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Genetic Diversity of Bread Wheat Genotypes (Triticum Aestivum L.) Revealed by Agromorphological Characteristics and Microsatellite SSR Markers

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***Abstract*— The present study was conducted to understand the genetic diversity of bread wheat's that grown in Algeria, and to evaluate polymorphism information content (PIC) of some wheat SSR primers. It was undertaken to examine the genetic diversity of ten bread wheat (*Triticum aestivum* L.) genotypes, using 16 microsatellite primer pairs (SSRs). SSR bands were scored across all genotypes, for presence (1) or absence (0) and transformed into 0/1 binary matrix. A pair-wise similarity matrix was generated with the software NTSYS. The Polymorphism Information Content (PIC) ranged from 0.23 to 0.70 respectively for the primer *WMC 21* and *WMC 50* with an average of 0.49 and 0.52 per primer pair. The similarity coefficient between cultivars ranged from 0.28 and 0.91 with an average of 0.60. Most of the genotypes showed a high degree of genetic similarity. The highest genetic distance value of 0.91 has been scored between Kauz/Pastor/Fiscal and Prl/2*Pastor and between Kauz/Pastor/Fiscal and Wbll1*2/Brambling. The lowest genetic distance value of 0.28 has been scored between Arz and Babax/Lr42//Babax*2/3/Kukuna. Cluster analysis based on microsatellite allelic diversity discriminated the varieties into different clusters. Ten wheat cultivars were grouped in three clusters. 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.**

Keywords*— Wheat (Triticum aestivum); PIC, cluster analysis; SSR markers; Genetic diversity.

INTRODUCTION

Common wheat *Triticum aestivum* L. (2n = 6x = 42), belonging to the family *Poaceae*, which is considered the most diverse and important family of the plant kingdom, produces large edible grains and provides about one-half of humans' food calories, a large part of their nutrient requirements and is among the most important grain crops in Algeria. The genetic variability in bread wheat is important for a better improvement of this crop and for the increase of cereal yield in the context of sustainable agriculture to face human needs in the next decades. The availability of genetic variability in wheat material is a pre-requisite for any breeding program aimed towards the improvement of wheat productivity [1]. Genetic manipulation is the best way to boost up wheat production. Therefore, it is necessary to estimate and study the genetic variation and mode of inheritance in different plant parameters to initiate productive wheat breeding programs [2]. Thus, preservation of genetic diversity of the national wealth could be used by breeders for a future variety development.

In order to reach a better characterization of the genetic diversity, it is imperative to use a reliable molecular biology tool for plant breeding and genetic resources management. The molecular diversity study allows us to understand the differences and similarities between the ecotypes, according to their genes. In addition, it describes the structure of genetic variability within populations. Several molecular techniques, to achieve these objectives, are currently available in several laboratories worldwide. Among these techniques, the Simple Sequence Repeats (SSRs) or microsatellites that have been proven to be the markers of choice during the last decade in plant research because of their hyper variability and ease of detection.

The diversity of germplasm has traditionally been described using morphological and agronomical traits. However, molecular markers such as microsatellites (SSRs) have been found to be complementary to morphological, pedigree, heterosis and biochemical data [3;4]. Microsatellites have a high potential use for the genetic analysis of self-pollinating crops because of their high degree of polymorphism and they are codominantly inherited [5; 6; 7]. Primers flanking the microsatellite locus in the selected DNA sequences even allow detecting multiallelic variation [8] . Previously microsatellites have been successfully used in diversity studies of wheat and barley [9;10;11;12].

In the present study, we used the SSR markers to investigate the diversity among 10 genotypes within the ITGC germplasm collection in Constantine/Algeria. The objectives of this study were to (*i*) use wheat microsatellite markers SSRs to assess levels and patterns of genetic variability among a representative sample of local wheat genotypes, (*ii*) compare these genetic diversity estimates with other international wheat cultivars, (iii) and use wheat microsatellite markers for the characterization and assessment of the genetic diversity of ten wheat varieties.

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MATERIALS AND METHODS

PLANT MATERIALS COLLECTION

This investigation was carried out at the experimental farm of ITGC (Technical Institute of High Culture) in Constantine/Algeria. Three local wheat varieties from ITGC, Constantine and seven introduce 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.

The genotypes were sown and grown under rain fed condition in a randomized complete-block design with two replicates per genotype, at the experimental field of ITGC. Each variety was sown along 2 m-long rows at a density of 40 seeds per line with 5 cm between seeds. This plot was used to study morphology from the seedling stage to maturity.

