THE USE OF SIMPLE SEQUENCE REPEATS FOR DETECTING GENETIC DIVERSITY IN EGYPTIAN AND EXOTIC BREAD WHEAT

M. Z. MATTAR

Botany Department, Faculty of Science, Menoufia University, Egypt.

The cultivated wheat (*Triticum aestivum* L.) belongs to tribe Triticeae of the family *Poaceae*. It is the first important cereal crop as the main source of human diet (FAO, 1985). Approximately, ninety five percent of wheat's grown today are hexaploid wheat which comprising three genomes A, B and D (Harti and Jones, 2001). Genetic diversity is considered one of the most important factors for crop improvement. Modern breeding process has dramatically narrowed the variation of important traits, especially among common wheat cultivars which are widely used in breeding programmes. It is important to investigate genetic diversity of wheat to assess its usefulness for breeding programmes. However, there are inherent problems with the use of data on morphological traits, the latter being limited in number and greatly influenced by the environment and by genotype X environment interactions. Molecular markers are a promising tool for evaluating genetic diversity among plant materials, as the phenotype and physiological markers are not accurate enough and often depend on environmental conditions. Many different DNA marker systems were used for diversity analysis. As far as the ability of polymorphism detection is concerned, the most useful markers were random amplified polymorphic (RAPDs) (Kuczynska *et al*., 2001; Mukhtar *et al*., 2002; Weber *et al*., 2005; Irzykowska *et al*., 2005) and simple sequence repeats (SSRs), also termed microsatellites, have been proposed as one of the most suitable markers for the assessment of genetic variation and diversity among wheat varieties, because they are multiallelic, chromosome-specific and distributed evenly throughout the plant genome (Prasad *et al*., 2000; Stachel *et al*., 2000; Huang *et al*., 2002; Roder *et al*., 2002, Landjeva *et al.*, 2006; Chabane *et al*., 2007; Salem *et al.*, 2008). Microsatellites were also successfully used to identify quantitative trait loci (QTLs) (Parker *et al.* 1998), to tag resistance genes (Peng *et al*., 1999; Borner *et al*., 2000) and to detect polymorphisms between the accessions of diploid and tetraploid wheat (Hammer *et al*., 2000; Pestsova *et al*., 2000; Fahima *et al*., 2002).

The objectives of this study are to (i) evaluate the application of microsatellite markers as a tool for detecting genetic diversity among Egyptian and exotic wheat varieties and (ii) compare these

genetic diversity estimates with other international wheat varieties.

MATERIALS AND METHODS

Plant material

A list of eleven bread wheat varieties with its origin and pedigree (if known) are presented in (Table 1). Since the information on allele sizes of 'Chinese Spring' (CS) is available (Huang *et al*., 2002), this cultivar has been used as a reference.

Genomic DNA isolation

Young leaves from eight-weeks-old plants were used to extract genomic DNA. The genomic DNA was isolated from these genotypes according to (Plaschke *et al*., 1995).

Microsatellite marker

Eleven simple sequence repeat markers (SSRs) for eleven loci were selected for genotyping. The basic information about the microsatellite markers such as name, chromosomal location, motif, annealing temperature (Tm°C) and fragment size in CS (bp) were presented in (Table 2). Microsatellite amplifications were carried out as reported by Roder *et al*. (1998).

Polymerase chain reactions (PCR)

DNA amplification was carried out in 25 µl reaction mixtures, each containing 5 µl wheat genomic DNA, 2.5 µl 10X PCR buffer (1 M KCl, 1.5 mM MgCl2 and 1M Tris-HCl, pH 8.3), 0.65 µl of each of the forward and reverse primers (250 nM), 50 µl dNTPs (0.2 nM) and 0.1 µl *Taq* DNA polymerase. 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 and 2 min at 72°C, with a final extension of 5 min at 72°C as described by Roder *et al.* (1998).

