 crosses) were grown on liquid MS medium supplemented with different concentrations of polyethylene glycol (PEG) 6000 (Average MW 5600-7000), as an osmoticum, for six weeks. Embryogenic calli were subcultured on a Cotton support placed in MS medium supplemented with 2% (w/v) sucrose, 1.0 mg/l 2,4-D and PEG concentrations of 0, 15 and 20% (w/v). The pH of the maintenance medium was adjusted to 5.8 before autoclaving. Five replicates of each treatment were used and calli were weighed every 2 weeks, and the initial callus weight used was 250 mg. All cultures were incubated in the dark at 27 °C.

Embryogenic calli were divided into small pieces and placed on a regeneration medium, i.e. MS medium free of glutamine, casein hydrolysate and 2,4-D, for shoot formation and kept under 16-h photoperiod for 8-10 days. At the end of this period, green areas indicative of shoot formation were visible to the naked eye. Green shoots were transferred, along with the callus as a unit to shoot elongation medium: MS salts, sucrose (15g), myo-inositol (50mg), and 2.5g/l gelrite. Green shoots (2cm in length) were transferred to root elongation medium: shoot elongation medium. After shoots and roots were developed (approximately after 21 days), regenerated plants were washed in tap water to remove gelrite from the roots and transplanted into small pots containing a mixture of one part peatmoss and one part fine vermiculite (v/v) (watered with 250 mg/l Ridomil MZ 72 wp) and covered with plastic bags in the growth chamber to maintain a relative humidity between 70% and 80%. After 3 days, the plastic bags were pored to decrease the humidity gradually for another 3 days, then the plastic bags were removed. The plants were transplanted to large plastic pots containing a mixture of one part clay soil and one part sand soil for 2 weeks and watered with tap water. Finally, wheat plants were transferred to the permanent sand clay soil until maturity.

The following measurements were determined:

1. Callus fresh weight (CFW) in grams.

2. Callus growth rate (CGR) in grams/day as follows:

CGR = (FinalCFW – InitialCFW)/Time in days (14 days)

3. Number of regenerated plants (RP) produced from 300-mg callus at the end of stress treatment.

Analysis of variance of a factorial experiment in a randomized complete block design including 13 wheat genotypes, three PEG concentrations and subcultures as well as all possible interactions for CFW, CGR and RP traits was performed according to Gomez and Gomes (1984).

RESULTS AND DISCUSSION

Callus induction

All genotypes induced callus with variation among them in the callus induction frequency (CIF). The CIF ranged from 72.22% to 100.00%. Among the six parents, Sids 1 cultivar (Fig. 1A) showed the highest percentage of CIF (100.00%), followed by Giza 164 (97.33%), whereas, Sakha 8 showed the lowest CIF percentage (73.33%). Among crosses, Sids 1 X Gemmeiza 5, Sids 1 X Giza 164 and Sids 1 X Giza 168 showed the highest callus induction rates (100.00%, 95.52% and 100.00%, respectively). Although Sahel 1 was a good callus inducer (97.33%), its three crosses, Sahel 1 X Gemmeiza 5, Sahel 1 X Giza 164 and Sahel 1 X Giza 168, exhibited lower percentages of CIF (75.00, 80.00 and 72.22%, respectively). On the other hand, Sakha 8 had the lowest percentage of callus induction among parents (73.33%), its crosses Sakha 8 X Gemmeiza 5 and Sakha 8 X Giza 164 showed a good callus induction frequency (88.75% and 90.00%, respectively).

Callus cultures showed the ability for maintenance on MS medium supplemented with 1 mg/l 2,4-D for all genotypes except for Giza 168 cultivar and Sakha 8 X Giza 168 cross which failed to be maintained owing to the browning of their calli rapidly.

Results of this study on the wheat genotypic variability in the frequency of immature embryos that responded to callus induction are in consistence with those of other investigators (Sears and Deckard 1982, Carman *et al* 1987, Barakat and Abdel-Latif 1995 b, Machii *et al* 1998, Rao and Chawla 1998, El-Wafa and Ismail 1999, and Abd El-Maksoud 2003).

