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Selection for Drought Tolerance Genotypes in Durum Wheat (*Triticum durum* Desf.) Under *In vitro* Conditions

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Abstract: Response of six genotypes of durum wheat (*Triticum durum* Desf.) to immature embryo culture, callus production and *in vitro* drought tolerance was the main objective of this study. The effect of water stress induced by polyethylene glycol (PEG 8000) on growth water content, necrosis of calli cultures and regeneration was investigated on selected genotypes of durum wheat. Whereas formed calli on MS medium supplemented with 4.0 mg/l of 2, 4- dichlorophenoxyacetic acid (2, 4-D) and 0.4 mg/l kinetin were subcultured on media containing different concentrations of PEG (0.0, 50, 100, 150, 200 and 250 g/l). After 4 weeks of culture, callus relative growth and callus water content were determined. The results showed that increasing PEG concentration in the medium causes a gradual decrease in callus growth parameters. Embryogenic calli showed best shoot regeneration on MS medium supplemented with 6.0 mg /l zeatin + 30 g /l sucrose + 2.5 g/l phytagel of six durum wheat genotypes. There was reduction in callus induction ability and plant regeneration efficiency with increasing levels of PEG (8000) stress. These results indicated that PEG (8000) can be used as water stress creating agent under *in vitro* conditions of durum wheat genotypes, Mexicali 75, Cham 5, Beni Suef 1 and Suhag 3 were relatively tolerant to drought stress as compared to Yavaros 79 and Altar 84. RAPD-PCR with four primers used to distinguish plantlets with regenerated from the PEG tolerant and control plantlets. Results showed that all durum wheat genotypes were not identical in their DNA ability to be amplified.

Key words: Durum wheat · Callus induction · Regeneration · PEG-8000 · Drought · RAPD marker

INTRODUCTION

Plant tissue culture plays an important role in the production of agricultural and ornamental plants and in the manipulation of plants for improved agronomic performance [1]. *In vitro* culture of plant cells and tissue has attracted considerable interest over recent years because it provides the means to study plant physiological and genetic processes in addition to offering the potential to assist in the breeding of improved cultivars by increasing genetic variability [2]. Improved yields of wheat depend on many factors, among which one of the most important factors is tolerance to environmental stress, particularly to water stress. Indeed, in durum wheat (*Triticum durum* Desf.), drought is a major non-biotic stress that causes severe yield loss. In the Mediterranean region, this loss ranges from 10 to 80% depending on the year [3-4]. Classical plant breeding for stressful environment is time consuming and inefficient because of multi-genic tolerance mechanism of plants, lack of well defined selection criteria [5] and undesirable genes incorporated during classical breeding [6]. One of the most supportive and promising breeding approaches to achieve stable drought tolerant wheat genotypes is to exploit natural diversity of the gene pool carrying desired genes for drought tolerance. In wheat species, different explant sources have been used for embryogenic callus formation and plant regeneration: mature and immature embryos [7], immature inflorescences and coleoptiles [8], shoot apical meristems [9] and anthers [10]. These tissues vary in their ability to regenerate whole plants [11]. Immature embryos and immature inflorescences gave the highest frequencies of regenerated plants *in vitro* [8]. Tissue culture responses

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using callus induction and regeneration capacity of wheat are influenced by the genotypes, explant source, geographical origin and physiological status of the donor plants, the culture medium and the interactions between them. Both mature and immature embryos have been used extensively in tissue culture protocols, but immature embryos are better explant source when regeneration is considered [1]. Polyethylene glycol (PEG), induced osmotic stress is used for *in vitro* selection of drought tolerant cell/callus of wheat [12]. Too high osmotic stress kills the tolerant cells while suboptimal level of osmoticum results in survival of non tolerant cell lines. In addition, embryogenic callus induction and regeneration of plantlets from selected calli is inversely proportional to increasing concentrations of PEG used in selection media [13].

The aim of this study was to exploit *in vitro* techniques for production, selection and regeneration of six durum wheat genotypes under different concentrations of PEG and test RAPD markers associated with plantlets were regenerated from the PEG tolerant calli.

MATERIALS AND METHODS

Plant Material and Explant Preparation: This study was carried out in the Tissue Culture and Biotechnology Labs, Marout Research Station, Desert Research Center (DRC), Alexandria, Egypt during the period from 2008 to 2011.

