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ORIGINAL ARTICLES

Genetic Characterizations Among Drought Tolerant Wheat Genotypes by Biochemical and Molecular Markers

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ABSTRACT

Wheat (*Triticum aestivum* L.) is the world's most important cereal crop and due to its different uses and nutritive value it is the staple food for more than one third of the world's population. Wheat production can be enchanced through the development of improved cultivars with wider genetic base capable of producing better yield under various agroclimatic conditions and stresses. This study aims to develop wheat genotypes, which have favorable response and tolerant for drought stress through *in vitro* culture and genetic polymorphism and genetic similarity of wheat genotypes by biochemical (SDS-PAGE) and RAPD-PCR as molecular marker. The genetic diversity of 25 wheat genotypes was estimated using germination characteristic under drought stress using two concentrations (-9 and -12 bar) from polyethylene glycol (PEG) *in vitro* and molecular analysis. The phenotypic data showed highly differences between genotypes for germination percentage. In addition, genotypes 2, 8, 13, 19, 22, 23 and 25 were more resistant for drought stress. Genetic similarity matrix was generated on the basis of Nei and Li's coefficients. The coefficients were used to make clusters using unweighted pair group method of arithmetic means (UPGMA) which separated the studied genotypes into three main groups depending on results SDS-PAGE and RAPD-PCR combination. The first group A (similarity range 0.69 to 0.93) contains on genotypes (1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12 and 13), the second group B (similarity range 0.73 to 0.93) comprised of genotypes (14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25), while group C includes on genotype-4 (similarity range 0.53 to 0.72) which was distantly related to other two groups**.** The information generated here along with the use of most advanced DNA fingerprinting techniques like RAPD-PCR would be helpful for future genome mapping programs, as well as for the application of intellectual breeder rights in Egypt. In addition, these tools could be used as complementary of traditional methods of identification of phenotypic traits for the control of registered cultivars in the trade market. This will help in collection and cataloguing of the germplasm in the form of a germplasm bank.

Key wrods: Polyethylene glycol, SDS-PAGE, polyphenol oxidase, RAPD-PCR.

Introduction

Wheat (*Triticum aestivum* L.) is the world's most important cereal crop and due to its different uses and nutritive value it is the staple food for more than one third of the world's population. Wheat production can be enchanced through the development of improved cultivars with wider genetic base capable of producing better yield under various agroclimatic conditions and stresses (Zhu *et al*., 2000).

Abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Boyer, 1982). One of the most important abiotic factors limiting plant germination and early seedling stages is water stress brought about by drought and salinity Almansouri, (2001), which are widespread problems around the world (Soltani *et al.,* 2006). Salinity and drought affect the plants in a similar way (Katerji *et al.,* 2004). Reduced water potential is a common consequence of both salinity and drought (Legocka and Kluk 2005). Water stress acts by decreasing the percentage and rate of germination and seedling growth (Delachiave and Pinho 2003). Germination of seeds, one of the most critical phases of plant life, is greatly influenced by salinity (Misra and Dwivedi 2004). Polyethylene glycol (PEG) widely used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly (Kawasaki *et al*., 1983). PEG has long been used *in vitro* culture to reduce water potential of nutrient solutions and to stimulate water stress without the risk of being taken up by the plants (Mer *et al*., 2000). Thus, cell cultures survive under water stress can be selected and raised as drought resistant cell lines. Dragiiska *et al.,* (1996) developed a system for *in vitro* selection during

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somatic embryogenesis in alfalfa using PEG as a selective agent for osmotolerance. Abdel Hady *et al*. (2001) subjected embryogenic calli of five wheat cultivars to *in vitro* selection for drought tolerance using 5, 10 and 20% PEG. One way of increasing productivity in stressful environments is to breed crops more tolerant to stress. However, success in breeding for tolerance has been limited because tolerance to stress is controlled by many genes and their simultaneous selection is difficult Flowers *et al*., (2000), complexity of the several tolerance mechanisms involved, tremendous effort is required to eliminate undesirable genes that are also incorporated during breeding Richards (1996) and there is a lack of efficient selection procedures particularly under field conditions (Ribaut *et al*., 1997).

Exposure of plants to abiotic stresses results in production of reactive oxygen species (ROSs) as by products, which damage the cellular components (Noctor and Foyer 1998). Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam and Tyagi 2004). The Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and glutathione reductase (GR) function in detoxification of superoxide and H_2O_2 (Yoshida *et al*., 2002; Gülen *et al*., 2008).

This study aims to develop wheat genotypes, which have favorable response and tolerant for drought stress through *in vitro* culture and genetic polymorphism and genetic similarity of wheat genotypes by biochemical and molecular markers.

