GENETIC DIVERSITY IN OLD AND MODERN EGYPTIAN BREAD WHEAT (*Triticum aestivum* L.) VARIETIES REVEALED BY SIMPLE SEQUENCE REPEATS

KH. F. M. SALEM[1] AND M. Z. MATTAR[2]

1. *Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), P. O. Box, 79, Sadat City, Sadat City University, Egypt*
2. *Department of Botany, Faculty of Science, Menoufia University, Egypt*

Wheat (*Triticum aestivum* L.) is the first and strategic important cereal crop in Egypt. National wheat breeding programs cultivated wheat represented essentially spring-type. Since the initiation of wheat breeding program in Egypt, new cultivars were developed by (1) selection from local populations, (2) introduction of new varieties and (3) crossing and selection for yield and its components. Several high-yielding bread wheat cultivars were produced. Current breeding objectives are aimed at improving productivity of wheat through increased resistance to biotic and abiotic stresses and to develop varieties with good milling and bread-making properties and a high nutritional value (Morgounov *et al.*, 2001). The development of such varieties requires a continuous supply of a source of desirable genes and/or gene complexes. The sources of such genes could be (i) bread wheat varieties which have not been used very intensively but have a higher general adaptation, (ii) landraces, (iii) wild relatives, and (iv) weedy species. A prerequisite for efficient utilization of the plant material is knowledge about the genetic diversity, within the Egyptian bread wheat germplasm.

Molecular markers that reveal polymorphism at the DNA level have been shown to be a very powerful tool for genotype characterization and estimation of genetic diversity. In this regard, microsatellites or simple sequence repeats (SSRs), due to their multiallelic nature, have been extensively used in several crops (Gupta and Varshney, 2000).

In recent years, due to the availability of SSR marker sequences for oligonucleotide synthesis, involvement of PCR amplification, the simplicity of protocol that produces reliable and highly detectable amplification products, their codominance and single localization constitutes their advantages over AFLP, RFLP and RAPD markers (Varshney *et al.*, 2005). Molecular markers developed from SSR resources for crop plants have been popularly called as genic molecular markers (Varshney *et al.*, 2007).

Wheat microsatellite markers (WMS) (Röder *et al.*, 1998), are known to

be abundant, highly polymorphic, reliable and relatively easy in application, have already been used in several studies to estimate the genetic diversity in wheat (Plaschke *et al.*, 1995; Ben Amer *et al.*, 2001; Chebotar *et al.*, 2002; Huang *et al.*, 2002; Alamerew *et al.*, 2004; Colomba and Gregorini, 2011; Sardouie-Nasab *et al.*, 2013; Akfirat and Uncuoglu, 2013).

The objectives of this study were to (i) assess the genetic diversity within old and modern bread wheat (*Triticum aestivum* L.) varieties cultivated in Egypt by using SSR markers and (ii) assess whether old Egyptian varieties could be a potential source for improving genetic diversity in modern wheat breeding in Egypt.

MATERIALS AND METHODS

Plant material

Thirty-three diverse bread wheat varieties (*Triticum aestivum* L) released from 1947 to 2004 and with Egyptian origin were used in this study. Grains of all Egyptian varieties were obtained from the Agricultural Research Center (ARC), Giza, Egypt. A List of the wheat varieties, year of release, pedigree and released group is presented in Table (1).

DNA extraction

Total genomic DNA was extracted from young leaves for five seedlings from 8-weeks-old seedlings of each genotype. Only one replication was sampled for DNA extraction. DNA extraction was performed according to Plaschke *et al.* (1995).

PCR amplification

PCR reaction contained 50-100 ng template DNA, 250 nM forward primer, 250 nM reverse primer, 0.2 mM dNTPs, 2.5 µl PCR buffer (10 X), 1.5 mM MgCl, 1 U *Taq* DNA polymerase in a total volume of 25 µl. Amplifications were carried out using the following programs: 5 min at 94°C followed by 35 cycles of 1 min 94°C, 1 min 50°C or 55°C or 60°C according to primer annealing temperature and 2 min at 72°C, with a final extension of 5 min at 72°C as described by Röder *et al.* (1998). The amplification products were resolved on 10% polyacrylamide denaturing gels (PAGE) (Röder *et al.*, 1998).

SSR analysis

Seventeen gatersleben wheat microsatellite (*Xgwm*) markers (Table 2) were selected from Röder *et al*. (1998). The microsatellite primers used were described by Röder *et al.* (1998). Fragment detection for SSR markers was carried out as given in Röder *et al*. (1998).