Six durum wheat (*Triticum durum* Desf.) genotypes Cham 5, Yavaros 79, Altar 84, Mexicali 75, Beni Suef 1 and Suhag 3 were used as the material for this study. The seeds were provided by ICARDI (Syria) and DRC (Egypt). The explant source was immature embryos collected from seeds in milky phase, approximately 14-18 days after anthesis.

Callus Induction: The grains were sterilized in 0.5% NaOCl for 15 min and then washed three times with sterile distilled water. Immature embryos were removed aseptically using forceps and placed on MS based [14] callus induction media supplemented with 4.0 mg/l 2, 4-D, 30 g/l sucrose and 2.5 g/l phytagel (MS1). The pH of the medium was adjusted to 5.7 prior to autoclaving at 121°C for 20 min. The explants were incubated in total darkness at 25±1°C temperature for four weeks. Afterward, induced calli were shifted to callus multiplication media supplemented with 4 mg/l 2, 4-D, 0.4 mg/l kinetin, 30 g/l sucrose and 2.5 g/l phytagel (MS2) for another period of four weeks. The culture media were refreshed every 14 to 18 days.

Selection of Drought Tolerant Callus: After four weeks of incubation on callus multiplication media, the obtained calli were separately subcultured in MS2 medium supplemented with different concentrations of polyethylene glycol (PEG 8000 from Sigma-Aldrich: CAS Number 25322-68-3) (0, 50, 100, 150, 200, 250 g/l). The callus relative growth (CRG) in stress and unstressed medium was measured in terms of percent increase in fresh weight. Calli were incubated for four weeks on selection media (MS2). After four weeks of culture the CRG is calculated by the following formula:

CRG = (final fresh weight-initial fresh weight)/ initial fresh weight

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

Callus dry weight was determined after a 48 h stay in the oven at 80°C and callus fresh weight determined just before drying.

Plant Regeneration Under PEG Treatments: The obtained calli were re-cultured on various callus selection media (MS2) with different concentrations of PEG8000 for 4 weeks and then shifted to regeneration medium supplemented with 30 g/l sucrose, 2.5 g/l phytagel and plant growth regulators (4 and 6 mg/l zeatin) or 2.0 mg/l BAP and 0.5 mg/l kinetin. The calli were incubated at 25±2°C temperature with 16 h light and 8 h dark photoperiod. The regeneration medium was refreshed every 15-21 days. Percentage of regeneration = (number of regenerated calli / total number of calli) x 100. Percentage of callus necrosis = (number of necrotic calli / total number of calli) x 100.

Rooting and Acclimatization: Four-to five-weeks old regenerated shoots cultures were transferred to rooting medium containing MS medium supplemented with 1.0 mg/l IAA. *In vitro* raised healthy plantlets were taken out from the culture bottles and washed gently with sterile water to remove the adhering medium completely. Thereafter, they were transferred to pots containing autoclaved soil mixture and vermiculite (3:1 v/v). The plantlets were covered with transparent polyethylene bags to prevent desiccation and were maintained in a greenhouse at temperature 28±2°C for one month. Completely developed plantlets were transferred to pots filled with 1:1 v/v mixture of soil and peatmoss and were successfully established in field conditions.

DNA Isolation: DNA was extracted from leaf tissue of wheat genotypes using a Cetyltrimethyl ammonium bromide (CTAB) method according to Maniatis *et al*. [15]. One gram of frozen durum wheat leaves were ground in cold pestle mortar with 1 ml buffer (100 mM Tris-HCl pH 8.0, 20mM EDTA, 1.4 M NaCl, 0.2% PVP40 (w/v), 0.2% (v/v) 2-mercaptoethanol), mixed, transferred to 500 µl eppendorf tube and incubate at 65ºC for one hours. After incubation the mixture was centrifuge for 20 minutes with 4000 rpm at room temperature. Supernatant was taken and RNAse 1:1000 dilution of RNAse (100 mg/ml) was added and kept at 37ºC at 30 minutes, then mixed with the same volume of chlorophorm-isomylalcohol (24:1) and centrifuged at 4000 rpm for 30 minutes. DNA was precipitated by the addition for 2/3 volume of cold isopropanol for overnight in 4ºC. Then supernatant removed from the tube and the pellet was washed with wash buffer (70 % ethanol), centrifuged again for 10 minutes with 1000 rpm at 20ºC and the pellet was dried under vacuum. The DNA pellet was resuspended in 100 µl of deionized H_2O and incubated at 50ºC for 15 min, centrifuged for 5 min with 1000 rpm at 20ºC then the solution was transferred to a new Eppendorf tube.

