

GENETIC DIVERSITY ANALYSIS OF DOMESTICATED WHEAT (*Triticum aestivum* L.) AND WILD WHEAT (*Aegilops* species)

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Genetic diversity is one of the key factors for improvement many crop plants including wheat. Plant breeders rely on the availability of genetic diversity during selection in cultivar development. The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity and or distance estimates among genotypes are helpful in the selection of parents to be used in a breeding program (Van Becelaere *et al.*, 2005). Varieties developed with wider genetic base may be helpful in enhancing the yield under various agro-climatic conditions (Asif *et al.*, 2005).

Wild relatives of common wheat, in which the genus *Aegilops* is one of them, have become an important genetic resource of both resistance to various diseases and tolerance against abiotic factors (Nelson *et al.*, 1995). Genus *Aegilops* L. (*Poaceae*) is one of the wheat relatives that is capable of making different complexes with each other and with *Triticum* L. (Bor, 1970). The wild species of *Triticeae* family, especially the genus *Aegilops* L. are valuable sources of genetic variation for wheat improvement since

they possess the genetic background of all the cultivated wheat having still unidentified important characters such as resistance to different biotic and abiotic stresses (Zaharieva *et al.*, 2004).

Aegilops is the source of several disease resistance genes that are of agronomic importance and have been successfully introgressed into wheat (Bariana and McIntosh, 1993). Genus *Aegilops* L. has been the most intensively studied group of grasses, especially since it is closely related to the cultivated wheat. The genus *Aegilops* contains 22 species comprising both diploids and polyploids that originated from center of origin (Van Slageren, 1994). The wild relatives of bread wheat, *T. aestivum* L., is a hexaploid ($2n = 6x = 42$; genome) that are considered as potential sources of useful alleles for bread wheat improvement. Common bread wheat (*Triticum aestivum*) is a case of a major crop that was most probably formed by hybridization in farmers' fields. Consequently, studying the genetic diversity of the genetic resources from such species may provide significant information regarding their potential for breeding purposes.

Genetic diversity can be assessed from pedigree analysis, morphological traits or using molecular markers (Pejic *et al.*, 1998). However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic (Fufa *et al.*, 2005). Genetic diversity estimates based on morphological traits, on the other hand, suffer from the drawback that such traits are limited in number and are influenced by the environment (Maric *et al.*, 2004).

Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment and do not require previous pedigree information. Among the molecular markers techniques, random amplified polymorphic DNA (RAPD) which introduced by Williams *et al.* (1990). This technique has the advantage of being easy to use and requiring a very small amount of genomic DNA without the need for blotting or radioactive detection (Atienzar *et al.*, 2000). Also, it is moderately reproducible. RAPD became useful tools to complement morphologic, agronomic and physiological characterization for better assessment of genetic diversity and towards associative mapping of traits. RAPD technique has been efficiently used by several researchers to study genetic diversity, for diploid wheat (Vierling and Nguyen, 1992; Doves and Gale, 1992; Chabane and Valkoun, 1998), tetraploid wheat (Joshi and Nguyen, 1993), intra- and inter-population genetic variability of cultivated and wild tertiary buckwheat accessions (Kump and Javornik, 2002). Also, it had been used to

make phylogenetic relationships among polyploid *Aegilops* species (Goryunova *et al.*, 2004) and to compare genetic distances between cultivars of wheat varieties (Kudryavtsev *et al.*, 2003; Khan *et al.*, 2005). The main objective of the present research is to detect the genetic diversity and relationships between domesticated and wild wheat cultivars via morphological traits, Peroxidase isozymes and RAPD technique.

MATERIALS AND METHODS

A- Plant material

Two wild wheat, *Ae. ventricosa* Tausch ($2n = 4x = 28$ chromosomes, DDNN) and *Ae. kotschy* Boiss ($2n = 4x = 28$ chromosomes, UUSS) were collected as random batches from natural habitats along the Marsa-Matroh/El Salom Road and Borg Al-Arab City respectively and four common wheat, *T. aestivum* L. ($2n = 6x = 42$ chromosomes, AABBDD) representing major cultivars harvested in Egypt, namely, cvs. Gemmieza 10, Sakha 93, Giza 168 and Sids 1 were sown at The Experimental Station of Faculty of Agriculture, Saba Basha, Abis Farm in one harvested seasons (November, 2011-2012) to study the following items.

B- Morphological variations

Six wheat cultivars were sown under standard conditions in four replicates in a completely randomized design to assess the difference in the morphological characteristics among the domesticated wheat cultivars and their relative's wild

wheat *Aegilops* species as follow: Heading to date (days), stem number/plant, number of spike/plant, grain number/spike and 1000 grains weight

C- Biochemical analysis

Study the profile of proxidase isozymes expressed in leaves of domesticated and wild wheat was used in the present study -as gene markers- for studying the genetic polymorphism. As conventional symbols in electrophoretic analysis, a pattern was first described in terms of Anodal (A) and Cathodal (C) zones according to their direction of mobility in the electrophoretic field. Each zone is assigned for a locus coding for a Peroxidase isozyme. Twenty different plants for each cultivar were examined individually for their isozyme patterns. A combination of agar-starch gel electrophoresis and enzyme activity attaining was used to screen for polymorphisms of peroxidase. The laboratory methods were performing according to Jonathan and Norman (1989).