Data analyses

Presence or absence of each amplified band was scored as 1 and 0, respectively, for all markers to generate a binary data matrix. The photographed gels were analyzed by gel pro-program to calculate the size range (minimum and maximum) of different alleles. The genetic diversity, expressed as PIC of each microsatellite locus was calculated by calculating the frequency of the microsatellite alleles based on polymorphic information content (PIC) according to formula of Nei (1973) modified by (Botstein *et al.,* 1980): $\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2$, where k is the total number of alleles detected for a locus of a marker and *Pi* is the frequency of the *i*th allele in the set of 11 varieties investigated. The statistical significance of the differences in gene diversity and the number of alleles between groups over loci was calculated. Genetic similarities were estimated from the allele binary format dataset using the Dice method (Nei and Li, 1979). The binary data were used to compute pair wise similarity coefficients and the similarity matrix obtained was subjected to cluster analysis using the

UPGMA (Unweighted Pair Group Method of Arithmetic Average) algorithm on NTSYS-pc version 2.1 software package (Rohlf, 2002). The data were used to examine the relationships between the numbers of alleles vs. the gene diversity.

RESULTS AND DISCUSSION

Eleven bread wheat varieties from different origin were evaluated using 11 microsatellite markers. These microsatellites were selected on the basis of their known chromosomal location to give a uniform coverage for all three wheat genomes (A, B, and D) (Table 2). A total number of 44 alleles were detected. The number of alleles per locus ranged from two for *Xgwm18* to 6 for *Xgwm46* with an average number of 4 alleles per locus (Table 3). The largest number of alleles per locus occurred in the B genome with 17, compared to 16 and 11 for genomes A and D, respectively (Table 4). The lowest number of alleles per locus among the six homoeologous groups was observed in homoeologous group one with two alleles (Table 4). Different number of alleles has been detected in wheat using microsatellite markers. 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 a*l. (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 fifteen European geographical areas. Salem *et al*. (2008) detected an average of 3.2 alleles in seven wheat varieties. In the present study, the average number of allele was 4 in eleven wheat varieties. The value 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 was high than the average of 3.2 alleles per locus in seven wheat varieties detected by Salem *et al.* (2008). Genetic distance within the wheat varieties was significantly different (Table 5). This result means that the variation was high.

To assess the genetic diversity of wheat varieties, marker data were converted into binary matrix, which in turn allowed calculating the genetic similarity index. A dendrogram was created with the use of these data (Fig. 1). The consensus tree showed that the eleven wheat varieties were divided into four main clusters; the first included the two wheat varieties Chinese Spring and Sahel l. The second main cluster was divided into two subclusters. The first sub-cluster included the two exotic wheat varieties Weaver and Bauerlang. The second one included four Egyptian wheat varieties namely Sakha 8, Gemmiza 9, Seds 1, Seds 9. The third main cluster included only one wheat variety Giza 167. The fourth main cluster included the two other exotic wheat varieties namely Apollo (German) and Rialto (UK).

Gene diversity, expressed as PIC for 11 microsatellite loci varied from 0.379 for the *Xgwm631* to 0.989 for

Xgwm165 with an average of 0.678 (Table 3). Gene diversity for the three genomes A, B and D was 0.680, 0.690 and 0.650, respectively (Table 4). Also, there is an increase in genetic diversity with the increase of allele number. The correlation coefficient between gene diversity and the number of alleles was high, $r = 0.779$ ($P < 0.01$). The linear relationship between them is shown in (Fig. 2). The obtained results agree with those of Huang *et al*. (2002) who reported that the PIC value was correlated with the number of alleles, and did not agree with those of Prasad *et al*. (2000).

In the present study, the average number of alleles was different for individual genomes, 4 for A genome, 4.25 for B genome and 3.67 for D genome. This might suggest that D genome is the most conserved. This may be due to the 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 are consistent with data achieved by Roder *et al*. (1998) and Huang *et al*. (2002) for SSR markers and also by Huang *et al*. (2000) for AFLP markers.

A genetic similarity (GS) matrix based on all possible pairs of lines ranged from 14.3% to 90.9% (Table 5). The lowest pairwise GS value was between the Egyptian wheat variety Seds 1 and the Chinese wheat variety Chines Spring, while the highest value was between the Egyptian wheat variety Gemmiza 9 and the German wheat variety and y Apollo (Table 5).

In conclusion, this study indicated that the microsatellites are very effective molecular markers for genotype identification and for estimation the genetic diversity among Egyptian and exotic wheat varieties. It concludes that on the basis of microsatellite markers, diverse parents can be selected.