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

N°	Genotypes	Pedigrees	Origin
V1	Ain abid	ITGC/ Algeria	Algeria
V2	Arz	ITGC/ Algeria	Algeria
V3	Hidhab	ITGC/ Algeria	Algeria
V4	Pastor/Wbll1	CMSA00Y00586-0POY 040M 040SY-030M-17ZT	CIMMYT (Mexico)
V5	Babax/Lr42//Babax*2/3vivitsi	CGSS01B00046T-099Y-099 M-099M-099Y-21Y-0B	CIMMYT (Mexico)
V6	Wbll1*2/Brambling	CGSS01B00062T-099Y 099 M-099M-099Y-099M-77Y-0B	CIMMYT (Mexico)
V7	Thelin//2*Attila*2/Pastor	CGSS02Y00089T-099B-099 M-099Y-099M-4Y-0B	CIMMYT (Mexico)
V8	Kauz/Pastor/Fiscal	CMSS02M00325S-030M-15 Y-0M-0Y	CIMMYT (Mexico)
V9	Prl/2*Pastor	CGSS97Y00034M099T0PB-027Y-099M-099Y-099M	CIMMYT (Mexico)
V10	Babax/Lr42//Babax*2/3/Kukuna	CGSS01B00048T-099Y099 M-099M-099Y-099M-31Y-0B	CIMMYT (Mexico)

DNA EXTRACTION AND SSR ANALYSIS

DNA ISOLATION

Total genomic DNA was extracted from frozen young leaves following a CTAB method modified and described by BEN NACEUR [13], and followed by an organic extraction in chloroform: isoamyl alcohol (24:1). DNA was purified by RNase (10 µg/ml) and its concentration was estimated using 0.8% agarose gel. DNA was dissolved and preserved in TE buffer (10 mMTris-HCl, 1 mM EDTA, pH 8.0).

DNA amplification

Amplification was performed using a thermocycler (Multigene optimax.), in a total volume of 25 µl containing 1.5Mm MgCl2, 0.2 mM dNTPs, 0.25 µM of forward and reverse primers, 1U of *Taq* DNA polymerase (Go Taq, Promega; http://www.promega.com), 1x buffer and 50 ng/µl DNA. PCR consisted of one round of perdenaturation at 94°C for 3 min followed by 35 amplification cycles of: 2 min denaturation at 94°C, 1 min hybridization at Ta between 52-64°C (Table 2), 2 min of extension at 72°C. These cycles were followed by a final extension for 1 min at 72°C. PCR products were separated on a 2% agarose gel. To better discern some fine bands, we also used a 40% polyacrylamide gel prepared with 70 ml distilled water; 10 ml TBE (5x); 20 ml of 40% acrylamide (19:1; acrylamide: bisacrylamide); 800 µl APS (ammonium persulfate (10x) and 80 µl TEMED (tetramethyl ethylene diamine). DNA concentrations were estimated by comparison with 100-bp DNA ladder (Promega). The amplified product was visualized under UV light on a gel documentation system after staining the gel with 5 µl Ethidium bromide.

MICROSATELLITE MARKERS ANALYSIS

The reaction of DNA amplification by PCR was performed with 16 microsatellite primer pairs (*WMC 14, WMC15, WMC16, WMC17, WMC18, WMC19, WMC20, WMC21, WMC22, WMC23, WMC24, WMC25, WMC27, WMC48, WMC50 and WMC283*) reported in table 2.

Table 2. Description of tested SSR primers (http:www. wheat.pw. usda.gov/ggpages/SSR/WMC).

