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IN VITRO STUDIES ON BREAD WHEAT GENOTYPES FOR DROUGHT TOLERANCE USING POLYETHYLENE GLYCOL

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ABSTRACT

Calli characters and its regeneration of 8 bread wheat genotypes were evaluated under water stress using PEG. Certain physiological aspects including ; chlorophylls, total sugars and proline content were also examined. The data showed a significant differences between the examined genotypes in response to drought stress conditions. However, genotypes Gemmeiza 10, Misr 1 and Gemmeiza 7 were found to be more stable against drought conditions in comparison with the other examined genotypes and genotypes Sakha 8 and Sakha 94 seemed to be more sensitive in this respect.

INTRODUCTION

Wheat is used mainly for human consumption and supported nearly 35% of the world population (Fao, 2012). It is nutritious, easy to store and transport and can be processed into various types of food. The demand for wheat is expected to grow faster than any other major Agricultural crops. To meet the needs of the growing world population the forecast demand for the year 2020 varies between 840 to 1050 million tons. Due to land limitation, the enhancement of wheat production must come from higher absolute yield, which can only be met by the concreted action of scientists involved in diverse agricultural disciplines and in particular by increased efforts in plant breeding .

Abiotic stress resulting from drought remains an ever-growing problem that has adverse effect on growth and crop production worldwide and causes important agricultural losses particularly in arid and semi-arid areas. The percentage of drought affected land areas more than doubled from the 1970_s to the early 2000_s in the world (Isendahl and Schmidt, 2006). Moreover, drought is multifaced stress conditions with respect to timing and severity, ranging from long drought seasons where rainfall is much lower than demand , to short period without rain where plants depend completely on available soil water (Lafitte *et al*., 2006). In addition, changing weather patterns and worldwide water shortage will likely result in irrigated wheat being grown with the loss of applied water and increasing the likelihood of a soil water deficit (Rebetzke *et al*.,2006).

Wheat as like as other cereal plants respond to drought through a variety of changes from morphological, physiological and metabolic modifications occurring in all organs to gene expression (Shao, *et al*., 2006). The physiological response to drought stress arises from the changes in the cellular gene expression profile and a number of genes are induced by exposure to such conditions (Shao *et al*., 2006 and Mafakheri *et al*., 2010).

Development of wheat cultivars with high yield is the main goal of water limited environments but success has been modest due to the varying nature of drought and the complexity of genetic control of plant responses (Mirbahar *et al*., 2009). Efforts have been focused mostly on induction of drought tolerance and exploiting high yield potential. Moreover, genotype selection for morphological, physiological and agronomic traits indicative of drought tolerance in field conditions is a major challenge to geneticists and breeders (Dhanda *et al*., 2004 and Mirbahar *et al*., 2009).

Selection of wheat varieties is always challenging and understanding of the genotyping characteristics and relationships of the germplasm is still very limited which stuck their effective exploitation.

The success of in vitro culture of wheat and other monocot plants depends mainly on the growth conditions of the source material (Delporte *et al*., 2001), medium composition and culture conditions (Saharan *et al*., 2004), and on the genotypes of donor plants. Among these factors, the genotype appears to be important factor influencing the efficiency of in vitro culture. In *Triticum*, for the explants with same age and the same growth regulator combination, callus production and plant regeneration capacity depend essentially on genotype (Zale *et al*., 2004). Immature embryo was reported in wheat as the best explant for callus induction and shoot regeneration (Arzani and Mirodjagh, 1999). However, the availability of immature embryo is limited by wheat growing seasons or requires sophisticated growth chambers.

In recent years, tissue culture based in vitro selection has emerged as a feasible and cost-effective tool for developing stress-tolerant plants. Plants tolerant to the abiotic stresses can be acquired by applying the selecting agents such as PEG or mannitol for drought tolerance in the culture media. The selection of somaclonal variations appearing in the regenerated plants may be genetically stable and useful in crop improvement (Rai *et al*., 2011).

The specific objective of this study was to evaluate eight bread wheat genotypes for drought tolerance, developed through in vitro studies.

MATERIALS AND METHODS

In vitro studies were carried out at the laboratories of plant tissue culture and Agric. Botany, Faculty of Agriculture, Mansoura Univ., Egypt during the period elapsed from 2010-2012.