Data collection and diversity

Gels were scored as binary data matrix. The presence (1) and absence (0) of alleles for each microsatellites marker were recorded for each variety. Gene diversity was calculated according to the formula of Nei (1973) using the equation

$PIC = 1 - \sum_{i=1}^{k} P_i^2$, where k is the total number of alleles detected for a locus of a marker and P_i is the frequency of the i^{th} allele in the set of thirty-three Egyptian wheat varieties investigated. Anderson *et al.* (1993) indicated that gene diversity is essentially the same as the polymorphism information content (PIC) as used by Botstein *et al.* (1980).

Genetic similarity estimation and cluster analysis

The data were analyzed using the SIMQUAL (Similarity for Qualitative Data) routine to generate Dice similarity coefficient (Dice, 1945). The similarity coefficient were used to construct dendrogram using Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm using the Numerical Taxonomy and Multivariate Analysis System (NTSYS), version 2.1 (Rohlf, 2002).

RESULTS AND DISCUSSION

Characteristics of SSR markers

All microsatellite markers used in this study yielded polymorphic fragments among the varieties tested. In total, 66 and 82 alleles were detected with an average of 3.88 and 4.82 alleles in both old and modern varieties, respectively (Table 3). The number of alleles per locus ranged from 3 to 6 with an average of 3.88 for old wheat varieties, while the number of alleles per locus ranged from 3 to 9 with an average of 4.82 for modern wheat varieties (Table 3). Huang *et al*. (2002) reported an average allele number of 18.1 in 998 gene bank accessions of hexaploid wheat originated from 68 countries of five continents. Khlestkina *et al*. (2004) found an average allele number of 6.6 in 54 Siberian old and modern common spring wheat varieties. Roussel *et al*. (2005) reported an average allele number of 16.4 in 480 wheat varieties originating from 15 European geographical areas and released from 1840 to 2000. Salem *et al*. (2008) detected an average of 3.2 alleles in seven wheat varieties. The data for the average number of alleles obtained in the present study of Egyptian bread wheat was lower than most previous studies, but it was comparable with Satchel's results, which detected 4.8 alleles per locus in wheat varieties (Satchel *et al*., 2000) and 3.2 alleles per locus in seven wheat varieties detected by Salem *et al.* (2008).

The correlation coefficient between gene diversity and the number of alleles for SSRs markers was high, r = 0.603 and r= 0.503, *(P<0.01),* for old and modern verities, respectively. The linear relationship between them is shown in Fig. (1). However, the correlation coefficient between gene diversity and the number of alleles for wheat genomes was r = 0.842 and r = 0.373, *(P<0.01),* for old and modern verities, respectively. The linear relationship between them is shown in Fig. (2). While, the correlation coefficient between gene diversity and the number of alleles for homologous groups was r = 0.710 and r = 0.029, *(P<0.01),* for old and modern verities, respectively. The linear

relationship between them is shown in Fig. (3). The value of gene diversity increased with the number of alleles at a given locus. There was significant correlation between gene diversity and the number of alleles. Therefore, the number of alleles can be used for the evaluation of genetic diversity. The data obtained in the present investigation agreed with those of Huang *et al*. (2002) who reported that the PIC value was correlated with the number of alleles.

Genetic diversity of A, B and D genomes

The 17 loci were distributed basically even on A, B & D genomes (6, 6 and 5, respectively) Table (4). Compared to old varieties, the modern groups showed the highest number of alleles of the three wheat genomes 30, 28 and 24 (B, A & D), respectively. Regarding to average genetic richness, the modern varieties showed higher number of alleles/loci 5, 4.8 and 4.67 (B, A & D), respectively than that in the old varieties (Table 4). As for genetic diversity, the modern varieties showed higher genetic diversity 0.703, 0.635 and 0.617 (B, A & D), respectively than that in the old varieties (Table 4). Indeed, the B genome showed the highest diversity. The number of alleles was different for individual genomes, (27 and 30) for B genome, (21 and 28) for A genome and (18 and 24) for D genome in old and modern wheat varieties, respectively. This might suggest that D genome is the most conserved. This may be due to the pattern of evolution of wheat genomes, as D genome was incorporated into hexaploid wheat much later than A and B genomes, so it may be less diverse. On the other hand, the number of SSR alleles located on B genome may reflect its greater variability sustained during evolution (Feldman, 2001). Those results were consistent with data achieved by Röder *et al*. (1998), Fahima *et al*. (1998), Huang *et al*. (2002), Alamerew *et al*. (2004), Colomba and Gregorini (2011), Li *et al*. (2012), Sardouie-Nasab *et al.* (2013) and Akfirat and Uncuoglu (2013) for SSR markers.