Primer	Sequences	Motifs	Chromosome location	Alleles size (pb)
WMC 14F	ACCCGTCACCGGTTTATGGATG	(CT) (CA)	7D	239
WMC 14R	TCCACTTCAAGATGGAGGGCAG			
WMC 15F	AGTCCGATTCGGACTCCTCAG	(CT) (CA)	4A	295
WMC 15R	GGACTAACCGAGGGTAGTTG			
WMC 16F	ACCGCCTGCATTCTCATCTAA	(CT)	4B	165
WMC 16R	GTGGCGCCATGGTAGAGATTG			
WMC 17F	ACCTGCAAGAAATTAGGAAC	(CA)	7A-7B	182
WMC 17R	CTAGTGTTTCAAATATGTCGA			
WMC 18F	CTGGGGCTTGGATCACGTCATT	(CA) (CT)	2D	237
WMC 18R	AGCCATGGACATGGTGTCCTTC			
WMC 19F	CTGACATGCGGCATTCACTTCC	(CA)	1A	153
WMC 19R	AGGCTTAGAACACACCGACACG			
WMC 20F	TTAAAAACACGCGGATCTTCTC	(CA)	1A	119
WMC 20R	GTACTCACATATTTCTCGGTCT			
WMC 21F	CGCTGCCGTGTAACTCAAAATC	$(GA)_{37}$		136
WMC 21R	AGTTAATTGGGCGCTCCAAGAA		-	
WMC 22F	ATCATTGGTTTCCTCTTCACTT	$(GT)_{24}$		169
WMC 22R	GTGGACTATTTAACATCTTCAT		-	
WMC 23F	ATTCGCTCATACGATAGGGTTG	$CT)_{22}$		314
WMC 23R	AGAGGCTGGTGTAGTTGGTTTG	$(CT)_{18}$	-	
WMC 24F	GTGAGCAATTTTGATTATACTG	$(GT)_{28}$	1A	136-155
WMC 24R	TACCCTGATGCTGTAATATGTG			
WMC 25F	TCTGGCCAGGATCAATATTACT	$(GT)_{26}$	2B	166
WMC 25R	TAAGATACATAGATCCAACACC			
WMC 27F	AATAGAAACAGGTCACCATCCG	$(GT)_{25}$	2B-5B	352-398
WMC 27R	TAGAGCTGGAGTAGGGCCAAAG			
WMC 48F	GAGGGTTCTGAAATGTTTTGCC	$(GA)_{9}$	4B	139-190
WMC 48R	ACGTGCTAGGGAGGTATCTTGC			
WMC 50F	CTGCCGTCAGGCCAGGCTCACA	$(GT)_{10}$	3A	219-236
WMC 50R	CAACCAGCTAGCTGCCGCCGAA	$(GT)_{16}$		
WMC 283F	CGTTGGCTGGGTTATATCATCT	$(CA)_{19}$	4A	
WMC 283R	GACCCGCGTGTAAGTGATAGGA	$(CA)_{8}$		

Diversity Analysis

Genetic Similarity Estimation and Cluster Analysis

The SSR profiles were transformed into a binary matrix where the presence of the generated band at a precise level is scored as 1 and its absence is scored as 0. A data matrix was prepared for the analyses. A pair-wise similarity matrix was generated with the software NTSYS pc-2.02j (NTSYS-Numerical Taxonomy and Multivariate Analysis [14].

Estimates of genetic similarity (GS) among all genotypes were also calculated using NEI and LI [15] coefficient of similarity between two individuals (i and j), according to the formula Nei and Li's coefficient = 2a/(b+c), where "a": is the number of shared bands in both samples i and j, "b": is the total number of bands of individual i and "c" the total number of bands of individual j. The similarity matrix was used to construct a dendrogram by the unweighted pair group method arithmetic averages (UPGMA) procedure (SOKAL and MICHENER, 1958). The goodness of fit of the clustering was tested using the MxCOMP program, which directly compares the original similarity matrix and the cophenetic value matrix, as suggested by RHOLF [14].

Marker Polymorphism

To measure the informativeness of the *SSR* markers, the polymorphism information content (PIC) for each *SSR* was calculated according to the formula:

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$$PIC = 1 - \sum_{i=1}^{k} Pi^2$$

where k is the total number of alleles detected for a locus of a marker and *P* the *I* frequency of the *i* th allele in the set of 10 genotypes investigated.

RESULTS AND DISCUSSION

A total of 16 SSR primers were tested, although only 11 (*WMC 14*, *WMC15*, *WMC17*, *WMC20*, *WMC21*, *WMC24*, *WMC25*, *WMC27*, *WMC48*, *WMC50*, *WMC283*) produced polymorphic bands (Table 3).

