Effect of Genotyps and Polyethylene Glycol on Immature Embryo Culture in Wheat

Rehab M. M. Habiba[1]; A. M. El-Adl[1]; S. A. Abd El-Mageed[2] and Kh. A. A. Gomaa[1]
[1]**Dept. of Genetics, Fac. of Agric., Mansoura University, Egypt**
[2]**Wheat Res. Dept., Field Crops Res. Inst., ARC, Egypt**

ABSTRACT

Seven wheat genotypes were used in this investigation to study the effect of genotypes, levels of polyethylene glycol (PEG) concentrations and their interaction on immature embryo induction, as well as, estimating heritability for *in vitro* traits. The genotypes were *in vitro* evaluated for immature embryos culture ability at four levels of PEG concentrations. Significant differences between genotypes were obtained for some traits at four PEG levels. Levels of mean squares were significant for some traits such as number of green point, plant length and number of tillers. Whereas, mean squares of interaction between genotypes × PEG levels was insignificant for all traits except number of tillers. The results demonstrated that the greatest percentages for green points and plant weight were recorded by the hybrid Sakha 93 × Gemmiza 9. In addition, Sakha 93 × Sids 1 was the best genotype for root length and number of tillers. Whereas, the greatest value for number of plantlets was observed in Sakha 93 and sakha 94. The addition of 10 g/l PEG to MS nutrient medium and 1 mg/l NAA and 0.5 mg/l Kinetin in regeneration medium could be the best concentration for immature embryo purpose in wheat. Furthermore, the genetic variations were highly positive for some traits such as number of green point at levels A and B, number of plants at levels A,B and D, plant weight at level A, plant length at level C, root length at level A and number of tillers at levels A,B and D. The values of heritability were ranged between 53.66 to 90.48% for number of tillers at levels D and A, respectively. The results indicated that the genes controlled growing traits in wheat were highly affected by media composition.

Keywords: Wheat, immature embryo culture, polyethylene glycol.

INTRODUCTION

Wheat is one of the most important cereal crops in the world. There are several species of *Triticum*. These species fall into three distinct groups, such as Diploids, Tetraploids and Hexaploids with 14, 28, and 42 chromosomes, respectively, according to (Martin & Leonard 1963).

In Egypt, 1.26 million hectares were planted with wheat in 2017 with average yielding 8.8 million tons. However, this is not enough for Egyptian population consumption, it is necessary decreasing the gap between the local production and the consumption. The improved cultivar is one of the main factors increases crop productivity.

The calli derived from immature embryo and immature inflorescences of *Triticum* have higher ability of differentiation and regeneration than those from other explant tissues (Bi and Wang 2008).

In plant cell cultures, water stress can occur by adding osmotic, such as polyethylene glycol, mannitol, sorbitol and sucrose. Polyethylene glycol is one of the most common methods that depends on the use of high molecular weight of osmotic substances (Rao and Jabeen 2013). These agents have no detrimental or toxic effects on the plant; they inhibit the plant's growth by reducing the water potential of the culture medium in a way similar to soil drying, so that cultured explants are unable to take up water (Bressan *et al.*, 1981).

Several studies have been reported on the effect of polyethylene glycol (PEG) on callus induction and plant regeneration from explants of graminious species (Farshadfar & Amiri 2016 and Kacem *et al.*, 2017). In most of these studies, an optimal concentration of PEG for callus induction and growth was investigated which varied with the species.

Thus, this investigation aimed to study the effect of genotypes, levels of PEG concentrations and their interaction on immature embryo induction in whea, as well as, estimating heritability for *in vitro* traits.

MATERIALS AND METHODS

Plant material

Four bread wheat genotypes (*Triticum aestivum* L.), which were chosen according to their degree of drought tolerance to be used in this study. They are included Sakha 93 (Tolerant), Sids 1 (Tolerant), Sakha 94 (Sensitive) and Gemmiza 9 (Sensitive), as well as, three crosses derived from these parents have been used as follows:

Cross 1 = (Sakha 93× Sids 1) Tolerant x Tolerant
Cross 2 = (Sakha 93×Gemmiza 9) Tolerant x Sensitive
Cross 3 = (Sakha 94×Gemmiza 9) Sensitive x Sensitive

All genotypes were supplied by Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Embryo culture procedure