Material and Methods

Effect of drought stress induced by different osmotic potential levels [(0 (control), -9 bar (222 g PEG) and -12 bar (251 g PEG)] of polyethylene glycol 6000 (PEG 6000) on germination and early seedling development of wheat were studied. Twenty-five wheat genotypes including were used in this experiment. This investigation was performed as factorial experiment under Randomized Complete Design (CRD) with three replications. In each experiment and each level of stress, hundred grains of each genotype were selected and sterilized in sodium hypochlorite (1%) and then washed in dsH_2O for two times. The grains of 25 genotypes were germinated in Petri dishes on two layers of filter paper in an incubator maintained at 25°C. Daily, germination rate was measured and need have replaced the filter papers and add the PEG soluble was performed. Every 24 hours after soaking, germination percentage and other traits were recorded daily. Seeds were considered germinated when the emergent radical reached 2 mm length. Rate of germination, was calculated using the following formulas:

Rate of germination = Number of germinated seeds x Total number of seeds x 100.

Every day the number of seeds germinated (2 mm radical) recorded for 10 days continued at the eleventh day was measured and recorded.

Polyphenol oxidase (PPO) isozyme extraction:

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted to identify isozyme variations among 25 wheat genotypes under drought stress compared to the control using polyphenyl oxidase isozymes according to (Stagemann *et al.,* 1985). 500 mg fresh leaves were homogenized in liquid N_2 and 100 µl of 0.2 M Phosphate buffer was added (pH 7.0 was adjusted by Potassium Phosphate, monobasic) and 10 µl of 2-Mercaptoethanol before centrifugation at 14000 rpm for 15 min at 4°C. The supernatant was stored at a temperature of -20°C until isozyme analysis. Polyphenol oxidase isozymes were detected according to (Baaziz *et al*., 1994), in which the gel was immersed in a solution containing 0.1% 1-dihydroxyphenyl alanine solubilized in 0.05 M phosphate buffer pH 7.5. Relative band mobility was measured in relation to the dye front and indicated by *Rf* values.

Electrophoretic analysis of protein by SDS-PAGE:

SDS-PAGE was used for detection of genetic variability among 25 wheat genotypes via determination quantitative and qualitative of the total proteins. This method was done according to Laemmli, (1970) as modified by Studier, (1973).

DNA extraction:

Young leaves of 25 wheat genotypes were collected and soaked in liquid nitrogen for DNA extraction using the 2% CTAB method modified by Agrawal *et al*., (1992).

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Random amplified polymorphism DNA-polymerase chain reaction (RAPD-PCR) analysis:

A total of five primers sequenced in (Table 3) were used to amplify DNA (manufactured by Bioneer, New technology certification from ATS Korea). The total reaction mixture was 15 µl contained 10× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP mixed, 10 *p*mol primer, 1.25 U *Taq* polymerase and about 150 ng genomic DNA. DNA amplification was obtained through 35 cycles in a DNA thermal cycler. The temperature profile was as follow: denature temperature 94°C for 30 sec.; annealing temperature 45°C for 1 min; and extension temperature 72 for 1 min, final extension at 72ºC for 5 min.

Amplification product analysis:

The amplified DNA (15 µl) for all samples was electrophoresed on 1% agarose containing ethedium bromide (0.5 µg/ml) in 1X TBE buffer (89 mM Tris-HCl, 89 mM Boric acid, 2.5 mM EDTA, pH 8.3) at 75 constant volt, and determine with UV transilluminator. The size of each fragment was estimated with reference to a size marker of 10 Kb DNA ladder (BioRoN, Germany).

Gel analysis:

Gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

Data analysis:

A matrix for SDS-PAGE, RAPD-PCR combined was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the lines. Genetic similarity coefficients were computed following Nei and Li (1979) as under:

$$\text{similarity} = \frac{2N_{x\,y}}{N_x + N_y}$$

where 'Nx' and 'Ny' are number of bands present in genotypes 'x' and 'y', respectively; and Nxy are the number of bands shared by the cultivar 'x' and 'y'. The data were subsequently used to construct a dendrogram using the unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal 1973) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.1 (Rohlf, 2000). Correlations coefficients were calculated using similarity coefficients obtained from combined SDS-PAGE and RAPD-PCR analysis.

Results and Discussion

Drought Stress Experiment:

The results indicated that percentage of germination varied under drought stress between 25 wheat genotypes (Fig. 1 and Table 1). The effect of drought stress level (-9 bar) revealed that all genotypes gave highly resistant, thus germination percentage (over 80%) except genotypes 4, 11, 12, 14, 16, 17 and 24 gave percentage of germination ranging from (61.11 to 77.36 %) (Table 1). As according to, drought stress level (-12 bar), it was observed that, genotypes 2, 8, 13, 19, 22, 23 and 25 scored the highest percentage of germination (over 80%), while the other genotypes showed (23.68 to 79.37% germination percentage) (Table 1). It can represent that genotypes 2, 8, 13, 19, 22, 23 and 25 was more resistant for drought stress. Results of the current study are in agreement with those obtained by Djibril *et al*., (2005); Hartmann *et al*., (2005); Van Den Berg and Zeng (2006) they found that water stress due to drought is probably the most significant abiotic factor limiting plant and also crop growth and development. Drought stresses is physiologically related, because induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent. Water deficit affects the germination of seed and the growth of seedlings negatively. Because of germination is one of the most important traits in early stage of growth in most plants, it seems that golden west in drought stress condition had more resistant than other cultivars and had more yield potential.

