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MOLECULAR CHARACTERIZATION AND GENETIC DIVERSITY IN SOME EGYPTIAN WHEAT (*Triticum aestivum* L.) USING MICROSATELLITE MARKERS

Ayman El-Fiki, Mohamed Adly

ABSTRACT
Wheat (*Triticum aestivum* L.) is the most important and strategic cereal crop in Egypt and has many bread wheat varieties. Seventeen Egyptian bread wheat varieties used in this study with a set of sixteen wheat microsatellite markers to examine their utility in detecting DNA polymorphism, estimating genetic diversity and identifying genotypes. A total of 190 alleles were detected at 16 loci using 16 microsatellite primer pairs. The number of allele per locus ranged from 8 to 20, with an average of 11.875. The polymorphic information content (*PIC*) and marker index (*MI*) average values were 0.8669, 0.8530 respectively. The (GA) n microsatellites were the highest polymorphic (20 alleles). The Jaccard's Coefficient for genetic similarity was ranged from 0.524 to 0.109 with average of 0.375. A dendrogram was prepared based on similarity matrix using UPGMA algorithm, divided the cultivars into two major clusters. The results proved the microsatellite markers utility in detecting polymorphism due to the discrimination of cultivars and estimating genetic diversity.

Keywords: DNA polymorphism; genetic diversity; heterozygosity; PIC; SSR; wheat

INTRODUCTION
Wheat (*Triticum aestivum* L.) is an important and strategic grain crop in the majority of the world. In Egypt, wheat is considered the most important and strategic cereal crop. It represents about 10 percent of the total agricultural production value and about 20 percent of all agricultural imports **(FAOSTAT, 2008)**. Wheat is a self-pollinating polypoid crop that has been bred for a wide array of specific end-use quality traits and various adaptive characteristics, resulting in the development of distinct cultivars tailored to specialized end uses and specific production environments.

Botanists have long used morphological characterization to classify and distinguish genotypes within plant species. The emergence of the Plant Variety Protection (PVP) Act in 1970 had an impact on the protection of plant varieties. Hence, it was necessary to develop the critical tools for classifying and distinguishing genotypes within plant varieties **(Rongwen et al., 1995)**. The polymorphisms of DNA provide a powerful tool for determining and discriminating the levels of genetic variation in plant germplasm. Molecular markers have thus become accurate and reliable tools for identifying and characterizing plant varieties and it has become effective tool for efficient selection of desired agronomic traits since it depends on genotype rather than phenotype. The advances in molecular genetics methodology have led to widespread use of co-dominant molecular markers, especially Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs) and the amplified fragment length polymorphisms (AFLPs) **(Röder et al., 1998; Bohn et al., 1999; Prasad et al., 1999; Prasad et al., 2000; Roy et al., 1999; Varshney et al., 2000;** for reviews see **Gupta et al., 1999; Gupta and Varshney, 2000; Bered et al., 2005)**.

The first one define microsatellite term was **(Litt and Lutty, 1989)**, as multilocus probes creating complex banding patterns and usually non- species specific occurring ubiquitously. They essentially belong to the repetitive DNA family. Microsatellite are repeated as of only a few bases, like two or three or five, and the whole repetitive region spans less than 150 bp. Therefore, it needs cloning and sequencing for designing the primers **(Weissenbach et al., 1992; Morgante and Olivieri, 1993; Powell et al., 1996)**. Furthermore, these markers have provided high reproducibility and genetic informativeness **(Coombs et al., 2004; Garcia et al., 2007)**.

Microsatellites produced from whole genome sequences, subgenomic sequences, ESTs and gene sequences have also been applied to DNA fingerprinting and the estimation of genetic diversity within a gene pool. Genetic diversity is defining the heritable variation within and between population's organisms **(Ramanatha and Hodgkin, 2002)**. Knowledge of the genetic diversity and population structure within germplasm collections is an

important foundation for crop improvement **(Thomason et al., 2007)**. Progress in plant breeding requires a broad genetic base with a rich and diverse germplasm collection being the backbone of every successful crop improvement program. In this study, we evaluate the potential of 16 microsatellite primer pairs in general and specific SSRs in particular for polymorphism determination, cultivars identification and to evaluate the level of microsatellite based genetic diversity between 17 Egyptian bread wheat cultivars that were potentially useful in wheat breeding programs.

Scientific hypothesis

It is expected that there will be a significant similarity between the wheat varieties used in this experiment due to their genetic resources.

MATERIAL AND METHODOLOGY

Seed Material

In this experiment, some elite Egyptian bread wheat cultivars (17) were selected in different regions in Egypt as shown in Table (1). These cultivars have been obtained from Gene Bank, Egyptian Ministry of Agriculture.

DNA isolation and microsatellite primers

Twenty five seeds of each wheat varieties have been planted in pots in greenhouse. After two weeks of planting, the plants were enough to get fresh leaves and were collected from each sample. The samples were immediately transferred to liquid nitrogen tank to prevent deterioration. Weighed about 1.5 g from plant leaves samples and ground by liquid nitrogen to obtain fine powder. Total genomic DNA was isolated from leaves of each of the seventeen varieties according to the protocol described by **Anderson et al. (1992)**, with a few modifications intended to improve the quality of DNA: two consecutive extractions with phenol: chloroform (1:1) were carried out by an additional wash of 97% (left at -20 °C for one hour) an 70% pre-cooled ethanol, respectively. The yield and quality of DNA were assessed by spectrophotometer and gel electrophoreses.