During winter season of 2016/2017, seeds of genotypes the studied varieties and crosses were cultivated at the Experimental Farm of Sids, Agricultural Research Station, Benysweif, Egypt. Young spikes were collected about 15 days post anthesis from each genotype. Immature caryopses were surface sterilized under sterile conditions by immersing them for one minute in 75% ethanol followed by immersion in 0.1% mercuric chloride solution with 2 drops of Tween 20 as a wetting agent for 20 minutes. Then, it was rinsed three times in sterile double distilled water. Immature embryos were excised from each grain aseptically and cultured with the scutellm side up on induction medium under sterile conditions. The induction medium used in this study was MS medium, which recommended by Murashige and Skoog (1962) containing 3%. The pH was adjusted to 5.8 before autoclaving at 121^0C for 20 minutes. The cultures were incubated in darkness at $25^oC \pm 2^oC$ for four weeks. The produced calli were transferred to regeneration medium with 1 mg/l NAA, 0.5 mg/l Kinetin and supplemented with four different concentrations of PEG as the following;

A: MS medium with 0 g/l PEG
B: MS medium with 10 g/l PEG
C: MS medium with 20 g/l PEG
C: MS medium with 30 g/l PEG

The cultures were kept under 16 hour's illumination (fluorescent light) at 22°C ± 2°C for four weeks. The regenerable calli, which have green shoot primordial (G.P) were counted and transferred to the same regeneration medium at the same condition for shoot development to four weeks. When the regenerated plants were about 5-8 cm length and have normal roots and shoots, they were carefully pulled up from the containers and their roots were washed with tap water to remove the remained parts of medium. Then, the total number of plantlet (NP), plant weight (PW), plant length (PL), root length (RL) and number of tillers (NT) were recorded according to Habiba *et al.,* (2013).

Experimental design

The experiments were designed as a complete randomized system for all genotypes used under four different levels of PEG. Sterilized specific MS medium autoclaved at 121°C, 1.5Ib/inch^2 for 20 min was distributed into culture Petri dishes (12 cm inner diameter), each one contained 20 ml. One Petri dish containing 10 sterilized immature embryo is considered as one experimental unit according to Habiba *et al.,* (2013).

Statistical analysis

All data were subjected to analysis of variance according to Steel and Torrie (1960). Subsequently partition of phenotypic variances to its components were done.

RESULTS AND DISCUSSION

Analysis of variance

As shown in Table 1, test of significance on the genotypes mean squares at the four levels of PEG indicated significant differences between genotypes for some *in vitro* traits with respect to the four PEG levels such as number of green point, plant weight and number of tillers at level A; number of green point and number of plants at level B, plant length at level C and number of plants at level D. In addition, the data of all genotypes obtained from PEG levels were set up in a combined analysis of variance and the obtained results are presented in Table 2. Genotypes mean squares were highly significant for all *in vitro* traits except plant weight and root length. These traits were: number of green point (GP), number of plants (NP), plant length (PL) and number of tillers (NT).

Table 1. Analysis of variance and genotypes mean squares at the four levels of PEG.

SOV	PEG levels	Df	GP	NP	PW (g)	PL (cm)	RL (cm)	NT
Replicates	A	2	0.110	0.057	0.023	6.588	0.355	0.011
	B		0.039	0.0004	0.285	68.260	1.646	1.622
	C		0.271	0.119	1.162	5.609	0.640	0.195
	D		0.155	0.001	0.005	4.028	0.163	0.021
Genotypes	A	6	1.873**	0.081	0.058*	5.539	0.614	0.188**
	B		1.887**	0.065**	0.143	40.090	0.454	1.959
	C		0.514	0.110	1.470	8.896*	0.793	0.124
	D		0.783	0.152**	0.017	6.689	0.119	0.121
Error	A	12	0.346	0.028	0.018	4.138	0.257	0.018
	B		0.257	0.011	0.100	26.580	1.002	0.725
	C		0.722	0.067	1.180	2.076	0.412	0.096
	D		0.613	0.027	0.011	6.448	0.258	0.620

Notes:
*,** Significant at 0.05 and 0.01 levels of probability, respectively.
A: MS medium with 0 g/l PEG.   B: MS medium with 10 g/l PEG.   C: MS medium with 20 g/l PEG.
D: MS medium with 30 g/l PEG.   GP: number of green point   NP: number of plants   PW: plant weigh
PL: plant length   RL: root length   NT: number of tillers

Table 2. Combined analysis of variance and genotypes mean squares, PEG levels and their interactions for all *in vitro* traits.