SUMMARY

The objectives of the current study were carried out to assess the genetic diversity and relationship among wheat varieties using SSR markers. Eleven wheat varieties were genotyped with 11 SSR markers. A total number of 44 alleles were detected. The number of alleles per locus ranged from two for *Xgwm18* to six for *Xgwm46* with an average number of 4 alleles per locus. The largest number of alleles per locus occurred in the B genome with 17, compared to 16 and 11 for genomes A and D, respecively. The value of polymorphism information content (PIC), a measure of gene diversity, was 0.379 for the *Xgwm631* and 0.989 for *Xgwm165* with an average of 0.678. Gene diversity for the three genomes A, B and D was 0.680, 0.690 and 0.650, respectively. Also, there is an increase in genetic diversity as the number of alleles increase. The correlation coefficient between gene diversity and the number of alleles was high, $r = 0.779$ ($P < 0.01$). A genetic similarity (GS) matrix based on all possible

pairs of lines ranged from 14.3% to 90.9%. The lowest pairwise GS value was between Seds 1 and Chines Spring, while the highest value was between the Egyptian wheat variety Gemmiza 9 and the German wheat variety Apollo. Clustering analysis based on GS grouped the 11 wheat varieties into 4 main clusters. Grouping based on clustering analysis was in good agreement with available pedigree and genetic background information. Information generated from this study can be used to select parents for hybrid development to maximize the yield and its components, and development of segregating populations to map genes controlling yield and its components in wheat.

REFERENCES

Borner, A., S. Chebotar and V. Korzun (2000). Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. Theor. Appl. Genet., 100: 494-497.

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.

Chabane, K., O. Abdalla, H. Sayed and J. Valkoun (2007). Assessment of EST-microsatellites markers for discrimination and genetic diversity in bread and durum wheat landraces from Afghanistan. Genet. Resour. Crop. Evol., 54: 1073-1080.

Fahima, T., M. S. Roder, K. Wendehake, V. M. Kirzhner, E. Nevo (2002). Microsatellite polymorphism in natural populations of wild emmer wheat, Triticum dicoccoides, in Israel. Theor. Appl. Genet., 104: 17-29.

FAO (1985). FAO production year book. 37: 107-122.

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

Hammer, K., A. A. Filatenko and V. Korzun (2000). Microsatellite markers: a new tool for distinguishing diploid wheat species. Genet. Res. Crop. Evol., 47: 497-505.

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

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

Huang, X. Q., F. J. Zeller, S. L. K. Hsam, G. Wenzel and V. Mohler (2000). Chromosomal location of AFLP markers in common wheat (*Triticum aestivum* L.) utilizing nullitetrasomic stocks. Genome, 43: 298-305.

Irzykowska, L., E. Zoltanska and J. Bocianowski (2005). Use of molecular and conventional techniques to identify and analyze genetic variability of *Rhizoctonia* spp. isolates. Acta Agrobot., 59: 19-32.

Khlestkina, E. K., M. S. Roder, 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.

Kuczynska, A., P. Milczarski, M. Surma, P. Masojc and T. Adamsk (2001). Genetic diversity among cultivars of spring barley revealed by random amplified polymorphic DNA (RAPD). J, Appl. Genet., 42: 43-48.

Landjeva, S., V. Korzun and G. Ganeva (2006). Evaluation of genetic diversity among Bulgarian winter wheat (*Triticum aestivum* L.) varieties during the period 1925–2003 using microsatellites. Genet. Res. Crop. Evol., 53: 1605-1614.

Mukhtar, M. S., M. Rahman and Y. Zafar (2002). Assessment of genetic diversity among wheat (*Triticum aestivum* L.) cultivars from a range of locations across Pakistan using random amplified polymorphic DNA (RAPD) analysis. Euphytica, 128: 417-425.

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

Nei, M. and W. H. Li (1979). Mathematical model for studying genetic variation in terms of endonucleases. Proc. Natl. Acad. Sci. USA, 76: 5269-5273.

Parker, G. D., K. J. Chalmers, A. J. Rathjen and P. Langride (1998). Mapping loci associated with flour colour in wheat (*Triticum aestivum* L.). Theor. Appl. Genet., 97: 238-245.

Peng, J. H., T. Fahima, M. S. Roder, Y. C. Li, A. Dahan, A. Grama, Y. I. Ronin, A. B. Korol and E. Nevo (1999). Microsatellite tagging of the stripe-rust resistance gene YrH52 derived from wild emmer wheat, Triticum dicoccoides, and suggestive negative crossover interference on chromosome 1B. Theor. Appl. Genet., 98: 862-872.

Pestsova, E., V. Korzun, N. P. Goncharov, K. Hammer, M. W. Ganal and M. S. Roder (2000). Microsatellite analysis of *Aegilops tauschii* germ-

plasm. Theor. Appl. Genet., 101: 100-106.