Polymerase Chain Reaction (PCR): After checking the concentration of genomic DNA by agarose gel electrophoresis for all six durum wheat genotypes which will use to detect a marker related to drought tolerance. Out of the 18 random primers screened, only four primers produced clear reproducible bands (sequences presented in Table 5).

Statistical Analysis: Analysis of variance (ANOVA) was executed and treatment means were compared by least significant difference (LSD) test at 5% probability level using MSTATC software [16].

RESULTS AND DISCUSSION

Callus Induction and Growth: Immature embryos cultured on MS medium supplemented with 4.0 mg/l 2, 4-D, 30 g/l sucrose and 2.5 g/l phytagel for six durum wheat genotypes and incubated in darkness at 25±1°C for four weeks. Induced calli were shifted to callus multiplication on MS medium supplemented with 4.0 mg/l 2,4-D, 0.4 mg/l kinetin, 30 g/l sucrose and 2.5 g/l phytagel for 4 weeks (Fig. 1). Good callus growth was observed on MS medium containing 4 mg/l 2, 4-D and similar result was reported by Tanzarella and Greco [17]. The growth of callus was evaluated during the growing period of 30 days on MS2 medium supplemented with different concentrations of PEG (Table 1). The fresh weights of callus for six genotypes Beni Suef 1, Cham 5, Yavaros 79, Altar 84, Suhag 3 and Mexicali 75 were 7.03, 6.23, 3.72, 4.65, 6.57 and 5.79 g / culture, respectively (Table 1). The fresh weights of callus after 30 day of growth of the six genotypes on different concentrations of PEG 8000 (0.0, 50, 100, 150, 200 and 250 g/L) were 9.29, 8.02, 6.79, 4.68, 3.31 and 1.89 g /culture, respectively.

Water stress is one of the limiting factors for plant growth and crop production. Upon exposure to water deficit, plants react by complex mechanisms involving morphological, physiological, biochemical and molecular factors, both at cellular and whole-plant levels [18, 19]. At the cellular level, the effect of water stress on the slowdown of cell divisions and elongation by the loss of turgor has been reported by Levitt [20]. This negative correlation between callus fresh weight and increasing the concentrations of PEG are agreement with previously studies such as Heyser and Nabors [21]. They indicated that the addition of PEG in the medium causes cell dehydration by reducing water availability to cells, which leads to a loss of cell turgor and hence a loss of growth.

Effect of PEG on Callus Relative Growth and Callus Water Content: The callus relative growth and callus water content were evaluated after 4 weeks of growing on callus MS2 medium with different concentrations of PEG. The results in Table 2 showed that increasing PEG in the medium significantly brings down the weight of calluses and therefore the relative growth. The highest callus relative growth (5.82) was noted in calluses grown on control medium MS without PEG and decreased gradually to 0.10 in medium MS2 with 25% PEG of Beni Suef 1 genotype and the lowest callus relative growth (0.02 g) of Yavaros 79 on MS2 with 25% PEG. While, the callus relative growth (4.58-5.62) was observed in calluses grown on control medium MS without PEG and decreased gradually to (0.03-0.12) in medium MS with 25% PEG of other durum wheat genotypes (Fig. 2). Callus water content decreased significantly with increasing PEG concentration in the medium. The highest values of water content (83.62, 83.58, 80.62, 81.32, 82.38 and 80.62) were recorded in the calluses under PEG-free media for Beni Suef 1, Cham 5, Yavaros 79, Altar 84, Suhag 3 and Mexicali 75, respectively. While, the lowest values of water contents (70.42, 65.38, 63.11, 60.35, 68.05 and 68.11) were observed in the calluses from media containing the highest PEG concentrations 25% of Beni Suef 1, Cham 5, Yavaros 79, Altar 84, Suhag 3 and Mexicali 75, respectively. Genotypes Mexicali 75, Cham 5, Beni Suef 1 and Suhag 3 more tolerant to high concentrations of

Table 1: Callus fresh weight in gram of six durum wheat genotypes after 4 weeks of growing on MS medium containing 4.0 mg/l 2,4-D with different concentrations of PEG8000.

