CYTOLOGICAL AND MOLECULAR CHARACTERIZATION OF SOME WHEAT (*Triticum aestivum* L.) CULTIVARS

MONA H. ABD EL-GAWWAD[1], H. EL RABEY[2], R.M. KHALAF[1], M. H. EL-HADARY[3] AND K. EL-HALAFAWY[3]

1. *National Gene Bank and Genetic Resources, Agricultural Research Center, Giza, Egypt*
2. *Bioinformatics Department, Genetic Engineering and Biotechnology Institute, Minufiya University, Sadat City, Egypt*
3. *Molecular Biology Department, Genetic Engineering and Biotechnology Institute, Minufiya University, Sadat City, Egypt*

Wheat (*Triticum aestivum* L.) belongs to *Poaceae* family. Ninety-five percent of wheats grown today are of the hexaploid type comprising three genomes A, B and D. Each of these genomes has seven chromosome pairs. The remaining 5% is durum (*Triticum turgidum* L., var. *durum*) tetraploid wheat (Heun *et al.*, 1997). The genomic formula of 2n=6x=42, AABBDD is believed to have been arisen as a result of allohexaploid between an AABB tetraploid, *Triticum turgidum* and with DD diploid genome, *Aegilops squarrosa* (*Triticum tauschii*) and the AA genome of hexaploid wheats has its origin from *Triticum urartu*, (Miller, 1987; Jahan and Vahidy, 1989). The wheat genome is 17000 Mb (Hartl and Jones, 2001).

The Karyotype analysis of plant chromosomes has various applications in plant systematic, cultivars identification and breeding purposes (Hussein, 2005; Fregonezi *et al.*, 2006; Chengqi, 2008). It includes the measurements of chromosomes i.e., total chromosome length, chromosome area, centromeric position, presence of satellites and long /short arm ratio (Fukui, 1988).

Biochemical evidences such as seed storage protein electrophoresis and isozyme polymorphisms are convenient evidences for assessing genetic relationships and identification of different cultivars (Nour El-Din *et al.,* 2004; Simova-Stoilova *et al.,* 2006).

The nonanchored ISSR (Inter Simple Sequence Repeats) markers are used for DNA fingerprinting and assessing the genetic diversity in *Poaceae* (Bornet and Branchard, 2004; Pharmawati *et al.* 2005; Bhuyan *et al.*, 2007), The ISSR-PCR markers are arbitrary multiloci markers produced by PCR amplification with a microsatellite primers. They are advantageous because no prior genomic information is required for their use and the technique is stable across a wide range of PCR parameters (Sivolap and Trbel'skiie, 2001; Du *et al.*, 2002; Hou *et al.*, 2005; Sofaliani *et al.*, 2008).

In the present study, the genetic diversity of six wheat cultivars was investigated based on karyotype analysis, α-esterase and peroxidase polymorphisms, seed storage protein electrophoresis and ISSR markers and the genetic relationships between these cultivars was addressed based on both protein and ISSR data and using NTSYS-pc2.0 (Numerical Taxonomy and Multivariate Analysis System) software (Rohlf, 1998).

MATERIALS AND METHODS

In this study, six wheat cultivars were carefully chosen to represent different drought tolerance potentials and were kindly provided by the National Gene Bank (NGB) at Agricultural Research Center (ARC), Giza, Egypt. These cultivars are: 1- Gemmiza 9, 2- Youkora, 3- Giza 168, 4- Sahel 1, 5- Sakha 93, 6- Sids 1.

Karyotype analysis

For karyotype analysis, seeds were germinated on moistened filter papers in Petri dishes at 25-30°C in an incubator. The lateral roots of about 1.5: 2.0 cm length were collected and treated with 0.05% colchicine for three h at room temperature, flamed by forceps and stained by aceto-orcein solution. The samples were examined using Image Processing Analysis System (Video Test - Karyo) in the c-metaphase. Measurements of the total lengths of chromosome (μ), area of chromosome (μ^2) and centromeric index percentage (length of short arm/length of chromosome) were taken for every chromosome. The three genomes of bread wheat (A, B and D) were judged according to Hussein (2005).

Isozyme analysis

α-Esterase (Est) was extracted according to Jonathan and Wendel (1990) and peroxidase (Px) was extracted according to Graham *et al.* (1964). Both isozymes were separated in 15% polyacrylamide gel electrophoresis according to Stegemann *et al*. (1985).

Total seed storage protein electrophoresis

SDS-PAGE of the total seed storage protein was achieved according to a modified method from Laemmli (1970). For molecular weight determination, a mixture of standard protein markers of the following molecular weights; 97.40 (phosphorylase) 66.00 KDa (Albumin, Bovine), 45.00 KDa (Albumin, egg), 31.00 KDa (carbonic anhydrous), 21.00 KDa (trypsin inhibitor) and 14.20 KDa (α-lactalbumin/lysozymes) was used. The banding profile was photographed analyzed using Gel Doc 2000, BioRad Densitometer scanner.

ISSR fingerprinting

DNA Isolation was performed according to Jonathan and Wendel (1990). ISSR-PCR reaction for the six genotypes of wheat was conducted using 10 specific primers, as presented in Table (1). Amplification was carried out in Stratagene thermocycler programmed as follows:

denaturation (one cycle) 94°C for 2 minutes, followed by 30 cycles (94°C for 30 second, 44°C for 45 Secs, 72°C for 1 minute and 30 Sec) and finally one cycle at 72°C for 20 minutes. The PCR product was separated against 100 bp DNA ladder in 1.2% agarose gel (Sambrook *et al*., 1989). The ISSR bands were detected on UV- transilluminator and photographed by Gel documentation system (UVP) 2000.

