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Assessing Wheat (*Triticum aestivum* L.) Genetic Diversity Using Morphological Characters and Microsatallite Markers

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Abstract: Genetic diversity of the seven wheat (*Triticum aestivum* L.) varieties was evaluated at the DNA level using 48 simple sequence repeats (SSR) alleles and 9 morphological characters. The wheat microsatellite markers (WMS) used determined 15 loci located on fifteen chromosomes and were capable of detecting 48 alleles with an average of 3.2 alleles per locus. The number of alleles per locus ranged from 2 to 7 and the allelic polymorphism information content (PIC) value ranged from 0.278 for the *Xgwm95* to 0.816 for the *Xgwm437* with an average of 0.548. The results revealed that the genotypes differed for morphological characters and SSR markers. The average genetic diversity based on morphological characters (23.49 with a range of 8.51-38.46) was higher than SSR markers (0.53 with a range of 0.42-0.63). Our results suggested that the classification based on morphological characters and genotypic markers of these wheat genotypes will be useful for wheat breeders to plan crosses for positive traits. The results obtained suggested that the wheat microsatellite primers can be used to distinguish all genotypes used and to estimate their genetic diversity. Genetic dissimilarity values between genotypes, calculated by the WMS derived data, were used to produce a dendrogram. The diversity within the analysed varieties is discussed. Hence, the identification of the genetic diversity between varieties should be a good tool of selecting these varieties in breeding programs. As a result of this study, genetically diverse parents can be identified, increasing the usefulness of varieties collections by broadening the genetic base of wheat varieties. The present study also indicates that microsatellite markers permit the fast and high throughout fingerprinting of accessions from a varieties collection in order to assess genetic diversity.

Key words: Wheat (*Triticum aestivum* L.) · Molecular markers · Microsatellite markers · Morphological characters · Genetic diversity

INTRODUCTION

Wheat, a self-pollinating crop, 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. Knowledge of genetic diversity in a crop species is fundamental to its improvement. Evaluation of genetic diversity levels among adapted, elite germplasm can provide predictive estimates of genetic variation among segregating progeny for pure-line cultivar development [1].

Criteria for the estimation of genetic diversity can be different: pedigree records, morphological traits or molecular markers [2]. The use of molecular markers for the evaluation of genetic diversity is receiving much attention. Many wheat scientists have studied genetic diversity in common wheat using different molecular markers [3-10] However, most of these marker systems show a low level of polymorphism in wheat, especially among cultivated lines and/or cultivars [11].

Because SSRs are multiallelic, they have high potential for use in evolutionary studies [12-16] and studies regarding genetic diversity and relationships. At present, microsatellites are one of the most promising molecular-marker types able to identify or differentiate genotypes within a species. Their co-dominant inheritance, high level of polymorphism and

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easy handling make them extremely useful for many different applications [17-20]. The objectives of this study were to (*i*) use SSRs and morphological characters to assess levels and patterns of genetic variability among a representative sample of wheat genotypes, (*ii*) compare these genetic diversity estimates with other international wheat cultivars, (iii) examine the genetic factors that affected microsatellite diversity and (iv) use wheat microsatellite markers for the characterization and assessment of the genetic diversity of seven wheat varieties.

MATERIALS AND METHODS

Plant Material: This investigation was carried out at the experimental farm of the Faculty of Agriculture, Shibin El-Kom, Menoufia University, Egypt, during the two wheat successive growing seasons, 2005/2006 and 2006/2007. Five wheat varieties from Agriculture Research Center (ARC), Giza, Egypt and two exotic varieties were used to establish the experimental materials for this investigation. All wheat varieties, along with their pedigree (if known) and country of origin, are listed in Table 1.

Table 1: Varieties name, country of origin and pedigree for the wheat varieties used in this study

No.	Varieties name	Origin	Pedigree
1	Sakha 69	Egypt	Inia-RL4220 x 7C/yr'S' CM1540-25.65.0S
2	Sakha 93	Egypt	Sakha 92/TR 810328
3	Gemmiza 3	Egypt	Bb/7C*2//Y50/Kal*3//Sakha8/4/Prv/WW/ 5/3/Bg"s"//On
4	Gemmiza 7	Egypt	CMH74 A. 630/5x//Seri 82/3/Agent
5	Seds 4	Egypt	Maya" S"/Mon" S"/CM1174.A592/3/ Giza157*
6	Baviacora	Europe	-------
7	Miriam	Europe	-------