Genetic diversity of the 7 homologous groups

Homologous group 7 possessed the highest average of allelic numbers, while group 2 was the lowest values for both modern and old varieties. The order from the highest to the lowest for modern varieties was 19 for group 7, (17) for group 4, (15) for group 1, (12) for group 5, (11) for group 3, (8) for group 2, while the order for old group was 14 (group 7), 13 (group 4), 12 (group 1), 10 (group 3=5) and 7 (group 2). Regarding to the average of genetic richness, the modern varieties had the highest values than the old varieties. In addition, the average of genetic richness from 1^{st} to 7^{th} homoeologous group for the modern varieties was 5, 4, 3.67, 5.67, 4.00 and 6.33, respectively. So, group 7 still hold the highest genetic richness and group 3 was the lowest in both modern and old varieties (Table 4). With regards to PIC, the highest value was 0.753 and 0.718 for group 4 in both modern and old varieties, respectively. Whereas, the lowest PIC values was 0.595 (group 5) and

0.528 (group 2) for both modern and old varieties, respectively. There were not large differences between A and D genomes in the average genetic richness for both old and modern wheat varieties, but average genetic richness for A genome was obviously lower. While, B genome had the highest average genetic richness. This indicated that there were more key genes/QTLs controlling important agronomic characteristics, and domestication and modern breeding provided much higher selective pressures to A genome (Peng *et al*., 2003). Among the 7 homologous groups, the genetic diversity of group 5 was much lower for both old and modern Egyptian wheat varieties. Therefore, it was estimated that breeding might have brought much higher selection pressure on genes conveyed by this group. This was consistent with the opinions of Börner *et al*. (2002) and Peng *et al*. (2003).

Cluster analysis

Similarity index and consensus tree were developed on the bases of the scorable banding patterns of the 6 released wheat groups which resulted from 33 wheat varieties using the 17 SSR markers as shown in Table (4 and 5). The similarity index showed that the two most closely related groups were C and B with the highest similarity index 0.588. On the other hand, the two most distantly related groups were (E and B) and (E and C) with the low similarity index 0.228.

Cluster analysis was conducted based on SSR data to group the wheat varieties and to construct a dendrogram as presented in Fig. (4). All 33 varieties were divided into six groups according to year of release. Two major clusters corresponded to the old and modern groups. Two groups can be distinguished by truncating the dendrogram at GS value of 0.25. With genetic distance (GD) < 0.588 as the standard of sub-cluster.

The consensus dendrogram showed that the Egyptian bread wheat varieties were divided into two main clusters (I and II). The first included two groups from four of the old wheat varieties (group A and C). Group (A) consisted of two varieties (Giza 139 and Giza 144), while group (C) consisted of two varieties (Giza 157 and Sakha 8). The second main cluster was divided into two sub-clusters (IIa and IIb). The first subcluster (IIa) included the other two old groups (group B and D). Group (B) contained two varieties (Giza 150 and Giza 155). However group (D) contained seven varieties (Sakha 61, Sakha 69, Giza 160, Sakha 92, Giza 162, Giza 163 and Giza 164). The second subclusters (IIb) included the modern Egyptian wheat varieties (group E and F). Group E had twenty varieties (Gemmiza 1, Sahel 1, Giza 167, Sids 1, Sids 2, Sids 3, Sids 4, Sids 5, Sids 6, Sids 7, Sids 8, Sids 9, Gemmiza 3, Gemmiza 5, Gemmiza 7, Gemmiza 9, Giza 168 and Sakha 93). However, group F contained only the two wheat varieties (Sakha 94 and Gemmiza 10).

The above discussion amply demonstrates the utility of microsatellites, which can be profitably utilized in wheat

not only for detecting polymorphism and tagging genes (Prasad *et al.*, 1999; Roy *et al*., 1999) but also for genotype identification and for estimation of genetic diversity. We conclude, therefore, that on the basis of microsatellite markers, diverse parents can be selected. In addition to provide new information about the relationships between the old and modern Egyptian bread wheat varieties analyzed. Also, the obtained data may be suggested that old Egyptian bread wheat varieties are a potential basis for genetic diversity in modern wheat breeding in Egypt.

SUMMARY

The objective of the present study was to assess genetic diversity within old and modern bread wheat varieties cultivated in Egypt and to find out whether old Egyptian varieties could be a potential source for genetic diversity in modern wheat breeding in Egypt. A set of 33 varieties was analyzed using 17 SSR markers, determining 17 loci located on 15 different chromosomes. A total of 66 and 82 alleles were detected with an average of 3.88 and 4.82 alleles in both old and modern wheat varieties, respectively. The average genetic diversity value was 0.617 in old varieties while in modern varieties it was 0.652. Compared to old varieties, the modern varieties showed the highest number of alleles for the three wheat genomes 30, 28 and 24 (genome B, A & D), respectively. Regarding the average genetic richness, the modern varieties showed higher number of alleles/locus 5, 4.8 and 4.67 (genome B, A & D), respectively than that in the old varieties. As for genetic diversity, the modern varieties showed higher genetic diversity 0.703, 0.635 and 0.617 (genome B, A & D), respectively. Indeed, the B genome showed the highest diversity. In generally, homologous group 7 possessed the highest average of allelic numbers, while group 2 was the lowest for both modern and old varieties. Cluster analysis was conducted based on SSRs data to group the bread wheat varieties and to construct a dendrogram. Two groups can be distinguished by truncating the dendrogram at GS value of 0.25.