In vitro selection for drought tolerance

1. Callus growth

Analysis of variance (Table 1) indicated highly significant mean squares due to wheat genotypes, PEG concentrations, subcultures and all possible interactions for callus growth characteristics.

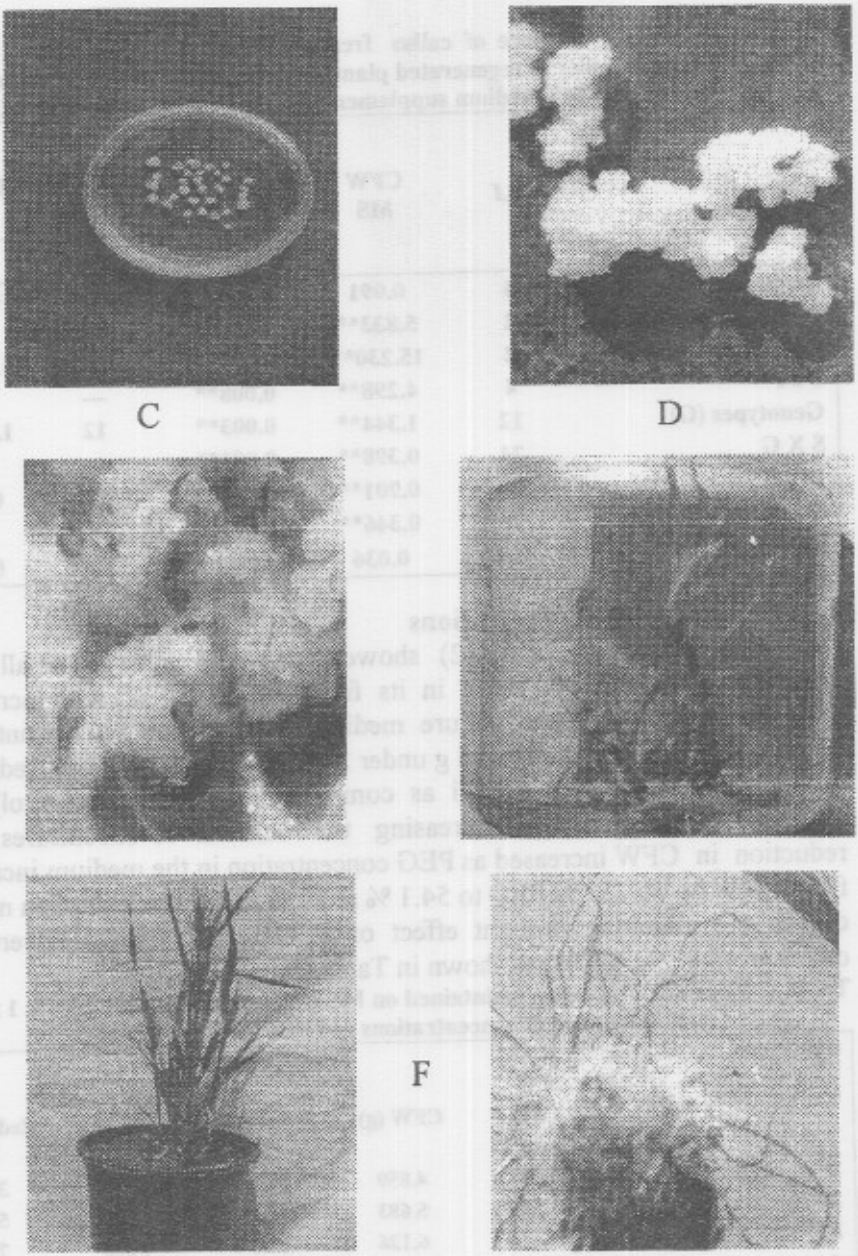

Fig. 1. Callus initiation (A), maintenance (B) and plant regeneration from (C to E) in the bread wheat, cultivar Sids 1. Embryogenic callus showing green regions (C) and developing shoots and roots (D). A regenerated plant at flowering stage (E). Failure to differentiate shoots prior to root formation resulted in root regenerates only (F) as shown by Gemmeiza 5 cultivar.