Table 2: Description of 15 wheat microsatellites markers, their chromosomal location and motif

Microsatellite marker name	Chromosome location	Motif
Xtaglgap	1B	(CAA)31
Xgwm18	1B	(CA)17GA(TA)4
Xgwm458	1D	(CA)13
Xgwm95	2A	(AC)16
Xgwm155	3A	(CT)19
Xgwm389	3B	(CT)14(GT)16
Xgwm3	3D	(CA)18
Xgwm165	*4A*	(GA)20
Xgwm513	4B	(CA)12
Xgwm186	5A	(GA)26
Xgwm408	5B	(CA)>22(TA)(CA)7(TA)9
Xgwm190	5D	(CT)22
Xgwm631	7A	(GT)23
Xgwm46	7B	(GA)2GC(GA)33
Xgwm437	7D	(CT)24

Evaluation of Morphological Characters: Plants were selected at random for 9 morphological characters measurements as follows: flowering time (days), plant height (cm), no. of tillers per plant, ear length (cm), no. of ears per plant, no. of spikelets per ear, no. of grain per ear, grain yield per ear and grain yield per plant.

DNA Isolation: Total genomic DNA was extracted from leaf tissue per each variety. Young leaves from eight-weeks-old plants were cut as tissue samples for DNA extraction. DNA was isolated from these genotypes as described by Plaschke *et al.* [18]

Microsatellite Markers Analysis: Fifteen wheat microsatellite markers for fifteen loci representing at least one microsatellite marker from chromosomes (1B, 1D, 2A, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 7A, 7B and 7D) (Table 2) were selected for genotyping [19]. The primer sequence of *Xtaglgap* was described by Devos *et al*. [17]. All Gatersleben Wheat Microsatellites (*Xgwm*) used were dinucleotide repeats, whereas *Xtaglgap* has a trinucleotide motif. Microsatellite amplifications were carried out as reported by Röder *et al*. [19]. Polymerase chain reaction and fragment analysis were performed according to Devos *et al*. [17] and Röder *et al*. [19] respectively. GWM designation, chromosomal location, motif and fragment size location in 'CS' (bp) of the amplified loci were reported by Röder *et al*. [19].

Data Collection and Diversity Analysis

Marker Polymorphism: To measure the informativeness of the *gwm* markers, the polymorphism information content (PIC) for each *gwm* was calculated according to the formula: $PIC = 1 - \sum_{i=1}^{k} P_i^2$, where k is the total number of alleles detected for a locus of a marker and P_i the frequency of the *i*th allele in the set of 7 varieties investigated.

Genetic Similarity Estimation and Cluster Analysis: Each SSR band was scored as present (1) and absent (0) for the different varieties. Genetic similarity (gs) between two varieties i and j was estimated following [21] by the formula: gsij = 2Nij/(Ni+Nj), where Nij is the number of

bands present in varieties i and j, Ni (resp. Nj) is the number of bands present in variety I (resp. j). Markers with missing observations for variety i and/or j not included in the calculation of gsij. Based on the genetic similarity matrix (denoted GS), Unweighted Pair Group Method of Arithmetic Average (UPGMA) i.e. cluster analysis were used to assess pattern of diversity among the wheat varieties. All calculations were performed using the NTSYS-pc version 2.1 software [22].

Statistical Analysis: The presence or absence of each single fragment amplified by microsatellite primers was coded by 1 or 0, respectively and scored for a binary data matrix. The genetic similarities (GS) were calculated for each pair of lines using the Dice similarity index [23].

Anderson *et al*. [24] referred to gene diversity as the polymorphic information content (PIC). The repeat number of the main allele was calculated according to the repeat number of the allele in 'CS' and by comparing the fragment sizes between 'CS' and the main allele of the different wheat varieties used in this concern..

RESULTS AND DISCUSSION

Microsatellite Polymorphism: Fifteen microsatellite markers for 15 loci were used to characterize and evaluate the genetic diversity of seven wheat genotypes. A total of 48 alleles were detected. The number of alleles per locus ranged from two for *Xgwm18, Xgwm95, Xgwm155, Xgwm3, Xgwm186 and Xgwm408* to 7 for *Xgwm437* with an average number of 3.2 alleles per locus (Table 3). The largest number of alleles per locus occurred in the B genome which is accounted to be 18, compared to 15 for genomes A and D (Table 3).