REFERENCES

Akfirat, F. S. and A. A. Uncuoglu (2013). Genetic diversity of winter wheat (*Triticum aestivum* L.) revealed by SSR markers. Biochem. Genet., 51: 223-229.

Alamerew, S., S. Chebotar, X. Huang, M. S. Röder and A. Börner (2004). Genetic diversity in Ethiopian hexaploid and tetraploid wheat germplasm assessed by microsatellite markers. Gen. Res. Crop Evol., 51: 559-564.

Anderson, J. A., G. A. Churchill, J. E. Antrique, S. D. Tanksley and M. E. Sorrels (1993). Optimizing parental selection for genetic linkage maps. Genome, 36: 181-188.

Ben Amer, I. M., A. Börner and M. S. Röder (2001). Detection of genetic diversity in Libyan wheat geno-

types using wheat microsatellite markers. Gen. Res. Crop Evol., 48: 579-585.

Börner, A., E. Schumann, A. Fürste, H. Cöster, B. Leithold, M. S. Röder and W. E. Weber (2002). Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.) Theor. Appl. Genet., 105: 921-936.

Botstein, D., R. L. White, M. Skolnick and R. W. Davis (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Hum. Genet., 32: 413-331.

Chebotar, S. V., M. S. Röder, A. Börner and Yu. M. Sivolap (2002). Characterization of Ukrainian bread wheat (*Triticum aestivum* L.) germplasm by using microsatellite markers. In: Proc. Int. Symp. Biotechnology Approaches for Exploitation and Preservation of Plant Resources, Yalta, Ukraine, 8-11.

Colomba, M. S. and A. Gregorini (2011). Genetic diversity analysis of the durum wheat Graziella Ra, *Triticum turgidum* L. subsp. *durum* (Desf.) Husn. (Poales, Poaceae). Biodiversity Journal, 2: 73-84.

Dice, L. R. (1945). Measures of the amount of ecologic association between species. Ecology, 26: 297-302.

Fahima, T. M. S. Röder, A. Grama and E. Nevo (1998). Microsatellite DNA polymorphism divergence in *Triticum dicoccoides* accessions highly resistant to yellow rust. Theor. Appl. Genet., 96: 187-195.

Feldman, M. (2001). Origin of cultivated wheat. In: Bonjean, A. P. and W. J. Angus. (eds). The World wheat book. A history of wheat breeding. Intercept, Paris, p 3-56.

Gupta, P. K. and R. K. Varshney (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica, 113: 163-185.

Huang, X. Q., A. Borner, M. S. Röder and M. W. Ganal (2002). Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet., 105: 699-707.

Khlestkina, E. K., M. S. Röder, T. T. Efremova, A. Borner and V. K. Shumny (2004). The genetic diversity of old and modern Siberian varieties of common spring wheat as determined by microsatellite markers. Plant Breed., 123: 122-127.

Li, H. Q., H. G. Zhang, B. L. Liu, D. C. Liu and B. Zhang (2012). Assessing genetic diversity and its changes of bread wheat in Qinghai Province, China, using agronomic traits and microsatellite markers.

Biological Agric. & Hort., 28: 120-128.

Morgounov, A., V. A. Zykin, G. A. Sereda and R. A. Urazaliev (2001). Siberian and North Kazakhstan wheat pool. In: A. P. Bonjean, and W. J. Angus (eds), The World Wheat Book. A History of Wheat Breeding, 755-772. Lavoisier Publishing, London, Paris, New York.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci., USA, 70: 3321-3323.

Peng, J., Y. Ronin, T. Fahima, M. S. Roder, Y. Li, A. Nevo E and A. Korol (2003). Domestication quantitative trait loci in *Triticum dicoccoides*, the progenitor of wheat. Proc. Nat. Acad. Sci., USA, 100: 147-159.

Plaschke, J., M. W. Ganal and M. S. Röder (1995). Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor. Appl. Genet., 91: 1001-1007.

Prasad, M., R. K. Varshney, A. Kumar, H. S. Balyan, P. C. Sharma, K. J. Edwards, H. Singh, H. S. Dhaliwal, J. K. Roy and P. K. Gupta (1999). A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. Theor. Appl. Genet., 99: 341-345.

Röder, M. S., V. Korzun, K. Wendehake, J. Plaschke, M. H. Tixier, P. Leroy and M. W. Ganal (1998). A microsatellite map of wheat. Genetics, 149: 2007-2023.

Rölf, F. J. (2002). NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.1, Applied Biostatistics, New York.