Statistical analysis

Isozymes, protein and ISSR gels were scored as 0/1 for absence/presence of the bands, respectively and the resulting data were analyzed using NTSYS-pc2.0 software (Rohlf, 1998). The karyotypes were analyzed using Image Processing Analysis System (Video Test - Karyo). Similarity coefficient matrices were calculated using simple matching similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrograms were constructed using the UPGMA method (Unweighted Pair-Group Method with Arithmetical mean; Sneath and Sokal, 1973).

RESULTS

Karyotype analysis

The karyotypes of the six wheat cultivars are shown in Fig. (1) and the karyotype analysis of the same cultivars are illustrated in Table (2). From the karyotype analysis of the six wheat cultivars (Table 2 and Fig. 1), it can be concluded that, the area of chromosome ranged from 0.09 μ^2 (in Sahel 1, chromosome 7B) to 0.57 μ^2 (in Youkora chromosome 2A). Chromosome length ranged from 0.51 μ (recorded in Giza 168, chromosome 7B) to 2.08 μ (in Youkora, chromosome 1A). The centromeric index ranged from 27.8% (in Sakha 93 chromosome 7D) to 50.5% (in Giza 168, chromosome 2A).

α-Esterase (α-Est)

The electrophoretic pattern of α-esterase (Table 3 and Fig. 2) revealed the polymorphisms among the studied cultivars. Bands Est 1 and Est 4 (Rf=0.05, 0.09) are characteristic to Sahel 1. Est 5 (Rf=0.33) is absent in Gemmiza 9 and Sakha 93 and present in the other cultivars under investigation. Sahel 1 is characterized by two positive markers; Est 1 and Est 4 (Rf= 0.05 and 0.09, respectively). Est 2, Est 3, Est 6, Est 7 and Est 8 (Rf=0.06, 0.08, 0.37, 0.72 and 0.75, respectively) were scored as common bands which are expressed in all wheat cultivars under investigation.

Peroxidase (Px.)

Peroxidase banding pattern (Pxs) of the six wheat genotypes is illustrated in Table (4) and Fig. (3). The zymogram pattern gave four bands (Rf= 0.5, 0.9, 0.94 and 0.97). The two bands Px 1 and Px 2 are expressed only in Sahel 1 and Sakha 93 genotypes, while Px 3 is expressed in all genotypes as a common band and Px 4 is absent in Youkora and Giza 168 genotypes and present in the other genotypes.

Total seed storage protein electrophoreses

Figure (4) shows the SDS-electrophoretic banding patterns of the total seed storage protein for the six wheat cultivars. The SDS banding pattern produced 27 bands distributed in all cultivars with molecular weights ranging from 15.62 KDa to 141.4 KDa. The distribution of these bands - in the studied cultivars - and their molecular weight are illustrated in Table (5). The occurrence of protein bands is as follows; 12 bands in Gemmiza 9, 27 bands in Youkora, 24 bands in Giza 168, 25 bands in Sahel 1, 16 bands in Sakha 93 and 20 bands in Sids 1. This result indicate that eight bands are monomorphic with molecular weights of 92.2, 87, 48.32, 39.19, 33.49, 32.72, 25.3 and 16.85 KDa and the other 19 bands are polymorphic bands (Table 5).

The current results indicated also that, some cultivars have some specific bands, which could be used to distinguish such cultivars from each others. For example, Gemmiza 9 had seven negative specific bands with molecular weights of 84.51, 78.34, 70.13, 61.36, 27.44, 22.67 and 21.89 KDa and could be distinguished from other cultivars by the absence of these bands while, Youkora had one positive specific band with molecular weight of 27.17 KDa. This band could be considered as a specific marker for distinguishing this cultivar from other cultivars. Moreover, Giza 168 had one specific band with molecular weight of 36.34 KDa and Sakha 93 had two negative markers with molecular weight of 30.34 KDa and 17.86 KDa.

The intercultivars relationships based on protein data analysis

The obtained seed storage protein data were applied to NTSYS-PC2 program to address the genetic relationships among the studied cultivars (Fig. 5). Gemmiza 9 was separated alone distant from all the other 5 samples under study. Sakha 93 and Sids 1 were clustered together in a subgroup, while Youkora, Giza 168 and Sahel 1 were clustered in another subgroup. Giza 168 and Sahel 1 are closely related to each other.

ISSR analysis

Table (6) and Fig. (6) illustrate the ISSR results of the six wheat cultivars. A total of 74 amplicons -amplified fragments ranged from 90 bp to 1280 bp were obtained from the tested primers with an average of 7 amplicons/primer. Primers HB11 and HB14 exhibited the highest number of fragments (10 and 16 amplicons, respectively), followed by primers HB9 and HB10 (9 and 8 amplicons respectively), while primers 814, 844A, 844B, HB8, HB15 and HB13 revealed the lowest number of fragments (3 - 6 amplicons for each) (Table 6). The total number of polymorphic bands was 40 with an average of 3.8 polymorphic amplicon/primer. The number of polymorphic markers varied among the different primers (see Table 6).

Nineteen out of the 74 ISSR-bands (about 25.6%) were found to be useful as cultivar-specific markers, which present in one cultivar and absent in the other cultivars under this investigation. Number of ISSR-PCR fragments that generated by using the ten primers, and could be used as cultivar-specific markers were arranged descending as, primer HB8 (five markers), primer HB11 (four markers), Primers HB10 (three markers), primers 814, HB13 and HB14 (two markers) and primer 844B (one marker). On the other hand, primers 844A, HB9 and HB15 showed no cultivar-specific markers. Thus, the wheat cultivars could be identified by cultivar-specific ISSR markers as follow; Gemmiza 9 is characterized by seven markers (AF01, AF19, AF38 and AF51) as a positive markers and (AF05, AF18 and AF36) as negative markers, Youkora is recognized by three negative markers (AF16, AF30 and AF60). Giza 168 is characterized by two negative markers (AF15 and AF17), Sahel 1 is characterized by three negative markers (AF44, AF46 and AF47), Sakha 93 is characterized by three positive markers (AF12, AF49 and AF67) and Sids 1 is distinguished by one positive marker (AF37) (Table 6).