The seven wheat genotypes of diverse origin were evaluated using 15 microsatellites. These microsatellites were selected on the basis of their known genetic locations to give a uniform coverage for all three wheat genomes (A, B and D) and a total of 48 polymorphic alleles were detected at 15 loci (Table 3). A wide range of allelic variants was observed for each locus (Table 3). The number of alleles per locus ranged from 2 to 7, with an average of 3.2. The maximum number of alleles was observed at *Xgwm437* and their size ranged from 91 to 123 bp. A similar pattern of allelic variation was also detected at other loci. The PIC values ranged from 0.278 for the *Xgwm95* locus to 0.816 for *Xgwm437* (Table 3).

An average of 3.2 alleles per locus was detected for the seven wheat varieties. This level of polymorphism is lower than the average of 10 alleles per locus reported by Fahima *et al*. [25] using wild emmer wheat accessions. This higher genetic variation in wild wheats could be attributed to the considerable amount of natural out crossing that occurs in these genotypes Tsegaye [26], also the landraces which are selected from local germplasm have a wide rang of diversity, however, cultivars which are product of repeated inbreeding would have lower genetic diversity than both of wild genotypes or landraces. Furthermore, the detected genetic diversity for the seven wheat varieties is also lower than that reported by Plaschke *et al*. [18] studying closely related European wheat cultivars having an average of 6.2 alleles per locus.

Table 3: Wheat microsatellite marker name, chromosomal location, no. of alleles, fragment in Chinese spring variety (CS) by bp, range of allele size (bp) and gene diversity for the microsatellite markers used in this study

Wheat microsatellite marker name	Chromo-somal location	No. of alleles	Frag-ment size in CS (bp)	Range of allele size (bp)	Gene diversity
Xtaglgap	1B	3	282	219-266	0.625
Xgwm18	1B	2	182	188-192	0.489
Xgwm458	1D	3	112	112-122	0.529
Xgwm95	2A	2	122	109-131	0.277
Xgwm155	3A	2	143	145-147	0.444
Xgwm389	3B	4	131	119-136	0.617
Xgwm3	3D	2	80	77-84	0.408
Xgwm165	4A	6	----	187-202	0.778
Xgwm513	4B	3	144	145-149	0.620
Xgwm186	5A	2	135	122-130	0.408
Xgwm408	5B	2	180	180-184	0.494
Xgwm190	5D	3	----	204-212	0.612
Xgwm631	7A	3	196	190-202	0.406
Xgwm46	7B	4	180	147-187	0.700
Xgwm437	7D	7	108	91-123	0.816
Total		48			8.220
Mean		3.2			0.548

Table 4: Similarity matrix for the seven bread wheat varieties based on their microsatellite markers

Variety	Gemmiza 3	Gemmiza 7	Seds 4	Sakha 69	Sakha 93	Mariam	Baviacora
Gemmiza 3	----------						
Gemmiza 7	0.375	---------					
Seds 4	0.325	0.478	--------				
Sakha 69	0.540	0.395	0.390	---------			
Sakha 93	0.562	0.443	0.406	0.688	-------		
Mariam	0.434	0.612	0.481	0.416	0.405	---------	
Baviacora	0.412	0.472	0.603	0.422	0.408	0.477	----------

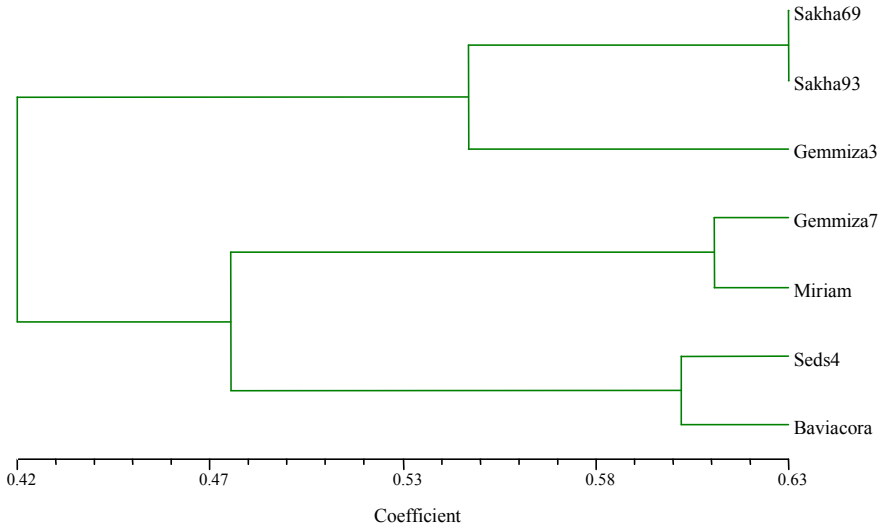

Fig. 1: A dendrogram based on genetic similarities discriminated all the wheat varieties used in this study.