Table 1. Analysis of variance of callus fresh weight (CFW), callus growth rate (CGR) and number of regenerated plants/300 mg callus (RP) of 13 wheat genotypes grown on MS medium supplemented with 3 concentrations of PEG.

S.V.	d.f	CFW MS	CGR MS	RP/300 mg callus	
				d.f	MS
Replications	4	0.091	0.0002	2	0.070
Subcultures (S)	2	5.833**	0.003**	—	—
PEG concentrations (P)	2	15.230**	0.035**	2	1.178**
S x P	4	4.298**	0.008**	—	—
Genotypes (G)	12	1.344**	0.003**	12	1.276**
S X G	24	0.398**	0.001**	—	—
P X G	24	0.901**	0.002**	24	0.173
S X P X G	48	0.346**	0.001**	—	—
Error	464	0.036	0.00007	76	0.088

1. 1. Effect of PEG concentrations

Data presented in Table (2) showed that wheat callus across all tested genotypes gradually decreased in its fresh weight (CFW) with increasing PEG concentration in the culture medium from 11.049 g under untreated control treatment (0%) to 4.178 g under 20% PEG concentration. Reduction in CFW of PEG-treated calli as compared to untreated (control) calli gradually increased with increasing the number of subcultures. This reduction in CFW increased as PEG concentration in the medium increased from 40.9 % at 15 % PEG to 54.1 % at 20 % PEG. The reduction may be due to the inhibiting drought effect of PEG treatment which lowered the callus growth rate (CGR) as shown in Table (4).

Table 2. Mean CFW of callus maintained on MS medium supplemented with 1 mg/l 2, 4-D and different concentrations of PEG.

Subculture	PEG concentration				
	0 %	15 %		20 %	
	CFW (g)	CFW (g)	Reduction %	CFW (g)	Reduction %
1st	5.843	4.859	16.8	4.085	30.1
2nd	9.555	5.683	40.5	4.195	56.1
3 rd	17.750	6.126	65.5	4.256	76.0
Average	11.049	5.556	40.9	4.178	54.1

LSD at 0.05:
between PEG concentrations (P) = 0.037
between subcultures (S) = 0.037
for P X S interaction = 0.065

Moreover, data in Table (3) indicated that callus growth rate (CGR) across all tested wheat genotypes was strongly reduced from 0.026 g/day under 0% PEG (control) to 0.005 g/day and 0.002 g/day under PEG-containing media, (15% and 20%), with reductions of 69.2 and 87.2 %, respectively.

Table 3. Mean CGR of callus maintained on MS medium supplemented with 1 mg/l 2, 4-D and different concentrations of PEG.

Subculture	PEG concentration				
	0 %	15 %		20 %	
	CGR (g/day)	CGR (g/day)	Reduction %	CGR (g/day)	Reduction %
1st	0.014	0.009	37.1	0.005	65.0
2nd	0.020	0.005	75.0	0.0006	97.0
3rd	0.045	0.002	95.5	0.0002	99.0
Average	0.026	0.005	69.2	0.002	87.2

LSD at 0.05
between PEG concentrations (P) = 0.000199
between subcultures (S) = 0.000199
for P X S interaction = 0.000344

The reduction in CGR of PEG-treated calli as compared to untreated (control) calli, was gradually increased as the number of subcultures increased, i.e. from the first through the third subculture. The minimal reduction in CGR (37.1%) was exhibited by the 15 % PEG treatment at the first subculture, while the maximal reduction (99.0%) was shown by the 20% PEG treatment at the third subculture (Table 3).

1.2. Role of Genotype

Data on genotypic differential responses to PEG-containing media for CFW and CGR traits are presented in Tables 4 and 5, respectively. The single cross Sids 1 X Giza 168 showed maximum reduction (73.4% and 86.2%) in CFW of its callus exposed to 15 and 20 % PEG-containing media, respectively as compared to their respective calli grown on PEG-free medium (Table 5). Sakha 8 cultivar followed by Sahel 1 X Giza 164 and Sids 1 exhibited minimal reduction in CFW under the conditions of 15% PEG-containing medium (21.2, 21.5 and 23.2%, respectively), whereas, Sids 1 cultivar only showed the minimal reduction under the condition of 20 % PEG-containing medium (37.7%).