SOV	df	GP	NP	PW (g)	PL(cm)	RL (cm)	NT
Levels (L)	3	1.900*	0.080	0.471	32.995*	0.856	0.932*
Reps / L	8	0.144	0.044	0.369	21.247*	0.701	0.462
Genotypes(G)	6	3.253**	0.284**	0.455	35.099**	0.939	1.031**
G × L	18	0.601	0.041	0.411	8.705	0.347	0.454*
Error	48	0.485	0.033	0.327	9.809	0.482	0.225

Notes:
*,** Significant at 0.05 and 0.01 levels of probability, respectively.
GP: number of green point   NP: number of plants   PW: plant weigh
PL: plant length   RL: root length   NT: number of tillers

These findings indicate the presence of real differences among these genotypes. Therefore, the planned comparisons between genotypes and the partition of the phenotypic variance to its components are valid. Furthermore, levels mean squares were significant with respect to some *in vitro* traits such as; number of green point, plant length and number of tillers. Whereas, genotype × levels mean squares were insignificant for all traits except

number of tillers. This indicated that all genotypes gave the same response at different PEG levels except number of tillers. In this respect, Khodadadi *et al.,* (2014) found that the effects of genotype and also drought stress levels on the regeneration percentage appeared significant effects at 1% probability level. In addition, Kacem *et al.,* (2017) observed significant differences among genotypes and PEG levels as well as their interactions for all traits.

Mean performance of genotypes for *in vitro* traits

Means of all *in vitro* traits at four levels of PEG are presented in Table 3. The results showed that no specific variety was superior or inferior for all studied traits at all levels of PEG. However, at level A, the greatest means of plant weight, plant length and number of tillers were observed in sakha 94 with means of 0.516, 8.260 and 0.889, respectively. Greatest means green point number and root length were observed in Sakha 93 × Gemmiza 9 with means of 3.167 and 1.924, respectively. The greatest mean value for number of plants was observed in sakha 93 with mean of 0.746. The lowest mean values for plant weight, plant length and number of tillers were observed in sakha 93 with means of 0.136, 4.130 and 0.230, respectively. The lowest mean value for number of green point was recorded in Sakha 94 with mean 1.000 and the lowest mean value for number of plants was observed in Sakha 93 × Sids 1 with mean of 0.285. Whereas, lowest mean value for root length was observed in Gemmiza 9 × Sakha 94 with mean of 0.810.

At level B, the greatest mean values for number of green point, root length and number of tillers were recorded in Sakha 93 × Sids 1 with means of 3.583, 1.833 and 2.500, respectively. The greatest mean values for plant weight and plant length were recorded in Gemmiza 9 × Sakha 94 with means of 0.685 and 13.500, respectively. Whereas, Sakha 93 gave the greatest value for number of plants with mean of 0.537. On the other hand, the lowest mean value for number of green point was recorded in Sakha94 with mean of 1.250, number of plants was recorded in Sakha 93 × Sids 1 with mean of 0.118, plant weight was recorded in Gemmiza9 with mean of 0.228, plant length was recorded in Sakha 93 × Gemmiza 9 with mean of 4.660, root length was recorded in Sids1 with

mean of 0.863 and number of tillers was recorded in Sakha 93 with mean of 0.333.

At level C, the greatest mean values for plant weight and number of tillers were recorded in Sakha 93 × Gemmiza 9 with means of 2.171 and 0.986; respectively. In addition, the greatest mean value for; plant length was recorded in Sakha 93 × Sids 1 with mean of 8.833, number of green point was recorded in Gemmiza 9 × Sakha 94 with mean of 2.417. Whereas, Sakha 93 gave the greatest value for number of plants with mean of 0.738, as well as, Gemmiza 9 gave the greatest value for root length with mean of 1.979. On the other hand, the lowest mean values for number of green point, plant weight, plant length, root length and number of tillers were recorded in Sakha 93 with means of 1.083, 0.214, 4.763, 0.510 and 0.400 respectively. While, the lowest mean value for number of plants was recorded in Gemmiza 9 × Sakha 94 with mean of 0.255.