D- DNA extraction

Genomic DNA was isolated through DNA isolation kit (Gene JET™, plant genomic DNA purification mini kit, Fermentas) and DNA was quantified by Gene quant at absorbance of 260/280 nm. The quality was further checked on 0.1% agarose gel.

E- RAPD analysis

Random amplified polymorphic DNA (RAPD), has been developed, in

which DNA is amplified using fourteen (10 mer) RAPD primers (Williams *et al.*, 1990). The PCR Operon primers used for RAPDs are listed in Table (1). These primers were selected from the Operon kits (Operon Technologies Inc., Alabameda CA). RAPD-PCR analysis was performed according to the method of Williames *et al.* (1990). The polymerase chain reaction mixture (25 µl) consisted of 0.8 U of Taq DNA polymerase; 25 pmol dNTPs; 25 pmol of primer and 50 ng of genomic DNA. PCR amplification was performed in a Biometra T1 gradient thermalcycler for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94°C for 1 min; annealing at 36°C for 1 min; extension at 72°C for 2 min and final extension at 72°C for 10 min (Soliman *et al.*, 2003). Amplification products were separated on 1.5% agarose gels at 100 volts for 1.30 hrs with 1 x TBE buffer. To detect ethidium bromide/DNA complex, agarose gels were examined on ultraviolet transilluminator and photographed. Using 100 bp DNA ladder (V-gene Biotechnology Limited, Shiqao, P. R. China), the lengths of the different DNA fragments were determined. The reproducible DNA fragments from two runs were scored for their presence (1) or absence (0) for each genome.

F- Data analysis

Data matrices were entered into the NTSYS program (Numerical Taxonomic and Multivariate Analysis System) software package, version 2.1, Applied Biostatistics Inc. (Rohlf, 2000). Similarity

coefficients were used to construct dendrograms using the UPGMA (unweighted pair group method with arithmetic average) and the SAHN (Sequential Agglomerative Hierarchical Nested clustering) routine in the NTSYS.

RESULTS AND DISCUSSION

A- Morphological variations

Results in Table (2) indicated high significant variations among the wild and domesticated wheat cultivars in the morphological characteristics. The four domesticated wheat cultivars was faster in heading to date compared with the wild species with range 20 to 31 days in average. Sakha 93 was as the earliest one of heading date in average 50.32 days, followed by Gemmieza 10, Giza 163 and Sids 1 in average 55.05, 58.29 and 61.30 (days), while wild wheat species were the lasted in average 72.00 and 81.16 (days), for *Aegilops kotschy* and *Aegilops ventricosa*, respectively.

Concerning the stem number/plant, the wild wheat species showed high values in compare with the other four domesticated cultivars in mean value reached to 12.00 stem in average, on the contrary, it was 3.00 for the domesticated wheat

Logically, the number of spike per plant is related to stem number per plant. The wild wheat showed high number of spike per plant (8 in average) compare with the wheat (2.5 in averages) On the other hand, the domesticated wheat cultivars significantly exceeded the two wild

species in the grain number/spike, and 1000 grain weight characteristics as shown in Table (2).

A number of researchers implied sets of morphological characters to establish genetic relationships between wild wheat tribes and cropping wheat cultivars such as Abdelsalam (2010) who pointed to significant genetic distance between domesticated wheat cultivars and the two different wild species (*Ae. ventricosa* and *Ae. kotschy*) especially in 50% time to heading. The author calculated the similarities among the wild/domesticated wheat cultivars based on their agro-morphological traits. Branlared *et al.* (1984) addressed 78 different varieties of bread wheat attempting to classify by three major criteria which involved pedigree, 26 agronomic and morphological characters and characterization of grain gliadine. Our data are consonant to the results of Hamada (1996) which assessed 13 *Aegilops* and 3 wild *Triticum* originally Turkish species by using morphological, pathological, qualitative and agricultural traits. As it was determined by the author, plant height might vary from 16.6 (*Aegilops juvenalis*) to 112.0 cm (*Aegilops mutica*), while spike length - from 2.4 (*Aegilops ovata*) to 23.3 cm (*Aegilops mutica*).

Our result is agreed with Karagoz *et al.* (2006) studied agro-morphological traits of certain wild *Aegilops* and *Triticum* species. In this study 112 populations of wild wheat and 12 populations of cultivated wheat were compared to

demonstrate evident agro-morphological variations across the populations examined. Singh (1994) used 12 yield parameters and 5 morphological traits of spring wheat to evaluate genetic divergence among 19 durum wheat genotypes. These genotypes were subsequently classified into seven separate clusters revealing high level of genetic divergence independent of original harvesting place.

B- Biochemical genetic analysis

The zymogram and photograph showing mobility pattern of peroxidase isozymes are illustrated in Fig. (1). It can be conducted that from this data the peroxidase patterns in the two wild and the four domesticated wheat plants leaves showed two kinds of banding profiles. First, it was evident that all plants expressed the Px.A₂, Px.C₂, and Px.C₄, and the four domesticated plants exhibited the same banding profile containing these three loci. Indicated that, these three common loci were consistently monomorphic expressed.