Table 3. PIC values, alleles number and annealing temperature generated by polymorphic primers

Primer	PIC	Ta (°C)	Nb Alleles	Primer	PIC	Ta (°C)	Nb Alleles
WMC 14	0.77	58	5	*WMC 25*	0.61	52	3
WMC 15	0.37	55	2	*WMC 27*	0.66	55	4
WMC 17	0.69	54	5	*WMC 48*	0.48	64	2
WMC 20	0.49	54	2	*WMC 50*	0.70	60	5
WMC 21	0.23	55	2	*WMC 283*	0.59	60.4	3
WMC 24	0.52	52	2				

Microsatellite Polymorphism

In this study eleven microsatellite markers for 11 loci were used to characterize and evaluate the genetic diversity of ten wheat genotypes (Table 3). Amplified microsatellite loci were analyzed for polymorphism using polyacrylamide gel electrophoresis and the result revealed that all the primer pairs detected polymorphisms among the wheat genotypes analyzed. A total of 34 alleles were detected. The number of alleles per locus ranged from two for *WMC 15*, *WMC 20*, *WMC 21*, *WMC 24*, *WMC 27*, and WMC *48* to 5 for *WMC 14*, *WMC 17* and *WMC 50* with an average number of 3.2 alleles per locus. The maximum number of alleles was observed at *WMC 14* (Figure 1), *WMC 17* and *WMC 50* and their size ranged from 182 to 239 bp.

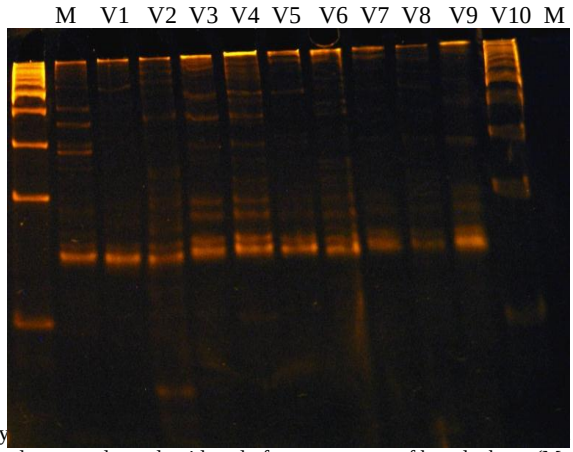

Fig. 1- Ty
DNA template on polyacrylamide gel of ten genotypes of bread wheat. (M: marker 100-bp DNA ladder. V1: AinAbid; V2: Arz; V3: Hidhab; V4: Pastor/Wbll1; V5: Babax/Lr42//Babax*2/3vivitsi; V6:Wbll1*2/Brambling; V7: Thelin//2*Attila*2/Pastor; V8: Kauz/ Pastor/Fiscal; V9: Prl/2*Pastor; V10: Babax/Lr42// Babax*2/3/ Kukuna).

Similar observation was reported by SALEM *et al.* [16] and ISLAM *et al.* [17] where they found that the number of alleles per locus ranged from 2 alleles to 7 alleles with an average of 3.2 alleles per locus. JAIN *et al.* [18] also reported similar observation of number allele per locus ranged from 3 to as high as 22 with an average of 7.8 alleles per locus. A similar pattern of allelic variation was also detected at other loci [2]. The landraces which are selected from local germplasm have a lower range of diversity; however, cultivars which are introduced would have a wide genetic diversity than both of wild genotypes or landraces. Furthermore, the detected genetic diversity for the ten bread wheat varieties is also lower than that reported by PLASCHKE *et al.* [6] and LEISOVA *et al.* [19] studying closely related European wheat cultivars having an average of 12 alleles per locus.

The Polymorphism Information Content (PIC) ranged from 0.23 to 0.70 respectively for the primer *WMC 21* and *WMC 50* with an average of 0.49 and 0.52 per primer pair (Table 3). These results are confirmed in two earlier studies on wheat where the PIC values ranged from 0.23 to 0.79 [20] and from 0.29 to 0.79 [6]. In another recent study, the PIC mean value (0.30) for SSRs was lower than the mean value of 0.48 and 0.49 observed in the present study [21]. However, these results are much higher in wheat Tunisian genotypes (PIC) ranged from 0.33 to 0.94 respectively for the primer *WMC 25* and *WMC 50* [2]. PIC also showed a significant, positive correlation with the number of alleles and allele size range for microsatellites evaluated in this study. The allele size range and the number of alleles were themselves also highly correlated.

Assessment of relationships between cultivars

According to NEI'S [15], the similarity coefficient between cultivars ranged from 0.28 and 0.91 with an average of 0.60 (Table 4). Most of genotypes showed a high degree of genetic similarity. The lowest genetic distance value of 0.28 has been scored between Arz and Babax/Lr42//Babax*2/3/Kukuna. Results indicating that they are genetically similar to each other. The highest genetic distance value of 0.91 has been scored between Kauz/Pastor/Fiscal and Prl/2*Pastor and between Kauz/Pastor/Fiscal and Wbll1*2/Brambling. The higher genetic distance between them indicates that genetically they are diverse compare to lower genetic distance value. Basically this value is an indication of their genetic dissimilarity.