Average CFW across all PEG concentrations ranged from 0.366 gm (Sakha 8) to 0.520 gm (Giza 164) for parents and from 0.418 gm (Sahel 1 X Giza 168) to 1.007 gm (Sids 1 X Giza 168) for crosses.

Table 4. Mean CFW in grams of 5 wheat parents and 8 F_1 crosses as affected by different concentrations of PEG supplemented to the MS medium.

Genotype	PEG concentration					Average
	0 %	15 %		20 %		
	CFW	CFW	Reduction	CFW	Reduction %	
Parents						
Sahel 1	0.633	0.381	39.8	0.296	53.2	0.436
Sids 1	0.639	0.491	23.2	0.398	37.7	0.509
Sakha 8	0.490	0.386	21.2	0.223	54.5	0.366
Gemmeiza 5	0.577	0.431	25.3	0.277	52.0	0.428
Giza 164	0.949	0.365	61.5	0.245	74.2	0.520
Mean (parents)	0.658	0.411	37.5	0.288	56.2	0.452
Crosses						
Sahel 1 X Gemmeiza 5	0.621	0.324	47.8	0.336	45.9	0.427
Sahel 1 X Giza 164	0.624	0.490	21.5	0.331	46.9	0.481
Sahel 1 X Giza 168	0.650	0.305	53.1	0.299	54.0	0.418
Sids 1 X Gemmeiza 5	1.216	0.544	55.3	0.471	61.3	0.744
Sids 1 X Giza 164	1.013	0.436	56.9	0.373	63.2	0.607
Sids 1 X Giza 168	2.153	0.573	73.4	0.296	86.2	1.007
Sakha 8 X Gemmeiza 5	0.574	0.426	25.8	0.338	41.1	0.446
Sakha 8 X Giza 164	0.910	0.388	57.4	0.309	66.0	0.536
Mean (crosses)	0.970	0.436	55.1	0.344	64.5	0.583
Average	0.850	0.426	43.2	0.322	56.6	

LSD at 0.05
between PEG concentrations (P) = 0.0377
between genotypes (G) = 0.0786
for P X G interaction = 0.136

Table 5. Mean CGR in gm/day as affected by genotypes and different concentrations of PEG supplemented to MS medium.

Genotype	PEG concentration					Average
	0 %	15 %		20 %		
	CGR	CGR	Reduction %	CGR	Reduction %	
Parents						
Sahel 1	0.015	0.004	73.33	0.001	93.33	0.006
Sids 1	0.013	0.009	30.77	0.004	69.23	0.008
Sakha 8	0.010	0.004	60.00	-0.001	90.00	0.004
Gemmeiza 5	0.013	0.005	61.54	0.000	100.00	0.006
Giza 164	0.034	0.004	88.23	0.000	100.00	0.012
Mean (parents)	0.017	0.0052	69.41	0.0008	95.29	0.0072
Crosses						
Sahel 1 X Gemmeiza 5	0.017	0.001	94.12	0.002	88.23	0.006
Sahel 1 X Giza 164	0.012	0.007	41.66	0.002	83.33	0.007
Sahel 1 X Giza 168	0.017	0.001	94.12	0.001	94.12	0.006
Sids 1 X Gemmeiza 5	0.045	0.009	80.00	0.006	86.66	0.020
Sids 1 X Giza 164	0.033	0.006	81.82	0.004	87.88	0.014
Sids 1 X Giza 168	0.092	0.010	89.13	0.001	98.91	0.034
Sakha 8 X Gemmeiza 5	0.014	0.005	71.43	0.002	85.71	0.007
Sakha 8 X Giza 164	0.029	0.003	89.65	0.001	96.55	0.011
Mean (crosses)	0.032	0.0053	83.44	0.0024	92.50	0.013
Average	0.026	0.005	73.52	0.001	90.30	

L.S.D at 0.05:
between PEG concentrations (P) = 0.000199
between genotypes (G) = 0.0004143
for P X G interaction = 0.0007175

The average CGR over PEG concentrations ranged from 0.004 g/day (Sakha 8) to 0.012 g/day (Giza 164) for parents and from 0.006 g/day (Sahel 1 X Gemmeiza 5 and Sahel 1 X Giza 168) to 0.034 g/day (Sids 1 X Giza 168) for crosses (Table 5).