Plant material:

Grains of wheat (*Triticum aestivum* L.) with different background were secured from the Field Crop Research Institute, Gemmeiza Research Station, ARC, Egypt.

The characteristics of these genotypes are presented in Table (1).

Table (1): The commercial name, identification characteristics and sources of the wheat (*Triticum aestivum* L.) genotypes evaluated in the present investigation.

No.	Commercial Name	Pedigree	Origin
1	Gemmeiza 7	CMH74 A.630/Sx11 Seri. 82/ Agent. C Gm 4611-2 Gm-3 Gm- 1 Gm-0 Gm	Gemmeiza Res. Stat.
2	Gemmeiza 10	Maya 74 "5" /on // 1160 147/31 BB/ Gll/4/ CHAt "5"/5/Crows CGm 5820-3 Gm-1 Gm-2 Gm- 0 Gm	Gemmeiza Res. Stat.
3	Gemmeiza 9	ALD "5"/HUAC // CMH 74 A. 630/Sx C Gm 4583-5 Gm-1 Gm-0 Gm	Gemmeiza Res. Stat.
4	Sakha 93	Sakha 29/TR 810328 S. 8871-IS- Sakha 2S-IS-OS	Sakha Res. Stat.
5	Sakha 94	OPAtA/Rayon // KAUZ. CUBW 90 Y 3180-OTO PM- 10 M-o15 Y- 0Y- 0AP-05.	Gemmeiza Res. Stat.
6	Sakha 8	Cno 67 // SN 64/KLRE/3/8156	Sakha Res. Stat.
7	Giza 168	MRL/BUE/SERI	Agric.Res.Cent**. G**iza
8	Misr 1	OASIS/SKAUZ // 4* BCN/3/2* PASTOR CMSS 00Y0 1881 T-0S0 M-030Y-030 M-03 WGY-33 M-0y-0S	Agric.Res.Cent**. G**iza

Micropropagation procedure:

Explant sources:

Grains of eight wheat genotypes were sown on 3rd Nov. 2010 at the Expt. Stat., Faculty of Agriculture, Mansoura Univ., Egypt. Young spikes were collected about 15 days post-anthesis from each genotype. Immature caryopsis were surface sterilized under sterilized condition by immersion them for 1 min. in 95% ethanol , followed by immersion in 0.1% mercuric chloride solution for 15 min. and rinsed three times with sterile distilled water (Abd El-Maksoud, 2003). The initial explants; immature embryos (2x3 mm) were excised aseptically with a sharp cutter sprayed with ethyl alcohol 70% and used for indirect embryogenesis. They were kept in a refrigerator at 4°C for 2h before culturing. The basal medium for culturing was the Murashige and Skoog; MS (1962) supplemented with 2mg/l of 2.4-Dichlorophenoxy acetic acid (2,4-D).

Drought treatments:

The MS basal nutrient solution was supplemented with or without PEG-MW 6000 (Polyethylene glycol, HO $(C_2H_4O)_nH$, mean mol mass 5000-7000, hydroxyl number 16-23, melting range 56-61 **°C** , India) at four concentrations denoted 0 as a control, 2000,4000 and 6000 ppm.

Ca**llus induction and initiation:**

Immature emberyos were used as explants. They were micropropagated in MS basal media supplemented with 2.0 mg/l 2,4-D. all the operations and inoculation were performed under strict aseptic conditions in a laminar airflow cabinet. The immature embryos were easily separated from the endosperm in imbibed grains and placed scutellum up on MS medium supplemented with 30g/l Sucrose and was adjusted to pH 5.7, solidified with 8 g/l agar and 2.0 mg/l 2,4-D (Merck, Germany). The medium was autoclaved at 121 °C for 20 min. and incubated at 25 °C for 28 days in growth chamber and in the darkness. Callus was maintained by subculturing every 21-28 days on the same MS medium. The specific nutrient media of

each treatment was dispensed into Petri dishes (11cm) at the rate of 20ml/dish. Each treatment was replicated 5 times (5 dishes, all culture dishes were maintained in a complete darkness in the controlled growth room at 25 ± 1 ^{o}C for 4 weeks. The incubated cultures were subcultured two times, 21-28 days intervals into corresponding fresh specific culture media up to the production of swelled and compact calli.

Embryo response percentage (before stress):

It was determined as follows: Number of callus formed / Number of embryos cultured x 100 .