The intercultivars relationships based on ISSR data analysis

The resulted ISSR data were analyzed using NTSYS-PC2 (Fig. 7) program to address the genetic relationship among the six wheat cultivars. The obtained dendrograms were 100% similar in both cases and the genetic relationship of the six wheat cultivars based on the ISSR data and analyzed using NTSYS-PC2 program was highly similar to that obtained from seed protein data analysis. Gemmiza 9 was separated distant from all the other cultivars under study followed by Youkora. Giza 168 and Sahel 1 appeared closely related to each others being clustered together. Similarly Sakha 93 and Sids 1 were also highly related to each other.

DISCUSSION

Application of cytological, biochemical and molecular genetics techniques have an important potential to provide a new tool for the study of both wild and domesticated species, in respect to investigation of evolution and migration of species from their gene pool centers (Badr *et al.*, 1996; Badr *et al.*, 2000; Fregonezi *et al*., 2006). Thus, identification and characterization of species become possible through “fingerprinting” for each species since DNA is a source of informative polymorphism (Heun *et al.*, 1997; El Rabey, 2006; El Rabey, 2008). Consequently, techniques of molecular genetic markers have an important potential for the detection of genetic differences among species and considering it as a basic reference for gene bank resources which will be of great value for executing further plant breeding programs through classical and new plant genetic engineering techniques such as tissue culture and protoplast fusion (Chengqi, 2008).

Earlier karyotype analysis of wheat chromosomes revealed that, chromosome

number 1, 2 and 3 are metacentric (55% ~ 45% of centromeric percentage), chromosomes numbers 4 and 5 are submetacentric (45% ~ 30% of centromeric percentage and chromosomes number 6 and 7 are subacrocentric (30% ~ 25% of Centromeric) (Jahan and Vahidy, 1989). Karyotype analysis of plant chromosomes has various applications in plant systematic, cultivars identification and breeding purposes (Badr *et al*., 1996; Cuadrado *et al*., 2000).

Accordingly, the karyotype analysis measurements of the six wheat cultivars of the current study as based on Hussein's (2005); the chromosome length, chromosome area and chromosome centromeric index of the studied cultivars could be used in differentiating these cultivars from each others (Fukui, 1988; Fregonezi *et al*., 2006 ; Chengqi, 2008).

Data of α-esterase and peroxidase isoforms showed differences between the six wheat cultivars in such characteristics as number, intensity and density of the bands. They can be believed to be specific markers for different cultivars under investigation because isoforms belonging to different genotypes of the same species collected from different locations may represent some degree of genetic variation among them (Abdel-Tawab *et al*., 1993; Ko *et al*., 1994; Abdelsalam *et al*., 1998). The intercultivars enzyme variation may be also due to the effect of environmental conditions (Loxdale, 1994). They are not reflected in the phenotypic appearance of the species and that may be a sign of genetic diversity of some isozyme loci. Therefore, electrophoresis can detect the most important variability in the structural genome via its expressed protein products (Michaud *et al.*, 1995). Isozymes polymorphism has been used in several laboratories to investigate origins of the cultivated wheats and other phylogenetic relationships among wheats and their close relatives (El-Saied and Afiah, 2004).

The present seed storage protein results could differentiate between the studied wheat cultivars producing some specific bands that can be used to distinguish such cultivars from each others. Furthermore, these results are quietly aligned with those obtained by many investigators which have been carried out on *Triticum* spp. (Kraic *et al.,* 1995; Simova-Stoilova *et al.,* 2006).

The genetic relationships of the six wheat cultivars based on seed storage protein data analysis using NTSYS-PC2 program was consistent with that resulted from the ISSR analysis, discriminating Gemmiza 9 from all the other cultivars under study. Sakha 93 and Sids 1 were clustered together in a subgroup, while Youkora, Giza 168 and Sahel 1 were clustered in another subgroup. Giza 168 and Sahel 1 are closely related to each other. The results of this study integrate the previously published cytological, biochemical and molecular investigation, emphasizing that these markers are powerful tools for cultivar identification (Abdel-

Tawab *et al.*, 1993; Radic–Miehle *et al.*, 1997; Dvoracek and Curn, 2003; El Rabey, 2004; El Rabey, 2008).

ISSR-PCR markers have an efficient value in determining of wheat variety/genotype-specific markers and wheat taxonomy, and also as tools for detection of similarities and dissimilarities of wheat genotypes (Bornet and Branchard, 2004; Bhuyan *et al*., 2007; Sofaliani *et al.,* 2008). Thus, the wheat cultivars under study could be identified by cultivar-specific ISSR markers that can differentiate these markers from each others.

From the current results it can be concluded that, isozyme analysis i.e α-esterase and peroxidase could partially differentiate between the six wheat cultivars under investigation, whereas total seed storage protein electrophoresis and ISSR-PCR markers could efficiently discriminate between all wheat germplasms under investigation. Therefore, they are helpful in characterization of genotypes due to the reproducibility of protein and ISSR markers and the inter cultivars relationships resulted from analyses of their data.

REFERENCES

Abdelsalam, A. Z. E., S. A. Ibrahim, F. M. A. Eldomyati and G. H. El-Nady (1998). Biochemical and molecular genetic characterization of Egyptian barley cultivars and a trial for their micropropagation. 3rd Arab Conference Modern Biotech & Areas of Application in the Arab World, 14-17 December, Cairo, Egypt, 583-604.

Abdel-Tawab, F. M., A. A. El Seoudy, M. A. Rashed and A. Bahiedin (1993). Enzyme activity and the identification of wheat cultivars. 4th Agric. Dev. Res., Ain Shams Univ., Cairo, Feb. 13-18, 1993. Annals Agric. Sci., Sp. Issue, 2, 465-475.