Although, Sids 1 X Giza 168 cross showed the highest absolute values of CFW and CGR under 0 and 15 % treatments, it was amongst those genotypes showing the lowest absolute values of the same traits under 20 % PEG treatment. This cross revealed, however, the highest reduction in CFW under 15 % and 20% PEG treatments (73.4 and 86.2%, respectively). Sids 1 X Gemmeiza 5 cross showed the highest absolute value of CFW under 20% PEG and ranked the second highest for CGR under 0 and 15% PEG treatments.

According to the *in vitro* screening of the tested wheat genotypes for drought tolerance, it could be concluded that the most drought tolerant genotypes, based on their absolute values of CFW and CGR traits under different PEG concentrations, are Sids 1 X Giza 168 followed by Sids 1 X Gemmeiza 5 and the parental cultivar Sids 1 under 15% PEG and Sids 1 X Gemmeiza 5 followed by Sids 1 and Sids 1 X Giza 164 under 20% PEG. On the average of the 15% and 20% PEG concentrations, the F_1 cross Sids 1 X Gemmeiza 5 could be considered the most drought tolerant genotype, followed by its parent Sids 1 and then the F_1 cross Sids 1 X Giza 168.

The *in vitro* screening showed that the F_1's Sahel 1 X Giza 168 and Sahel 1 X Gemmeiza 5 under 15% PEG and the cultivar Sakha 8 under 20% PEG could be regarded as the most drought sensitive genotypes in this study; expressed in both CFW and CGR traits (Tables 4 and 5).

It is worthnoting that, for either CFW or CGR traits, the average of all crosses is higher than that of all parents under all PEG concentrations, indicating that heterozygotes exhibited higher estimates of CFW and CGR than homozygotes and suggesting the role of heterosis (and other factors) for such traits in vitro.

On average, F_1's were higher than parents by 18.6% for CFW and 80.6% for CGR. Such superiority was more pronounced at 20% PEG concentration, especially for CGR, while it was less pronounced at 15% PEG for both studied traits.

Wheat genotypic differences in drought tolerance at the cellular level expressed in CFW and CGR under water stress conditions *in vitro* were also reported by other investigators (Al-Naggar 1989, Galiba *et al* 1989, Trivedi *et al* 1991, Barakat and Abdel Latif 1995a , c and El-Wafa 1999).

2. Plant regeneration

Calli derived from immature embryos of 13 wheat genotypes that maintained for 210 days on MS solid medium supplemented with 1 mg/l 2, 4-D and subsequently grown on MS liquid medium supplemented with three different concentrations of PEG (0, 15 and 20%) for six weeks were transferred to MS hormone-free medium for plant regeneration induction. Regenerated plantlets from Sids 1 cultivar are shown in Figure 1 (from C to E).

Analysis of variance (Table 1) for number of regenerated plants indicated highly significant mean squares due to wheat genotypes and PEG concentrations.

2.1. Effect of polyethylene glycol

In this experiment wheat plants were regenerated from calli of 7, 6 and 4 genotypes maintained on media contained polyethylene glycol of 0, 15 and 20% concentration, respectively (Table 6). Increasing the polyethylene glycol concentration in the maintenance medium caused a significant reduction in the regeneration ability across responded wheat genotypes. At 0%, 15% and 20% PEG, an average of 1.33, 0.87 and 0.33 plants/300 mg callus were regenerated, respectively (Table 6). The mean number of regenerated plants/300 mg callus at 0, 15 and 20% PEG concentrations for crosses was 1.79, 1.38 and 0.50, while it was 0.60, 0.06 and 0.06, respectively for parents.