Prasad, M., R. K. Varshney, J. K. Roy, H. S. Balayan, P. K. Gupta (2000). The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor. Appl. Genet., 100: 584-592.

Roder, 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

Roder, M. S., K. Wendehake, V. Korzun, G. Bredemeijer, D. Laborie, L. Bertrand, P. Isaac, S. Rendell, J. Jackson, R. J. Cooke, B. Vosman and M. W. Ganal (2002). Construction and analysis of a microsatellite-based database of European wheat varieties. Theor. Appl. Genet., 106: 67-73.

Rolf, 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.

Salem, K. F. M., A. M. El-Zanaty and R. M. Esmail (2008). Assessing wheat (*Triticum aestivum* L.) genetic diversity using morphological characters and microsatallite markers. World J. Agri. Sci., 4: 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.

Weber, Z., L. Irzykowska and J. Bocianowski (2005). Analysis of mycelial growth rates and RAPD-PCR profiles in a population of Gaeumannomyces graminis var. tritici originating from wheat plants grown from fungicide treated seed. J. Phytopathol., 153: 318-324.

Table (1): List of wheat genotypes used in the study with their origin and pedigree.

No.	Genotype Name	Origin	Pedigree
1	Chinese Spring	China	LV/Sichuan
2	Apollo	Germany	Unknown
3	Rialto	UK	Unknown
4	Weaver	Unknown	Unknown
5	Bauerlang	Unknown	Unknown
6	Sakha 8	Egypt	Indus/Norteno “s”
7	Gemmiza 9	Egypt	Ald”s”/Huac”\s”//CMH74A.630/5x
8	Giza 167	Egypt	Au/Up301//Gll/Sx/Pew”s”/4/Mai”s”/May”s” //Pew”s”
9	Seds 1	Egypt	HD 2172/Pavon”s”//1158.57/Maya 74 “s”
10	Seds 9	Egypt	Unknown
11	Sahel 1	Egypt	N.S.732/Pim//Vee”s”

Table (2): Description of 11 wheat microsatellite markers, their chromosomal location, mtif, annealing temperature and fragment size.

No.	Microsatel-lite	Chromosomal location	Motif	Annealing Tm (ºC)	Fragment size in CS (bp)
1	*Xgwm3*	3D	(CA)18	55	79
2	*Xgwm 46*	7B	(GA)3GC(GA)33	60	179
3	*Xgwm160*	4A	(GA)21	60	182
4	*Xgwm165*	4A	(GA)20	60	190
5	*Xgwm186*	5A	(GA)26	60	135
6	*Xgwm190*	5D	(CT)22	60	209
7	*Xgwm261*	2D	(CT)21	55	189
8	*Xgwm389*	3B	(CT)14 (GT)16	60	129
9	*Xgwm513*	4B	(CA)12	60	140
10	*Xgwm631*	7A	(GT)23	60	196
11	*Xgwm18*	1B	(CA)17GA(TA)4	50	183

Table (3): Description of 11 wheat microsatellites, their position, size range of alleles, number of alleles and gene diversity (expressed PIC).

Locus	Position	Size range of alleles (bp)		Number of alleles	Gene diversity
		Min Allele	Max Allele		
Xgwm3	3D	75	79	3	0.562
Xgwm 46	7B	145	179	6	0.770
Xgwm160	4A	178	186	4	0.698
Xgwm165	4A	187	202	5	0.989
Xgwm186	5A	124	140	4	0.677
Xgwm190	5D	198	210	4	0.720
Xgwm261	2D	165	192	4	0.681
Xgwm389	3B	117	140	4	0.722
Xgwm513	4B	141	151	5	0.765
Xgwm631	7A	190	200	3	0.379
Xgwm18	1B	186	190	2	0.500
Total				44	------
Mean				4.00	0.678

Table (4): Genetic diversity (expressed PIC) in different genomes and chromosomes across eleven loci in eleven wheat varieties.

Locus	Number of alleles	Average number of alleles	Gene diversity
Genome			
A	16	4.00	0.680
B	17	4.25	0.690
D	11	3.67	0.650
Chromosome			
1	2	2.00	0.500
2	4	4.00	0.681
3	7	3.50	0.642
4	14	4.70	0.817
5	8	4.00	0.699
7	9	4.50	0.575

Table (5): Similarity matrix (%) of wheat genotypes based on their microsatellite alleles.