SSR markers and protocols

Sixteen microsatellite primer pairs (SSR) were selected from **(Röder et al., 1998)**, Table 2. Amplification reactions were carried out in a total volume of 25 µL, which contained 250 nM of each primer (Metabion GmbH, Germany), 0.2 mM of each deoxynucleotide, 1.5 mM $MgCl_2$, 1 unit *Taq* polymerase (Bioline, GmbH, Germany) and 50 – 100 ng of template DNA. All reaction volumes were 25 µL overlaid with a drop of mineral oil. The thermocycling program (MJ Research PTC-100 thermal cycler) used was: one cycle at 94 °C for 3 min, 45 cycles at 94 °C for 1 min at either 50, 55 or 60 °C (depending on the individual microsatellite), 2 min at 72 °C, and the final extension step of 10 min at 72 °C. Electrophoresis was done to visualize the PCR amplified product. It was carried out on 2.0% agarose gel and amplified fragments were visualized by staining wit ethidium bromide. Fragment sizes were determined with PyElph 1.4 software, is the commercial program Quantity One from Bio-Rad **(Pavel and Vasile, 2012)**. The fragment size in "Chinese Spring" was taken as standard **Röder et al., (1998)**.

Statistic analysis

The fragment(s) sizes in 'Chinese Spring' were taken as standard, and the size differences of the fragments in other genotypes were considered to be the result of alterations in the repeat number of the simple sequences at the corresponding site(s). All distinct DNA fragments scored as present (1) or absent (0) were used to compute pair-wise similarity coefficients **(Jaccard, 1908)** for each of the markers for the purpose of assessing genetic diversity leading to cluster analysis. PowerMarker software (v3.0, 2004) is statistical software for genetic marker data analysis **(Liu and Muse, 2005)** was used for estimating of allele number, allele frequencies, genotype, heterozygosity and polymorphic information content (PIC). However, Marker index (MI) was calculated according to **Powell et al. (1996)**, MI = Average polymorphic information content (PIC) x Proportion of polymorphic bands x Average number of loci per assay unit.

Cluster analysis

For phylogenetic analysis, data only from the polymorphic SSR loci were subjected to MVSP software statistical software (Kovach Computing Services, Pentraeth, Wales, U.K). All the 17 wheat varieties were clustered based on the estimated genetic distance. The phylogenetic analysis was carried out with the clustering method of the unweighted Pair Group Method Using Arithmetic Average (UPGMA).

Principal component analysis (PCA)

The original 1 – 0 data matrix was used for calculating a correlation matrix between pairs of markers. The correlation matrix was employed for the calculation of eigenvalues, which were then used for determining the coordinates for each genotype that were used for PCA.

RESULTS AND DISCUSSION

DNA polymorphism and genotype identification

The results of PCR amplification of a number of microsatellite loci in seventeen Egyptian bread wheat cultivars using sixteen microsatellite primer pairs are summarized in (Table 2). Altogether, 190 alleles at 16 loci were obtained with average 11.875 alleles per locus. The maximum number of alleles detected at *Xgwm32-3A* belonging to (GA) n was 20 alleles with size ranged from 163-179 bp. However, the minimum number of alleles detected at *Xgwm156-5A* belonging to (GT) n and *Xgwm157-2D* (CT) n was 8 alleles with size from 270-299 bp and 107-113 bp respectively, Table 2. Allele frequency per locus varied from eight (*Xgwm156-5A* and *Xgwm157-2D*) to twenty *Xgwm32-3A*. It is known that microsatellite primer pairs are locus specific and that is meant to be a single locus marker comparing with other molecular markers as RFLP probes, RAPD, ISSR and SCoT primers which multilocus **(Vivodík et al., 2016)**. In the present study, the 16 loci that were assigned to specific chromosomes were able to distinguish between 17 Egyptian bread wheat and thus useful for detecting polymorphism.

Table 1 List of the elite Egyptian wheat cultivars and region of cultivation.

No.	Wheat cultivars	Cultivation region	No.	Wheat cultivars	Cultivation region
1	Misr 1	North Delta - Central & South Delta - New Land - North & East Coast	11	Sids 13	Central Egypt
2	Misr 2	New Land - North and East Coast	12	Sakha 93	North Delta - Central and South Delta - Upper Egypt
3	Gimmasa 7	Nubaria Area - New Land	13	Sakha 94	North Delta - Central and South Delta
4	Gimmasa 9	Nubaria, North Delta - Central and South Delta	14	Sakha 69	North Delta - North Coast Areas
5	Gimmasa 10	Nubaria, North Delta - Central and South Delta	15	Shandaweel 1	Central Egypt - Upper Egypt
6	Gimmasa 11	Nubaria, North Delta - Central and South Delta	16	Bani Sweef 1	Central Egypt - Upper Egypt
7	Gimmasa 12	Nubaria and most of the governorates of the Delta	17	Bani Sweef 4	Central Egypt
8	Giza 168	North Delta - Central and South Delta - Middle Egypt - Upper Egypt - New Land - Nubaria area			
9	Sids 8	Central and Upper Egypt - South Valley			
10	Sids 12	North Delta - Central Egypt - Upper Egypt - New Land - North Coast - Nubaria area			

Table 2 Primers of SSR, locus, repeat motif and annealing temperature (Ann. Temp.) were used.