Callus fresh weight was determined expressed as mg F.wt. during the callus induction stage for all genotypes used.

For shoot multiplication, the regenerated embryogenic calli were re-cultured in the same specific media with two sub-culturing (21-28 days) intervals on fresh specific media. The formed primordia were sub-cultured under the same conditions previously mentioned (18/6h day/night). Shoot multiplication was took place till forming shoots having 2-3 foliage leaves.

Visual characterization of callus:

The calli were frequently observed and characterized as follows: A, No browning (excellent) ; B, callus with slightly brown surface (very good); C, whole callus tissue brown with optimum growth (good); D, entire callus tissue deeply brown with suboptional growth (moderate) ; E, whole callus tissue deeply brown with no growth at all (poor) according to Mahmood *et al*., (2012).

Regeneration of percentage (%) of calli after stress:

The calli were cultured on various callus selection media then shifted to generation medium (MS+30g/l sucrose + 8g/l agar + 0.2 mg/l 2,4-D + 0.5 mg/l Kinetin)**.** The calli were incubated at 24^{o}C temperature with (18/6h day / night). Regeneration was took place till forming shoots, having 2-3 foliage leaves and regeneration percentage of calli was computed as follows:

Callus regeneration (green points)% = No. of calli regenerated / Total No. of calli cultured X 100.

The primary calli from each treatment were separated from the initiated explants used. They were re-sub-cultured two times, two weeks intervals. For the embryogenic friable callus stage and green points, the same specific callus initiation media were also used.

The growing morphogenic calli derived from immature embryos of each genotype were exposed to drought stress conditions. They were cultured on the specific media supplemented with 0.5 mg/l Kinetin as the following treatments:

1- MS basal medium (control).
2- MS basal medium + 2000 mg/l PEG.
3- MS basal medium + 4000 mg/l PEG.
4- MS basal medium + 6000 mg/l PEG.

Photosynthetic pigments estimation:

Photosynthetic pigments were extracted for 24h at room temperature in methanol after adding traces of sodium carbonate. Photosynthetic pigment concentrations were determined from fresh material of the examined genotypes Gemmeiza 7, Gemmeiza 10 and Misr 1 whose showed more

tolerance to drought conditions under the present investigation. Determination was done Spectrophotometrically (Spekol 11,UK) according to Wellburn (1994) and expressed as mg/g F.W.

Total soluble carbohydrates: (Total sugars) were extracted from1.0g fresh material taken during the multiplication stage of the examined genotypes cultured under either control or PEG treatments by ground in a mortar with 20 ml ethanol 70% and kept over-night at room temperature (Kayani *et al*., 1990)before being filtered. The residue was extracted three times by heating at 70^{o}C for one hour in water bath with 20 ml ethanol 70%. Filtration took place through filter paper Whatman No.1. activated charcoal was added to the combined extract and evaporated in water bath at 55 ^{o}C till dryness. The dried Film was dissolved in 10 ml of 10% aqueous isopropanol and the filtrate was made up to a known volume, 50 ml, with ethanol.

Total sugars were determined using the method of Sadasivam and Manickam (1996). Briefly, 1ml of supernatant was put into glass tube, and 1ml of phenol solution and 5ml concentrated sulfuric acid were added. The mixture was shaken, and absorbance was read at 490 nm Spectrophotometerically by Milton Roy Spectronic 1201.

Proline determination:

Half gram of the fresh material taken during the shoot multiplication stage was homogenized ground in a morter with salphosalicylic acid 3% w/v. The homogenate was filtered through filter paper Whatman No.1. The extract volume was raised up to 10ml with salphosalicylic acid (Bates *et al*., 1973). Proline was determined by the modified ninhydrine method (Troll and Lindsley, 1955); omitting phosphoric acid to avoid interference with concentrated sugars (Magne and Larker, 1992). Plant materials were placed into test tubes containing distilled water. The tubes were kept for 30 min. in a boiling water bath then cooled at room temperature. To obtain 150 µl of the corresponding water extract, 1ml of ninhydrine reagent was added and maintained in a boiling water bath for 20 min. The mixture was cooled and the product formed was extracted with toluene. Absorbance was measured at 520 nm on a Spectrophotometer by Milton Roy Spectronic 1201. A calibration curve was made with L-proline as a standard.