Second, the two wild types *Ae. ventricosa* and *Ae. kotschyi* displayed extra three common loci (Px.A₁, Px.C₁, and Px.C₃). The banding pattern activity of *Ae. Ventricosa* displayed a unique marker band at Px.C₅ locus indicating that (Px.A₁, Px.C₁, Px.C₃ and Px.C₅) loci are polymorphic specifically to the wild wheat.

The confirmation of obtaining limited number of polymorphic isozyme marker in wheat had been shown by Hart *et al.* (1983) who indicated that, within

Triceae several amphiploids, and especially the hexaploid wheats, often produce complex electrophoretic patterns that are difficult to interpret because of the presence of multi locus isozymes.

Peroxidase isoenzyme assay was implied as most appropriate technique for the evaluation of *Aegilops ventricosa* Tausch. Assessed and classified peroxidase patterns were ascribed to different phenotypes under control of four genetic loci Tanksley *et al.* (1983). Two out of detected iso-enzyme bands shifted, as a rule, to the cathode, while the resting bands migrated in anodic direction. Zhang *et al.* (1993) surveyed isozymes in two hundred and sixty eight accessions of wild barley from diverse eco-geographical zones of Israel and Iran. This study revealed highly polymorphic iso-enzymes as within each population and across wild barley populations.

C- Molecular studies

Fourteen, RAPD-PCR primers were used in screening the diversity between different genomic-DNA of wild and domesticated wheat. For each primer-DNA combination, the amplification was repeated at least twice. As shown in Tables (4 and 5) and Plate (1), the number of reproducible bands/primer varied between 18 for primer OPC-12 and 56 for primer OPH-11 with a total of 550 bands.

The results in Table (5) clearly indicated that in all studied wheat, 397 (72%) of the 550 fragments were polymorphic and 153 (28%) were monomor-

phic. In the meantime, all used primers generated 51 specific markers (Tables 4 and 5).

The largest number of these markers was specific for wild wheat, *Ae. ventricosa* and *Ae. Kotchyi* (20 and 12 markers, in respect). Furthermore, two specific large markers (1801 and 2332 bp) were observed in the two wild types. Also, two specific markers (280 and 987 bp) were reported for domesticated wheat Sakha 93. While, Giza 168 showed 5 specific marker (209, 311, 578, 873 and 2510 bp) and finally Sids 1 and Gemmeiza 10 exhibited 6 specific marker ranged from (400 to 1108 bp).

Manifesto *et al.* (2001) found some specific RAPD marker while examining genetic diversity in spring wheat cultivars grown in the Yaqui Valley of Mexico and the Punjab of Pakistan. Also, Sajida Bibi *et al.* (2009) indicated many specific RAPD markers among commercially grown lines of wheat in Pakistan. Due to different obtained data from the studied cultivars using RAPD marker further studies will be necessary to identify the genetic constitutions of specific markers.

Molecular markers provide a good estimate of genetic diversity since they are independent of confounding effects by environmental factors (Powell *et al.*, 1996). This will led to identify their interrelation especially with the biotic and abiotic stress in order to enhance the domesticated wheat structure. Hoping to use them as gene constructs for improving

these cultivars using their relatives of wild wheat.

D- Genetic similarity and Dendogram

Genetic similarity values generated from RAPD marker varied between 0.31 and 0.89 with an average of 0.6. Dendrogram based on similarity values (Table 6) from RAPD was constructed to reveal similarities between the six different wild and domesticated wheat. The dendrogram (Fig. 2) demonstrated that the six wheat cultivars fall into two main groups. The first one was divided into two clusters containing *Ae. ventricosa* and *Ae. Kotchyi* wild types with genetic similarity of (57%). The second one divided into two subclusters. According to similarity, the first one contained Gemmeiza 10 and the second continue Giza 168, Sakha 93 and Sids 1 in similarity from 79 to 89%.

These results are in agreement with results obtained by Guadagnuolo *et al.* (2001a) who indicated that similarity matrices clearly separated wild species of wheats obtained from Switzerland, Austeria and England from cultivated ones. In the meantime, Naghavi *et al.* (2009) reported a genetic similarity value of 0.67 in wheat based on RAPD markers. While, Basel (2012) obtained (GS) values from RAPD marker in Syrian wheat varied between 0.769 and 0.989 with an average of 0.888.

For the molecular markers employed in the present study, the fourteen different RAPD primers had generated a high level of polymorphism and conse-

quently large number of genomic-specific markers than that of isozymes assay. The results of Guadagnuolo *et al.* (2001a) confirmed that only two among 22 enzyme systems tested provided marker useful for differentiating closely related and essential autogamous species of wheat.

The random nature of the random amplified polymorphic DNAs (RAPDs) analysis complements isozyme variation. Where, it only reflects differences in protein-coding genes, which are probably eliminated during the introgression process if they do not confer adaptive advantages (Guadagnuolo *et al.*, 2001b).

The dendrogram generated by isozymes only are poor in its discrimination of population's similarity than RAPD. These differences might be based on the kind of information provided by each type of markers. RAPD can detect diversity in both coding and non-coding regions of the genome. Where, small repeated random sequence mutations may be accumulated in non-coding sequences and then diversity can be better revealed by RAPD than isozymes (Heun *et al.*, 1994; Lanner-Herrera *et al.*, 1996); Nybom and Bartish (2000); Hemeida and Hassan (2001).