No.	SSR primers				
	Locus	Forward primer	Revers primer	Repeat motif	Ann. Temp. °C
1	*Xgwm2-3A*	CTG CAA GCC TGT GAT CAA CT	CAT TCT CAA ATG ATC GAA CA	(CA)18	50
2	*Xgwm32-3A*	TAT GCC GAA TTT GTG GAC AA	TGC TTG GTC TTG AGC ATC AC	(GA)19	55
3	*Xgwm33-1A*	GGA GTC ACA CTT GTT TGT GCA	CAC TGC ACA CCT AAC TAC CTG C	(GA)19	60
4	*Xgwm47.1-2A*	TTG CTA CCA TGC ATG ACC AT	TTC ACC TCG ATT GAG GTC CT	(CT)7TT(CT)16	60
5	*Xgwm71.2-2A*	GGC AGA GCA GCG AGA CTC	CAA GTG GAG CAT TAG GTA CAC G	(GT)20	60
6	*Xgwm95-2A*	GAT CAA ACA CAC ACC CCT CC	AAT GCA AAG TGA AAA ACC CG	(AC)16	60
7	*Xgwm113-4B*	ATT CGA GGT TAG GAG GAA GAG G	GAG GGT CGG CCT ATA AGA CC	(GT)12	55
8	*Xgwm114-3B*	ACA AAC AGA AAA TCA AAA CCC G	ATC CAT CGC CAT TGG AGT G	(GA)53	60
9	*Xgwm130-7A*	AGC TCT GCT TCA CGA GGA AG	CTC CTC TTT ATA TCG CGT CCC	(GT)22	60
10	*Xgwm131-1B*	AAT CCC CAC CGA TTC TTC TC	AGT TCG TGG GTC TCT GAT GG	(CT)22	60
11	*Xgwm155-3A*	CAA TCA TTT CCC CCT CCC	AAT CAT TGG AAA TCC ATA TGC C	(CT)19	60
12	*Xgwm156-5A*	CCA ACC GTG CTA TTA GTC ATT C	CAA TGC AGG CCC TCC TAA C	(GT)14	60
13	*Xgwm157-2D*	GTC GTC GCG GTA AGC TTG	GAG TGA ACA CAC GAG GCT TG	(CT)14	60
14	*Xgwm159-5B*	GGG CCA ACA CTG GAA CAC	GCA GAA GCT TGT TGG TAG GC	(GT) 15	60
15	*Xgwm160-4A*	TTC AAT TCA GTC TTG GCT TGG	CTG CAG GAA AAA AAG TAC ACC C	(GA)21	60
16	*Xgwm161-3D*	GAT CGA GTG ATG GCA GAT GG	TGT GAA TTA CTT GGA CGT GG	(CT)15	60

Such a discriminatory set should also ensure the uniform distribution of the microsatellite primers of this set across the three genomes of bread wheat since in several studies, including the present study, microsatellites have been shown to be more frequent in the A and B genomes, than in the D genome **(Röder et al., 1998; Stephenson et al., 1998)**.

Table 3 Locus, expected product size (bp), products size range (bp), allele No., allele frequency, genotype No., gene diversity, heterozygosity, polymorphic information content (PIC) and marker index (MI) of 16 SSR markers assayed in 17 Egyptian bread wheat.

No.	Locus	Expected product size(bp)	Product sizes range (bp)	Allele No.	Allele fre-quency	Genotype No.	Gene diversity	Hetero-zygosity	PIC	MI
1	*Xgwm2-3A*	128	131-145	15	0.2059	15.0000	0.9048	1.0000	0.8979	0.8883
2	*Xgwm32-3A*	169	163-179	20	0.1176	17.0000	0.9377	1.0000	0.9342	0.9231
3	*Xgwm33-1A*	116	105-124	17	0.1471	16.0000	0.9187	1.0000	0.9131	0.8881
4	*Xgwm47.1-2A*	-	159-193	18	0.1176	17.0000	0.9291	1.0000	0.9246	0.9029
5	*Xgwm71.2-2A*	120	105-126	16	0.1765	17.0000	0.9048	0.9412	0.8974	0.8533
6	*Xgwm95-2A*	128	112-133	12	0.1471	14.0000	0.9014	1.0000	0.8929	0.8486
7	*Xgwm113-4B*	148	144-164	10	0.2353	13.0000	0.8685	0.9412	0.8553	0.8324
8	*Xgwm114-3B*	168	138-173	10	0.2059	13.0000	0.8720	1.0000	0.8589	0.8517
9	*Xgwm130-7A*	126	118-133	10	0.1765	14.0000	0.8772	1.0000	0.8645	0.8470
10	*Xgwm131-1B*	165	142-173	9	0.2059	12.0000	0.8547	1.0000	0.8377	0.8299
11	*Xgwm155-3A*	143	124-149	9	0.2059	12.0000	0.8547	1.0000	0.8377	0.8464
12	*Xgwm156-5A*	300	270-299	8	0.2059	10.0000	0.8443	1.0000	0.8251	0.8338
13	*Xgwm157-2D*	106	107-113	8	0.2647	10.0000	0.8339	1.0000	0.8137	0.8198
14	*Xgwm159-5B*	189	177-198	9	0.2059	13.0000	0.8581	1.0000	0.8423	0.8408
15	*Xgwm160-4A*	184	182-200	9	0.2059	12.0000	0.8685	1.0000	0.8543	0.8400
16	*Xgwm161-3D*	154	141-159	10	0.3235	13.0000	0.8356	0.8235	0.8202	0.8019
Mean				11.875	0.1967	13.6250	0.8790	0.9816	0.8669	0.8530

The SSRs relative with them, more alleles detected at (CA) n and (AC) n one loci each, however, (GA) n loci 4 loci, (CT) n and (GT) n loci5 loci each. **Röder et al. (1995)** reported that (GT) n repeats to be more polymorphic than other simple repeats such as (GA) n in wheat. However, in barley more alleles were detected for (GA) n repeats than for (GT) n repeats **(Struss and Plieske 1998)**. The results were obtained agreement with **(Dreisigacker et al., 2004; Dvojkovic et al., 2010; Spanic et al., 2012)** and opposed with **Kumar et al. (2016)**. The loci of microsatellite are multi-allelic and the alleles co-dominant, proved that the microsatellite marker strict in determined DNA polymorphism and highly informative genetic markers **(Devos et al., 1992; Devos et al., 1993; Xie et al., 1993; Mason, 2015)** than other markers as single nucleotide polymorphisms (SNPs) which are biallelic and dominant. The earlier studies proved that four microsatellite markers set each had the ability to distinguish between 24 genotypes in barley and 16 genotypes in tomato **(Rusell et al., 1997; Bredemeijer et al., 1998)**. Furthermore, the microsatellite set could be used to distinguish each genotype in a set of more than 100 wheat genotypes **(Manifesto et al., 1999)**.