Badr, A., K. Mueller, R. Schaefer-Pregl, H. El Rabey, S. Effgen, H. H. Ibrahim, C. Pozzi, W. Rohde and F. Salamini (2000). On the origin and domestication history of barley (*H. vulgare*). Molecular Biology and Evolution, 17: 499-510.

Badr, A., M. A. Hammoud and H. A. El Rabey (1996). Chromosomal studies in the Egyptian flora V. Chromosomal relationships in the genus *Astragalus* L. (*Fabaceae*) and their taxonomic inferences. Cytologia, 61: 105-111.

Bhuyan, N., B. K. Borah and R. N. Sarma (2007). Genetic diversity analysis in traditional lowland rice (*Oryza sativa* L.) of Assam using RAPD and ISSR markers. Current Science, 93: 967-972.

Bornet, B. and M. Branchard (2004). Use of ISSR fingerprints to detect microsatellites and genetic diversity in several related *Brassica* taxa and *Arabidopsis thaliana*. Hereditas, 140: 245-248.

Chengqi, A. O. (2008). Chromosome numbers and karyotypes of *Allium Przewalskianum* Populations. ACTA Biological Cracviensia Series Botanica, 50: 43-49.

Cuadrado, A., T. Schwarzacher and N. Jouve (2000). Identification of different chromatin classes in wheat using *in situ* hybridization with simple sequence repeat oligonucleotides. Theor. Appl. Genet., 101: 711-717.

Du, J. K., Y. Y. Yao, Z. F. Ni, H. R. Peng and Q. X. Sun (2002). Genetic diversity revealed by ISSR molecular marker in common wheat, spelt, compactum and progeny of recurrent selection. Yi Chuan Xue Bao, 29: 445-452.

Dvoracek, V. and V. Curn (2003). Evaluation of protein fractions as biochemical markers for identification of spelt wheat cultivars (*Triticum spelta* L.). Plant Soil Environ., 49 : 99-105.

El Rabey, H. (2004). DNA and seed protein fingerprinting of Egyptian crop plants. IV. The genetic relationships among barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.). The international conference of genetic engineering and its applications, Sharm El Sheikh City, South Sinai, Egypt, 8-11 April, Vol. (1): 329-344.

El Rabey, H. (2006). DNA and seed protein fingerprinting of Egyptian crop plants. V. Differentiation between Giza 168 and Sakha 69 wheat cultivars (*Triticum aestivum* L.) using RAPD markers and seed protein electrophoresis. The 4th international conference on biolological sciences, Faculty of Science, Tanta University, Proc. Botany, 171-178.

El Rabey, H. (2008). Molecular and biochemical studies on *Egyptian Hordeum murinum* L. complex as revealed by RAPD-PCR and seed storage protein electrophoresis (2007). Taeckholmia, 28: 145-156.

El-Saied, F. M. and S. M. Afiah (2004). Genetic and Biochemical variants of some halophytes from Shalateen region, the South-Eastern portion of Egypt. Arab Univ. J. Agric. Sci., Ain Shams Univ., 15: 523-530.

Fregonezi, J. N., T. Fernandes, T. Domingues, A. Vieira and A. L. Vanzela (2006). Karyotype differentiation of four *Cestrum* species (*Solanaceae*) based on the physical mapping of repetitive DNA. Genetics and Molecular Biology, 29: 97-104.

Fukui, K. (1988). Analysis and utility of Chromosome Information by using the chromosome Image analyzing System, CHIAS. Bull. Nat1. Inst. Agrobiol., 4: 153-176.

Graham, R. C., U. Lundholm and M. J. Karnovsky (1964). Cytochemical demonstration of peroxidase activity with 3 amino 9 ethylcarbazole. Histochem. Cytochem. J., 13: 150-152.

Hartl, D. L. and E. W. Jones (2001). Genome evolution in the grass family. In, genetics and analysis of genes and genomes. Fifth edition, Jones and Bartlett publishers/ Sudbury, Massachusetts, US.

Heun, M., R. Schäfer-Pregl, D. Klawan, R. Castagna, M. Accerbi, B. Borghi and F. Salamini (1997). Site of Einkorn Wheat Domestication Identified by DNA Fingerprinting. Science, 278: 1312- 1314.

Hou, Y. C., Z. H. Yan, Y. M. Wei and Y. L. Zheng (2005). Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genet. News, 35: 9-22.

Hussein, S. (2005). C-banding analysis of chromosome translocations in doubled haploid wheats. African J. of Biotechnology, 4: 541-547.

Jahan, Q. and A. A. Vahidy (1989). Karyotype analysis of hexaploid wheat, *Triticum Aestivum* L. CV. 'SARSABZ'. Islamic Academy of Sciences J., (2-3): 179-181.

Jonathan, F. W. and N. F. Wendel (1990). Visualization and interpretation of plant isozyme. In: Isozymes in Plant Biology. D. E. Sdtis and P.S. Sottis (eds.). London Champan and Hall, 5 - 45.

Ko, H. L., R. J. Henry and J. A. Ronalds (1994). Rapid cereal genotype analysis. Improvement of cereal quality by genetic engineering. Proceedings of the Royal Australian Chemistry Institute, Cereal Chemistry Division on Improvement of Cereal Quality by genetic Engineering, Sydney, Australia, 153-157.

Kraic, J., L. Horvath, E. Gregova and I. Zak (1995). Standard methods for electrophoretic separation of wheat glutenins and gliadins by SDS-PAGE and A-PAGE. Rostlinna Vyroba, 41: 219-223.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680-685.

Loxdale H. D. (1994). Isozyme and protein profiles of insects of agricultural and horticultural importance. In: Hawksworth DL (ed.). The Identification and Characterization of Pest Organisms, Wallingford, CAB INTERNATIONAL, 337-375.