Furthermore, an additional factor affecting genetic diversity assayed by different marker techniques is the number of markers used in the analysis (Smith *et al.*, 1992). Demeke *et al.* (1996) indicated that RAPD marker analysis provides virtually unlimited number of markers to compare

individual genotypes. Generally, most variability/taxonomic affinity studies in wheat focused mainly on morphology and nuclear DNA diversity (Basel, 2012). In most cases, parental selection for developing a wheat pure line or a hybrid is carried out according to performance of the parents and complementation for important agronomic traits. Yet, genetic diversity among parents is critical derive transgressive segregant from a cross (Rharrabti *et al.*, 2001).

Finally, the high resolution, polymorphism and reproducibility of RAPD-PCR assays shown in this study, could provides a simple, rapid and cost-effective system to the researchers to assess the genetic diversity and its utilization in breeding programs. RAPD markers based fingerprinting could be used to characterize large number of wild wheat and cultivars. Research can be pursued to look for marker association with important traits/genes/QTLs in wheat. This technology could also be used for testing the purity of genetic stocks.

SUMMARY

Diversity of four domesticated wheat cultivars (Sids 1, Sakha 93, Giza 168 and Gemmeiza 10 and two wild wheat (*Aegilops ventricosa* and *Aegilops kotchyi*) were analyzed by morphological, biochemical and molecular analysis. Five morphological characteristics i.e. Heading to date (days), stem number/plant, number of spike/plant, grain number/spike and

1000 grains weight (g) were calculated to show the difference among wheat cultivars and their relatives *Aegilops* species. High significant variations were observed among the wild and domesticated wheat cultivars. The four domesticated wheat cultivars was earlier in heading to date compared with the wild species with range 20 to 31 days in average. Biochemical analysis for peroxidase isozymes profile exhibited three marker bands (Px_{A1}, Px_{C1} and Px_{C3}) for the wild type cultivars, also *Ae. ventricosa* expressed unique marker band at Px5c locus. Fourteen (10 mer) RAPD-PCR were used to detect the genetic diversity. In Total of 550 amplified fragments, 51 DNA specific markers were detected. The number of reproducible bands/primer varied between 18 for primer OPC-12 and 56 for primer OPH-11 with a total of 550 bands. The largest number of these markers was specific for wild wheat, *Ae. ventricosa* and *Ae. kotchyi* (20 and 12 markers, in respect). Furthermore, two specific large markers (1801 and 2332 bp) were observed in the two wild types. Also, two specific markers (280 and 987 bp) were reported for domesticated wheat Sakha 93. While, Giza 168 showed 5 specific marker (209, 311, 578, 873 and 2510 bp) and finally Sids 1 and Gemmeiza 10 exhibited 6 specific marker ranged from (400 to 1108 bp). High similarity between the two wild wheat types was recorded. The four domesticated wheat cultivars were clustered in one group.

REFERENCE

- Abdelsalam, N. R. (2010). Specific properties of hybridisation among common wheat (*Triticum aestivum* L.) and wild wheat species *Aegilops* under experimental field conditions. *J. Appl. Sci. Res.*, 6: 2068-2073.
- Asif, M., M. Rahman and Y. Zafar (2005). DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphic DNA (RAPD) analysis. *Pakistan J. Bot.*, 37: 271-277.
- Atienzar, F., A. Evenden, A. Jha, D. Savva and M. Depledge (2000). Optimized RAPD analysis generates high-quality genomic DNA profiles at high annealing temperatures. *Bio/Techniques*, 28: 52-5
- Bariana, H. S. and R. A. Mcintosh (1993). Cytogenetic studies in wheat XIV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. *Genome*, 36: 476-482.
- Basel, S. (2012). Biochemical and genetic variation of some Syrian wheat varieties using NIR, RAPD and AFLPs techniques. *J. Plant Biol. Res.*, 1: 1-11
- Bor, N. L. (1970). *Gramineae*, In: Rechinger, K. H. (ed.) *Flora Iranica*: Vol. 70. Graz, Austria:

- Akademische Druk-Und
Verlagsanstalt. Wien.
- RAPD analysis. Russian J. Genet-
ics, 40: 515-523.
- Branlared, G. and A. Chevalet (1984).
Diversity of bread wheats cultivat-
ed in France. *Agronomie*, 4: 933-
938.
- Chabane, K. and J. Valkoun (1998).
Standardization of RAPD marker
techniques to determine the diver-
sity of diploid wheat: *Triticum*
urartu. p. 155-158, in A. A. Jaradat
(Ed.) *Triticeae III*. Science Pub-
lishers, Inc., Enfield, N. H., USA,
pp 478.
- Demeke, T., A. Laroche and D. A. Gaudet
(1996). A DNA marker for the *Bt*-
10 common bunt resistance gene in
wheat. *Genome*, 39: 51-55.
- Devos, K. M. and M. D. Gale. (1992). The
use of random amplified polymor-
phic DNA markers in wheat.
Theor. Appl. Genet., 84: 567-572.
- Fufa, H., P. S. Baenziger, B. S. Beecher, I.
Dweikat, R. A. Graybosch and K.
M. Eskridge (2005). Comparison
of phenotypic and molecular mark-
er based classifications of hard red
winter wheat cultivars. *Euphytica*,
145: 133-146
- Goryunova, S. V., E. Z. Kochieva, N. N.
Chikida and V. A. Pukhalskyi.
(2004). Phylogenetic relationships
and intraspecific variation of D-
genome *Aegilops* L. as revealed by
- Guadagnuolo, R., D. Savova-Bianchi and
F. Felber (2001a). Specific genetic
markers for wheat, spelt, and four
wild relatives: comparison of
isozymes, RAPDs, and wheat mi-
crosatellites. *Genome*, 44: 610-621.
- Guadagnuolo, R., D. Savova-Bianchi, J.
Keller-Senften and F. Felber
(2001b). Search for evidence of in-
troggression of wheat (*Triticum*
aestivum L.) traits into sea barley
(*Hordeum marinum* s. str. Huds.)
and bearded wheatgrass (*Elymus*
caninus L.) in Central and North-
ern Europe, using isozymes, RAPD
and microsatellite markers. *Theor.*
Appl. Genet., 103: 191-196.
- Hamada, A. A. (1996). Genetical analyses
of diallel cross in bread wheat un-
der different environmental condi-
tions in Egypt. *Ind. J. Genet. Plant*
Breed., 56: 34-48.
- Hart, G. E. (1983). Genetic and evolution
of multilocus isozymes in
hexaploid wheat. *Curr. Top. Boil.*
Med. Res., 10: 365-380
- Hemeida, A. Alaa and Hassan Th. Mo-
hamed (2001). Genetic diversity in
five *Acacia* species as revealed by
isozyme and RAPD markers. *J.*
Adv. Agric. Res., 6: 777-796.

- Heun, M. and B. Friebe (1990). Introgression of powdery mildew resistance from rye into wheat. *Phytopathology*, 80: 242-245.
- Jonathan, F. W. and F. W. Norman (1989). Isozymes in plant biology: Visualization and interpretation of plant isozymes. Chapter 1: 5-45.
- Joshi, C. P. and H. T. Nguyen (1993). Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats. *Genome*, 36: 602-609.
- Karagoz, A., N. Planali and T. Polat (2006). Agro-morphological characterization of some wild wheat *Aegilops* L. and *Triticum* L. species *Turk J Agric For.*, 30: 387-398.
- Khan, I. A., F. S. Awan, A. Ahmad, Y. Fu and A. Iqbal (2005). Genetic diversity of Pakistan wheat germplasm as revealed by RAPD markers. *Genetic Resources and Crop Evolution*, 52: 239-244.
- Kudryavtsev, A. M., S. P. Martynov, M. Broggio and V. A. Pukhalskiy (2003). Relevance of RAPD analysis for revealing phylogenetic relationships between cultivars of durum wheat *Triticum durum* Desf. *Russian Journal of Genetics*, 39: 1043-1051.
- Kump, B. and B. Javornik (2002). Genetic diversity and relationships among cultivated and wild accessions of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as revealed by RAPD markers. *Genetic Resources and Crop Evolution*, 49: 565-572.
- Lanner-Herrera, C., M. Gustafeson, A. S. Filt and T. Bryngelsson (1996). Diversity in natural populations of wild *Brassica oleracea* as estimated by isozyme and RAPD analysis. *Genetic Resources and Crop Evolution*, 43: 13-23.
- Manifesto, M. M., A. R. Schlatter, H. E. Hopp, E. Y. Suárez and J. Dubcovsky (2001). Quantitative evaluation of genetic diversity in wheat germplasm using molecular markers. *Crop Sci.*, 41: 682-690.
- Maric, S., S. Bolaric, J. Marcic, I. Pejic and V. Kozumplink (2004). Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. *Plant Breeding*, 123: 366-369.
- Naghavi, M. R., M. Malaki, H. Alizadeh, M. Pirseiedi and M. Mardi (2009). An assessment of genetic diversity in wild diploid wheat *Triticum boeoticum* from West of Iran using RAPD, AFLP and SSR markers. *J. Agric. Sci. Techno.*, 11: 585-598.
- Nelson, J. C., M. S. Sorrels, A. E. Van Deynze, L. U. Yun Hal, M. Atkin-