Genetic diversity

Polymorphism Information Content (PIC) was estimated for 16 loci (Table 2). The PIC values ranged from 0.8137 of locus Xgwm157-2D to 0.9342 of locus Xgwm32-3A with an average of 0.8669. As well as, the heterozygosity values were estimated as well, ranging from 0.8235 to 1.00 with average 0.9816. The marker index (MI) value over all 16 microsatellite markers was 0.8530. The heterozygosity was detected in three loci (Xgwm71.2-2A, Xgwm113-4B and Xgwm161-3D) with values 0.9412, 0.9412 and 0.8235 respectively, (Table 3). The genotype number per locus was estimated based on allelic frequency data, where the highest number detected 17 of locus Xgwm32-3A and lowest number founded 10 of locus Xgwm156-5A and Xgwm157-2D with an average 13.6250 (Table 2). The data of microsatellite loci and the corresponding alleles were used to calculate the polymorphic information content (PIC) and heterozygosity (H) to evaluate a marker system for its ability to detect high levels of DNA polymorphism in an analysis of genetic diversity. In earlier studies on bread wheat, the PIC values were ranged from 0.23 to 0.79 **(Röder et al., 1995)** and from 0.29 to 0.79 **(Plaschke et al., 1995)** and 0.21 to 0.90 **(Prasad et al., 2000)**. On the other hand, the PIC values obtained by **(Bohn et al., 1999)** were very low (0.30). However, **(Saeidi et al., 2006; Khalighi et al., 2008; Tahernezhad et al., 2010; Mir et al., 2012; Arora et al., 2014)** studies were the opposite of the previous results and consistent with our results where PIC value > (0.7). The marker index is also used to measure the efficiency of polymorphism **Khodadadi et al., (2011)**.

Table 4a The matrix of Jaccard's similarity to 17 Egyptian bread wheat is indicated in Table 1.

	Misr1	Misr2	Gim7	Gim9	Gim10	Gim11	Gim12	Giza168	Sids8
Misr 1	1.000								
Misr 2	0.409	1.000							
Gimmasa 7	0.391	0.348	1.000						
Gimmasa 9	0.391	0.292	0.524	1.000					
Gimmasa 10	0.240	0.429	0.348	0.476	1.000				
Gimmasa 11	0.333	0.348	0.333	0.524	0.476	1.000			
Gimmasa 12	0.391	0.476	0.391	0.333	0.240	0.391	1.000		
Giza 168	0.138	0.185	0.138	0.222	0.231	0.320	0.320	1.000	
Sids 8	0.154	0.261	0.200	0.250	0.261	0.304	0.364	0.192	1.000
Sids 12	0.222	0.231	0.320	0.269	0.231	0.500	0.500	0.308	0.348
Sids 13	0.103	0.148	0.185	0.231	0.240	0.333	0.280	0.320	0.500
Sakha 93	0.143	0.148	0.103	0.231	0.148	0.333	0.280	0.222	0.429
Sakha 94	0.269	0.185	0.222	0.179	0.103	0.222	0.222	0.133	0.292
Sakha 69	0.103	0.033	0.185	0.231	0.069	0.143	0.067	0.138	0.154
Shandawel1	0.103	0.069	0.143	0.103	0.107	0.103	0.067	0.138	0.111
Bani Sweef3	0.065	0.000	0.138	0.138	0.032	0.065	0.065	0.214	0.107
Bani sweef4	0.032	0.033	0.067	0.067	0.069	0.032	0.032	0.031	0.071

Table 4b The matrix of Jaccard's similarity to 17 Egyptian bread wheat is indicated in Table 1.

	Sids12	Sids13	Sakha93	Sakha94	Sakha69	Shandawel1	Sweef3	Sweef4
Sids 12	1.000							
Sids 13	0.435	1.000						
Sakha 93	0.435	0.391	1.000					
Sakha 94	0.308	0.375	0.320	1.000				
Sakha 69	0.269	0.280	0.280	0.375	1.000			
Shandawel1	0.100	0.103	0.103	0.179	0.143	1.000		
Bani Sweef3	0.172	0.222	0.138	0.259	0.320	0.269	1.000	
Bani sweef4	0.065	0.103	0.143	0.138	0.185	0.333	0.375	1.000