	Callus fresh weight in gram on different; PEG concentrations in medium						
Genotypes	0.0	50	100	150	200	250	Mean
Beni Suef 1	12.56	9.84	8.23	5.14	4.28	2.18	7.03[a]
Cham 5	9.23	8.73	8.12	5.02	4.12	2.13	6.23[a]
Yavaros 79	6.36	5.12	4.05	3.78	1.72	1.24	3.72[d]
Altar 84	7.52	6.74	5.08	4.16	3.36	1.08	4.65[c]
Suhag 3	10.25	9.32	8.14	5.11	3.82	2.83	6.57[a]
Mexicali 75	9.86	8.36	7.16	4.88	2.56	1.92	5.79[b]
Mean	9.29[a]	8.02[b]	6.79[c]	4.68[d]	3.31[e]	1.89[f]	--

Table 2: Effect of MS medium supplemented with 4 mg/l 2,4-D, 0.4 mg/l kinetin and different PEG concentrations on callus relative growth and callus water content in six durum wheat genotypes after 4 weeks

	Callus Relative Growth (CRG)						Callus water content (CWC %)					
Genotypes	0.0	50	100	150	200	250	0.0	50	100	150	200	250
Beni Suef 1	5.82[a]	4.58[b]	2.24[d]	1.26[e]	0.52[f]	0.10[h]	83.62[a]	82.72[a]	80.75[b]	79.16[b]	77.13[c]	70.42[d]
Cham 5	5.24[a]	3.36[c]	1.81[e]	1.11[e]	0.61[f]	0.11[h]	83.58[a]	82.68[a]	80.66[b]	78.14[b]	72.02[c]	65.38[e]
Yavaros 79	4.64[b]	3.81[c]	2.58[d]	1.02[e]	0.55[f]	0.02[i]	80.62[b]	79.73[b]	78.64[b]	77.12[c]	71.23[d]	63.11[e]
Altar 84	5.62[a]	4.42[b]	2.16[d]	0.98[f]	0.42[g]	0.03[i]	81.32[a]	78.13[b]	74.25[c]	68.76[d]	65.13[e]	60.35[e]
Suhag 3	5.33[a]	3.01[c]	2.22[d]	1.23[e]	0.63[f]	0.12[h]	82.38[a]	81.32[a]	79.43[b]	78.44[b]	76.23[c]	68.05[d]
Mexicali 75	4.58[b]	3.63[c]	1.48[e]	0.68[f]	0.68[f]	0.08[i]	80.62[b]	79.73[b]	78.64[b]	76.12[c]	70.23[d]	68.11[d]
Mean	5.21[a]	3.80[b]	2.08[c]	1.05[d]	0.57[e]	0.08[f]	82.02[a]	80.72[ab]	78.72[b]	76.29[bc]	71.99[c]	65.90[d]

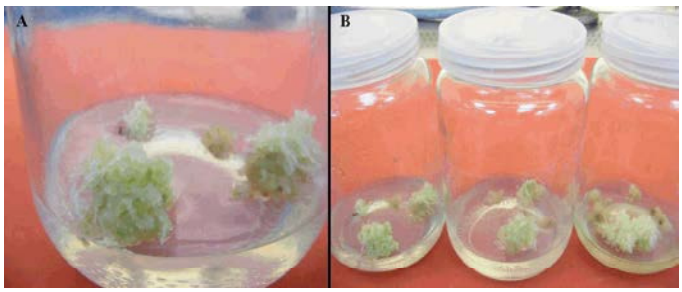

Fig. 1: Callus growth in *Triticum durum* of immature embryos Beni Suef 1 genotype on MS medium supplemented with 4 mg/l 2,4-D (A) and callus multiplication on MS medium supplemented with 4 mg/l 2,4-D and 0.4 mg/l kinetin without PEG concentrations of Beni Suef 1, Cham 5 and Yavaros 79 genotypes (B)

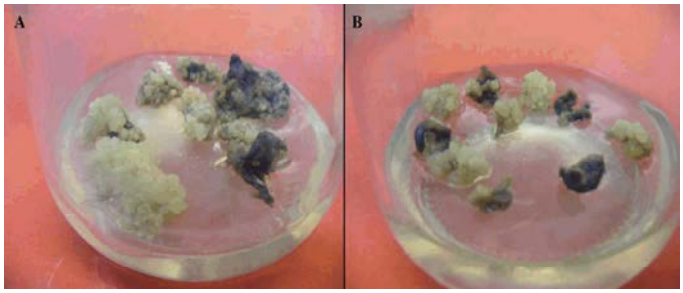

Fig. 2: Callus growth from immature embryos of Mexicali 75 genotype cultured on MS medium containing 4 mg/l 2,4-D and 0.4 mg/l kinetin with different PEG concentration (A) 200 g/l PEG; (B) 250g/l PEG