At level D, the greatest mean values for number of green point and plant length were recorded in Sids1 with means of 2.500 and 7.670, respectively. The greatest mean value for; number of plants was recorded in Sakha 93 with mean of 0.889, root length was recorded in Gemmiza9 with mean of 1.088. Whereas, Sakha 93 × Gemmiza 9 gave the greatest value for plant weight with mean of 0.326, Sakha 93 × Sids 1 gave the greatest value for number of tillers with mean of 0.796. On the other hand, the lowest mean values for number of green point, plant length and root length were recorded in Sakha 93 with means of 1.083, 4.370 and 0.593, respectively. The lowest mean values for plant weight and number of tillers were recorded in Gemmiza 9 with means of 0.167 and 0.278, respectively. Whereas, Sids1 gave the lowest mean value for number of plants with mean of 0.230.

Table 3. Mean performance of genotypes for all *in vitro* traits at different PEG levels.

Genotypes	Plant length (cm)				Root length (cm)				Number of tillers			
	A	B	C	D	A	B	C	D	A	B	C	D
Sids 1	5.340[a]	5.380[a]	5.074[b]	7.670[a]	0.979[ab]	0.863[a]	0.815[ab]	0.917[a]	0.597[b]	0.539[b]	0.407[a]	0.583[abc]
Sakha 93	4.130[a]	5.920[a]	4.763[b]	4.370[a]	0.945[ab]	0.917[a]	0.510[b]	0.593[a]	0.230[c]	0.333[b]	0.400[a]	0.319[bc]
Sakha 94	8.260[a]	10.940[a]	8.398[b]	7.440[a]	1.593[ab]	1.639[a]	0.880[ab]	0.778[a]	0.889[a]	0.750[b]	0.519[a]	0.556[abc]
Gemmiza 9	5.520[a]	6.330[a]	6.646[ab]	4.370[a]	0.981[ab]	1.222[a]	1.979[a]	1.088[a]	0.289[c]	0.370[b]	0.500[a]	0.278[c]
Sakha 93 × Gemmiza 9	4.890[a]	4.660[a]	4.786[b]	5.170[a]	1.924[a]	1.116[a]	1.653[ab]	1.083[a]	0.639[b]	0.664[b]	0.986[a]	0.750[ab]
Sakha 93 × Sids 1	6.830[a]	12.170[a]	8.833[a]	6.700[a]	1.731[ab]	1.833[a]	1.333[ab]	1.000[a]	0.734[ab]	2.500[a]	0.667[a]	0.796[a]
Gemmiza 9 × Sakha 94	5.510[a]	13.500[a]	7.370[ab]	4.420[a]	0.810[b]	1.667[a]	1.009[ab]	0.667[a]	0.326[c]	1.667[ab]	0.528[a]	0.667[abc]

Genotypes	Number of green point				Number of plants				Plant weight (g)			
	A	B	C	D	A	B	C	D	A	B	C	D
Sids 1	2.083[bc]	2.500[b]	1.833[a]	2.500[a]	0.499[ab]	0.395[ab]	0.483[a]	0.230[b]	0.295[abc]	0.317[a]	0.320[a]	0.228[a]
Sakha 93	1.583[cd]	1.583[c]	1.083[a]	1.083[a]	0.746[a]	0.537[a]	0.738[a]	0.889[a]	0.136[c]	0.240[a]	0.214[a]	0.174[a]
Sakha 94	1.000[d]	1.250[c]	1.917[a]	1.250[a]	0.500[ab]	0.389[ab]	0.304[a]	0.450[b]	0.516[a]	0.680[a]	0.339[a]	0.335[a]
Gemmiza 9	3.000[ab]	2.500[b]	2.000[a]	2.083[a]	0.329[b]	0.308[bc]	0.328[a]	0.450[b]	0.235[bc]	0.228[a]	0.329[a]	0.167[a]
Sakha 93 × Gemmiza 9	3.167[a]	2.833[ab]	2.167[a]	2.083[a]	0.289[b]	0.274[bcd]	0.622[a]	0.255[b]	0.337[abc]	0.245[a]	2.171[a]	0.326[a]
Sakha 93 × Sids 1	2.750[ab]	3.583[a]	2.000[a]	1.500[a]	0.285[b]	0.118[d]	0.261[a]	0.384[b]	0.476[ab]	0.614[a]	0.410[a]	0.308[a]
Gemmiza 9 × Sakha 94	2.000[bcd]	2.833[ab]	2.417[a]	1.917[a]	0.474[ab]	0.147[cd]	0.255[a]	0.290[b]	0.216[bc]	0.685[a]	0.340[a]	0.183[a]

Notes:
Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.
A: MS medium with 0 g/l PEG.   B: MS medium with 10 g/l PEG.
C: MS medium with 20 g/l PEG.   D: MS medium with 30 g/l PEG.