Michaud, H., L. Toumi, R. Lumaret, T. X. Li, F. Romone and F. D. Giusto (1995). Effect of geographical discontinuity on genetic variation in Quercus ilex L. (holm oak). Evi-

dence from enzyme polymorphism. Heredity, 74: 590-606.

Miller, T. E. (1987). Systematics and evolution in wheat breeding by F.G.H. Lupton. Chapman and Hall, 1-30.

Nour El-Din, M. Nahed, F. M. El-Saied and A. M. Ahmed (2004). Ecophysiological responses and genetic variants in some species of the genus *zygophyllum*. J. of Agric. Sci. Mansoura Univ., 29: 2345-2362.

Pharmawati, M., G. Yan and P. M. Finnegan (2005). Molecular variation and fingerprinting of *Leucadendron* cultivars (*Proteaceae*) by ISSR markers. Ann. Bot. (Lond), 7: 1163-1170.

Radic-Miehle, H., C. Saam, R. Huels, I. Kling and C. Heseman (1997). Characterization of spelt (*Triticum spelta* L.) forms by gel electrophoretic analyses of seed storage proteins. II. The glutenins. Theor. Appl. Genet., 94: 882-886.

Rohlf, F. J. (1998). NTSYSpc. Numerical taxonomy and multivariate analysis system, version 2.02c. Exeter Software, New York.

Sambrook, J., E. F. Fritsch and T. Maniatis (1989). Molecular cloning. A laboratory manual, Second edition., 6: 3-9.

Simova-Stoilova, L., V. Vassileva, T. Petrova, N. Tsenov, K. Demirevska and U. Feller (2006). Proteolytic Activity in wheat leaves during drought stress and recovery. Gen. Appl. Plant Physiology, Special ISSUE, 91-100.

Sivolap, I. M. and D. I. Trebel'skii (2001). Identification of maize genotypes by PCR analysis. Tsitol Genet, 35: 14-21.

Sneath, P. H. A. and R. R. Sokal (1973). Numerical taxonomy. Freeman, San Francisco.

Sofaliani, O., N. Chaparzadeh, A. Javanmard and M. S. Hejazi (2008). Study the Genetic Diversity of Wheat Landraces from Northwest of Iran Based on ISSR Molecular Markers. International J. of Agriculture & Biology, 1560-8530.

Sokal, R. R. and P. H. A. Sneath (1963). Principles of Numerical Taxonomy. Freeman. San Francisco, 359.

Stegemann, H., A. M. R. Afifiy and K. R. F. Hussein (1985). Cultivar Identification of dates (*Phoenix dactylifera*) by protein patterns. 2nd international Symposium of Biochemical Approaches to Identification of Cultivars. Braunschweig, West Germany, 44: 141-147.

Table (1): ISSR primers names and sequences.

Primer name	Sequence	Primer name	Sequence
ISSR-814	$(CT)_8$ TG	ISSR-HB10	$(GA)_6$ CC
ISSR-844A	$(CT)_8$ AC	ISSR-HB11	$(GT)_6$ CC
ISSR-844B	$(CT)_8$ GC	ISSR-HB13	$(GAG)_3$ GC
ISSR-HB 8	$(GA)_6$ GG	ISSR-HB14	$(CTC)_3$ GC
ISSR-HB 9	$(GT)_6$ GG	ISSR-HB15	$(GTG)_3$GC

Table (2): Mean chromosome length, area and centromery index percentage of the 21 chromosomes for the six wheat cultivars. A= Area, L= Length and CI= centrometry index percentage. Light and heavy shadow indicates the minimum and maximum values, respectively.

	Gemmiza 9			Youkora			Giza 168			Sahel 1			Sakha 93			Sids 1		
	A	L	CI	A	L	CI	A	L	CI	A	L	CI	A	L	CI	A	L	CI
1A	**0.42**	**1.35**	46.5	0.53	**2.08**	47.8	**0.20**	**1.07**	45.5	**0.45**	**1.47**	47.3	0.32	**1.32**	46.8	**0.36**	**1.68**	47.5
2A	0.33	1.30	46.0	**0.57**	1.92	47.5	0.13	0.95	**50.5**	0.33	1.33	44.6	0.22	1.18	44.5	0.32	1.52	47.0
3A	0.35	1.23	47.0	0.34	1.62	46.8	0.17	0.81	45.5	0.17	0.96	**50.0**	0.19	1.15	44.9	0.22	1.25	46.5
4A	0.19	1.18	37.6	0.34	1.06	39.7	0.16	0.81	38.6	0.24	0.92	37.0	0.23	1.13	41.0	0.25	1.07	40.7
5A	0.19	1.15	37.8	0.26	1.01	39.6	0.15	0.76	36.0	0.17	0.87	34.6	0.22	0.94	37.0	0.18	1.05	37.0
6A	0.19	1.00	31.1	0.26	0.88	32.5	0.12	0.74	29.5	0.22	0.84	30.9	0.16	0.92	32.0	0.23	0.82	32.0
7A	0.15	0.85	30.7	0.23	0.83	34.0	0.16	0.70	30.2	0.17	0.78	32.2	0.17	0.83	33.5	0.17	0.76	31.8
1B	0.33	1.29	46.0	0.45	1.55	48.5	0.17	0.71	47.5	0.37	1.33	46.5	0.31	1.25	**48.5**	0.34	1.15	**50.0**
2B	0.17	1.19	46.7	0.33	1.35	48.4	0.18	0.65	49.0	0.37	1.27	48.0	0.27	1.09	46.8	0.17	1.08	47.0
3B	0.23	1.08	46.1	0.25	1.25	**49.4**	0.14	0.64	46.8	0.33	1.06	49.4	0.21	1.05	46.5	0.23	0.94	46.8
4B	0.18	0.96	38.7	0.35	1.10	38.0	0.10	0.62	32.9	0.26	1.00	34.9	0.19	1.01	35.6	0.21	0.90	39.5
5B	0.11	0.84	40.7	0.25	1.02	39.7	0.11	0.59	39.0	0.17	0.93	38.5	0.16	0.88	30.3	0.17	0.87	37.4
6B	0.21	0.72	30.6	0.19	0.90	29.0	0.12	0.54	30.0	0.18	0.61	29.0	0.13	0.81	29.6	0.21	0.80	28.9
7B	0.40	0.52	28.1	0.13	0.63	29.4	0.11	0.51	31.5	0.09	0.53	28.4	0.10	0.73	30.0	0.15	0.60	31.0
1D	0.33	1.29	**49.6**	0.38	1.94	46.5	0.13	0.96	46.5	0.26	1.38	46.5	**0.34**	1.16	45.5	0.24	1.19	45.9
2D	0.25	1.24	49.9	0.37	1.78	48.0	0.17	0.91	46.9	0.26	1.28	47.3	0.33	1.02	47.4	0.26	1.11	49.0
3D	0.27	1.20	46.4	0.35	1.49	44.6	0.12	0.86	47.0	0.26	1.25	46.5	0.18	0.94	38.4	0.22	0.99	45.0
4D	0.18	1.19	36.0	0.25	1.30	36.5	0.13	0.83	37.1	0.22	1.15	40.5	0.19	0.89	37.5	0.27	0.90	41.2
5D	0.22	1.11	39.5	0.33	1.11	36.0	0.12	0.68	37.5	0.22	1.04	37.6	0.17	0.80	38.6	0.16	0.85	35.1
6D	0.14	1.07	33.1	0.25	1.04	30.4	0.13	0.61	32.0	0.23	0.95	28.7	0.17	0.78	29.5	0.21	0.83	29.0
7D	0.12	0.95	32.5	0.32	0.87	29.3	0.12	0.55	30.6	0.15	0.75	31.3	0.18	0.69	27.8	0.14	0.74	28.5