Polyphenol oxidase (PPO) activity:

Isozyme patterns were investigated in 25 genotypes of wheat under drought stress using two concentrations from PEG *in vitro*. PPO-patterns displayed a total of eight bands at different *Rf* values varying from 0.432 to 0.870, whereas five bands were polymorphic with 62.5 polymrphism. The other three with *Rf* (0.700; 0.809 and 0.870) were monomorphic bands at two PEG concentrations compared with the control as presented in (Table 2 and Fig. 2). The relative front (*Rf*) value of each band was calculated depending on this value.

It was observed that, the total density of PPO-containing fractions was higher in the drought stress (two PEG concentrations) tolerant wheat genotypes of 7, 8, 9 and 18 except genotypes of 2, 3, 5, 12 and 14 were the lowest in PPO-activity. Moreover, genotypes of 4, 6, 13 and 21 were not change in PPO-activity with both two PEG concentrations compared with the control. On the other hand, one isozyme marker with *Rf* 0. 622 appeared in -9 bar concentration from PEG of 22 and 25 genotypes. In addition, one biochemical marker at *Rf* 0.432 scored in -12 bar concentration of PEG for 1, 10, 20 and 23 genotypes. Also, one marker with *Rf* 0.515 showed in the same the concentration of genotypes 16, 18 and 24 (Table 2 and Fig. 2). These results were in an agreement with Mišan *et al*., (2006) found that the level of POD activity is an important antioxidant parameter of different wheat lines, supposing that other oxidative stress scavenging enzymes (catalase, glutathione, peroxidase) are working in an appropriate level. The modifications of gene expression due to environmental stress are a common response in the metabolism of plant cells. Gene activation due to environmental stimuli plays an extremely important role in the adaptation of plants to unfavorable conditions and promotes the appearance of specific proteins Naqvi *et al*., (1995). In addition, isozyme polymorphism is good indicator of response to biotic and abiotic stresses (Doebley, 1989). The results of Italianskaya, (1995) found that some drought-resistant bread wheat variants through cell selection for osmotic stress resistance with PEG as selective agent and electrophoretic analysis of esterase and peroxidase revealed the specific appearance of new isozyme bands and lack of several isozymes in drought-resistant somaclones as compared with the parental cultivars.

Protein banding patterns:

The SDS-PAGE for total proteins of twenty five genotypes is illustrated in (Fig. 3). A total number of 24 bands were detected with molecular weights (MWs) ranging from 55.66 to 7.61 KDa, whereas 19 bands were polymorphic with 79.17% polymrphism. With regard to protein banding patterns, five common (monomorphic) bands with MWs (55.66, 43.55, 27.98, 20.39, and 17.07 KDa) were recorded in all genotypes. The highest number of bands (15) was recorded in genotype No. (9) and the lowest number (7) were recorded in genotypes No. 24 and 25. However, (1, 2, 6, 7, 11, 12 and 14), (5, 8, 10 and 13), (16, 20, 21 and 23) and (17, 24 and 25) genotypes were equally in number of bands (13), (12), (9) and (7), respectively. Five unique bands were recorded in genotypes No. 15, 12, 9, 21, and 13 with MWs (30.90, 25.08, 13.97, 12.95, and 9.90 KDa), respectively. These bands could be considered as specific markers. These results were in an agreement with, Chen *et al*., (1996); Perovic *et al*., (1998); Shehata (2004), They found differences in electrophoretic patterns of soluble proteins and suggested that these differences could be used effectively in the identification of wheat cultivars. Ahmed *et al*., (2010); Zarghani1 and Imamjomeh (2011) reported that the possible reasons for the high polymorphism obtained for SDS-PAGE could be due to assessment of genetic diversity based on the total seed protein. Shehata, (2004) mentioned that the electrophoresis separation of water soluble protein indicate the presence of a wide genetic variations among the studied wheat cultivars. These results agreed with Chen *et al*., (1996). Found differences in electrophoresis patterns of soluble proteins and suggested that these differences could be used effectively in the identification of wheat cultivars. Our results are also, in accordance with those of Perovic *et al.,* (1998) who used water soluble protein electrophoretic patterns in identifying different barley and wheat genomes A, B and D in addition to, the Rye genome (R), Six protein markers were found to distinguish R genome, one marker for cultivars belonging to A genome and three markers for cultivars representing B and D genome. The analysis of a large number of wheat cultivars by means of SDS-PAGE has proved that all loci display a large number of allelic variations. He also demonstrated that in hexaploid wheat there are three alleles: the allele a controls the synthesis of the subunit1, the allele b for the sunthesis of the subunit 2 and the allele c is not translated into a protein visible on gel. Our results show that protein patterns obtained by SDS-PAGE may be used as a sufficient tool for differentiation of the studied cultivars by comparing amount of protein bands.