	1	2	3	4	5	6	7	8	9	10
1-Chinese Spring										
2-Apollo	33.3									
3-Rialto	46.2	72.7								
4-Weaver	28.6	25.0	23.1							
5-Bauerlang	29.6	26.1	24.0	66.7						
6-Sakha 8	22.2	17.4	16.0	37.1	38.5					
7-Gemmiza 9	15.4	90.9	25.0	23.1	24.0	32.0				
8-Giza 167	28.6	83.3	38.5	21.4	22.2	14.8	38.5			
9-Seds 1	14.3	25.0	15.4	35.7	37.0	44.4	53.8	35.7		
10-Seds 9	20.0	76.9	71.4	33.3	34.5	41.4	50.0	33.3	66.7	
11-Sahel 1	42.9	83.3	76.9	21.4	37.0	37.0	38.5	28.6	28.6	53.3

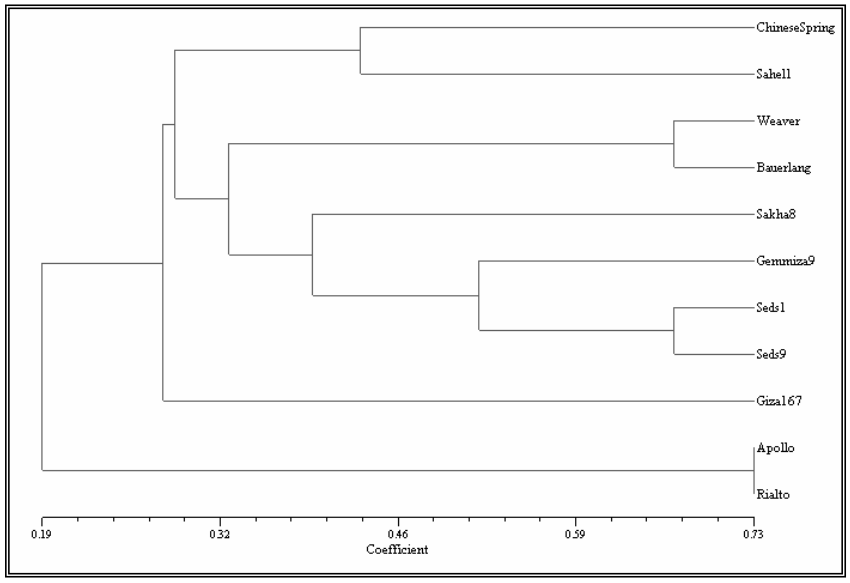

Fig. (1): Dendrogram of similarities between 11 wheat varieties, based on data of 11 microsatellites.

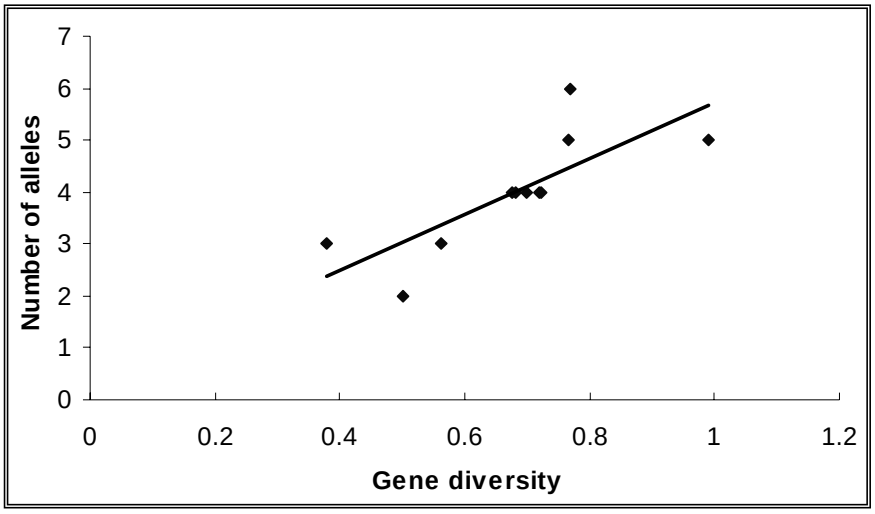

Fig. (2): Relationship between gene diversity and the number of alleles detected at 11 microsatellite loci, described by the function:
$Y = 0.3486 + 5.3818X$, $R^2 = 0.6068$.