PEG compared Yavaros 79 and Altar 84 which were least tolerant to PEG. Decline in CGR% on media containing higher PEG-8000 induced osmotic stress might be due to reduced cell division, shrinking cytoplasmic volume and loss of cell turgor, nutritional imbalance due to reduced up take of water, an increase in electrolyte leakage and decrease in cell water contents with increasing stress [22]. Reduced cell growth is the most immediate and sensitive

Table 3: Effect of MS medium supplemented with different growth regulators on regeneration percentage of six durum wheat genotypes after 6 weeks

	Regeneration percentage					
Culture media	Beni Suef1	Cham5	Yavaros79	Altar84	Suhag3	Mexicali75
MS+ 4 mg/l zeatin	53.28^{c}	61.54^{c}	52.82^{c}	69.63^{c}	48.13^{c}	60.15^{c}
MS+ 6 mg/l zeatin	89.34^{a}	66.42^{a}	68.72^{a}	82.64^{a}	52.21^{a}	64.53^{a}
MS+2 mg/l BAP and 0.5 mg/l kinetin	62.81^{b}	63.13^{b}	57.25^{b}	63.24^{b}	50.08^{b}	61.32^{b}
Mean	68.48^{b}	63.69^{c}	59.61^{d}	71.84^{a}	50.14^{e}	62.00^{c}

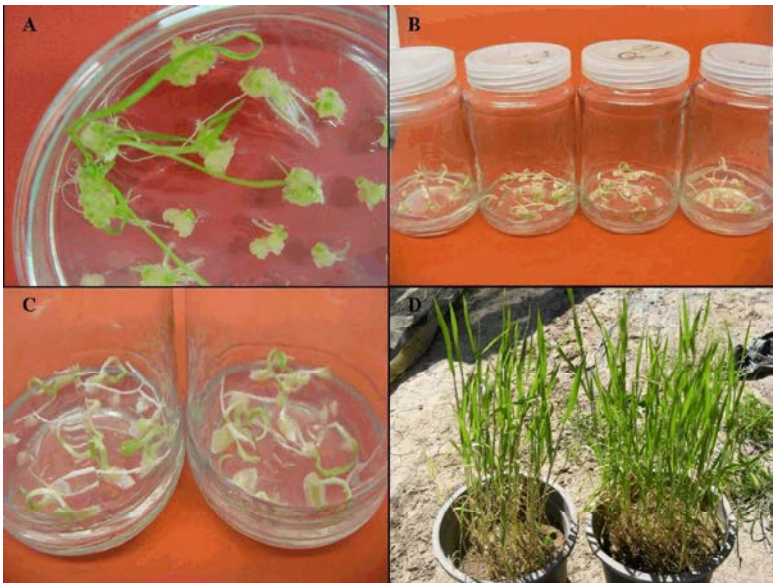

Fig. 3: Regeneration of Beni Suef 1 genotype on MS medium supplemented with 6 mg/l zeatin without PEG concentrations (A), Regeneration on MS medium supplemented with 6 mg/l zeatin with different PEG concentrations of Beni Suef 1 and Mexicali 75 genotypes; 200 g/l (B), 250 g/l (C), and acclimatization of drought-tolerant plants of Beni Suef 1 and Mexicali 75 genotypes (D)

response of the plants to osmotic stress [20]. The growth of the calli is significantly restricted by increasing and continuous presence of PEG in the media [23] over time. Earlier, similar results were reported in soft wheat [24], sunflower [25] and rice [26].

Regeneration *via* Somatic Embryos and Transplanting: Regeneration started with the appearance of green dots on callus after 4 weeks of incubation on regeneration medium and generally produced normal stem and leaves. The result showed that best regeneration percentage was observed on MS medium supplemented with 6 mg/l zeatin as compared to MS supplemented with 2 mg/l BAP and 0.5 mg/l kinetin of six durum white genotypes. The minimum number of regenerated plantlets was recoded in MS medium supplemented with 4 mg/l zeatin of six durum wheat genotypes (Table 3). The best results of number of regenerated plantlets were observed on MS medium supplemented with 6 mg/l zeatin of Beni Suef 1 genotype (Fig. 3A).