Since, genotypes gave different performance with different PEG levels as observed above for most of studied *in vitro* traits. So, the combined data over the four PEG levels could be more precise to present information concerning the behavior of these genotypes. Therefore, the

average means of seven genotypes were determined from the combined data over PEG levels and the results are shown in Table 4. The results revealed that there are significant differences between paired of means in most traits. The results cleared that greatest values for green

points and plant weight were recorded in Sakha 93 × Gemmiza 9 with means 2.563 and 0.770, respectively. In addition, Sakha 93 × Sids 1 was the best genotype for root length and number of tillers. Whereas, the greatest value for number of plantlets was observed in Sakha 93 with mean of 0.728, however sakha 94 was the best genotype for plant length with mean of 8.762. On the other hand, Sakha 93 appeared to be the wariest genotype for immature embryos culture purpose, which gave poorest response to this process for all studied traits except for number of plantlets among four levels of PEG. Abdelsamad *et al.,* (2007) found that the greatest mean value for percentage of regeneration was recorded at 10% PEG in Sakha 93 and DH-1 with means of 38.2 and 30.1%, respectively, while the lowest mean values were recorded in DH-2 and Giza 168 with means of 32.4 and 12.5%, respectively. At 20% PEG levels were significantly decreased in regeneration mean percentages in all genotypes. Sakha 93 gave the greatest value for plant regeneration with mean of 24.3%. Helaly *et al.,* (2103) found that Misr 1 showed the highest green point percentage at 6000 mg/l PEG , whereas Sakha 94 was the lowest in this respect.

Table 4. Mean performance of genotypes for all *in vitro* traits from the combined data of different levels of PEG.

Genotypes	GP	NP	PW (g)	PL (cm)	RL (cm)	NT
Sids 1	2.229[a]	0.402[b]	0.290[ab]	5.865[bc]	0.894[ab]	0.532[bcd]
Sakha 93	1.333[b]	0.728[a]	0.191[b]	4.796[c]	0.741[b]	0.321[d]
Sakha 94	1.354[b]	0.411[b]	0.467[ab]	8.762[a]	1.222[ab]	0.678[bcd]
Gemmiza 9	2.396[a]	0.354[b]	0.240[b]	5.718[bc]	1.318[ab]	0.359[cd]
Sakha 93 × Gemmiza 9	2.563[a]	0.360[b]	0.770[a]	4.877[c]	1.444[a]	0.760[bc]
Sakha 93 × Sids 1	2.458[a]	0.262[b]	0.452[ab]	8.633[a]	1.475[a]	1.174[a]
Gemmiza 9 × Sakha 94	2.292[a]	0.291[b]	0.356[ab]	7.698[ab]	1.038[ab]	0.797[a]

Notes: Means followed by the same letter in the same column are not significantly different at 0.05 level of probability.
GP: number of green point   NP: number of plants   PW: plant weight
PL: plant length   RL: root length   NT: number of tillers

Effect of PEG levels

Owing to PEG effect as observed earlier, it could be more informative to average the performances of all genotypes over each level. Therefore, the means of PEG levels for all genotypes *in vitro* traits are presented in Table 5. The results appeared the greatest values at level B for all traits except number of plantlets and plant weight. These findings revealed that the addition of 10 g/l PEG to MS nutrient medium and 1 mg/l NAA and 0.5 mg/l Kinetin in regeneration medium could be the best concentration for immature embryo culture purpose in wheat. This agreed with Jin Kang *et al.,* (2003), who found that the low PEG level resulted in more green plants than either the control (zero PEG) or high PEG level, although no significant effect was found. Whereas, Kacem *et al.,* (2017) found that increase in PEG levels reduced the number of calli. While, increase in PEG levels increased the time required for callus induction as well as frequency of embryogenic structures and number of plants.

Table 5. The PEG levels averaged overall genotypes for *in vitro* traits.