Table (3): Presence (1) versus absence (0) of α-esterase isoforms of the six wheat cultivars genotypes, as analyzed using gel documentation program software, RF= relative mobility of bands.

Band No	Rf	wheat cultivars					
		Gemmiza 9	Youkora	Giza 168	Sahel 1	Sakha 93	Sids 1
		α-Esterase (α-Est.)					
α-Est. 1	0.05	0	0	0	1	0	0
α-Est. 2	0.06	1	1	1	1	1	1
α-Est. 3	0.08	1	1	1	1	1	1
α-Est. 4	0.09	0	0	0	1	0	0
α-Est. 5	0.33	0	1	1	1	0	1
α-Est. 6	0.37	1	1	1	1	1	1
α-Est. 7	0.72	1	1	1	1	1	1
α-Est. 8	0.75	1	1	1	1	1	1
Total		5	6	6	8	5	6

Table (4): Presence (1) versus absence (0) of peroxidase isoforms of the six wheat cultivars genotypes, as analyzed using gel documentation program software. RF= relative mobility of bands.

Band No	Rf	wheat cultivars					
		Gemmiza 9	Youkora	Giza 168	Sahel 1	Sakha 93	Sids 1
		Peroxidase (Px.)					
Px. 1	0.5	0	0	0	1	1	0
Px. 2	0.9	0	0	0	1	1	0
Px. 3	0.94	1	1	1	1	1	1
Px. 4	0.97	1	0	0	1	1	1
Total		2	2	2	4	4	2

Table (5): Polymorphic and monomorphic SDS-PAGE cultivar and bands of total proteins bulk samples of the six wheat cultivars, 1 = band present and 0 = band absent.

Band No	MW (KDa)	wheat cultivars					
		Gemmiza 9	Youkora	Giza 168	Sahel 1	Sakha 93	Sids 1
1	141.40	0	1	1	1	0	0
2	117.75	0	1	1	1	0	0
3	102.40	0	1	1	1	0	0
4	92.20	1	1	1	1	1	1
5	87.00	1	1	1	1	1	1
6	84.51	0	1	1	1	1	1
7	78.34	0	1	1	1	1	1
8	70.13	0	1	1	1	1	1
9	61.36	0	1	1	1	1	1
10	48.32	1	1	1	1	1	1
11	45.32	0	1	0	0	0	1
12	40.58	1	1	1	1	0	0
13	39.19	1	1	1	1	1	1
14	36.34	1	1	0	1	1	1
15	33.49	1	1	1	1	1	1
16	32.72	1	1	1	1	1	1
17	30.34	1	1	1	1	0	1
18	28.12	0	1	1	1	0	1
19	27.44	0	1	1	1	1	1
20	27.17	0	1	0	0	0	0
21	25.03	1	1	1	1	1	1
22	22.67	0	1	1	1	1	1
23	21.89	0	1	1	1	1	1
24	18.71	0	1	1	1	0	0
25	17.86	1	1	1	1	0	1
26	16.85	1	1	1	1	1	1
27	15.62	0	1	1	1	0	0
Total		12	27	24	25	16	20

Table (6): ISSR fragments per primer, fragment length, polymorphic fragments and percentage of polymorphism in the six wheat cultivars based on ISSR-PCR analysis.