Statistical analysis:

All data were subjected to analysis of variance according to Steel and Torrie (1980). Subsequently partition of phenotypic variances to its components were done.

RESULTS AND DISCUSSION

Embryo response percentage (before stress):

Data presented in Table (2) indicated that all studied genotypes responded positively forming callus tissues depending on the genotype. Genotype Misr 1 was the most responsive whereas genotype Gemmeiza 9 proved to be less efficient to induce callus as compared to all other genotypes. Similar results were reported by other investigators on different plant responses. Gonzales *et al*., (2001) indicated that wheat calli type and

their products as well as the regeneration of plantlets were affected depending upon genotype and induction medium. The success of in vitro culture depends mainly on the growth conditions of the source material (Delporte *et al*., 2001), medium composition and culture conditions (Saharan *et al*., 2004)and on the genotypes of donor plants. In *Triticum,* for the explants with same age and the same growth regulator combination, callus production and plant regeneration capacity depend essentially on genotype (Zale *et al*., 2004). Results of Mitic *et al*., (2006) indicated factor genotype as the most important for determining regeneration potential in wheat.

Callus fresh weight:

Data presented in Table (2) show that genotypes Gemmeiza 7 followed by Misr 1 was able to produce more callus fresh weight in comparison with all other studied genotypes. However, genotype Sakha 94 was the lowest in this respect. In this connection, wheat calli type and their products were affected depending upon genotype and induction medium (Gonzales *et al*., 2001). The success of in vitro culture depends mainly on the growth conditions of the source material (Delporte *et al*., 2001), medium composition and culture conditions (Saharan *et al*., 2004) and on the genotypes of donor plants. Explants with same age and the same growth regulator combination, callus production depend essentially on wheat genotype (Zale *et al*., 2004).

Table (2): Mean performance for the in vitro wheat immature embryo culture ability.

Parameters / Genotypes	Embryo response % ER%	Callus fresh weight (mg F.Wt.)
Giza 168	0.884 ab	80.35 cd
Sakha 8	0.961 ab	63.25 ed
Gemmeiza 10	0.974 ab	80.08 cd
Gemmeiza 9	0.846 c	36.75 ef
Sakha 93	0.981 ab	106.25 ab
Gemmeiza 7	0.974 ab	144.80 a
Misr 1	1.000 a	132.28 ab
Sakha 94	0.916 ab	28.20 f
LSD at 5%	0.104	27.18

Table (3): Effects of various callus selection media on callus health of wheat studied genotypes (Visual observation).

Genotypes	MS1 control	MS2 2000 mg/l	MS3 4000 mg/l	MS4 6000 mg/l
Giza 168	A(Excellent)	B(very good)	B(good)	C(moderate)
Sakha 8	A(Excellent)	B(very good)	C(good)	D(poor)
Gemmeiza 10	A(Excellent)	A(Excellent)	A(Excellent)	B(very good)
Gemmeiza 9	A(Excellent)	B(very good)	BIgood)	C(moderate)
Sakha 93	A(Excellent)	B(very good)	B(good)	C(moderate)
Gemmeiza 7	A(Excellent)	A(Excellent)	A(Excellent)	B(very good)
Misr 1	A(Excellent)	A(Excellent)	A(Excellent)	B(very good)
Sakha 94	A(Excellent)	B(very good)	C(good)	D(poor)

Visual observation (Callus health):

Generally, increase in callus browning was recorded with increasing level of stress condition. Calli cultured on stress free medium (MS_1) were found to be healthier with unstricted growth (excellent) than those on the stressed media for all studied genotypes. Calli subjected to MS_4 for four weeks exhibited poor health in both genotypes Sakha 8 and Sakha 94 whereas those of genotypes Gemmeiza 10, Gemmeiza 7 and MIsr 1 were maintained very good healthy under such highest stress level. Genotypes Giza 168, Gemmeiza 9 and Sakha 93 showed varying good health in a descending order depending on the stress level used. Callus cultured on MS_1 or MS_2 did not affect callus health and callus looked light yellow to whitish in color with no browning. Whole callus tissues were brown when calli were cultured on MS_4 except those related to genotypes Gemmeiza 10, Gemmeiza 7 and Misr 1 with optimum callus growth .