The lower level of polymorphism in the seven wheat genotypes may be attributed to a narrow genetic diversity of these seven wheat cultivars, five of them are local varieties which are product of the same location. Ablett *et al*. [27] stated that SSRs have a potentially high polymorphic frequency and of 126 markers investigated, polymorphisms were found in 33 (26%) when tested in 10 wheat varieties.

For the genome A, B and D an average of 3, 3.6 and 3.75 alleles per locus, respectively, was detected considering the seven wheat genotypes. These results are comparable with the results reported by Plaschke *et al*. [18], excluding *Xgwm174* located on 5D with 16 alleles, in terms of the contribution of each genome to genetic variation observed. The present results indicated that lower average number of alleles than that obtained by Fahima *et al*. [25] in the two wheat genomes A and B which are accounted to 10.9 and 9 alleles respectively. Yifru *et al*. [28] collected 133 Ethiopian tetraploid wheat accessions and eight introduced cultivars and used 29 wheat microsatellite markers to explaine the genetic diversity of these 133 wheat accessions. They found that a total of 383 alleles were detected with an average value of 13.14 alleles per locus. Relatively more alleles were observed in the B genome than in the A genome. Gene diversity indices ranged from 0.08 to 0.95, with a mean value of 0.72. Nevertheless, these results confirm the conclusion of Plaschke *et al*. [18] that the small number of markers is sufficient to distinguish closely related wheat genotypes and carry out phylogenetic studies, hence could select genotypes for highest genetic diversity.

Similarity indices and consensus tree were developed on the basis of the scorable banding patterns of the seven wheat varieties using the fifteen SSR primers as shown in Table 4 and Fig. 1.

The similarity indices showed that the two most closely related cultivars were Sakha 69 and Sakha 93 with the highest similarity index (0.688). On the other hand, the two most distantly related cultivars were Gemmiza 3 and Seds 4 with low similarity index (0.325). The consensus tree showed that it divided the wheat genotypes into two main clusters, the first included the wheat varieties Sakha 69, Sakha 93 and Gemmiza 3. The second main cluster was divided into two sub-clusters.

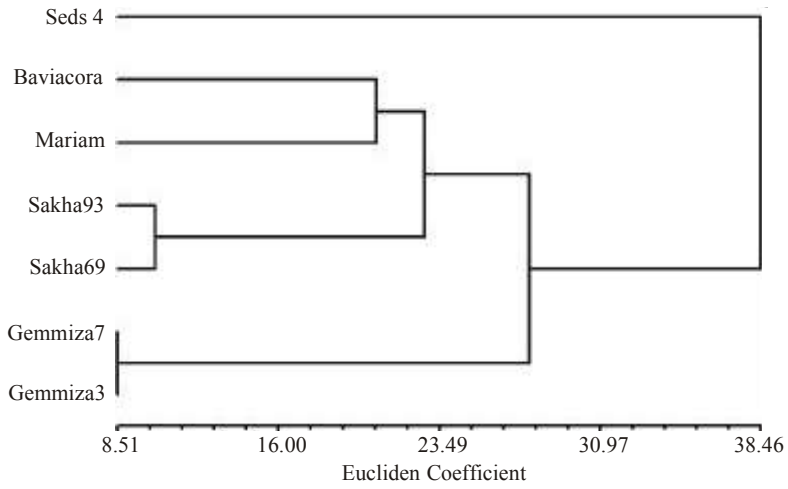

Fig. 2: A dendrogram of seven wheat varieties based on 9 morphological characters.