Table 4 shows Effect of MS medium supplemented with 6 mg/l zeatin and different concentrations of PEG on necrosis and regeneration percentage in six durum wheat genotypes. The highest necrotic calluses percentage was observed in calluses from medium MS containing 25% PEG of six durum wheat genotypes. Calli from the control MS medium without PEG showed no necrosis. The highest regeneration percentage (89.3) was observed in callus from MS medium without PEG and decreased gradually to (16.4) in medium MS with 25% PEG of Beni Suef 1 genotype (Fig. 3B and 3C). While, the lowest regeneration percentage (52.2) was observed in callus from MS medium without PEG and decreased gradually to (8.24) in medium MS with 25% PEG of Altar 84 genotype as compared with other treatments. The progressive increase of PEG in the culture medium caused a gradual decrease in callus water content in the six genotypes. The regenerated plants were successfully established in the pots (98%) under field conditions. Plant height was observed at the time of hardening,

Table 4: Effect of MS medium supplemented with 6 mg/l zeatin and different concentrations of PEG on necrosis and regeneration percentage in six durum wheat genotypes

	Necrosis%						Regeneration%					
Genotypes	0.0	50	100	150	200	250	0.0	50	100	150	200	250
Beni Suef 1	0.0	3.28^{i}	9.18^{g}	32.2^{e}	56.7^{c}	68.5^{a}	89.3^{a}	68.3^{b}	65.1^{b}	48.2^{c}	28.4^{e}	16.4^{f}
Cham 5	0.0	4.68^{h}	10.1^{f}	28.1^{e}	48.2^{d}	72.2^{a}	66.4^{b}	61.6^{b}	59.3^{c}	42.3^{d}	21.6^{e}	9.13^{g}
Yavaros 79	0.0	5.18^{h}	11.2^{f}	30.2^{e}	52.2^{c}	65.4^{b}	68.7^{b}	62.3^{b}	62.2^{b}	44.2^{d}	37.2^{d}	8.84^{g}
Altar 84	0.0	2.28^{i}	12.1^{f}	38.2^{d}	48.9^{d}	72.9^{a}	52.2^{c}	50.2^{c}	50.2^{c}	41.4^{d}	25.2^{e}	8.24^{g}
Suhag 3	0.0	3.92^{i}	9.23^{g}	33.4^{e}	58.3^{b}	62.7^{b}	82.6^{a}	78.5^{a}	72.3^{b}	51.3^{c}	28.8^{e}	9.28^{g}
Mexicali 75	0.0	6.38^{h}	17.8^{f}	35.3^{e}	52.8^{c}	68.4^{a}	64.5^{b}	61.5^{b}	51.3^{c}	38.8^{d}	23.7^{e}	11.3^{f}
Mean	0.0^{f}	4.32^{e}	11.60^{d}	32.90^{c}	52.85^{b}	68.35^{a}	70.62^{a}	63.73^{ab}	60.07^{b}	44.37^{c}	27.48^{d}	10.53^{e}

15 days after hardening and after transferring to the pots. It was observed that somaclones developed from PEG-treated calli attained greater height than control plants (Fig. 3D). Similar results were reported on rice by Al-Bahrany [27]. Heyser and Nabors [21] showed that osmotic stress increase, due to the addition of PEG in the medium, was accompanied by a sharp decrease in water content of tissues. This water stress induces also a cell osmotic adjustment by accumulation of solutes such as endogenous proline, which contributes to the protection against cellular damage caused by dehydration, hence triggering an adaptive response [28]. Our results showed that increasing the concentration of PEG in the medium caused an increase in the callus necrosis percentage in the six genotypes studied. Similar results were also reported in soft wheat by Mahmood *et al.* [29]. Necrosis observed in calli of wheat subjected to osmotic stress of PEG-6000 (-1.2 MPa) is generally accompanied by growth stop.

The PEG-8000 in solid media lowers water potential of the medium that adversely affect cell division leading to reduced callus growth and consequently influences regeneration [30]. Also, regeneration ability of explants was usually decreased by repeated subculture over time in many plants [31]. A parallel decrease in plantlet regeneration with increasing *in vitro* osmotic stress was reported in rice [26], wheat [32] and tomato [4]. Drought stress causes profuse mutation in cellular metabolism including protein functioning and alteration in amount of proteins [33]. The decrease in regeneration frequency on media with higher osmotic stress may be due to altered gene expression [34] controlling this trait or the genes may express themselves but the resultant proteins may be denatured 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 [35].