These results agreed with those obtained by Al-Naggar (1989) who found that the higher the concentration of PEG the lower was the regenerability of wheat embryogenic callus, and by El-Wafa (1999) who found that the proliferation of embryogenic calli declined markedly with increasing osmotic stress and the number of green spotted calli was significantly affected by osmotic pressure.

Table 6. Mean number of wheat regenerated plants/300 mg of callus fresh weight as affected by genotype and PEG concentration.

Genotype	PEG concentration			Mean
	% 0	% 15	% 20	
Parents				
Sahel 1	0	0	0	0.00
Sids 1	1.67	0.33	0.33	0.78
Sakha 8	0	0	0	0.00
Gemmeiza 5	0	0	0	0.00
Giza 164	1.33	0	0	0.44
Mean (parents)	0.60	0.06	0.06	0.24
Crosses				
Sahel 1 X Gemmeiza 5	0	0	0	0.00
Sahel 1 X Giza 164	1.67	0.66	0.66	1.00
Sahel 1 X Giza 168	0	0	0	0.00
Sids 1 X Gemmeiza 5	3.33	3.33	2.33	3.00
Sids 1 X Giza 164	3.33	1.67	1.00	2.00
Sids 1 X Giza 168	3.33	2.67	0	2.00
Sakha 8 X Gemmeiza 5	0	0	0	0.00
Sakha 8 X Giza 164	2.67	2.67	0	1.78
Mean (crosses)	1.79	1.38	0.50	1.22
Average	1.33	0.87	0.33	

L.S.D at 0.05
between PEG concentrations (P) = 0.1338
between genotypes (G) = 0.2785
for P X G interaction = 0.4824

2.2 Role of genotype

Plant regeneration from callus was achieved in this experiment only in 7 out of the 13 wheat genotypes tested, i.e 6 genotypes did not respond to regeneration (Table 6). Sears and Deckard (1982) observed variability among wheat genotypes tested for plant regeneration after long term subcultures. Out of 39 genotypes, tested by them, 18 genotypes were capable of regenerating plants after 4 subcultures (90 to 125days) and cultures from 5 genotypes remained totipotent after 240 days.

Among the 5 wheat parents used in this study, only Sids 1 and Giza 164 showed an ability of regeneration. Five out of 8 F_1 crosses regenerated plants from their calli. It was observed that Sids 1, Giza 164 parents and their F_1 crosses showed regenerability. The highest regeneration ability (3 plantlets) was recorded by the Sids 1 X Gemmeiza 5 cross, while the lowest (0.44 plantlets) was shown by Giza 164 cultivar. It is worthy to note that at each PEG as well as across PEG concentrations, the F_1's exhibited higher number of regenerated plants/300 mg callus than their parents. The F_1 crosses showed higher regeneration ability of 198.3, 2200.0, 733.3% than

parents at 0, 15 and 20% PEG concentrations, respectively, with an average (across concentrations) of 408.0% higher regenerability than parents. This could be attributed to the hybrids effect of the F_1 crosses. Moreover, such effect in regenerability of the F_1's was more pronounced under water stress (15 and 20% PEG) than that under non-stress (0% PEG). Variability among wheat genotypes in regenetration ability was also reported by many investigators (Sears and Deckard 1982, Maddock *et al* 1983, Agarwal and Tiwari 1995, Barakat and abdel-Latif 1995b , c, Fennell *et al* 1996, Machii *et al* 1998, Rao and Chawla 1998).

Regenerated plants derived from calli maintained on PEG-containing media were grown to maturity. Their progenies will be tested for the ability to tolerate different levels of water stress with the aim of obtaining some wheat cell lines that tolerate drought.

REFERENCES

Abd El-Maksoud, M. M. (2003). Heterosis and genetic control of immature embryo culture ability in Egyptian wheat (*Triticum aestivum* L.). Egypt J. Plant Breed. 7 (1): 363-373.

Agarwal, D. K. and S. Tiwari (1995). Effect of genotypes and nutrient media on immature embryo culture of wheat. Indian J. of Genet. and Plant Breed. 55 (1): 50-57.