The first sub-cluster included varieties Gemmiza 7 and Mariam. The second one included cultivars Seds 4 and Baviacora. UPGMA cluster analysis of genomic SSR genetic similarity (gs) matrix resulted in the dendrogram in Figure 1. Three groups can be distinguished by truncating the dendrogram at gs value of 0.49. The major group (denoted group I) consists of 3 genotypes and includes three Egyptian wheat cultivars (Sakha 69, Sakha 93 and Gemmiza 3). Another group (Group II) includes one Egyptian wheat cultivar (Gemmiza 7) and one exotic variety (Miriam). Group III includes one Egyptian wheat cultivar (Seds 4) and one exotic variety (Baviacora). The cultivars, (Sakha 69, Sakha 93), (Seds 4' and 'Baviacora), (Gemmiza 7 and Miriam) were closely grouped, which indicated their genetic similarities. The dendrogram presented in Figure 1 demonstrate the ability of microsatellites to detect large amount of genetic diversity in genotypes with expected narrow genetic pool.

A wide range of genetic diversity among all genotypes was observed. The results have shown that it is possible to both classify the genetic diversity of elite genotypes and select genotypes or cultivars for the highest genetic diversity using SSRs, as indicated by cluster analysis. In previous studies, a higher number of wheat genotypes from the same origin have been analyzed using different DNA marker systems that produced the genetic diversity or similarity levels within a specific group of genotypes [7-9]. In this study, a different approach was taken by analyzing a smaller number of elite wheat genotypes of diverse origin using a higher number of SSRs to provide better genome coverage. These findings clearly demonstrate the reliability, usefulness and efficiency of SSRs in analyzing genomic diversity. Thus, it should be possible to establish a collection of highly polymorphic SSRs for genetic diversity studies cultivar identification and plant variety protection in wheat as has been proposed for soybean [29].

SSRs show a much higher level of polymorphism and are more informative in hexaploid wheat than any other marker system [4-6,18]. Genotypes with the most distinct DNA profiles are likely to contain the greatest number of novel genes and are likely to carry unique and potentially agronomically useful genes. Also, Hayden *et al*. [30] stated that increased SSR density on the published wheat genetic map over the last few years will further enhance breeding and research efforts.

The genetic diversity levels observed in this study would be useful indicators if such an approach is planned for the wheat genome. This makes genomic diversity estimates a potentially valuable predicting source for selecting diverse parent genotypes for favourable heterotic combinations in a wheat improvement program that aims to broaden the genetic basis and progeny performance for complex traits such as yield or partial disease resistance [31].

Morphological Characters Analyses: Distance estimates based on 9 morphological characters ranged from 8.51 to 38.46 with an average of 23.49 (Fig. 2). Cluster analysis based on the morphological data assigned the genotype into two groups (Fig. 2). The first cluster include Seds 4, originating from Egypt, while the second group was divided into two sub-groups, the first sub-group included Gemmiza 3 and Gemmiza 7.

While the second sub-group divided into two sub-sub-groups, the first one included Baviacora and Mariam. On the other hand the second branches included Sakha 93 and Sakha 69.

The knowledge about the genetic relationships of genotypes provides useful information to address breeding programmes and germplasm resource management [32]. In this study, morphological data analysis of the bread wheat genotypes was coupled with molecular analyses (SSR markers) to investigate the genetic relationships among 5 Egyptian bread wheat genotypes and two exotic varieties. The genotypes showed diverse morphological traits and distinct SSR markers patterns (Fig. 1 & Fig. 2).

The range of genetic distance based on morphological traits was on average higher than SSR markers, which may reflect the influence of the environment on the performances of the materials. Therefore, the DNA markers and morphological traits will not necessarily gain closely matching results [10, 33]. Semagn [34] suggested two reasons for low correlation between DNA markers and morphological as well as protein data: (a) DNA markers cover a larger proportion of the genome, including coding and noncoding regions, than the morphological markers and (b) DNA markers are less subjected to artificial selection compared with morphological markers. Martı´nez *et al*. [33] believed that the correspondence between different methods might be improved by analysing more morphological characters and DNA markers.

In summary, our data showed significant variation in morphological traits and microsatellite DNA polymorphisms among wheat varieties. This study using wheat microsatellite markers and morphological characters revealed considerable amount of genetic diversity among seven wheat varieties. The WMS data can be used in selecting diverse parents in breeding programme and in maintaining genetic variation in the germplasm, is crucial in utilizing the genetic potential of these genotypes for improvement of traits needed for adaptation to various stress conditions. Also, this study also shows that analyzing higher numbers of genotypes may not add much practical value to a general plant improvement program, unless a specific crossing program is aimed towards the improvement of specific traits. It is therefore suggested that a focused breeding scheme should be adopted while analyzing genome diversity estimates for parent selection to gain maximum value and practical impact on a breeding program.

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