Molecular Genetic Marker: Genomic DNA of the wheat genotypes were extracted further used in performing Randomly Amplified polymorphic DNA (RAPD). Four arbitrary oligonucleotide primers, number of fragment amplified using these different primers showed that: the number of amplified fragments different from one genotype to another indicating that all durum wheat genotypes are not always identical in their DNA ability to be amplified and these primers have amplified 115 PCR bands (Table 6). A maximum of 47 fragments were amplified with primer OPO-03 and minimum of 16 fragments were amplified with primer B-19. All four used RAPD primers generated polymorphic bands; the OPO-03 primer recorded the percentage polymorphism (25.5%) as it revealed 12 polymorphic bands in 47 amplified fragments (Table 6). The size of amplified ranged from 1350bp to 300bp approximately (Fig. 4). When the oligonucleotide OPO-03 was used it produced amplified DNA of 1350 in plantlet produced from 25% PEG of Altar 84 genotype, 1650 bp, 1000bp, 450bp and 350bp in plantlets produced from 25% PEG of Mexicali 75 genotype, 1000bp and 650bp in plantlet produced from 25% PEG of Cham 5 genotype. The OPO-03 primer was produced only PCR product with four genotypes, eleven bands in Cham5, twelve bands in Altar 84, thirteen bands in Mexicali 75 and eleven bands in Yavaros 79 (Table 5). The B-19 primer recorded the least percentage (12.5%) by revealing two polymorphic bands in 16 amplified fragments. The B-19 primer amplified 16 bands in two genotypes only. The primer produced eight bands in Beni Suef 1 with molecular weight range 700-100bp and eight bands in Suhag 3 with molecular weight range 1200-200 bp. Using olgonucleotide B-19 produced amplified DNA of 250bp in Suhag 3 and absent band in Beni Suef 1 whereas; this primer produced zero band in other genotypes (Fig. 4). The results of RAPD analysis using primer OPO-06 are illustrated in Figure (5). The OPO-06 primer amplified 31 bands in 4 genotypes only (Table 5).

Table 5: List of primer, their nucleotide sequences and amplification results with six durum wheat genotypes

		Number of amplified fragment in each genotypes					
Primer code	Sequence (5' to 3')	Cham5	Yavaros79	Altar 84	Mexicali 75	Beni Suef 1	Suhag 3
OPO-05	CCCAGTCACT	8	0	0	0	6	7
OPO-06	CCACGGGAAG	5	9	8	9	0	0
OPO-03	CTGATACGCC	11	11	12	13	0	0
B-19	ACCCCCGAAG	0	0	0	0	8	8
Total		24	20	20	22	14	15

Table 6: Polymorphism rate for the six durum wheat genotypes using OPO-05, OPO-06, OPP-03 and B-19 random primers

Primer code	Sequence (5' to 3')	Total amplified band	Polymorphic band	Polymorphism (%)
OPO-05	CCCAGTCACT	21	5	23.8
OPO-06	CCACGGGAAG	31	11	35.5
OPO-03	CTGATACGCC	47	12	25.5
B-19	ACCCCCGAAG	16	2	12.5
Total		115	30	--

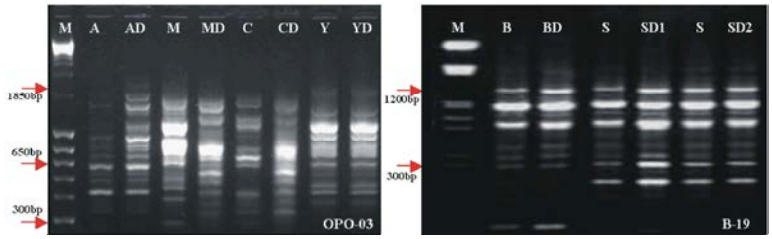

Fig. 4: DNA banding pattern generated by RAPD-PCR with different primers OPO-03 and B-19 in six durum wheat genotypes. Lane M: DNA marker; (A) Altar 84 control, (AD) Altar 84 with 25% PEG, (M) Mexocali 75, (MD) Mexocali 75 with 25% PEG, (C) Cham 5 control , (CD) Cham 5 with 25% PEG, (Y) Yavaros 79 control, (YD) Yavaros 79 with 25% PEG (B) Beni suef 1 control, (BD) Beni suef 1 with 25% PEG, (S) Suhag 3 control (SD1) Suhag 3 with 20% PEG, and (SD2) Suhag 3 with 25% PEG