Levels of PEG added to MS medium	GP	NP	PW (g)	PL (cm)	RL (cm)	NT
A	2.226[ab]	0.446[a]	0.316[a]	5.783[b]	1.280[a]	0.529[b]
B	2.440[a]	0.310[a]	0.430[a]	8.414[a]	1.322[a]	0.975[a]
C	1.917[ab]	0.427[a]	0.589[a]	6.553[ab]	1.168[a]	0.572[b]
D	1.774[b]	0.421[a]	0.246[a]	5.734[b]	0.875[a]	0.564[b]

Notes: Means followed by the same letter in the same column are not significantly different at 0.05 level of probability.
GP: number of green point   NP: number of plants   PW: plant weight
PL: plant length   RL: root length   NT: number of tillers

Genetic parameters

Genetic variation and heritability in broad (H_b) sense were estimated within each PEG level for all traits as shown in Table 6. The results revealed that the magnitude of genetic variation was positive for all *in vitro* traits at four levels of PEG except for number of green point at level C and root length at levels B and D. The results showed that genetic variation was high for some traits at four levels of PEG concentration such as number of green point at levels A and B, number of plants at levels A,B and D, plant weight at level A, plant length at level C, root length at level A and number of tillers at levels A,B and D. These results confirmed by the values of heritability, which ranged from 53.66 to 90.48% for number of tillers at levels D and A, respectively.

The relative magnitudes of the parameters under investigation were estimated for all *in vitro* traits from the combined data over PEG levels and the obtained results are shown in Table 7. The results revealed that the genetic variation were positively decreased in all traits except for number of green point and number of plants. In addition, genetic the values by interaction variations were positively decreased in all traits except for plant length and root length which give negative values. This finding explain the low values of heritability in these traits, as well as, the genes controling these traits are highly affected by media composition. Hamada *et al.,* (2002) studied the diallel and cluster analysis for some quantitative characters in bread wheat and found that broad sense heritability was high for all traits. However, narrow sense heritability was high for most traits. Darwish and Ashoush (2003) tested two crosses and their six populations of bread wheat for grain yield, yield components and some growth attributes. Heritability in broad sense was high to moderate in magnitude, however, in narrow sense the values were high to low in its magnitude. Hendawy (2003) studied the genetic architecture of yield and its components and some other agronomic traits in bread wheat and reported that

high heritability values in broad sense were recorded for most traits in the three crosses, although, high to moderate heritability values in narrow sense were also obtained for most traits in the three crosses. El-Borhamy (2004) studied performance of six genotypes and their 15 F_1 hybrids of wheat under drought stress for plant height, grain yield and yield components. He found that heritability in narrow sense was low in most traits. Tammam (2005) studied six generation in wheat under different environmental conditions and reported that heritability in broad sense was high to moderate for studied traits under both treatments, however, heritability in narrow sense was low to moderate for the some traits under normal and drought treatments.

El-Hag (2006) estimated of genetic parameters for earliness and some agronomic characters in three crosses of bread wheat and obtained moderate to high broad and narrow sense heritability estimates were obtained for most traits in three crosses. Abd El-Rahman and hammad (2009) estimated of some genetic parameters for some agronomic characteristics in three crosses of bread wheat and found that heritability in broad and narrow sense were generally moderate to high in most traits in three crosses. Aboshosha and Hammad (2009) estimated of genetic parameters for yield and yield components as well as some agronomic characters in wheat and found that heritability in narrow sense was moderate to low in all traits.

Table 6. Estimates of relative magnitudes of different genetic parameters for *in vitro* traits at different levels of PEG.

Genetic Parameters	Number of green point				Number of plants				Plant weight (g)			
	A	B	C	D	A	B	C	D	A	B	C	D
$\sigma^2 g$	0.520	0.554	-0.048	0.078	0.016	0.018	0.012	0.043	0.013	0.005	0.098	0.002
$\sigma^2 e$	0.104	0.075	0.219	0.183	0.011	0.003	0.025	0.008	0.006	0.042	0.392	0.003
$\sigma^2 ph$	0.624	0.629	0.171	0.261	0.027	0.021	0.037	0.051	0.019	0.047	0.490	0.005
Hb %	83.33	88.08	0.00	29.89	59.26	85.71	32.43	84.31	68.42	10.64	20.00	40.00