RAPD-PCR fingerprinting:

Seven primers RAPD-PCR were used to identify the studied 25 genotypes wheat as shown in (Fig. 4 and Table 3). The number of bands amplified per primer varied between 7 (OPA-11 and OPA-18) and 16 (OPA-05). A total of 58 bands were amplified of which 27 monomorphic and 31 bands were polymorphic resulting in a polymorphism of 53.45%. The size of bands varied between 2000 bp (in Genotype-6) and 100 bp (into

Genotype-25). The extent of polymorphism per primer ranged from 20 (OPA-02) to 85.71% (OPA-18), On the other hand, both primers OPA-05 and OPA-18 scored one unique marker with molecular sizes 2000 and 600 bp, respectively (Table 3). The present study revealed a low level of polymorphism has been previously reported in wheat using RAPD. Sun *et al*., (2003) reported 63% polymorphism in 35 wheat varieties using RAPD-PCR. Mukhtar *et al*., (2002) observed 64% polymorphism using 50 RAPD primers in 20 Pakistani wheat genotypes. In the present study, 53.45% polymorphism was observed for 25 wheat genotypes using seven RAPD-PCR primers.

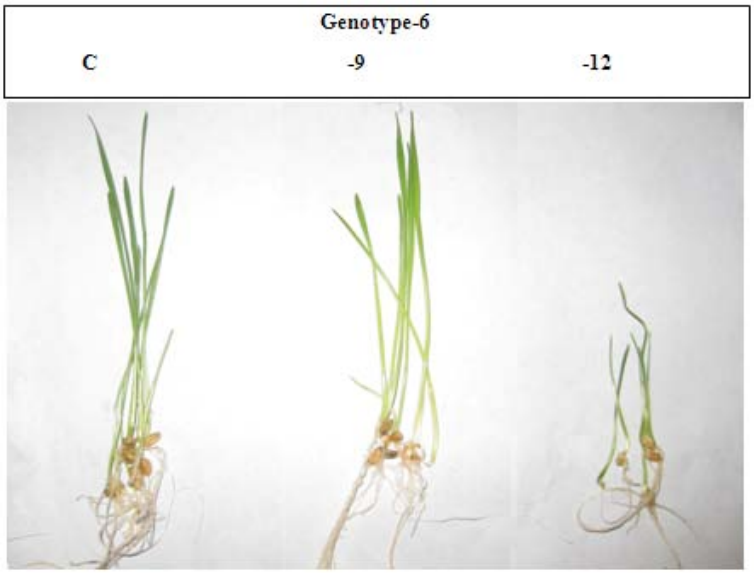

Fig. 1: Illustrate effect of the treatment with two concentrations from PEG on wheat genotype-6. C= (The control).

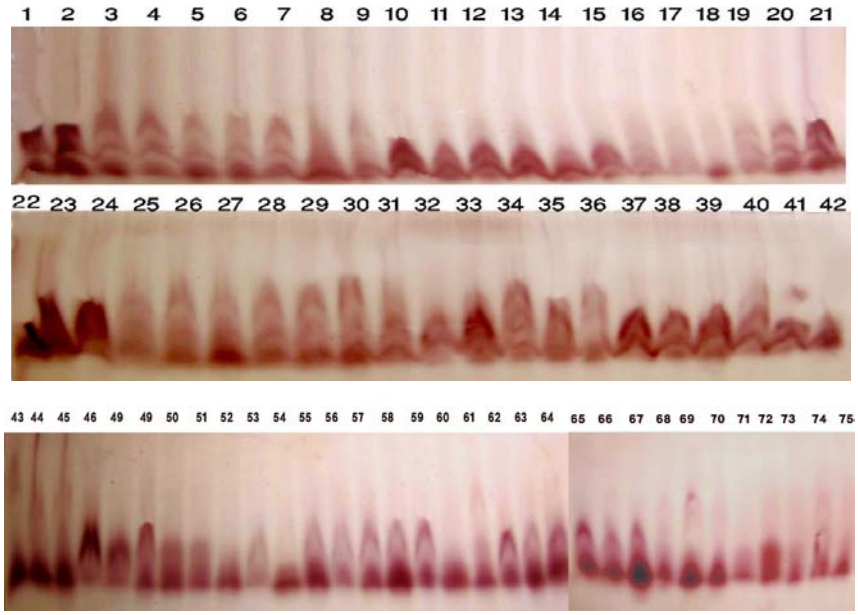

Fig. 2: Zymogram of polyphenol oxidase banding patterns for the 25 genotypes from wheat under drought stress.

1, 4, 7, 10, 13, 16, 19, 22, 25, 28, 31, 34, 37, 40, 43, 46, 49, 52, 55, 58, 61, 64, 67, 70, 73= 1----25 genotypes wheat ,respectively (untreated).