Callus browning was found to be good indicator of callus sensitivity to PEG-6000 induced osmotic stress. It seemed that tolerant calli had lower browning at higher levels of osmotic stress and survival. At higher level of osmatic stress, tixixity in addition to water paucity may deter cell growth (Mahmood *et al*., 2012).

Exposure of calli for four weeks to MS_4 medium spoiled them with evident necrosis:Necrosis was more on the surface of the callus facing medium. In this connection, Hassan *et al*., (2004) reported that in vitro osmotic stress of -1.0 MPa for eight months of culture onto PEG-6000 containing media is lethal for sunflower calli with evident necrotic tissues on callus surface.

It can be revealed that callus culture of wheat for three to four weeks on MS based media containing PEG-6000 induced osmotic stress of 4000 mg/l had sub-lethal effect on callus health and is selective for calli of genotypes Gemmeiza 10, Gemmeiza 7 and Misr 1, allowing drought tolerant calli to survive. Similar results were reported by Mahmood *et al*., (2012).

Regeneration (%) of stressed calli and plantlets:

The regeneration potential of wheat calli of the different studied genotypes after exposure to PEG-6000 induced osmotic stress is presented in Table (4). The interaction of callus selection media and genotypes was significant. The regeneration potential of calli decreased significantly with increasing osmotic stress in culture media (Table 4). The highest regeneration was witnessed when calli were exposed for media devoid of PEG-6000 . However, in case of Gemmeiza 7 the plantlets percentage was found to be increased as PEG level was raised. The least regeneration was recorded for calli cultured on M_4 medium (6000 ppm) for 21-28 days. However, Giza 168 was the lowest in this respect.

Furthermore Misr 1 showed the highest green point percentage at 6000 mg/l PEG , whereas Sakha 94 was the lowest in this respect.

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Addition of PEG-6000 in solid media lowers water potential of the medium that adversely affect cell division leading to reduced callus growth and consequently influences regeneration (Ehsanpour and Razavizadeh, 2005). Also, regeneration ability of explants is usually decreased by repeated subculture over time in many plants (Dragiiska *et al*., 1996; Mohamed *et al*., 2000).

Regeneration plantlets %:

Data presented in Table (4) show that there were variation response for studied genotypes with reference to plantlet production. However, genotype Gemmeiza 7 was the best one forming green points under stress conditions as compared to all other studied genotypes. Moreover, genotypes Gemmeiza 7 showed more regeneration ability represented by plantlets percentage under the highest PEG level and the response was a PEG – concentration dependent. However, Sakha 94 and Giza 168 were the least efficient in this respect.

Drought stress causes profuse mutation in cellular metabolism including protein functioning and alteration in amount of proteins (Plomion *et al*., 1999). The decrease in regeneration frequency on media with higher osmotic stress may be due to altered gene expression (Visser, 1994) controlling this trait or the genes may express themselves but the resultant proteins may be denaturated due to increased stress. The motives behind regenerative potential loss are not clearly legitimated and may be consequence of malfunctioning or loss of substances supporting regeneration of tissues, epigenetic changes or somaclonal variations (George, 1993).

Chlorophylls content:

Data presented in Table (5) clearly show that Chl.a,b and total Chls as well as a/b ratio were significantly decreased in the three selected genotypes under PEG-induced higher osmotic stress.

Table (5): Effect of high PEG level on photothynthetic pigments (Chls.) concentrations (mg/g F.Wt.) of callus wheat genotypes Gemmeiza 10, Gemmeiza 7 and Misr 1.

Parameters / Genotypes	PEG level mg/l	Chl. a	Chl. b	Total Chl.	Chl. a/b	Chl. a/b%
Gemmeiza 10	0	2.883	0.611	3.494	4.718	471.8
	6000	0.580	0.212	0.792	2.735	277.5
Gemmeiza 7	0	2.846	1.077	3.923	2.642	264.2
	6000	0.699	0.318	1.017	2.198	219.8
Misr 1	0	2.436	0.964	3.400	2.526	252.6
	6000	1.239	0.502	1.741	2.468	246.8
LSD at 5%		0.155	0.121	0.038	0.070	6.998

In the frame of "physiological window" mild drought induces in plants regulation of water loss and uptake allowing maintenance of their leaf relative water content within the limits where the photosynthetic capacity shows no or little changes. But severe drought induces in plants unfavourable changes leading to inhibition of photosynthesis and growth (Yordanov *et al*., 2003). They added that the most severe drought stress is desiccation. On the basis

of presence or absence of bulk water, the mechanisms of protection are different.