Genetic Parameters	Plant length (cm)				Root length (cm)				Number of tillers			
	A	B	C	D	A	B	C	D	A	B	C	D
$\sigma^2 g$	0.350	2.497	2.105	0.195	0.114	-0.213	0.116	-0.042	0.057	0.369	0.005	0.022
$\sigma^2 e$	1.496	10.867	0.860	2.034	0.090	0.365	0.148	0.082	0.006	0.284	0.037	0.019
$\sigma^2 ph$	1.846	13.364	2.965	2.229	0.204	0.152	0.264	0.040	0.063	0.653	0.042	0.041
Hb %	18.96	18.68	70.99	8.75	55.88	0.00	43.94	0.00	90.48	56.51	11.90	53.66

Notes:

A: MS medium with 0 g/l PEG

B: MS medium with 10 g/l PEG

C: MS medium with 20 g/l PEG

D: MS medium with 30 g/l PEG

$\sigma^2 g$: genotypic variance

$\sigma^2 e$: environment variance

$\sigma^2 ph$: phenotypic variance

Hb%: heritability in broad sense

Table 7. Estimates of relative magnitudes for different genetic parameters from the combined data over PEG levels.

Genetic Parameters	GP	NP	PW (g)	PL (cm)	RL (cm)	NT
$\sigma^2 g$	0.221	0.020	0.004	2.200	0.049	0.048
$\sigma^2 Gl$	0.041	0.002	0.030	-0.542	-0.052	0.067
$\sigma^2 e$	0.159	0.012	0.107	3.443	0.168	0.084
$\sigma^2 ph$	0.391	0.032	0.118	5.508	0.204	0.149
Hb %	56.58	62.47	3.10	39.94	24.18	32.29

Notes:

GP: number of green point

NP: number of plants

PW: plant weigh

PL: plant length

RL: root length

NT: number of tillers

REFERENCES

Abd El-Rahman, E. and S. M. Hammad. (2009). Estimation of some genetic parameters for some agronomic characteristics in three crosses of bread wheat . J. Agric. Sci., Mansoura Univ., 34(2): 1091-1100

Abdelsamad, A; O. E. El-Sayed and Hayam, F. Ibrahim (2007). Development of drought tolerant double haploid wheat using biochemical genetic markers on *In vitro* culture. J. of Applied Sci. Research, 3 (11): 1589-1599.

Aboshosha, A.A.M. and S.M. Hammad (2009). Estimation of parameters for yield and yield components and some agronomic characters in two crosses of bread wheat (*Triticum aestivum* L.). J. Agric. Sci., Mansoura Univ., 34(5): 4293-4300.

Bi, R. and H. Wang (2008). Primary studies on tissue culture from mature embryos in diploid and tetraploid wheat. Front. Agric. Chin. 2: 262–265.

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.

Darwish, I.H.I. and H.A. Ashoush (2003). Hetersois, gene effects, heritability and genetic advance in bread wheat. Minufiya J. Agric. Res.28: 433-444.

EL-Borhamy, H.S. (2004). Genetic analysis of some drought and yield related characters in spring wheat varieties (*Triticum aestivum* L.). J. Agric. Sci., Mansoura Univ., 29 (7):3719-3729.

El-Hag, A.A. (2006). Estimation of genetic parameters for earliness and some agronomic characters in three crosses of bread wheat, (*Triticum aestivum* L.). J. Agric. Sci., Mansoura Univ., 31(7): 4271-4280.

Farshadfar, E. and R. Amiri (2016). *In vitro* application of integrated selection index for screening drought tolerant genotypes in common wheat. Acta agriculturae Slovenica. 107: 335 – 344.

Habiba, Rehab M. M.; M. M. Abd El- Maksoud and Kh. A. A. Gomaa (2013). Effect of genotyps and their interaction with 2,4 dichlorophenoxy acetic acid (2,4-d) levels on wheat́s immature embryo culture response. Egypt. J. Genetic. Cytol., 42: 37-52.

Hamada, A. A.; N.A. Mohamed and F. Ashmawy (2002). Diallel and cluster analysis for some quantitative characters in bread wheat (*Triticum aestivum* L.). J. Agric. Sci., Mansoura Univ., 27: 5719-5734.

Helaly, M. N.; Z. A. Mohamed; R. A. Fouda and Sally A. A. Arafa (2013). In vitro studies on bread wheat genotypes for drought tolerance using polyethylene glycol. J. Plant Production, Mansoura Univ., 4 (4): 605 – 620.

Hendawy, H.I. (2003). Genetic architecture of yield and its components and some other agronomic traits in bread wheat. Minufiya J. Agric. Res., 28: 71-86.