Roussel, V., L. Leisova, F. Exbrayat, Z. Stehno and F. Balfourier (2005). SSR allelic diversity changes in 480 European bread wheat varieties released from 1840 to 2000. Theor. Appl. Genet., 111: 162-170.

Roy, J. K., M. Prasad, R. K. Varshney, H. S. Balyan, T. K. Blake, H. S. Dhaliwal, H. Singh, K. J. Edwards and P. K. Gupta (1999). Identification of a microsatellite on chromosome 6B and a STS on 7D of bread wheat showing association with preharvest sprouting tolerance. Theor. Appl. Genet., 99: 336-340

Salem, K. F. M., A. M. El-Zanaty and R. M. Esmail (2008). Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatellite markers. World J. Agri. Sci., 5: 538-544.

Stachel, M., T. Lelly, H. Grausgruber and J. Vollmann (2000). Application of microsatellites in wheat (*Triticum aestivum* L.) for studying genetic differentiation caused by selection

for adaptation and use. Theor. Appl. Genet., 100: 242-248.

Sardouie-Nasab, S., Gh. Mohammadi-Nejad and B. Nakhoda (2013). Assessing genetic diversity of promising wheat (*Triticum aestivum* L.) lines using microsatellite markers linked with salinity tolerance. J. Plant Molecular Breeding, 1: 28-39.

Varshney, R. K., S. N. Nayak, G. D. May and S. A Jackson (2005). Next-generation sequence technologies and their implications for crop genetics and breeding. Trends in Biotechnology, 27: 522-530.

Varshney, R. K., T. Mahender, R. K. Aggrawal and A. Borner (2007). Genic molecular markers in plants: development and applications. In: Varshney R. K. and R. Tuberosa. (eds) Genomics assisted crop improvement, vol I: genomics approaches and platforms. Springer, Dordrecht, p 13-30.

Table (1): List of bread wheat cultivars released by the Wheat Research Department, ARC, Giza, Egypt during the last 50 years*.

No	Varieties	Year of release	Pedigree	Released Group
1	Giza 139	1947	Hindi 90/ Kenya B256	Group A
2	Giza 144	1958	Rgent/2* Giza 139	Group A
3	Giza 150	1960	Mida-Cadet/2* Giza 139	Group B
4	Giza 155	1968	Regent/2* Giza 139//Mida-Cadit /2* Hindi 62	Group B
5	Giza 157	1977	Giza 155//Pit 62 /LR 64/3/Tzpp/Knott	Group C
6	Sakha 8	1977	Indus/Norteno “s”	Group C
7	Sakha 61	1980	Inia/RL 4220//7C/Yr “s”	Group D
8	Sakha 69	1980	Inia/RL 4220//7C/Yr “s”	Group D
9	Giza 160	1982	Chenab70/Giza 155	Group D
10	Sakha 92	1987	Napo 63/Inia 66//Wern “s”	Group D
11	Giza 162	1987	Vcm//Cno67/7C/3/Kal/BbCM8399-D-4M-3Y-1M-1Y-1M-0Y	Group D
12	Giza 163	1987	*T.aestivum*/Bon//Cno/7C CM33009-F-15M-4Y-2M-1M-1M-1Y-0M	Group D
13	Giza 164	1987	Kvz/Buha “s”//Kal/Bb CM33027-F-15M-500y-0M	Group D
14	Gemmiza 1	1991	Maya 74/On//1160.147/3/Bb/Gall/4/Chat”s” CM58924-1GM-OGM	Group E
15	Sahel 1	1994	N.S.732/Pim//Vee”s”	Group E
16	Giza 167	1995	Au/Up301//Gll/Sx/Pew”s”/4/Mai”s”/May”s”//Pew”s”CM67245-C-1M-2Y-1M-7Y-1M-0M	Group E
17	Sids 1	1996	HD 2172/Pavon”s”//1158.57/Maya 74 “s” SD46-4SD46-4Sd-2SD-1SD-0SD	Group E
18	Sids 2	1996	HD 2206/Hork”s”/3/Napo63/Inia66//Wern “s” SD635-4SD-1SD-1SD-0SD	Group E
19	Sids 3	1996	Sakha 69/Giza155 SD723-7SD-1SD-0SD	Group E
20	Sids 4	1994	Maya "s"/Mon "S"/CM H74.A592/3/Giza 157*2	Group E
21	Sids 5	1994	Maya "s"/Mon "S"/CM H74.A592/3/Giza 157*2 SD10001-7sd-4SD-2SD-0SD	Group E
22	Sids 6	1994	Maya "s"/Mon "S"/CM H74.A592/3/Sakha 8*2 SD10002-4SD-3SD-1SD-0SD	Group E
23	Sids 7	1994	Maya "s"/Mon "S"/CM H74.A592/3/Sakha 8*2 SD10002-8SD-1SD-1SD-0SD	Group E
24	Sids 8	1994	Maya "s"/Mon "S"/CM H74.A592/3/Sakha 8*2 SD10002-14SD-3SD-1SD-0SD	Group E
25	Sids 9	1994	Maya "s"/Mon "S"/4//CM H72.428/MRC//jip/3/CMH74A582/5/Giza157*2SD10003	Group E
26	Gemmiza 3	1997	Bb/7C*2//Y50/Kal*3//Sakha8/4/Prv/WW/5/3/Bg”s”//OnCGM.4024-1GM13 GM2GM-0GM	Group E
27	Gemmiza 5	1998	Vee”s”/SWM 6525 CGM.4017-1GM-6 GM-3 GM-0GM	Group E
28	Gemmiza 7	2000	CMH74 A. 630/5x//Seri 82/3/Agent CGM.4611-2GM-3GM-1GM-0GM	Group E
29	Gemmiza 9	2000	Ald”s”/Huac”\s”//CMH74A.630/5x CGM.4583-5GM-1GM-0GM	Group E
30	Giza 168	1999	Mil/Buc//Seri	Group E
31	Sakha 93	1999	Sakha 92/TR 810328	Group E
32	Sakha 94	2004	Opata/Rayon//Kauz	Group F
33	Gemmiza 10	2004	Maya 74 “s”/On//1160-147/3/Bb/4/Chat”s”/5/Ctow	Group F