Al-Naggar, A. M. M. (1989). Selection for drought tolerance in wheat tissue culture. Proc. of the 4th Int. Conf. Stat. Comp. & Demo. Res., Cairo, 25-30 March. pp. 1-18.

Barakat, M. N. and T. H. Abdel-Latif (1995a). *In vitro* selection for drought tolerant lines in wheat. I. Effect of polyethylene glycol on the embryogenic cultures. Alex. J. of Agric. Res. 40 (1): 97-112.

Barakat, M. N. and T. H. Abdel-Latif (1995b). Somatic embryogenesis and plant regeneration in callus from mature and immature embryo cultures of wheat. Alex. J. of Agric. Res. 40 (1): 113-129.

Barakat, M. N. and T. H. Abdel-Latif (1995c). *In vitro* selection for drought-tolerant lines in wheat. II. *In vitro* characterization of cell lines and plant regeneration. Alex. J. of Agric. Res. 40 (3): 167-190.

Bressan, R. A., P. M. Hasegawa and A. K. Handa (1981) Resistance of cultured higher plant cells to polyethylene glycol-induced water stress. Plant Sci. Lett. 21: 23-30.

Carman, J. G., N. E. Jefferson and W. F. Campbell (1987). Induction of embryogenic (*Triticum aestivum* L.) calli. I. Quantification of genotype and culture medium effects. Plant Cell, Tissue and Organ Culture 10 (2): 101-113.

Dolgikh, Yu., S. N. Larina, Z. B. Shamina, N. E. Zhdanova, and T. N. Pustovoitova (1994). Drought tolerance of maize plants obtained from the cell lines resistant to osmotic stress production by polyethylene glycol. Russian J. of Plant Physiol. 41 (6): 748-753.

El-Wafa, A. A. (1999). Response of immature embryos *in vitro* regeneration of some wheat (*T. aestivum*) genotypes under different osmotic stress of mannitol. Assiut J. of Agric. Sci. 30 (3): 25-34.

El-wafa, A. A. A. and A. E. A. Ismail (1999). Callus induction and plant regeneration from culture of immature embryos of spring wheat. Assiut J. of Agric. Sci. 30 (3): 13-23.

Fennell, S.; N. E. Bohorova, J. Crossa, D. A. Hoisington and M. Van-Ginkel (1996). Plant regeneration from immature embryos of 48 elite CIMMYT bread wheats. Theor. Appl. Genet. 92 (2): 163-169.

Galiba, G., L. Simon-Sarkadi, A. Salgo, and G. Kocsy (1989). Genotype dependent adaptation of wheat varieties to water stress *in vitro*. J. Plant Physiol. 134: 730-735.

Gomez, K. A. and A. A. Gomez (1984). Statistical procedure for agricultural research, 2nd edition (Wily Interscience Publication) John Wily and Sons Inc. New York.

Machii, H., H. Mizuno, T. Hirabayashi, H. Li, and T. Hagio (1998). Screening wheat genotypes for high callus induction and regeneration capability from anther and immature embryo cultures. Plant Cell, Tissue and Organ Culture 53 (1): 67-74.

Maddock, S. E.,V. A. Lancaster, R. Risiott and J. Franklin (1983). Plant regeneration from cultured immature embryos and inflorescences of 25 cultivars of wheat (*Triticum aestivum*). J. of Experimental Botany 34 (144): 915-926.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant . 15: 433-497.

Özgen, M., S. Türet, S. Özcan and C. Sancak (1996). Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breed. 115 (6): 455-458.

Rao, C. S. and H. S. Chawla (1998). Interaction of genotype, culture medium, growth regulators and levels of 2,4-D on the regeneration response of wheat. Acta Agronomica Hungarica 46 (2): 105-112.

Santoz-Diaz, M. S. and N. Ochoa- Alejo (1994). PEG tolerant cell clones of chili pepper: accumulation. Plant Cell, Tissue and Organ Culture 37: 1-8.

Sears, R. G. and E. L. Deckard (1982). Tissue culture variability in wheat: callus induction and plant regeneration. Crop Sci. 22: 546-554.