- son, M. Bbernard and P. Leroy (1995). Molecular mapping of wheat major genes and rearrangements in homologous groups 4, 5 and 7. *Genetics*, 141: 721-726.
- Nybom, H. and I. Bartish (2000). Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. *Perspectives in Plant Ecology, Evolution and Systematics*, 3: 93-114.
- Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplicl, P. Castiglioni, G. Taramino and M. Motto (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. *Theor. Appl. Genet.*, 97: 1248-1255.
- Powell, W., M. Morgante, C. Andre, M. Hanafey, J. Vogel, S. Tingey and A. Rafalski (1996). The comparison of RFLP, RAPD, AFLP and SSR (Microsatellite) markers for germplasm analysis. *Mol. Breed.*, 2: 225-238
- Rharrabti, Y., D. Villegas, L. E. García del Moral, N. Aparicio. S. Elhani and C. Royo (2001). Environmental and genetic determination of protein content and grain yield in durum wheat under Mediterranean conditions. *Plant Breed.*, 120: 381-388.
- Rohlf, F. J. (2000). On the use of shape spaces to compare morphometric method. *Hystrix Italian J. Mammology*, (n.s.), 11: 8-24.
- Sajida Bibi, M. U., A. Imtiaz, A. Khan, and M. H. Naqvi (2009). Study of genetic diversity in wheat (*Triticum aestivum* L.) using random amplified polymorphic DNA (RAPD) markers. *Pak. J. Bot.*, 41: 1023-1027.
- Siugh, P. K. (1994). Genetic diversity in durum wheat germplasm. *Ann. Agric. Res.*, 15: 418-422.
- Smith, O. S. and J. S. C. Smith (1992). Measurement of genetic diversity among hybrids: A comparison of isozymic, RFLP, pedigree and heterosis data. *Medica*, 37: 53-60.
- Soliman, S. S., A. A. Bahy and M. M. Mohamed (2003). Genetic comparisons of Egyptian date palm cultivars (*Phoenix dactylifera* L.) by RAPD-PCR. *African Journal of Biotechnology*, 2: 86-86.
- Tanksley, S. D. and T. J. Orton (1983). *Isozymes in plant genetics and breeding (Part A)*. Elsevier Science Publishers B. V., Amsterdam.
- Van Becelaere, G., E. L. Lumblers, A. H. Paterson and P. W. Chee (2005). Pedigree-vs. DNA marker-based genetic similarity estimates in cotton. *Crop Sci.*, 45: 2281-2287.

- Van Slageren, M. W. (1994). Wild wheats: a monograph of *Aegilops* L. and *Amblyopyrum* (Jaub. & Spach) Eig (*Poaceae*). Wageningen Agricultural University Papers 94-97, Wageningen, the Netherlands.
- Vierling, R. A. and H. T. Nguyen (1992). Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes. *Theor. Appl. Genet.*, 84: 835-838.
- Williams, J. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531-6535.
- Zaharieva, M., J. M. Prospero and P. Monneveux (2004). Ecological distribution and species diversity of *Aegilops* L. genus in Bulgaria. *Biodivers. Conserv.*, 13: 2319-2337.
- Zhang, Q. F., M. A. S. Mariif and A. Kleinhofs (1993). Comparative diversity analysis of RELPs and isozymes within and among populations of *Hordeum vulgare* spp. *spontaneum*. *Genetics*, 134: 909-916.

Table (1): The nucleotide sequences of primers used for RAPD analysis.

Primer code	Sequence (5'-3')	Primer code	Sequence (5'-3')
1- OPA-05	AGG GGT CTT G	8- OPD-04	TCT GGT GAG G
2- OPA-10	GTG ATC GCA G	9- OPD-08	GTG TGC CCC A
3- OPA-15	TTC CGA ACC C	10- OPD-11	AGC GCC ATT G
4- OPB-07	GGT GAC GCA G	11- OPH-11	AGC GCC ATT G
5- OPC-05	GAT GAC CGC C	12- OPR-01	CTT CCG CAG T
6- OPC-12	TGT CAT CCC C	13- OPR-02	GGT GCG GGA A
7- OPC-16	CAC CAT CCA G	14- OPR-03	GAC CTA GTG G

Table (2): Mean of morphological characteristics for the wild and domesticated wheat cultivars.

Parents	heading date (days)	Stem number /plant	Number of spike /plant	Grain Number /Spike	1000 Grains Weight
<i>Aegilops ventricosa</i>	81.16	14.33	08.11	32.17	29.95
<i>Aegilops kotschyi</i>	72.00	10.10	7.91	17.55	19.14
Gemmeiza. 10	55.05	03.00	2.54	46.11	48.11
Sakha 93	50.32	03.30	2.98	38.32	40.00
Giza 163	58.29	02.95	2.00	40.06	46.14
Sids 1	61.30	04.67	3.01	39.10	45.19

Table (3): Unique DNA markers for the different domesticated wheat (*Triticum aestivum* L.) and wild wheat (*Aegilops* species) resulting from PCR-RAPD analysis.

Cultivars	DNA specific Marker Fragment Length (bp)	Total
Sids 1	400, 480, 530, 901, 1031 & 1089	6
Sakha 93	280 & 987	2
Giza 168	209, 311, 578, 873 & 2510	5
Gemmeiza 10	404, 647, 609, 742, 905 & 1108	6
<i>Ae. Kotschyi</i>	264, 313, 369, 395, 416, 422, 698, 786, 855, 901, 1040 & 2332	12
<i>Ae. Ventricosa</i>	132, 177, 213, 248, 307, 312, 361, 363, 378, 388, 392, 403, 454, 545, 557, 557, 779, 1237, 1486 & 1801	20
Total		51

Table (4): Similarity indices (%) among domesticated wheat (*T. aestivum* L.) and their wild wheat (*Ae.* species) based on fourteen RAPD primers.

Cultivars	Sids 1	Sakha 93	Giza 168	Gemmeiza 10	<i>Ae. Kotschyi</i>
Sakha 93	0.89				
Giza 168	0.77	0.84			
Gemmeiza 10	0.67	0.73	0.79		
<i>Ae. Kotschyi</i>	0.41	0.42	0.45	0.51	
<i>Ae. Ventricosa</i>	0.31	0.32	0.33	0.38	0.57

Table (5): Number of amplified fragments and specific marker for domesticated wheat (*Triticum aestivum*) and their relative's wild wheat (*Aegilops* species) based on RAPD analysis.