2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, 68, 71, 74= 1----25 genotypes wheat treated with -9 bar from PEG, respectively.

3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36, 39, 42, 45, 48, 51, 54, 57, 60, 63, 66, 69, 72, 75= 1----25 genotypes wheat treated with -12 bar from PEG, respectively.

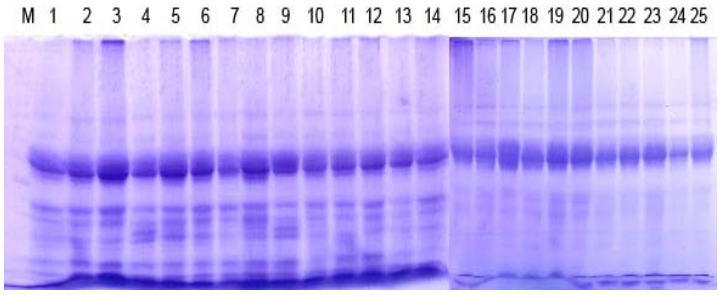

Fig. 3: SDS-binding patterns of 25 wheat genotypes (*Triticum aestivum* L.). Lane M= protein marker with molecular weights (10, 15, 20, 25, 30, 40, 50, 60, 70, 85, 100, 120, 150 and 200 KDa).

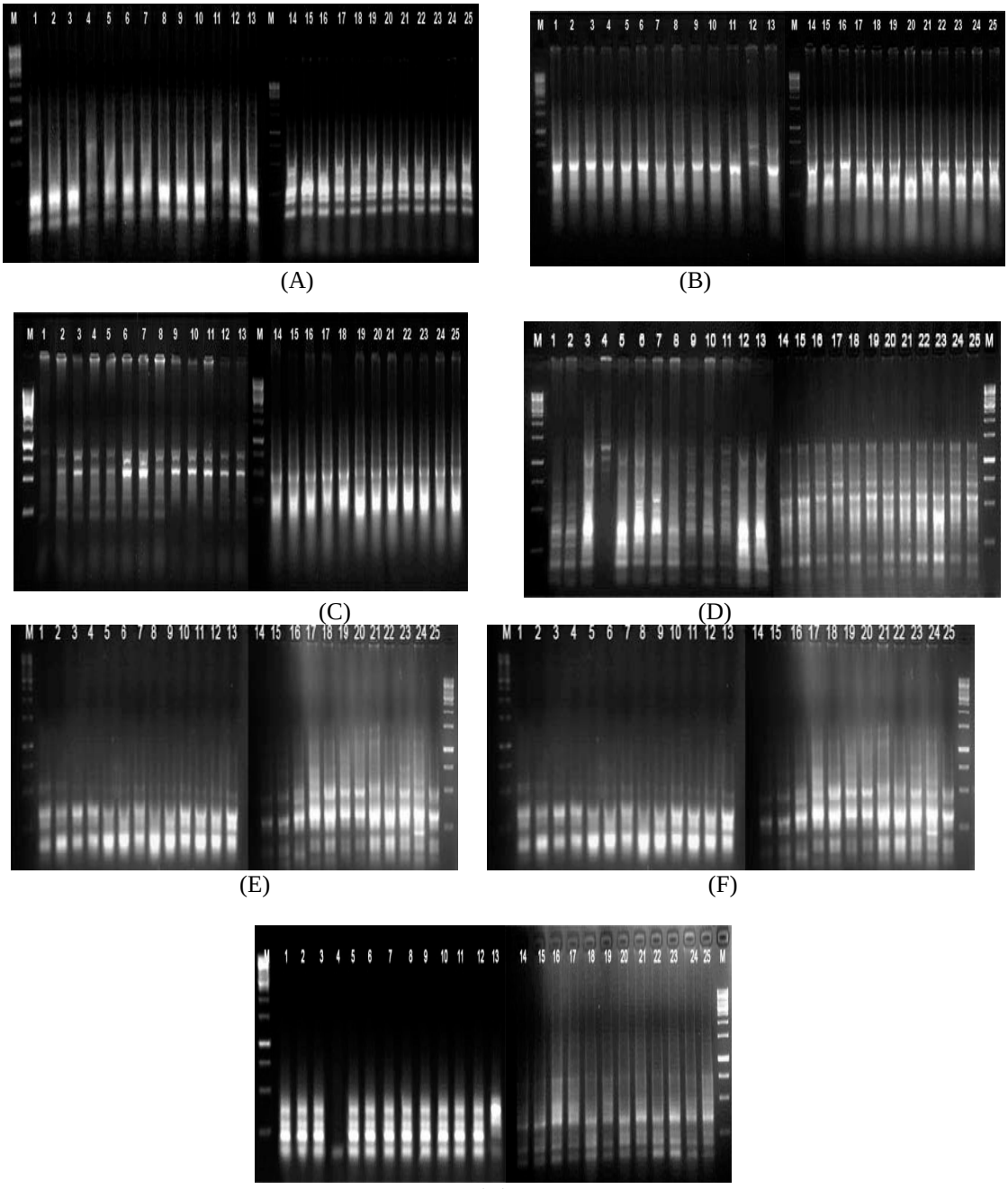

Fig. 4: RAPD-PCR analysis of 25 wheat genotype with seven arbitrary primers. (A) Primer OPA-1, (B) Primer OPA-2, (C) Primer OPA-4, (D) Primer OPA-5, (E) Primer OPA-11, (F) Primer OPA-16 and (G) Primer OPA-18. Lane M = 10 Kb DNA ladder with molecular sizes (250, 500, 750, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 5000, 6000, 8000 and 10000 bp).

Table 1: Comparison of reaction of genotype and stress levels on germination.

No. of Genotype	The percentage of Germination (Control)	The percentage of Germination (-9 bar)	The percentage of Germination (-12 bar)	No. of Genotype	The percentage of Germination (Control)	The percentage of Germination (-9 bar)	The percentage of Germination (-12 bar)
1	100	91.11	46.51	14	63.33	62.50	50.33
2	100	93.75	88.10	15	96.97	88.89	59.52
3	100	80.56	79.37	16	98.08	73.53	54.84
4	97.73	77.36	73.33	17	90.70	61.11	51.75
5	98.04	83.78	76.67	18	95.24	92.54	48.94
6	96.67	83.33	23.68	19	88.89	88.89	83.33
7	80.95	87.06	70.22	20	96.67	84.85	72.45
8	97.73	86.33	84.21	21	90.32	81.82	71.43
9	94.05	84.85	59.21	22	100	90.00	85.71
10	100	94.12	60.00	23	100	97.22	80.00
11	100	64.00	50.83	24	88.00	68.34	28.81
12	94.59	74.19	70.59	25	97.37	92.59	86.67
13	86.05	84.78	80.24				

Table 2: PPO-profiles of 25 wheat genotypes under drought stress using two concentrations from PEG

No. bands	***Rf***	**Genotype-1**			**Genotype-2**			**Genotype-3**			**Genotype-4**			**Genotype-5**			**Genotype-6**			**Genotype-7**		