*****Thanks are due to ARC, Ministry of Agric., Egypt**.**

Table (2): Characteristics of 17 wheat SSR markers, their chromosomal location, primer sequence, motif, annealing temperature and fragment size.

No.	SSR markers And their chromosomal location	Primer sequence (L) Left (R) right		Motif	Annealing Tm (°C)	Fragment size in CS (bp)
1	*Xgwm3* -3D	GCA GCG GCA CTG GTA CAT TT AAT ATC GCA TCA CTA TCC CA	(L) (R)	$(CA)_{18}$	55	79
2	*Xgwm18- 1B*	TGG CGC CAT GAT TGC ATT ATC TTC GGT TGC TGA AGA ACC TTA TTT AGG	(L) (R)	$(CA)_{17}GA$ $(TA)_4$	55	183
3	*Xgwm 46-7B*	GCA CGT GAA TGG ATT GGA C TGA CCC AAT AGT GGT GGT CA	(L) (R)	$(GA)_3GC$ $(GA)_{33}$	60	179
4	*Xgwm 95-2A*	GAT CAA ACA CAC ACC CCT CC AAT GCA AAG TGA AAA ACC CG	(L) (R)	$(AC)_{16}$	60	179
5	*Xgwm155-3A*	CAA TCA TTT CCC CCT CCC AAT CAT TGG AAA TCC ATA TGC C	(L) (R)	$(CT)_{19}$	60	144
6	*Xgwm160-4A*	TTC AAT TCA GTC TTG GCT TGG CTG CAG GAA AAA AAG TAC ACC C	(L) (R)	$(GA)_{21}$	60	182
7	*Xgwm165-4A*	TGC AGT GGT CAG ATG TTT CC CTT TTC TTT CAG ATT GCG CC	(L) (R)	$(GA)_{20}$	60	190
8	*Xgwm186-5A*	GCA GAG CCT GGT TCA AAA AG CGC CTC TAG CGA GAG CTA TG	(L) (R)	$(GA)_{26}$	60	135
9	*Xgwm190-5D*	GTG CTT GCT GAG CTA TGA GTC GTG CCA CGT GGT ACC TTT G	(L) (R)	$(CT)_{22}$	60	209
10	*Xgwm261-2D*	CTC CCT GTA CGC CTA AGG C CTC GCG CTA CTA GCC ATT G	(L) (R)	$(CT)_{21}$	55	189
11	*Xgwm389-3B*	ATC ATG TCG ATC TCC TTG ACG TGC CAT GCA CAT TAG CAG AT	(L) (R)	$(CT)_{14}$ $(GT)_{16}$	60	129
12	*Xgwm408-5B*	TCG ATT TAT TTG GGC CAC TG GTA TAA TTC GTT CAC AGC ACG C	(L) (R)	$(CA)>_{22}(T)$ $(CA)_7(TA)_9$	55	176
13	*Xgwm437-7D*	GAT CAA GAC TTT TGT ATC TCT C GAT GTC CAA CAG TTA GCT TA	(L) (R)	$(CT)_{24}$	50	107
14	*Xgwm458-1D*	AAT GGC AAT TGG AAG ACA TAG C TTC GCA ATG TTG ATT TGG C	(L) (R)	$(CA)_{13}$	60	113
15	*Xgwm513-4B*	ATC CGT AGC ACC TAC TGG TCA GGT CTG TTC ATG CCA CAT TG	(L) (R)	$(CA)_{12}$	60	140
16	*Xgwm631-7A*	---		$(GT)_{23}$	60	196
17	*Xtaglgap-1B*	GCA GAC CTG TGT CAT TGG TC GAT ATA GTG GCA GCA GGA TAC G	(L) (R)	$(CAA)_{31}$	60	282

Table (3): Characteristics of SSR markers used with the chromosomal location, marker name, allele size range, number of alleles per locus and gene diversity calculated over a set of 33 old and modern wheat varieties.