Cultivars	Total	Primers														
		OPA-05	OPA-10	OPA-15	OPB-07	OPC-05	OPC-12	OPC-16	OPD-04	OPD-08	OPD-11	OPH-11	OPR-01	OPR-02	OPR-03	
<i>Ae. Ventricosa</i>	AF	90	6	6	4	10	9	3	7	3	11	6	9	5	5	6
	Sm	20	0	0	1	3	0	2	1	0	7	2	2	0	1	1
	PF(%)	63(70)	2(33)	2(33)	4(100)	7(70)	8(89)	2(67)	7(100)	3(100)	8(73)	4(67)	6(67)	3(60)	3(60)	4(67)
<i>Ae. Kotchyi</i>	AF	89	7	7	3	9	10	3	5	4	7	5	10	7	4	8
	Sm	12	0	0	1	3	0	1	0	1	2	0	2	1	0	1
	PF(%)	62(69)	3(42)	3(42)	3(100)	6(67)	9(90)	2(67)	5(100)	4(100)	4(57)	3(60)	7(70)	5(71)	2(50)	6(75)
Gemmeiza 10	AF	100	6	6	11	8	9	4	8	2	8	6	9	9	4	10
	Sm	6	1	0	2	0	0	1	0	0	0	0	0	1	0	1
	PF(%)	73(73)	2(33)	2(33)	11(100)	5(63)	8(89)	3(75)	8(100)	2(100)	5(63)	4(67)	6(67)	7(78)	2(50)	8(80)
Giza 168	AF	97	5	5	9	9	10	3	7	8	9	5	9	6	4	8
	Sm	5	0	0	0	1	1	0	0	3	0	0	0	0	0	0
	PF(%)	70(72)	1(20)	1(20)	9(100)	6(67)	9(90)	2(67)	7(100)	8(100)	6(67)	3(60)	6(67)	4(67)	2(50)	6(75)
Sakha 93	AF	89	5	5	8	7	10	2	4	5	9	5	9	7	5	8
	Sm	2	0	0	0	0	1	0	0	0	0	0	1	0	0	0
	PF(%)	62(69)	1(20)	1(20)	8(100)	4(57)	9(90)	1(50)	4(100)	5(100)	6(67)	3(60)	6(67)	5(71)	3(60)	6(75)
Sids 1	AF	85	4	6	7	7	7	3	2	5	8	5	10	8	5	8
	Sm	6	0	0	1	0	2	1	0	1	0	0	0	1	0	0
	PF(%)	58(68)	Zero	2(33)	7(100)	4(57)	6(86)	2(67)	2(100)	5(100)	5(63)	3(60)	7(70)	6(75)	3(60)	6(75)
Total	AF	550	33	35	42	50	55	18	33	27	52	32	56	42	27	48
	Sm	51	1	0	5	7	4	5	1	5	9	2	5	3	1	3
	PF(%)	397(72)	9(27)	11(31)	42(100)	32(64)	49(89)	12(67)	33(100)	27(100)	43(65)	20(63)	38(68)	30(71)	15(55)	36(75)

*AF= No. Amplified Fragments; Sm: Specific marker fragments and PF(%): Polymorphic fragments and Percentages of polymorphism are in parentheses.

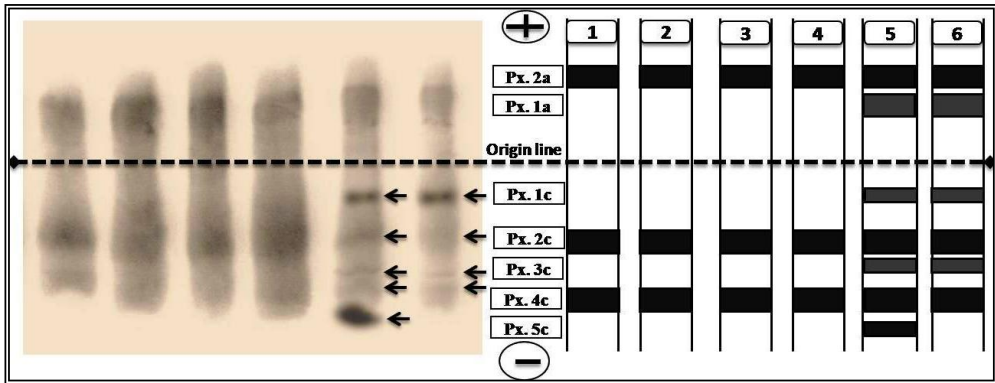


Fig. (1): Zymograms showing electrophoretic profiles of Peroxidase isozyme in wild and domesticated wheat as flow: (1) Gemmeiza 10, (2) Sakha 93, (3) Giza 168, (4) Sids 1, (5) *Ae. ventricosa* and (6) *Ae. Kotschyi*, respectively