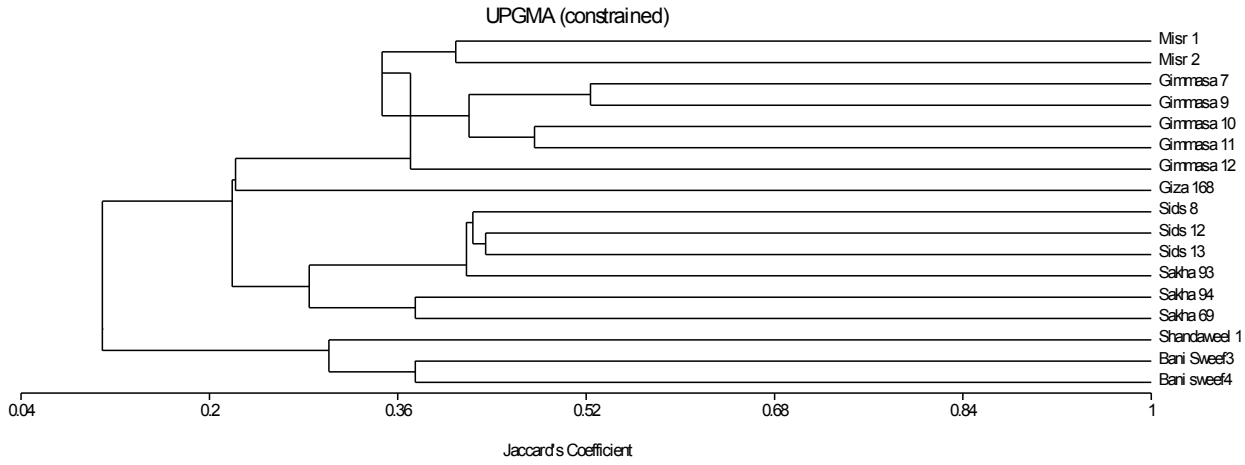

Figure 1 Dendrogram of 17 Egyptian bread wheat based on data on allelic profiles generated using 16 microsatellite primer pairs.

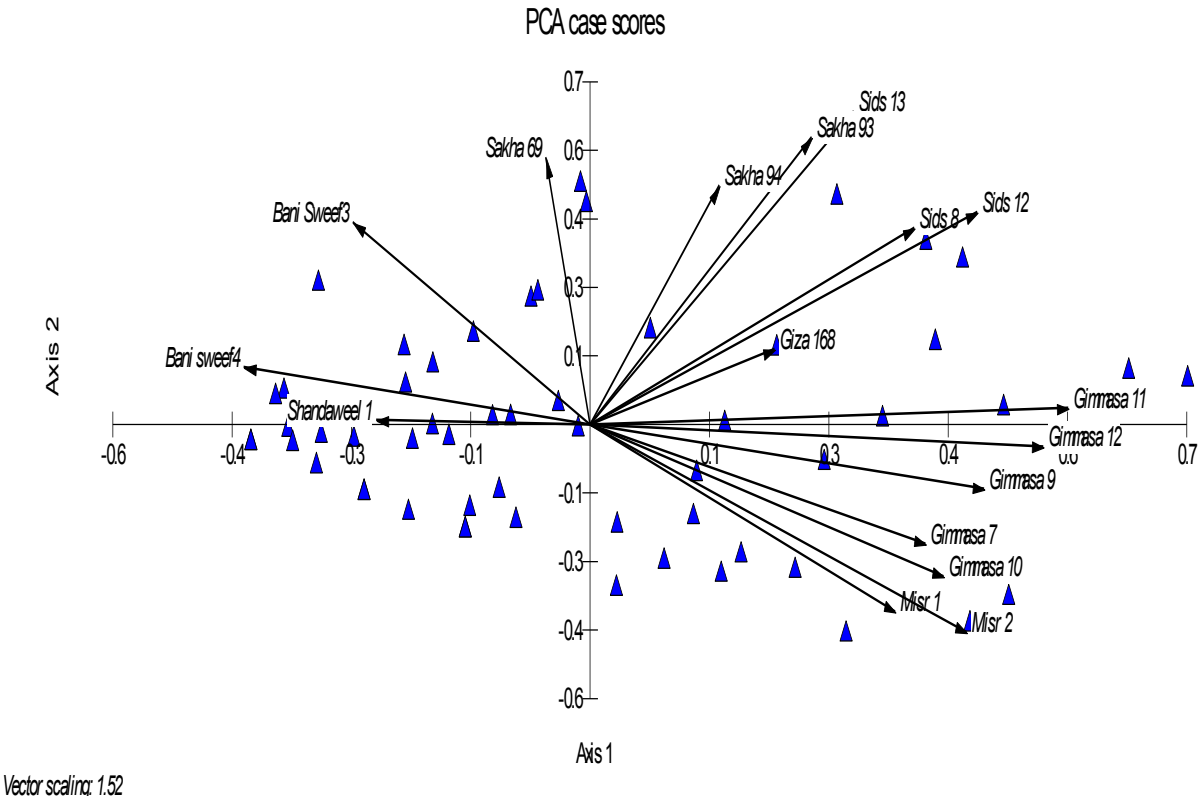

Figure 2 Matrix plot among 17 Egyptian bread wheat cultivars revealed by principle component analysis based on SSR data.

The marker index (MI) value was calculated also for used markers and was close to (0.8530) the MI value (0.70) reported by **(Prasad et al., 2000)**, however those results were higher than the MI value (0.21) obtained by **(Bohn et al., 1999)** on SSRs wheat. When comparing the MI value with other markers found that, SAMPL (9.61) and AFLP (3.41) in bread wheat **(Bohn et al., 1999)**. However, MI value (6.14) that is intermediate between the above two contrasts was also available for AFLPs in soybean **(Powell et al., 1996)**.