ISSR primers code	Amplified fragmenets			
	Total amplified fragments	Length range (bp)	Polymorphic fragments	Percentage of polymorphism (%)
ISSR-814	6	103-339	2	33.0%
ISSR-844A	5	445-1240	4	80.0%
ISSR-844B	3	510-654	2	67.0%
ISSR-HB 8	6	430-735	6	100.0%
ISSR-HB 9	9	365-1165	2	22.0%
ISSR-HB 10	8	275-1145	7	87.5%
ISSR-HB 11	10	90-695	5	50.0%
ISSR-HB 13	5	300-1230	4	80.0%
ISSR-HB 14	16	100-1280	6	37.5%
ISSR-HB 15	6	240-540	2	33.0%
Total	74	90-1280	40	54.0%

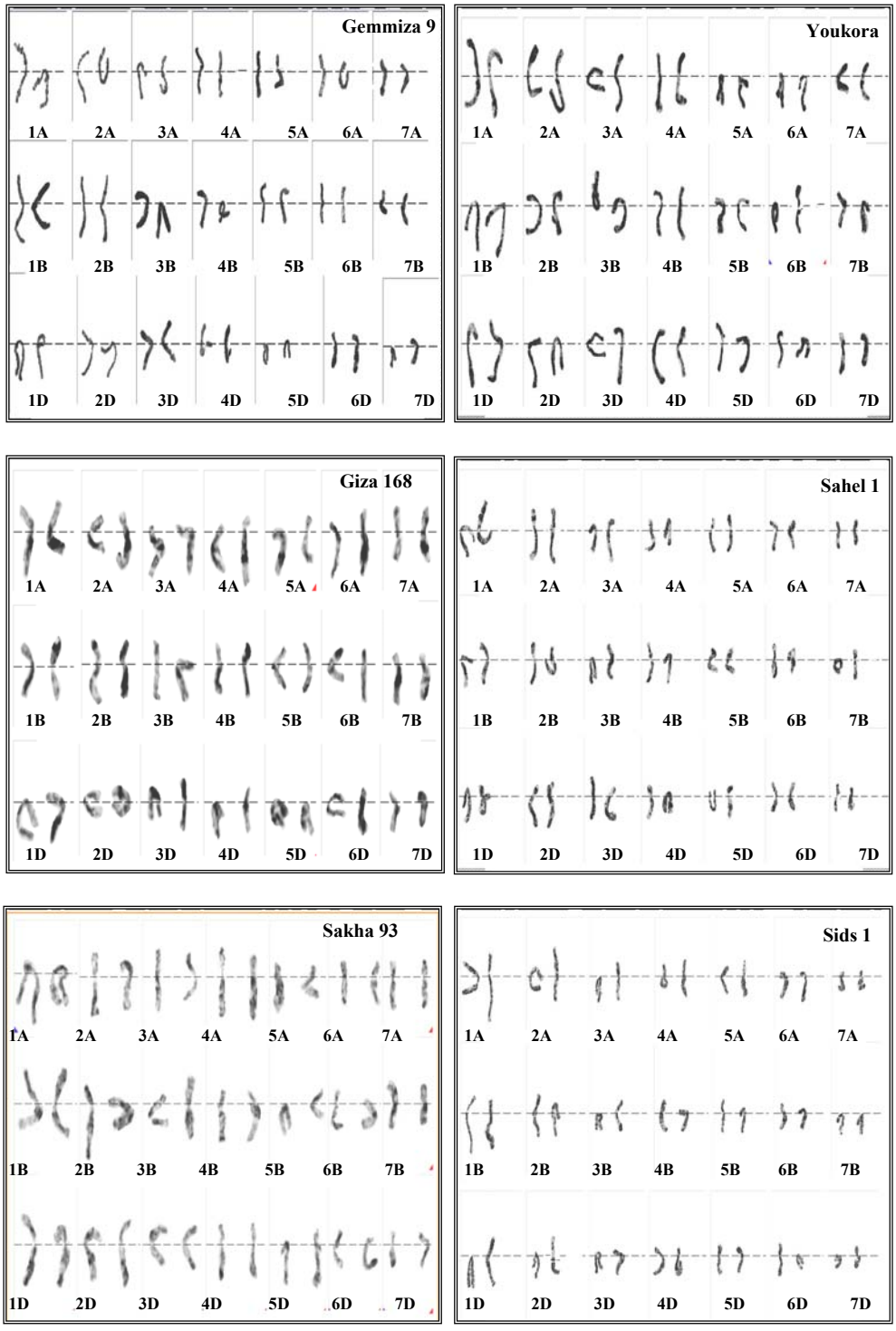


Fig. (1): Karyotypes of Gemmiza 9, Youkora, Giza 168, Sahel 1, Sakha 93 and Sids 1 wheat cultivars imaged with video digital camera and analyzed by video test karyotyping software program.

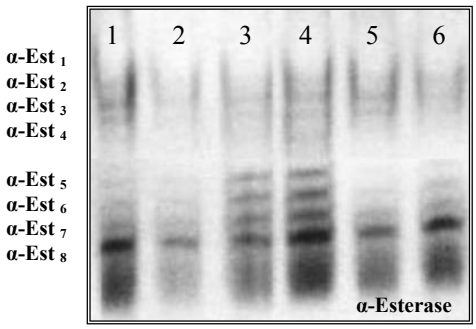


Fig. (2): Zymogram of α-esterase banding patterns for the six wheat cultivars. **1-Gemmiza 9, 2-Youkora, 3-Giza 168, 4-Sahel 1, 5-Sakha93, 6- Sids 1.**

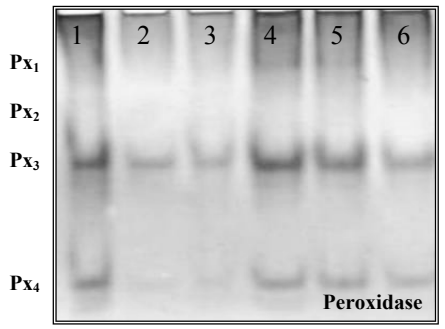
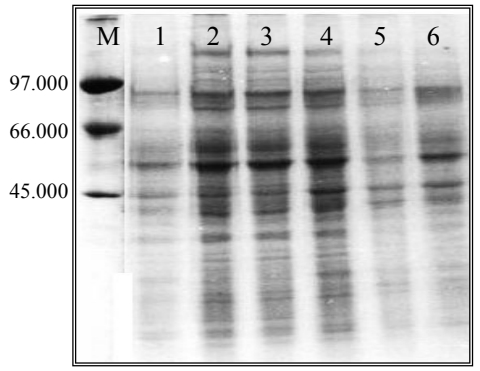


Fig. (3): Zymogram of peroxidase banding patterns for the six wheat cultivars. **1-Gemmiza 9, 2-Youkora, 3-Giza 168, 4-Sahel 1, 5-Sakha93, 6- Sids 1.**

Fig. (4): SDS-PAGE of protein banding patterns for the six wheat cultivars. M-Marker 1-Gemmiza 9, 2-Youkora, 3-Giza 168, 4-Sahel 1, 5-Sakha93, 6- Sids 1.