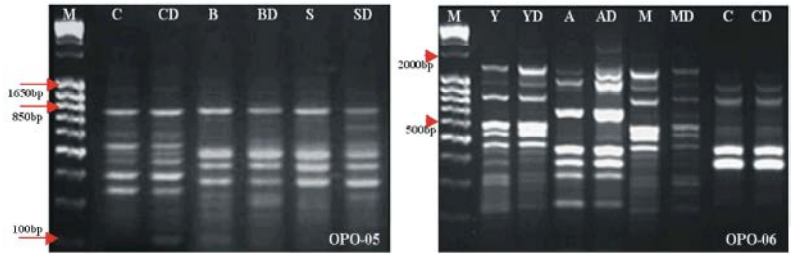

Fig. 5: DNA banding pattern generated by RAPD-PCR with different primers OPO-05 and OPO-06 in six durum wheat genotypes. Lane M: DNA marker; (C) cham 5 control , (CD) Cham 5 with 25% PEG, (B) Beni suef 1 control, (BD) Beni suef 1 with 25% PEG, (S) Suhag 3 control, (SD1) Suhag 3 with 25% PEG, (Y) Yavaros 79 control, (YD) Yavaros 79 with 25% PEG, (A) Altar 84 control, (AD) Altar 84 with 25% PEG, (M) Mexocali 75, (MD) Mexocali 75 with 25% PEG

The OPO-06 primer recorded the highest percentage polymorphism (35.5%) as it revealed 11 polymorphic bands in 31 amplified fragments; nine bands in Yavaros 79 with molecular weight range 2200bp -200bp, eight bands in Altar 84 with molecular weight range 2200-150bp, nine bands in Mexicali 75 with molecular weight range 2000-200bp and five bands in Cham 5 with molecular weight range 1650- 280bp (Table 5).

The results of RAPD analysis using primer OPO-05 are illustrated in Fig. 5. The OPO-05 primer amplified 21 bands in three genotypes only. The primer produced eight bands in Cham 5 with molecular weight range 700-100bp, six bands in Mexicali 75 with molecular weight range 600-100 bp and seven bands in Suhag 3 with molecular weight range 700-200bp while, this primer produced zero band in other genotypes. The oligonucleotide of OPO-05

produced one amplified DNA segment of 650bp in drought tolerant Suhag 3 genotype. These results are in agreement with those obtained by Rashed *et al.* [36], they reported that six primers only gave a polymorphism with wheat genotypes, which four primers out of them developed molecular markers of drought tolerant wheat. The five primers produced multiple band profiles with a number of amplified DNA fragment ranging from Zero to eleven. Three primers P18, P29 and P39 were reacted and generated PCR product with all genotypes, whereas primer P24 and P86 reacted only with twelve and fourteen genotypes, respectively. Malik *et al.* [37] used RAPD markers to detect DNA polymorphism between two wheat genotypes as a drought resistant and drought-susceptible. They revealed that RAPD technique has a great potential to find DNA-based polymorphisms between the genotypes of the same species. Abdel-Tawab *et al.* [38] detected five positive and negative RAPD markers for drought tolerance in Egyptian bread wheat. Abdel-Bary *et al.* [39] detected eight positive and negative RAPD markers for salinity tolerance in maize. Moreover, our results are in agreement with those reported by Nachit *et al.* [3], who associated yield-related traits as grain yield, yield components and stress physiological traits with some molecular markers in durum wheat. Several markers showed strong relationships with grain yield, yield components and stress physiological traits, indicating that there are potential markers for use in marker-assisted selection to improve abiotic stresses tolerance by molecular breeding.

CONCLUSION

In vitro tissue culture could be an important means of improving crop tolerance and yield through genetic transformation as well as by induced somaclonal variation. Therefore, it is important to devise an efficient protocol of callus proliferation to start *in vitro* selection for drought tolerance and to broaden opportunities for genetic manipulation of wheat through tissue culture, including trying various explants and media. The results of this study indicated that effect of callus selection media comprising PEG-8000 induced drought stress on callus growth rate, survival and regeneration for screening drought tolerant callus lines of durum wheat genotypes. Differential genotypic response was also noted in callus ability to proliferate and regenerate plantlets under concentrations of PEG. Also, the results showed that all durum wheat genotypes were not always identical in their DNA ability to be amplified. RAPD analysis is useful molecular tools to indicate genetic polymorphism between the durum wheat genotypes under drought stress.

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