Locus	Position	Allele size range (bp)		Number of alleles		Gene diversity	
		Min allele	Max allele	Old	Modern	Old	Modern
Xgwm 3	3D	77	84	3	3	0.615	0.535
Xgwm 190	5D	204	212	3	3	0.569	0.558
Xgwm 261	2D	165	192	3	4	0.500	0.575
Xgwm 437	7D	91	130	6	9	0.694	0.835
Xgwm 458	1D	109	122	3	5	0.569	0.645
Xgwm 18	1B	186	192	4	3	0.513	0.605
Xgwm 46	7B	147	187	5	7	0.722	0.780
Xgwm 389	3B	119	136	4	5	0.694	0.750
Xgwm 408	5B	178	194	4	3	0.583	0.595
Xgwm 513	4B	141	150	5	5	0.778	0.725
Xtaglgap	1B	212	280	5	7	0.769	0.765
Xgwm 631	7A	190	200	3	3	0.500	0.244
Xgwm 95	2A	109	131	4	4	0.556	0.803
Xgwm 155	3A	129	147	3	3	0.486	0.515
Xgwm 160	4A	177	189	3	5	0.611	0.740
Xgwm 165	4A	187	202	5	7	0.764	0.795
Xgwm 186	5A	122	134	3	6	0.569	0.605
Total				66	82	--------	--------
Mean				3.88	4.82	0.617	0.651

Table (4): Genetic diversity between the old and modern varieties in different genomes and homologous chromosome groups.

Location	Number of loci checked	Number of alleles		Average genetic richness		Gene diversity	
		Old wheat varieties	Modern Wheat varieties	Old wheat varieties	Modern Wheat varieties	Old wheat varieties	Modern Wheat varieties
Genome							
A	6	21	28	3.50	4.67	0.581	0.617
B	6	27	30	4.50	5.00	0.677	0.703
D	5	18	24	3.60	4.80	0.589	0.635
Homologous chromosome Group							
1	3	12	15	4.00	5.00	0.617	0.672
2	2	7	8	3.50	4.00	0.528	0.689
3	3	10	11	3.33	3.67	0.598	0.600
4	3	13	17	4.33	5.67	0.718	0.753
5	3	10	12	3.33	4.00	0.574	0.595
7	3	14	19	4.67	6.33	0.639	0.620

Table (5): Genetic similarity matrix values for the six groups of wheat varieties based on SSR markers.

	Group A	Group B	Group C	Group D	Group E
Group B	0.294				
Group C	0.352	0.588			
Group D	0.235	0.457	0.571		
Group E	0.231	0.228	0.228	0.319	
Group F	0.261	0.294	0.294	0.236	0.400

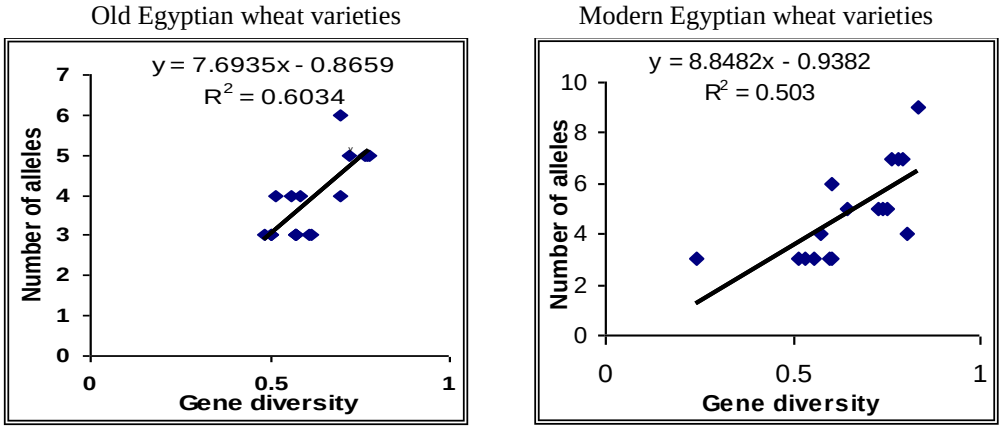

Fig. (1): Correlation between gene diversity and the number of alleles over 17 SSR loci in total of 33 old (left) and modern (right) bread wheat varieties.