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

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
V1	1,000									
V2	0,696	1,000								
V3	0,640	0,417	1,000							
V4	0,583	0,435	0,640	1,000						
V5	0,696	0,545	0,667	0,870	1,000					
V6	0,667	0,522	0,640	0,667	0,696	1,000				
V7	0,500	0,522	0,640	0,500	0,522	0,833	1,000			
V8	0,583	0,435	0,640	0,583	0,609	0,917	0,833	1,000		
V9	0,583	0,522	0,720	0,667	0,696	0,833	0,750	0,917	1,000	
V10	0,455	0,286	0,696	0,455	0,476	0,727	0,636	0,818	0,818	1,000

A dendrogram was constructed based on the NEI'S genetic distance calculated from 10 alleles generated from 12 wheat varieties. All 12 wheat cultivars could be easily distinguished. The UPGMA cluster tree analysis led to the grouping of the 10 wheat varieties in three major clusters (Fig. 2).

The consensus tree showed that it divided the wheat genotypes into three main clusters, the first included landrace wheat varieties Ain Abid and Arz (Algerian cultivar), The second main cluster was divided into two sub-clusters. The first sub-cluster included only landrace wheat varieties Hidhab. The second one included the

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largest cluster of 5 wheat varieties among selected 10 wheat varieties. Some popular wheat varieties included in this group are Kauz/Pastor/Fiscal, Wbll1*2/Brambling. Babax/ Lr42// Babax*2/3/Kukuna, Thelin//2*Attila*2/Pastorand Prl/2*Pastor (introduce cultivar). Kauz/Pastor/FiscalandWbll1 *2/Brambling were closely grouped, which indicated their genetic similarity. The last group is formed by wheat cultivars from Mexico Babax/Lr42//Babax*2/3vivitsi and Pastor/Wbll1. The dendrogram presented in Figure 2, demonstrate the ability of microsatellites to detect large amount of genetic diversity in genotypes with expected narrow genetic pool.

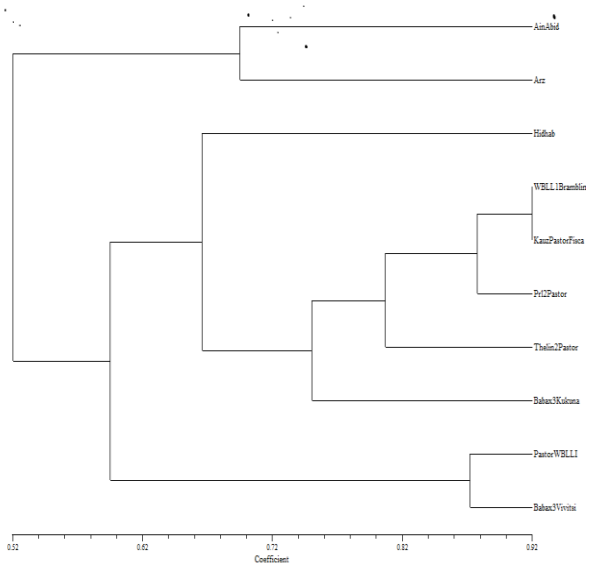

Fig. 2- Dendrogram based on data of 11 microsatellite primers from 10 bread wheat genotypes constructed by UPGMA.

The cophenetic coefficient was r = 0.82, indicating that there is good fit between dendrogram clusters and the similarity matrix.

In this study, a different approach was taken by analyzing a smaller number of 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 [16]. Microsatellite markers are popular for different applications in wheat breeding due to their high level of polymorphism and easy handling [22; 7; 23; 24] and are used to evaluate genetic diversity of hexaploid wheat [25]. In the present study SSR markers almost succeeded in discrimination wheat cultivars. But these markers could not separate wheat cultivars with different growth types completely.

Most of the primers that have been used in this study revealed a high polymorphism. So they can be used for screening, evaluation of genetic diversity and molecular mapping studies in bread wheat. In general, diversity measurements were higher in the cultivars at which such a high level of genetic similarity may be used for selection of the materials in the breeding programs where cultivars with high genetic distance can be used for this purpose. It can be concluded that more polymorphic wheat SSR markers could be used for efficient screening of the germplasm by saturating more regions of wheat genome.

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