Genetic similarity

In order to investigate, genetic relationships between 17 Egyptian wheat genotypes cluster analysis based on Jaccard's similarity coefficients and UPGMA algorithm were calculated for the 43 durum wheat germplasm. A Jaccard's genetic similarity matrix is presented in Table 4a and 4b. The average similarity among 17 Egyptian bread wheat was 0.357. The nearest neighbor cluster analysis obtained from Jaccard's similarity coefficient (Figure 1) illustrated the variability between 17 Egyptian bread wheat. The detected of DNA polymorphism by 16 microsatellite markers allowed of estimates genetic distance and clustering of 17 Egyptian bread wheat cultivars in two major groups. The first group (Group I) included Misr 1, Misr 2, Gimmasa 7, Gimmasa 7, Gimmasa 9, Gimmasa 10, Gimmasa 11, Gimmasa 12 and Giza 168. The second group (Group II) included the other cultivars Sids 8, Sids 12, Sids 13, Sakha 93, Sakha 94, Sakha 69, Shandaweel 1, Bani Sweef 3 and Bani Sweef 4. The similarities between 17 Egyptian bread wheat based on 16 microsatellite markers were ranged from 0.109 in Shandweel 1, Bani Sweef 3 and Bani Sweef 4 cultivars to 0.524 in Gimmaza 7 and Gimmaza 9 with average of 0.357. A Jaccard's genetic similarity matrix was estimated between pairs of Egyptian wheat cultivars using 16 microsatellite markers through cluster analysis. This study used UPGMA cluster analysis based on genetic similarity values for SSR alleles from all the wheat cultivars to construct a dendrogram (Figure 1). The similarity value between 17 Egyptian wheat cultivars was ranged from 0.109 to 0.524 with average value 0.357. This average of genetic similarity value (0.357) can be compared with other studies, whereas SSR-based genetic similarity coefficient values of 0.31 **(Plaschke et al., 1995)** and 0.57 **(Bohn et al., 1999)**. However, STS-based genetic similarity coefficient value of 0.81 **(Chen et al., 1994)** were reported. In these different studies on genetic diversity in bread wheat, undertaken using a variety of molecular markers, the variation in genetic similarity coefficient values may be attributed either to the differences in number of genotypes and the probes/primers used (e.g. 119 RFLP probes were used by **Paull et al. 1998**) or to the relative superiority of microsatellites to detect DNA polymorphism. An unusually low value of RFLP-based genetic similarity (0.18) reported by **Paull et al. (1998)** is certainly due to the larger sample of 124 diverse genotypes and bigger set of 119 RFLP probes used in this study. From the previous study it illustrated the importance and usefulness of the microsatellites (SSR) technique in wheat **(Prasad et al., 1999; Roy et al., 1999)**. Based on the results obtained are considered the

microsatellites technique was sensitive and critical in differentiating between the different varieties of Egyptian wheat under study, as well as in determining the polymorphism, genotype identification, genetic similarity and estimation of genetic diversity.

The principle component analysis was used to visualize the genetic relationships among genotypes shown in Figure 2. Of the total polymorphism, only 40.44% was accounted for by the first two components, implying that the used markers possessed a suitable dispersion of markers in the genome. The 17 Egyptian wheat cultivars were clustered into two groups. The principle component analysis thus is largely compatible to those from cluster analysis obtained from UPGMA.

CONCLUSION

Microsatellites or SSR markers are one of the most common genetic markers and used in many genetic applications. Microsatellites are codominant, highly polymorphic, and Mendelian inherited, all these qualities made it very suitable for such study and the ability to accurately identify differences between wheat varieties in this study. The species specific markers identified would be utilized in future introgression breeding programs.

REFERENCES

Anderson, J. A., Ogihara, Y., Sorrells, M. E., Tanksley, S. D. 1992. Development of a chromosomal arm map for wheat based on RFPL markers. *Theoretical Applied Genetics*., vol. 83, no. 8, p. 1035-1043. https://doi.org/10.1007/BF00232969

Arora, A., Kundu, S., Dilbaghi, N., Sharma, I., Tiwari, R. 2014. Population structure and genetic diversity among Indian wheat varieties using microsatellite (SSR) markers. *Australian Journal of Crop Science*, vol. 8, no. 9, p. 1281-1289. Available at: http://www.cropj.com/tiwari_8_9_2014_1281_1289.pdf

Bered, F., Terra, T. F., Spellmeier, M., Neto, J. F. B. 2005. Genetic variation among and within sweet corn populations detected by RAPD and SSR markers. *Crop Breeding and Applied Biotechnology*, vol. 5, no. 4, p. 418-425. https://doi.org/10.12702/1984-7033.v05n04a07

Bohn, M., Utz, H. E., Melchinger, A. 1999. Genetic Similarities among Winter Wheat Cultivars Determined on the Basis of RFLPs, AFLPs, and SSRs and Their Use for Predicting Progeny Variance. *Crop Science*, vol. 39, no. 1, p. 228-237. https://doi.org/10.2135/cropsci1999.0011183X003900010035xb

Bredemeijer, G. M. M., Arens, P., Wouters, D., Visser, D., Vosman, B. 1998. The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. *Theoretical Applied Genetics*, vol. 97, no. 4, p. 584-590. https://doi.org/10.1007/s001220050934

Chen, H. B., Martin, J. M., Lavin, M., Talbert, L. E. 1994. Genetic diversity in hard red spring wheat based on sequence tagged site PCR markers. *Crop Science*, vol 34, no. 6, p. 1628-1632. https://doi.org/10.2135/cropsci1994.0011183X003400060037x

Coombs, J. J., Frank, L. M., Souches, D. S. 2004. An applied fingerprinting system for cultivated potato using simple sequence repeats. *American Journal of Potato Research*, vol. 81, no. 4, p. 243-250. https://doi.org/10.1007/BF02871765

Devos, K. M., Atkinson, M. D., Chinoy, C. N., Liu, C. J., Gale, M. D. 1992. RFLP based genetic map of the homoeologous group 3 chromosomes of wheat and rye. *Theoretical Applied Genetics*, vol. 83, no. 8, p. 931-939. https://doi.org/10.1007/BF00232953

Devos, K. M., Millan, T., Gale, M. D. 1993. Comparative RFLP maps of the homoeologous group-2 chromosomes of wheat, rye and barley. *Theoretical Applied Genetics*, vol. 85, no. 6-7, p. 784-792. https://doi.org/10.1007/BF00225020

Dreisigacker, S., Zhang, P., Warburton, M. L., Van Ginkel, M., Hoisington, D., Bohn, M., Melchinger, A. E. 2004. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different mega environments. *Crop Science*, vol. 44, no. 2, p. 381-388. https://doi.org/10.2135/cropsci2004.0381

Dvojkovic, K., Šatovic, Z., Drezner, G., Somers, D. J., Lalic, A., Novoselovic, D., Horvat, D., Maric, S. Šarcevic, H. 2010. Allelic variability of Croatian wheat cultivars at the microsatellite locus Xgwm261. *Poljoprivreda*, vol. 16, p. 32-37.