Jin Kang, T.; M. Sik Yang and E. L. Deckard (2003). The effect of osmotic potential on anther culture in spring wheat (*Triticum aestivum*). Plant Cell, Tissue and Organ Culture 75: 35–40.

Kacem, N. S.; F. Delporte; Y. Muhovski; A. Djekoun and B. Watillon (2017). *In vitro* screening of durum wheat against water stress mediated through polyethylene glycol. Journal of Genetic Engineering and Biotechnology 15: 239–247.

Khodadadi, E.; L. fahmideh and B. Fakheri (2014). Evaluation of osmotic stress tolerance in wheat genotypes and solidarity of assessed traits using *in vitro* mature embryos culture. International Journal of Geology, Agriculture and Environmental Sciences 2: 1-7.

Martin, J. H. and W. H. Leonard (1963). Cereal Crops. The Macmillan Company New York. pp. 275-287.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15: 473-497.

Rao, S. and F. T. Z. Jabeen (2013). *In vitro* selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (Saccharum officinarum L.). Physiol. Mol. Biol. Plants 19: 261–268.

Steel, R. G. and J. H. Torrie (1960). Principles and procedures of statistics. Mc-Graw Hill book Company, INC. New York.

Tammam A. M. (2005). Generation mean analysis in bread wheat under different environmental conditions. Minufiya J. Agric. Res. 30 (3): 937-956.

تأثير التراكيب الوراثية والبولى إيثيلين جليكول على زراعة الأجنة الغير ناضجة فى القمح

رحاب محمد محمد حبيبة[1]، على ماهر العدل[1]، صلاح الدين أحمد عبد المجيد[2] و خالد أحمد أحمد جمعة[1]

[1] قسم الوراثة ـ كلية الزراعة ـ جامعة المنصورة ـ مصر

[2] قسم بحوث القمح ـ معهد بحوث المحاصيل الحقلية ـ مركز البحوث الزراعية ـ مصر

فى هذه الدراسة تم استخدام سبعة تراكيب وراثية لدراسة تأثير التراكيب الوراثية والتركيزات المختلفة من (البولى إيثيلين جليكول PEG) والتداخل بينهما على قابلية زراعة الأجنة غير الناضجة فى القمح ، وكذلك قياس معامل التوريث للصفات المعملية. وقد قيمت هذه التراكيب الوراثية معملياً لقابليتها لزراعة الأجنة غير الناضجة على بيئة MS مع إضافة أربعة تركيزات مختلفة من PEG. وجود اختلافات معنوية بين التراكيب الوراثية لبعض الصفات فى التركيزات الأربعه من PEG. كانت مستويات PEG معنوية فيما يتعلق ببعض الصفات المدروسة مثل عدد البقع الخضراء ، طول النبات وعدد الأشطاء. فى حين كان التداخل بين التراكيب الوراثية ومستويات PEG غير معنوى لكل الصفات ماعدا عدد الأشطاء. أوضحت النتائج أن أفضل قيم لعدد البقع الخضراء ووزن النبات سجلت للهجين سخا ٩٣ × جميزه ٩. بالإضافة إلى ذلك ، سخا ٩٣ × سدس ١ كان أفضل تركيب وراثى لطول الجذر وعدد الأشطاء. فى حين كانت أفضل قيم لعدد النباتات لوحظت فى سخا ٩٣ ، وكان سخا ٩٤ أفضل تركيب وراثى لطول النبات. إضافة ١٠ جم/لتر من PEG للبيئة المغذية MS مع إضافة ١مجم NAA و ٠.٥ مجم Kinetin فى بيئة Regeneration هى أحسن تركيزات لغرض زراعة الجنين غير الناضج فى القمح. علاوة على ذلك كانت قيم التباين الوراثى موجبة وعالية لبعض الصفات المدروسة مثل عدد البقع الخضراء فى التركيز الأول والثانى ، وعدد النباتات فى التركيز الأول والثانى والرابع ، ووزن النبات فى التركيز الاول ، طول النبات فى التركيز الثالث وطول الجذر فى التركيز الأول ، وعدد الأشطاء فى التركيز الأول والثانى والرابع. قيم معامل التوريث كانت تتراوح مابين ٥٣.٦٦ إلى ٩٠.٤٨ لعدد الأشطاء فى التركيز الرابع والأول على التوالى. أشارت النتائج أن الجينات التي تتحكم فى صفات النمو في القمح تأثرت بشدة بواسطة مكونات البيئة.