		C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12
1	0.432			++	++		+													+	++	++
2	0.515	+++	+++	++	++	+	+	+	+	+							+	+	+	+	++	++
3	0.622										++	++	++	++								
4	0.700	+++	+++	++	++	+	+	+	+	+	++	++	++	++	+	++	+	+	+	+	++	+++
5	0.809	+++	+++	++	++	++	++	++	+	+	++	++	++	++	+	+	+	+	+	+	++	+++
6	0.870	+++	+++	++	++	++	++	++	+	+	++	++	++	++	+	+	+	+	+	+	++	+++
Total bands= 6		**4**	**4**	**5**	**5**	**4**	**5**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**3**	**4**	**4**	**4**	**5**	**5**	**5**

C= Control + = presence of bands (faint) ++= dark +++= very dark

Table 2: Continuous

No.	**Rf**	**Genotype-8**			**Genotype-9**			**Genotype-10**			**Genotype-11**			**Genotype-12**			**Genotype-13**			**Genotype-14**		
		C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12
1	0.432									++	+			++		+						
2	0.515							++	++	++	+		++	++		+				++	++	
3	0.622		+++	+++	+	+	+	++	++	++	++		+++	++	++	+				++		
4	0.700	+++	+++	+++	+	++	+	++	++	++	++	++	+++	++	++	+	+++	++	++	++	++	++
5	0.809	+++	+++	+++	+	++	++	++	++	++	++	++	+++	++	++	++	+++	++	++	++	++	++
6	0.870	+++	+++	+++	+	++	+++	++	++	++	++	++	++	++	++	++	+++	++	++	++	++	+++
Total bands= 6		**3**	**4**	**4**	**4**	**4**	**4**	**5**	**5**	**6**	**6**	**3**	**5**	**6**	**4**	**6**	**3**	**3**	**3**	**5**	**4**	**3**

C= Control + = presence of bands (faint) ++= dark +++= very dark

Table 2: Continuous

No.	**Rf**	**Genotype-15**			**Genotype-16**			**Genotype-17**			**Genotype-18**			**Genotype-19**			**Genotype-20**			**Genotype-21**		
		C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12
1	0.432	+	+	+															1			
2	0.515	++	++				+++						++	+	++	++	+			+++	++	+++
3	0.622		++	++	+++	++	+++		++													
4	0.700	+++	+++	+++	+++	+++	++	++	++	+	+	+	++	++	++	+++	++		++	++	++	+++
5	0.809	+++	+++	+++	++	++	++	++	++	++	+	++	+++	++	++	+++	++	++	++	+++	+++	+++
6	0.870	+++	+++	+++	++	++	+++	+++	++	++	+	+++	+++	++	++	+++	++	++	++	+++	+++	+++
Total bands= 6		**5**	**6**	**5**	**4**	**4**	**5**	**3**	**4**	**3**	**3**	**3**	**4**	**4**	**4**	**4**	**4**	**2**	**4**	**4**	**4**	4