Cha-um and Kirdmanee (2008) reported that osmotic pressure in the culture medium caused low water use efficiency and chlorophyll degradation. Chl. a, Chl. b and total carotenoids concentrations in the osmotic stressed sugarcane plantlets decreased especially in 4000 mM mannitol treatment. They concluded that sugarcane variety K84-200 was very sensitive to water deficit as it had a maximum pigment degradation, low photosynthetic abilities and maximum growth reduction the relationship between biochemical and physiological characters and growth of osmotic stressed plantlets was found to be positive and could be applied as effective indices for screening elite sugarcane varieties for drought tolerance.

Total sugar content:

Data presented in Table (6) indicate that total sugar content significantly increased in genotypes Gemmeiza 10, Gemmeiza 9 and Sakha 94 under the lower drought stress treatment thereafter decreased as the concentration of stress was raised. However, total sugar content markedly decreased in genotypes Sakha 8 and Gemmeiza 7 and the effect was a concentration dependent. Genotypes Giza 168, Sakha 93 and Gemmeiza 7 showed a significant decrease in total sugar content under the highest concentrations of PEG.

In this connection, Mohamed *et al*., (2000) reported that soluble sugars in in vitro drought tolerant clone of *Tagetes minuta* were significantly increased in either clones when 30 mM mannitol was added to growth medium. They added that both drought-tolerant-selected clone PM_3 and the non-stress-selected clone P_4 grown on medium with 30 mM mannitol had higher soluble sugars, but not significantly so, than the same plants grown on mannitol –free medium. The accumulation of soluble sugars in osmotically stressed plants of neither clone appears insufficiant to relieve water stress.

Table (6) : Effect of drought concentrations on sugars and proline of wheat genotypes.

Parameters / Genotypes	Sugars					Proline				
	Drought concentrations (mg/l)									
	Control	2000	4000	6000	Mean	Control	2000	4000	6000	Mean
Giza 168	29.12	29.26	28.64	27.98	28.77	15.07	17.80	21.87	30.12	21.21
Sakha 8	29.70	25.94	29.30	26.66	27.90	17.86	18.38	15.88	14.77	16.72
Gemmeiza 10	28.58	30.30	33.96	28.16	30.25	19.31	25.59	48.09	67.62	40.15
Gemmeiza 9	29.40	52.62	28.48	27.36	34.46	33.03	27.92	27.51	69.14	39.40
Sakha 93	30.40	29.14	28.82	28.82	29.29	19.02	32.57	42.34	16.23	27.54
Gemmeiza 7	44.00	26.72	28.40	29.74	32.21	5.59	10.71	18.91	26.29	15.37
Misr 1	30.62	28.50	28.44	28.42	28.99	9.66	16.35	21.17	27.86	18.76
Sakha 94	28.56	31.24	30.17	28.98	29.74	23.44	24.83	21.35	20.30	22.48
Mean	31.31	31.71	29.53	28.26	30.20	17.87	21.77	27.14	34.41	25.21
LSD at 5% for:										
Genotypes	0.28					0.14				
Drought	0.20					0.10				
Gen. x Drought	0.56					0.28				

Hassan *et al*., (2004) recorded that sugar contents of both Sunflower callus lines significantly increased with increasing PEG concentration. Moreover, PEG treatment causes a decrease in the polysaccharides content in both selected and non-selected callus lines. The decrease in polysaccharide may be explained by its degradation into disaccharide and monosaccharide, which were increased under water stress. It is possible that sugars may be involved in osmotic adjustment under water deficit.

Lutts *et al*., (2004) showed that no soluble sugar accumulation fully accounted for lowering the internal osmotic potential in PEG-durum wheat – treated calli and total soluble sugars did not conspicuously accumulate to high levels in stressed calli comparatively to controls. It should, however, be mentioned that, because of the availability of sucrose in the external medium, control calli already contained high amounts of sugars and that the range of soluble sugars concentration in the collected cell cap was far higher than for proline. Consequently, even a small increase in soluble sugar concentration from a relative point of view, may contribute to osmotic adjustment in absolute terms.