Sumaryati, S., I. Negrutiu and M. Jacobs (1992). Characterization and regeneration of salt and water stress mutants from protoplast culture of *Nicotiana pulumbagini* folic (Viviani). Theor. Appl. Genet. 83: 613-619.

Trivedi, S., G. Galiba, , N. Sankhlaand L. Erdei (1991). Responses to osmotic and NaCl stress of wheat varieties differing in drought and salt tolerance in callus cultures. Plant Sci. 73: 227-232.

Vasil, I. K. and V. Vasil (1999). Transformation of wheat *via* particle bombardment. From: Methods in molecular biology, Vol. 111: Plant cell culture protocols. Edited by: R. D. Hall ©Humana Press Inc., Totowa, NJ.

الإستيلاد من كالوسات القمح المصري المعرضة للبولي اثيلين جليكول محدث الإجهاد المائي

أحمد مدحت محمد النجار[1] سوسن سامى يوسف[2] عبد الشافى ابراهيم رجب[3] محمد رشاد البكرى[3]

قسم المحاصيل١- قسم الوراثة٢-كلية الزراعة-جامعة القاهرة

وحدة التقنية الحيوية النباتية-قسم البحوث النباتية-مركز البحوث النووية-هيئة الطاقة الذرية٣

تم دراسة الاستجابة لزراعة الأجنة غير الناضجة وتأثير تعريض الكالوس لتركيزات مختلفة من البولى اثيلين جليكول المحدث للاجهاد المائى واستيلاد النباتات الخضراء من هذه الكالوسات فى بعض أصناف قمح الخبز المصرى (سدس ١ وساحل ١ وسخا ٨ وجميزة ٥ وجيزة ١٦٤ وجيزة ١٦٨) وهجنها. أعطت كل التراكيب الوراثية تحت الدراسة إستجابة لتكوين الكالس من الأجنة غير الناضجة مع الإختلاف بينها فى النسبة المئوية للإستجابة. أظهر الصنف سدس١ والهجينين سدس١ × جميزة ٥ وسدس ١ × جيزة ١٦٨ أعلى نسبة إستجابة (١٠٠%). أظهرت مزارع الكالس قدرة على الإستجابة للإدامة والنمو على بيئة موراشيج وسكوج مضافاً إليها ١ مليجرام/ لتر D-٢،٤ لكل التراكيب الوراثية بإستثناء جيزة ١٦٨ وهجينه سخا ٨ × جيزة ١٦٨ حيث فشلا فى ذلك.

بصفة عامة حدث تناقص تدريجى فى كل من صفتى الوزن الطازج ومعدل نمو الكالس فى كل التراكيب الوراثية بزيادة تركيز البولى إيثلين جليكول المضاف للبيئة. كانت أفضل التراكيب الوراثية تحملاً للإجهاد المائى معملياً إعتماداً على قيم وزن الكالس الطازج ومعدل نموه عند تركيزات مختلفة من البولى إيثلين جليكول هى الهجين سدس١ × جميزة ٥ يليه الصنف سدس١ ثم الهجين سدس١ × جيزة ١٦٤. كان متوسط عدد النباتات المستولدة من كالوسات معرضة لتركيزات صفر و ١٥% و ٢٠% بولى إيثلين جليكول للهجن هو ١،٧٩، ١،٣٨، ٠،٥٠ ، بينما كان للآباء ٠،٠٦، ٠،٠٦، ٠،٠٦ ، على التوالى. أظهر صنفين وخمسة هجن قدرة على إستيلاد النباتات الخضراء. وسجلت أعلى قدرة على الإستيلاد من ٣٠٠ مليجرام كالوس (٣ نباتات) بواسطة الهجين سدس١ × جميزة ٥، بينما الأقل قدرة (٠،٤٤ نبات) سجلت بواسطة الصنف جيزة ١٦٤. وتعتبر هذه النتائج مساهمة فى إمكانية استحداث تراكيب من القمح تتحمل الجفاف.

المجلة المصرية لتربية النبات ٨: ٢٧٣-٢٨٦ (٢٠٠٤)