FAOSTAT. 2008. FAO Statistical Databases. Food and agriculture organization of the United Nations, Statistics Division. Available at: http://faostat.fao.org/site/339/default.aspx

Garcia, A. F., Alberini, J. L., Zucchi, M. I., De Souza, A. P. 2007. Microsatellite molecular markers in the cultivar identification of Brazilian soybean for human consumption. *Crop Breeding and Applied Biotechnology*, vol.7, p. 155-164. https://doi.org/10.12702/1984-7033.v07n02a07

Gupta, P. K., Varshney, R. K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, vol. 113, no. 3, p. 163-185. https://doi.org/10.1023/A:1003910819967

Gupta, P. K., Varshney, R. K., Sharma, P. C., Ramesh, B. 1999. Review Molecular markers and their applications in wheat breeding. *Plant Breeding*, vol. 118, no. 5, p. 369-390. https://doi.org/10.1046/j.1439-0523.1999.00401.x

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale (New research on floral distribution). *Bulletin de la Societé Vaudoise des Sciences Naturelles*, vol. 44, p. 223-270. https://doi.org/10.5169/seals-268684. (In French)

Khalighi, M., Arzani, A., Poursiahbidi, M. A. 2008. Assessment of genetic diversity in *Triticum* spp. and *Aegilops* spp. using AFLP markers. *African Journal of Biotechnology*, vol. 7, p. 552-546.

Khodadadi, M., Fotokian, M. H., Miransari, M. 2011. Genetic diversity of wheat (*Triticum* aestivum L.) genotypes based on cluster and principal component analyses for breeding strategies. *Australian Journal of Crop Sciences*, vol. 5, no. 1, no.1, p. 17-2. Available at: http://www.cropj.com/khodadadi_5_1_2011_17_24.pdf

Kumar, S., Kumar, V., Kumari P., Kirti, Singh, A. K. Singh, R. 2016. DNA fingerprinting and genetic diversity studies in wheat genotypes using SSR markers. *Journal of Environmental Biology*, vol. 37, p. 319-326.

Litt, M., Lutty, J. A. 1989. A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics*, vol. 44, no. 3, p. 397-401.

Liu, K., Muse, S. V. 2005. PowerMarker: Integrated analysis environment for genetic marker data. *Bioinformatics*, vol. 21, no. 9, p. 2128-2129. https://doi.org/10.1093/bioinformatics/bti282

Manifesto, M. M., Schlatter, A. R., Hopp, H. E., Suarez, E. Y., Dubcovsky, J. 1999. Bread wheat (*Triticum* aestivum)

fingerprinting using microsatellites. Plant Anim Genome 7th Conf., p.371. Available at: http://www.intl-pag.org/

Mason, A. S. 2015. SSR Genotyping. Plant Genotyping. In: Batley J. *Plant Genotyping. Methods in Molecular Biology (Methods and Protocols).* Humana Press, New York, NY, p 77-89. https://doi.org/10.1007/978-1-4939-1966-6_6

Mir, R. R., Kumar, J., Balyan, H. S. Gupta, P. K. 2012. A study of genetic diversity among Indian bread wheat (*Triticum aestivum* L.) cultivars released during last 100 years. *Genetic Resources and Crop Evolution*, vol. 59, no. 5, p. 717-726. https://doi.org/10.1007/s10722-011-9713-6

Morgante, M., Olivieri, A. M. 1993. PCR-amplified microsatellites as markers in plant genetics. *The Plant Journal*, vol. 3, no. 1, p. 175-182. https://doi.org/10.1111/j.1365-313X.1993.tb00020.x

Paull, J. G., Chalmers, K. J., Karakousis, A., Kretschmer, J. M., Manning, S., Langridge, P. 1998. Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. *Theoretical Applied Genetics*, vol. 96, no. 3-4, p. 435-446. https://doi.org/10.1007/s001220050760

Pavel, A. B., Vasile, C. I. 2012. PyElph – a software tool for gel images analysis and phylogenetics. *BMC Bioinformatics*, vol. 13, no. 9, p. 13-19. https://doi.org/10.1186/1471-2105-13-9

Plaschke, J., Ganal, M. W., Röder, M. S. 1995. Detection of genetic diversity in closely related bread wheat using microsatellite markers. *Theoretical Applied Genetics*, vol. 91, no. 6-7, p. 1001-1007. https://doi.org/10.1007/BF00223912

Powell, W., Morgante, M., Andre, C., Hanafey, M., Vogel, J., Tingey, S., Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding*, vol. 2, no. 3, p. 225-238. https://doi.org/10.1007/BF00564200

Prasad, M., Varshney, R. K., Kumar, A., Balyan, H. S., Sharma, P. C., Edwards, K. J., Singh, H., Dhaliwal, H. S., Roy, J. K., Gupta, P. K. 1999. A microsatellite marker associated with a QTL for grain protein content on chromosome arm 2DL of bread wheat. *Theoretical Applied Genetics*, vol. 99, no. 1-2, p. 341-345. https://doi.org/10.1007/s001220051242

Prasad, M., Varshney, R. K., Roy, J. K., Balyan, H. S., Gupta, P. K. 2000. The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. *Theoretical Applied Genetics*, vol. 100, no. 3, p. 584-592. https://doi.org/10.1007/s001220050077

Ramanatha, V. R., Hodgkin, T. 2002. Genetic diversity and conservation and utilization of plant genetic resources. *Plant Cell Tissue Organ Culture*, vol. 68, no. 1, p. 1-19. https://doi.org/10.1023/A:1013359015812