Javed and IKram, (2008) stated that total soluble carbohydrates content significantly increased (more negative) in two wheat genotypes callus as concentration of sucrose increased in the culture medium. It is concluded that increasing sucrose concentrations in the medium above control caused osmotic stress and it also been found that accumulation of total soluble carbohydrates accumulated in greater amount responsible for turgor maintenance and increase in callus dry weights.

Moreover, total soluble carbohydrates accumulation increased many folds upon exposure to abiotic stress (Ahmad *et al*., 2007). Javed and IKram, (2008) revealed that sucrose induced osmotic stress increased total soluble carbohydrates content in both wheat genotypes callus tissues (Al-Khayri and Al-Bahrany, 2002). Under drought stress the osmotic potential in tolerant plants is reduced (more-ve) with the increasing intensity of stress. This reduced osmotic potential helps the plants to uptake more water and maintain growth (Almansouri *et al*., (2000). The increase in water potential and accumulation of osmotic like total soluble carbohydrates was to accelerate the water uptake and hence enhance growth (Ahmad *et al*., 2007). Generally, reduction in growth and accumulation of free proline and total soluble carbohydrates in callus tissues of both the wheat genotypes, responsible for the increase (more-ve) in osmotic and water potentials while, decreased in tugor potential and collectively showed impact on the enhacement of callus dry weights. It has also been found that excessive production of total soluble carbohydrates was responsible for turgor maintenance in the callus tissues during sucrose induced osmotic stress.

Proline content:

Data in Table (6) show the effect of different applied treatments on the proline content in the studied genotypes. The response of the studied genotypes varied from genotype to another depending on the applied treatment. However, proline content was increased under stress condition and genotypes Gemmeiza 7, Gemmeiza 10 and Misr 1 were more responsive in this respect.

Ahmad *et al*., (2007) recorded that higher levels of stress increased the proline content in one-month old calli of two Indica rice genotypes many folds with more increase being under PEG stress. They added that at cellular level mechanism of PEG induced osmotic stress tolerance with the accumulation of free proline, as an important osmolyte in the cytosol.

Farshadfar *et al*., (2012) stated that analysis of variance for bread wheat callus growth rate and proline content exhibited highly significant differences among the genotypes for all the characters in the stress conditions (15% PEG).

Bano and Yasmeen (2010) reported that with decrease in soil moisture content under induced drought stress, the percentage increase in proline was greater in leaves and spikes of potted plants.

Moreover, Al-Bahrany (2002) added that increasing PEG concentration was also associated with a progressive reduction in rice callus water content which caused increase in proline accumulation reaching significant increase over the control at 100 g/l PEG.

However, proline content is closely correlated with plant anti-drought especially under soil water deficits. The related difference in physiological indicators and proline content for different soil water treatments among wheat with different genotypes is not clear (Hong-Bo *et al*., 2006).

Vendruscolo *et al*., (2007)reported that drought resulted in the accumulation of proline in wheat plants transformed with the *Vigna aconitifolia* and the tolerance to water deficit observed in transgenic plants was mainly due to protection mechanisms against oxidative stress and not caused by osmotic adjustment.

Mohamed *et al*., (2000) reported that both the drought-tolerant – selected clone PM_3 and the non-stress –selected clone P_4 of *Tage*tes *minuta* grown on medium with 30 mM mannitol had higher proline content but not significantly so, than the same plants grown on mannitol-free medium. The accumulation of proline in osmotically stressed plants of neither clone appears insufficient to relieve water stress. Alian *et al*., (2000) recorded that there was no correlation between the accumulation of proline and drought stress tolerance in tomato. Therefore, the drought-tolerant – selected clone of *T. minuta* may have another strategy to tolerate water stress.

On the other hand, Hassan *et al*., (2004) revealed that the proline showed a positive correlation with the degree of tolerance to water stress in *Helianthus annuus,* which suggests that proline accumulation accompanies survival and growth in drought environment.

However, proline concentration increased in the stressed wheat calli but it probably did not play a key role in osmotic adjustment because (i) from a quantitative point of view, proline accumulation only contributed to a few percent to total osmotic adjustment, (ii) proline accumulation was of the same order of magnitude in the presence of the three stressing agents while obvious differences were observed in terms of ψs, and (iii) the relative behavior of the three different cultivars for proline accumulation did not reflect their relative behavior in terms of osmotic adjustment (Lutts *et al*., 2004).