+ = Presence of bands
C= control +++= very dark ++= dark += faint

Table 3 Continuous

No.	**Rf**	**Genotype-22**			**Genotype-23**			**Genotype-24**			**Genotype-25**		
		C	-9	-12	C	-9	-12	C	-9	-12	C	-9	-12
1	0.432						+						
2	0.515	+++	+++		+++		+			+	+	+	
3	0.622		++		++							+	
4	0.700	+++	+++	++	+++	+	++	++	+	++	++	++	
5	0.809	+++	+++	++	+++	++	++	++	+	++	++	++	++
6	0.870	+++	+++	+++	+++	++	+++	++	+	++	++	++	++
Total bands=6		**4**	**5**	**3**	**5**	**3**	**5**	**3**	**3**	**4**	**4**	**5**	**2**

+ = Presence of bands
C= Control ++= dark +++= very dark

The information gathered here would be helpful in genome mapping studies and for the development of wheat cultivars with wider and diverse genetic background to obtain improved crop productivity. The data obtained in this experiment confirmed the efficiency of the RAPD technique for determination and estimation of genetic distances and relatedness among different plant genotypes. The RAPD analysis has been found to a valuable DNA marker system to evaluate genetic diversity. The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform. Because of the simple experimental procedures, the requirement of minimal amount of plant tissue and the possibility of automation

(Terzi, 1997; Asif *et al*., 2005; Abdul and Tahir 2010), RAPD analysis should be very useful in breeding for rapid and early identification of most diverse individuals in large seedling populations, allowing the detection of true to type genotypes for the improvement of our crop breeding programs. Keeping in view the useful information about the close genetic relationship, it is suggested that mission oriented breeding programs with the help of DNA fingerprinting technology will be helpful to produce distinct cultivars/ genotypes with diverse genetic background and improved productivity.

Table 3: Polymorphism of the RAPD-PCR primers among *25* wheat genotypes.

Primer Code No.	Sequence (5`→3`)	Size range of the scorable Bands (bp)	Total bands	No. of monomorphic bands	No. of polymorphic bands	Unique bands	% Polymorphism
OPA-01	CAGGCCCTTC	460-145	6	3	3	0	50
OPA-02	TGCCGAGCTG	590-180	5	4	1	0	20
OPA-04	AATCGGGCTG	870-230	5	3	2	0	40
OPA-05	AGGGGTCTTG	2000-100	16	4	12	1	75
OPA-11	CAATCGCCGT	700-170	7	7	0	0	0
OPA-16	AGCCAGCGAA	1000-150	12	5	7	0	58.33
OPA-18	AGGTGACCGT	750-150	7	1	6	1	85.71
Total		2000-100	58	27	31	2	53.45

The dendrogram resulted from the combination between the banding patterns of protein SDS-PAGE and RAPD-PCR (Table 4 and Fig. 5) revealed that 25 wheat genotypes can be clustered in three distinct groups. Group A (similarity range 0.69 to 0.93) contains on genotypes (1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12 and 13), Group B (similarity range 0.73 to 0.93) comprised of genotypes (14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 and 25), while group C includes on genotype-4 (similarity range 0.53 to 0.72). Molecular markers using RAPD-PCR show better resemblance compared to biochemical markers using SDS-PAGE and isozymes. Thus, their disadvantages include a low level of polymorphism to have few alleles per locus, especially when the genetic base is narrow (Kesseli *et al*., 1991). In addition, proteins can be affected qualitatively and quantitatively in their expression level by environmental factors and tissue type. On the contrary, molecular markers are not environmentally influenced, which means that the same banding profiles can be expected at all times for the same genotype (Kumar *et al*., 2009).

Table 4: Similarity indices among the 25 wheat genotypes as estimated using SDS-PAGE and RAPD-PCR.