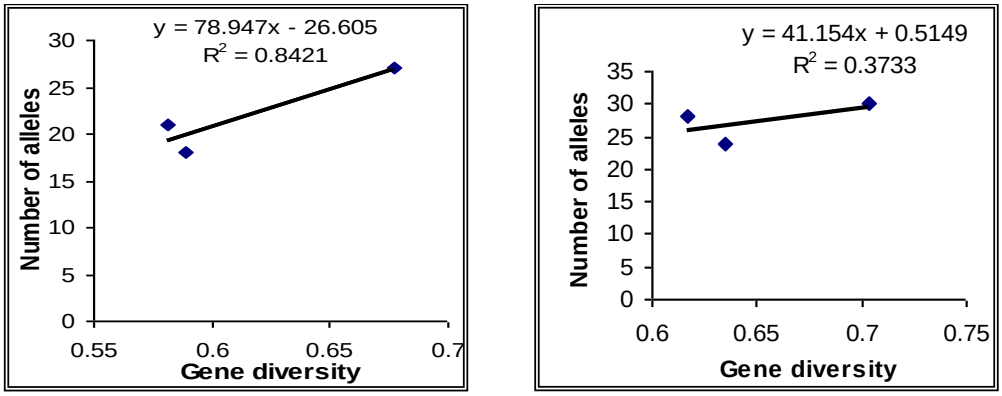

Fig. (2): Correlation between gene diversity and the number of alleles over all wheat genomes in total of 33 old (left) and modern (right) bread wheat varieties.

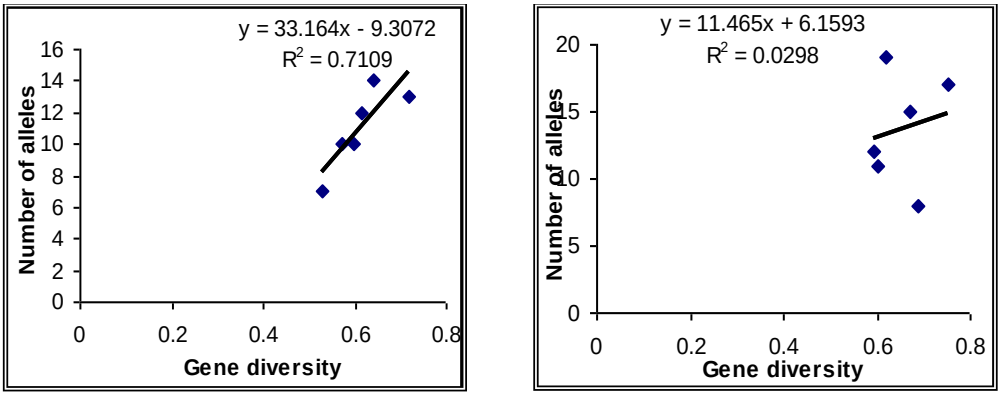

Fig. (3): Correlation between gene diversity and the number of alleles over 6 homologous groups in total of 33 old (left) and modern (right) bread wheat varieties.

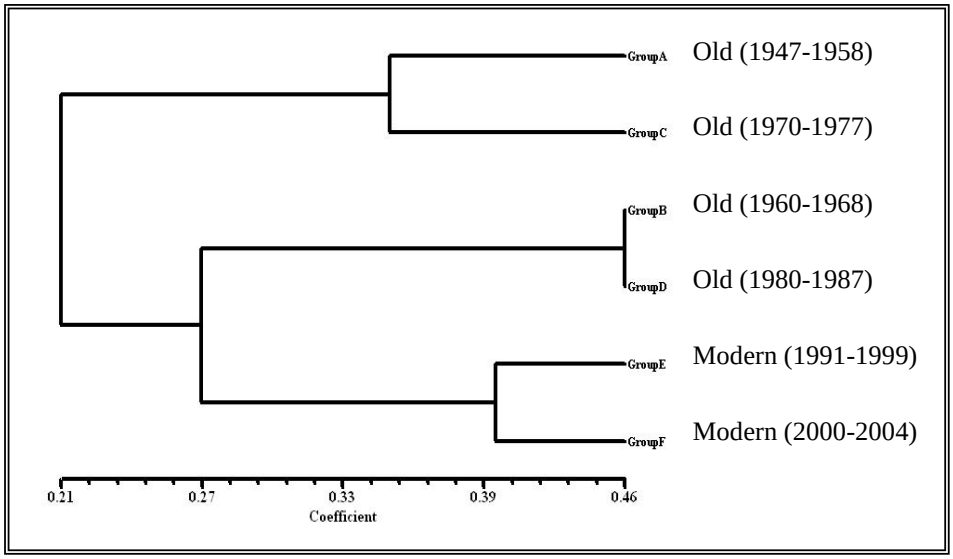

Fig. (4): Dendrogram reflecting genetic similarity between 33 Egyptian bread wheat, based on the analysis of 17 microsatellite loci.