Röder, M. S., Plaschke, J., Konig, S. U., Borner, A., Sorrells, M. E., Tanksley, S. D., Ganal, M. W. 1995. Abundance, variability and chromosomal location of microsatellites in wheat. *Molecular and General Genetics*, vol. 246, no. 3, p. 327-333. https://doi.org/10.1007/BF00288605

Röder, M. S., Korzun, V., Wendehake, K., Plaschke, J., Tixier, M. H., Leroy, P., Ganal, M. W. 1998. A microsatellite map of wheat. *Genetics*, vol. 149, no. 4, p. 2007-2023. https://doi.org/10.1007/BF00279889

Rongwen, J., Akkaya, M. S., Bhagwat, A. A., Lavi, U., Cregan, P. B. 1995. The use of microsatellite DNA markers for soybean genotype identification. *Theoretical and Applied Genetics*, vol. 90, no. 1, p. 43-48. https://doi.org/10.1007/BF00220994

Roy, J. K., Prasad, M., Varshney, R. K., Balyan, H. S., Blake, T. K., Dhaliwal, H. S., Singh, H., Edwards, K. J., Gupta, P. K. 1999. Identification of a microsatellite on chromosome 6B and a STS on 7D of bread wheat showing association with preharvest sprouting tolerance. *Theoretical Applied Genetics*, vol. 99, no. 1-2, p. 336-340. https://doi.org/10.1007/s001220051241

Russell, J., Fuller, J., Young, G., Thomas, B., Taramino, G., Macaulay, M., Waugh, R., Powell, W. 1997. Discriminating between barley genotypes using microsatellite markers. *Genome*, vol. 40, no. 4, p. 442-450. https://doi.org/10.1139/g97-059

Saeidi, H., Rahiminejad, M. R., Vallian, S., Heslop-Harrison, J. S. 2006. Biodiversity of diploid D-genome Aegilops tauschii Coss. in Iran measured using microsatellites. *Genetic Resources and Crop Evolution*, vol. 53, p. 1477–1484. https://doi.org/10.1007/s10722-005-7110-8

Spanic, V., Buerstmayr, H. Derezner, G. 2012. Assessment of genetic diversity of wheat genotypes using microsatellite markers. *Periodicum Biologorum*, vol. 114, no. 1, p. 37-42.

Stephenson, P., Bryan, G., Kirby, J., Collins, A., Devos, K., Busso, C., Gale, M. 1998. Fifty new microsatellite loci for the wheat genetic map. *Theoretical Applied Genetics*, vol. 97, no. 5-6, p. 946-949. https://doi.org/10.1007/s001220050975

Struss, D., Plieske, J. 1998. The use of microsatellite markers for detection of genetic diversity in barley populations. *Theoretical Applied Genetics*, vol. 97, no. 1-2, p. 308-315. https://doi.org/10.1007/s001220050900

Tahernezhad, Z., Zamani, M. J., Solouki, M., Zahravi, M., Imamjomeh, A. A., Jafaraghaei, M., Bihamta, M. R. 2010. Genetic diversity of Iranian Aegilops tauschii Coss. using microsatellite molecular markers and morphological traits. *Molecular Biology Reports*, vol. 37, no. 7, p. 3413-3420. https://doi.org/10.1007/s11033-009-9931-6

Thomason, M. J., Septiningsih, E. M., Suwardjo, F., Santoso, T. J., Silitonga, T. S., McCouch, S. R. 2007. Genetic diversity analysis of traditional and improved Indonesian rice (*Oryza sativa* L.) germplasm using microsatellite markers. *Theoretical Applied Genetics*, vol. 114, no. 3, p. 559-568. https://doi.org/10.1007/s00122-006-0457-1

Varshney, R. K., Prasad, M., Roy, J. K., Kumar, N., Harjit-Singh, Dhaliwal, H. S., Balyan, H. S., Gupta, P. K. 2000. Identification of eight chromosomes and a microsatellite marker on 1AS associated with QTLs for grain weight in bread wheat. *Theoretical Applied Genetics*, vol. 100, no. 8, p. 1290-1294. https://doi.org/10.1007/s00122005143

Vivodík, M., Gálová, Z., Balážová, Z., Petrovičová, L. 2016. Start codon targeted (SCOT) polymorphism reveals genetic diversity in European old maize (*Zea mays* L.) genotypes. *Potravinarstvo Slovak Journal of Food Sciences*, vol. 10, no. 1, p. 563-569. https://doi.org/10.5219/660

Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G., Lathrop, M. 1992. A second-generation linkage linkage map of the human genome. *Nature*, vol. 359, p. 794-801. https://doi.org/10.1038/359794a0

Xie, D. X., Devos, K. M., Moore, G., Gale, M. D. 1993. RFLP-based genetic maps of the homoeologous group 5 chromosomes of bread wheat (*Triticum aestivum* L.). *Theoretical Applied Genetics*, vol. 87, no. 1-2, p. 70-74. https://doi.org/10.1007/BF00223747

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Contact address:
*Ayman El-Fiki, National Centre for Radiation Research and Technology, Department of Natural products, Biotechnology Div., Atomic Energy Authority Egypt, Ahmed El-Zomur St., P.O.Box: 29, Nasr City, Cairo, Egypt, Tel.: (+202) 22746791, E-mail: aymana.elfiki@eaea.org.eg

Mohamed Adly, National Centre for Radiation Research and Technology, Department of Natural products, Biotechnology Div., Atomic Energy Authority Egypt, Ahmed El-Zomur St., P.O.Box: 29, Nasr City, Cairo, Egypt, Tel.: (+202) 22746791, E-mail: adly4000@yahoo.com

Corresponding author: *