N0.	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	G25
G1	1.00																								
G2	0.93	1.00																							
G3	0.91	0.88	1.00																						
G4	0.69	0.69	0.70	1.00																					
G5	0.89	0.93	0.88	0.72	1.00																				
G6	0.86	0.86	0.88	0.69	0.93	1.00																			
G7	0.89	0.86	0.88	0.67	0.89	0.86	1.00																		
G8	0.88	0.88	0.89	0.68	0.91	0.91	0.91	1.00																	
G9	0.84	0.84	0.83	0.65	0.91	0.91	0.85	0.89	1.00																
G10	0.88	0.91	0.82	0.67	0.91	0.84	0.84	0.86	0.89	1.00															
G11	0.89	0.89	0.91	0.69	0.93	0.89	0.89	0.91	0.91	0.91	1.00														
G12	0.86	0.86	0.88	0.69	0.93	0.90	0.90	0.88	0.88	0.84	0.93	1.00													
G13	0.82	0.85	0.83	0.66	0.88	0.82	0.82	0.83	0.80	0.86	0.88	0.85	1.00												
G14	0.80	0.83	0.81	0.61	0.86	0.83	0.80	0.82	0.82	0.78	0.83	0.83	0.76	1.00											
G15	0.77	0.77	0.78	0.59	0.77	0.75	0.72	0.73	0.76	0.78	0.80	0.77	0.76	0.80	1.00										
G16	0.83	0.83	0.84	0.58	0.83	0.80	0.80	0.81	0.81	0.81	0.86	0.80	0.79	0.80	0.86	1.00									
G17	0.77	0.77	0.78	0.53	0.77	0.74	0.77	0.75	0.73	0.75	0.77	0.74	0.76	0.83	0.80	0.86	1.00								
G18	0.77	0.77	0.78	0.56	0.80	0.77	0.77	0.75	0.75	0.75	0.77	0.77	0.76	0.86	0.86	0.86	0.93	1.00							
G19	0.75	0.75	0.77	0.55	0.78	0.76	0.79	0.74	0.74	0.74	0.75	0.79	0.72	0.91	0.79	0.78	0.88	0.91	1.00						
G20	0.78	0.78	0.79	0.56	0.81	0.78	0.78	0.77	0.77	0.76	0.78	0.78	0.74	0.91	0.78	0.81	0.91	0.91	0.96	1.00					
G21	0.77	0.77	0.78	0.56	0.80	0.77	0.77	0.76	0.76	0.75	0.77	0.77	0.73	0.80	0.77	0.83	0.89	0.89	0.85	0.88	1.00				
G22	0.78	0.75	0.80	0.55	0.75	0.73	0.79	0.74	0.71	0.74	0.75	0.73	0.72	0.82	0.76	0.81	0.95	0.88	0.86	0.89	0.91	1.00			
G23	0.75	0.72	0.76	0.55	0.75	0.72	0.75	0.71	0.71	0.70	0.72	0.72	0.71	0.78	0.84	0.83	0.86	0.90	0.82	0.82	0.87	0.88	1.00		
G24	0.73	0.73	0.74	0.50	0.73	0.70	0.76	0.71	0.69	0.71	0.73	0.73	0.75	0.73	0.79	0.84	0.88	0.84	0.80	0.80	0.85	0.86	0.88	1.00	
G25	0.71	0.71	0.75	0.51	0.71	0.69	0.69	0.73	0.70	0.69	0.74	0.71	0.70	0.80	0.77	0.77	0.83	0.80	0.81	0.84	0.77	0.81	0.75	0.75	1.00
G=Genotype																									

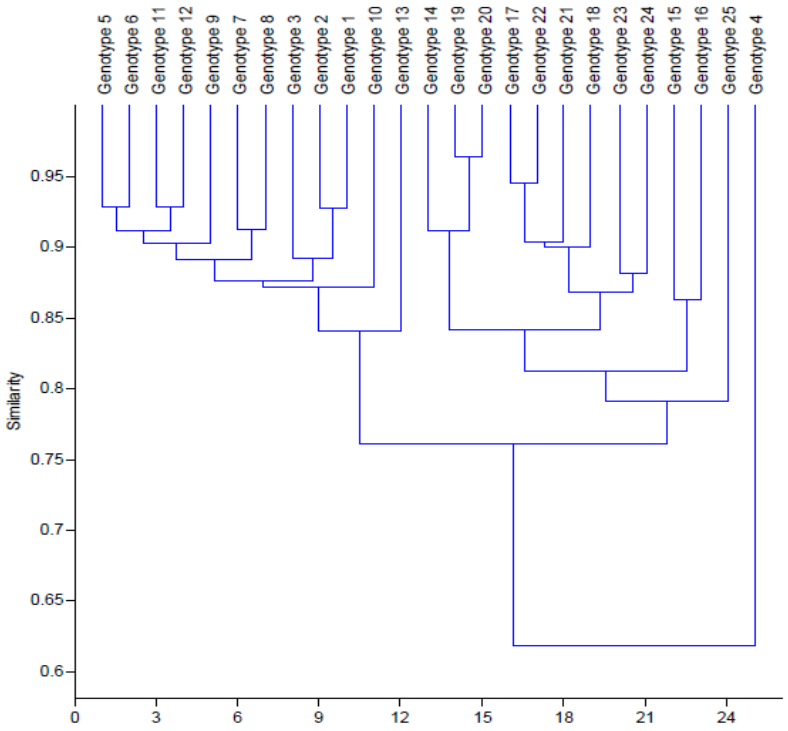

Fig. 5: Dendrogram of 25 wheat genotypes based on SDS-PAGE and RAPD-PCR combination by UPGMA algorithm using Jaccard's similarity coefficient.

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