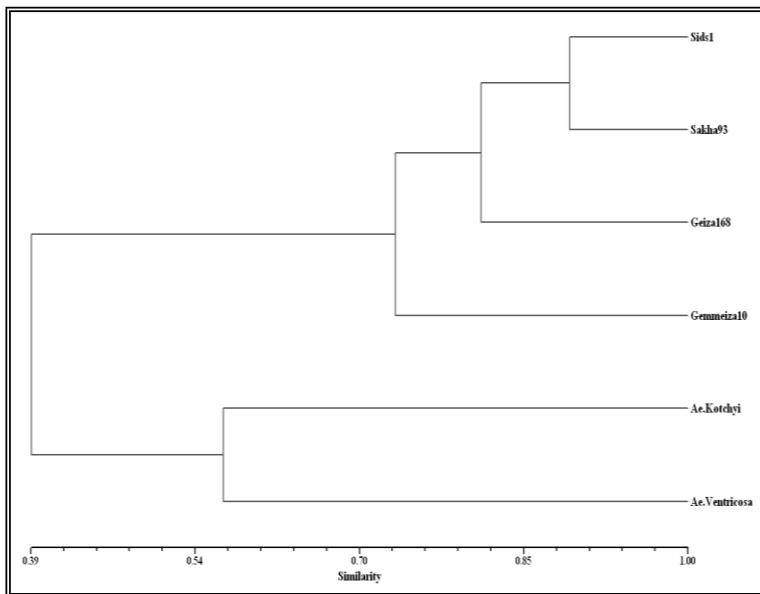


Fig. (2): Dendrogram of different domesticated wheat (*Triticum aestivum* L.) and wild wheat (*Aegilops* species) based on RAPD primers.

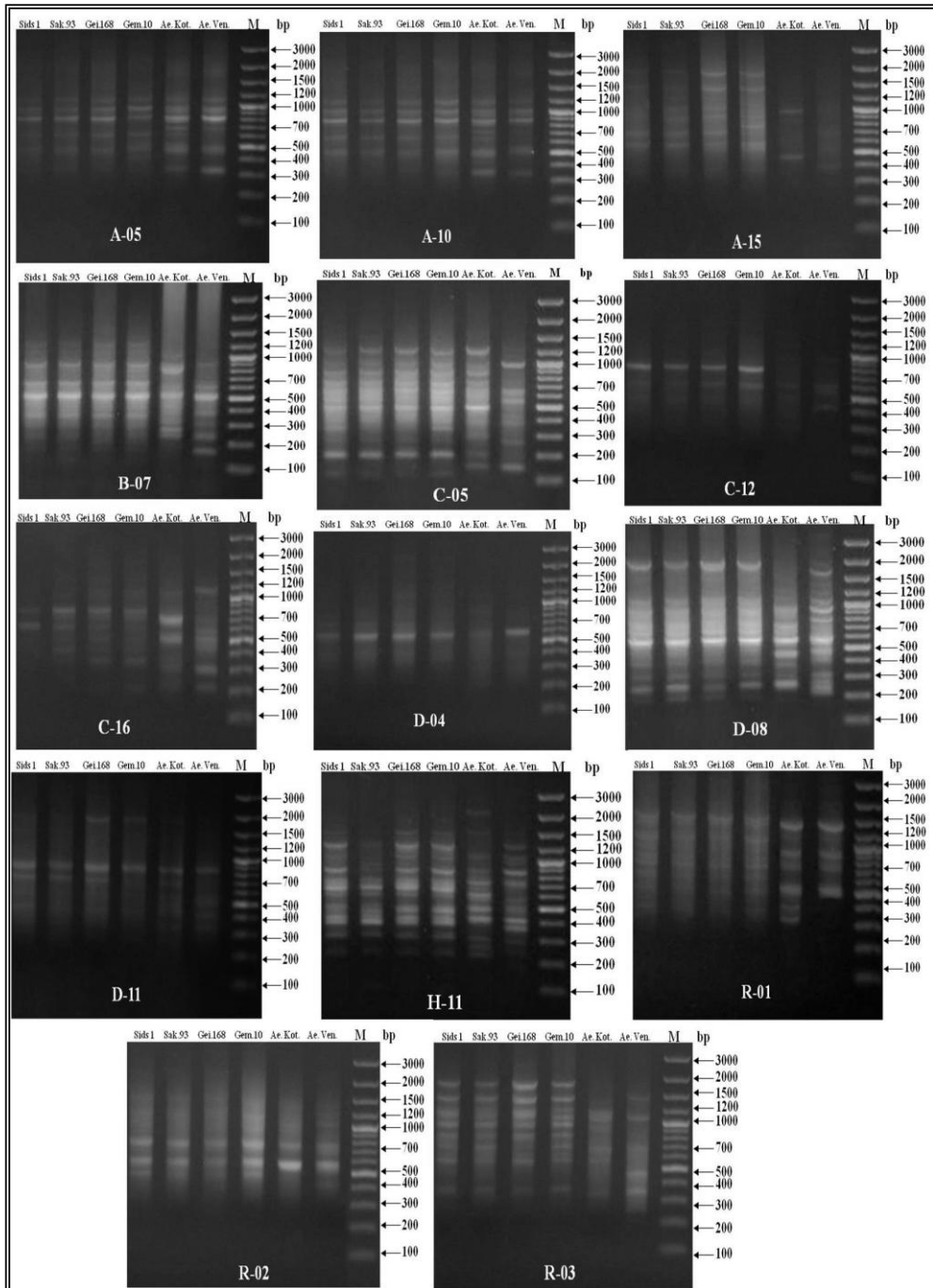


Plate (1): Photographs illustrating DNA fingerprinting of different domesticated wheat (*Triticum aestivum* L.) and their relative's wild wheat (*Aegilops* species) based on RAPD techniques.