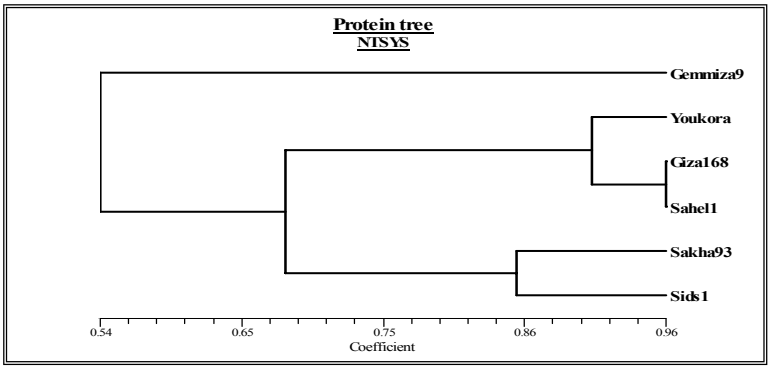


Fig. (5): Dendrogram representing the relationship among the six the wheat cultivars under study based on seed storage protein data using NTSYS-PC2 program.

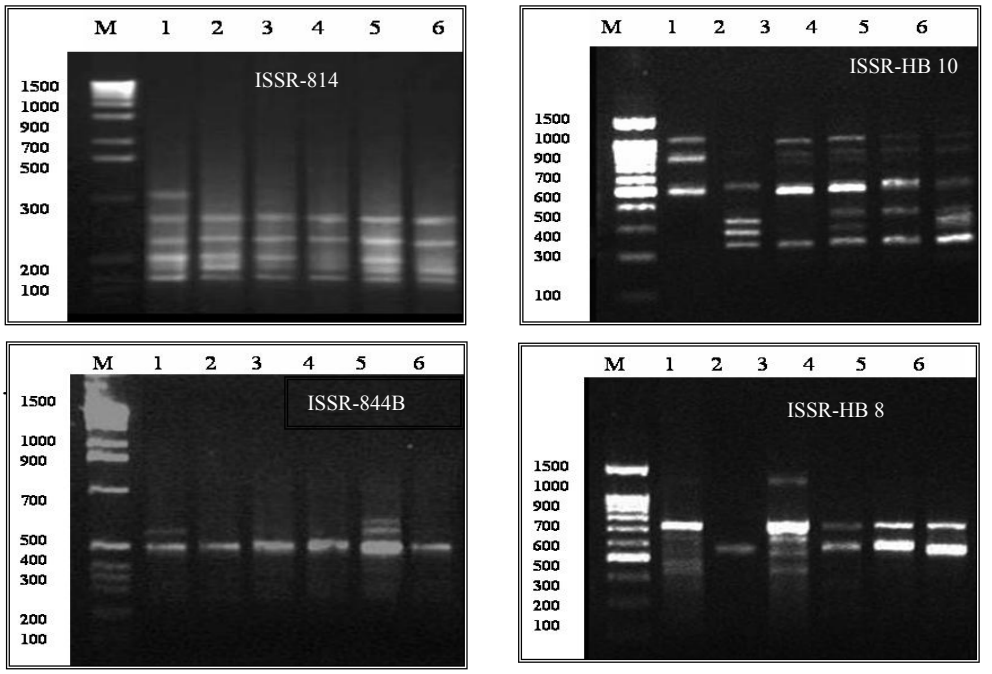


Fig. (6): Examples of ISSR-PCR amplified fragments of the six wheat cultivars using ten primers, M= 100 pb DNA ladder (Stratagene®), 1= Gemmiza 9, 2= Youkora, 3= Giza 168, 4=Sahel 1, 5=Sakha 93 and = Sids 1.

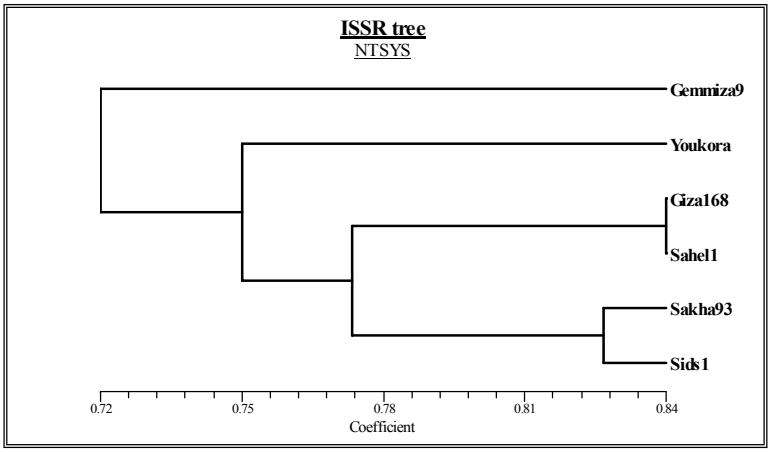


Fig. (7): Dendrogram indicating the genetic relationships among the six wheat cultivars based on ISSR-PCR data analysis using NTSYS-PC2 program.