Javed and Ikram (2008) stated that relative growth rate decline constantly in both wheat genotypes, while dry weights of callus tissues of

both the wheat genotypes increased consistently. The increase in dry weight of callus tissue was due to the more accumulation of proline and total soluble carbohydrates in the callus tissues. Osmotic adjustment through the accumulation of cellular solutes, such as proline has been suggested as one of the possible means for overcoming osmotic stress caused by the loss of water (Shankhadar *et al*., 2000).

Free proline accumulation increased many folds upon exposure to abiotic stresses (Al-Khayri and Al-Bahrany, 2002; Ahmad *et al*., 2007). Javed and IKram (2008) revealed that sucrose induced osmotic stress increased free proline content in both wheat genotypes callus tissues. Under drought stress the osmotic potential in tolerant plants is reduced with the increasing intensity of stress. This reduced osmotic potential helps the plants to uptake more water and maintain growth (Almansouri *et al*., 2000). The increase in water potential and accumulation of osmotic like, proline were to accelerate the water uptake and hence, enhance growth (Ahmad *et al*., 2007). It has also been found that excessive production of free proline and total soluble carbohydrates were responsible for turgor maintenance in the callus tissues during sucrose induced osmotic stress (Javed and IKram, 2008).

It could be concluded that genotypes Gemmiza 7, Gemmiza 10 and Misr 1 seemed to be more stable against PEG induced drought stress in comparison with the other examined wheat genotypes and can be selected for further drought investigation.

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دراسات معملية على طرز جينية من قمح الخبز تحت ظروف الجفاف باستخدام بولى ايثلين جليكول .

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تم تقييم صفات العكس وسلوك الإكثار الدقيق لثمانية طرز جينية من قمح الخبز تحت ظروف الجفاف ، باستخدام بولى إيثيلين جليكول . كما درست العلاقة بين سلوك الإكثار وبعض الصفات الفسيولوجية ؛ مثل محتوى الكلوروفيلات والسكريات الكلية والبرولين ، حيث أشارت النتائج الى وجود إختلافات معنوية بين الطرز المختبرة ، من حيث إستجابتها لظروف الجفاف ، كانت طرز القمح الجينية جميزة ١٠ ، مصر ١ ، جميزة ٧ أكثر ثباتا تحت ظروف الجفاف مقارنة بالطرز الجينية الأخرى محل الدراسة . كما أظهرت النتائج أن طرازى سخا ٨ وسخا ٩٤ كانا أكثر حساسية لظروف الجفاف عن غيرهما من الطرز المختبرة .

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Table (4): Mean performance of wheat genotypes from the combined data over four PEG levels on green points and leaflets Percentages.

Parameters	Green points %				Plantlets %			
	PEG level (mg/l)							
Genotypes	0	2000	4000	6000	0	2000	4000	6000
Giza 168	2.5710 def	1.5255 ij	1.2965 jk	0.5105 mno	0.0001 i	0.0001 i	0.6660 cde	0.0001 i
Sakha 8	2.8155 de	1.8275 hi	0.7550 lmn	0.4690 no	0.4870 def	1.0000 b	0.3330 fgh	0.3330 fgh
Gemmeiza 10	3.6990 b	2.8530 d	2.0265 gh	1.4715 ij	0.3470 fgh	1.3330 a	0.2770 fghi	0.2500 fghi
Gemmeiza 9	3.7375 b	1.7650 hi	0.9900 kl	0.3040 op	0.1500 ghi	0.4630 defg	0.2610 fghi	0.3770 efgh
Sakha 93	2.2950 c	2.0400 gh	1.2050 jk	0.4840 mno	0.3330 fgh	0.0001 i	0.0001 i	0.3330 fgh
Gemmeiza 7	4.8375 a	3.6150 bc	2.4600 ef	1.4770 ij	0.3330 fgh	0.9280 bc	0.7500 bcd	1.4660 a
Misr 1	4.8740 a	3.5000 bc	2.4350 f	1.6825 hi	0.1110 hi	0.8330 bc	0.3660 efgh	0.2080 fghi
Sakha 94	2.3425 fg	0.8375 lm	0.2625 op	0.0550 p	0.0001 i	0.2220 fghi	0.1660 ghi	0.1330 hi
L.S.D. at 5%	0.3587				0.3140			