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Investigation of Genetic Diversity among Bread Wheat Cultivars (*Triticum aestivum* L.) Using SSR Markers

Kara Karima[1], Rached-Kanouni Malika[2] and Ben Naceur M'barek[3]

[1] Département de Biologie et Ecologie Végétale, Faculté des Sciences de la Nature et de la Vie, Université des Frères Mentouri, Constantine, Algérie

Département de Biologie, Faculté des Sciences Exactes et Sciences de la Nature et de la Vie, Université Larbi Ben M'Hidi, Oum El Bouaghi, Algérie

Banque Nationale des Gènes de Tunis, Rue Yasser Arafat, 1080, Tunis, Tunisie

(Email : karima_k2006@yahoo.fr)

***Abstract*:** *The present study was conducted to understand the genetic diversity of bread wheat's that grown in Algeria. 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 and transformed into 0/1 binary matrix. The Polymorphism Information Content (PIC) ranged 0.13 to 0.70 respectively for the primer WMC 24 and WMC 50 with an average of 0.48 and 0.49 per primer pair. The similarity coefficient between cultivars ranged from 0.33 and 0.90 with an average of 0.63. Most of the genotypes showed a high degree of genetic similarity. The highest genetic distance value of 0.90 has been scored Angi4 and between Milan/S87230/babax. The lowest genetic distance value of 0.33 has been scored between Hammam1 and Attila2Pastor. The genotypes were clustered in four clear groups according to their origin, pedigree and characters similarities*

Keywords: *Cluster analysis, Genetic diversity, SSR markers, Wheat (Triticum aestivum)*

1. Introduction

Bread wheat (Triticum aestivum L.) is a staple food and the major source of calories and protein for a large world population, 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]. 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. 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. 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 [3]. Previously microsatellites have been successfully used in diversity studies of wheat and barley [4]. 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 investigation genetic diversity of ten wheat varieties.

2. Materials and Methods

2.1. 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 I.

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 I
Genotypes 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	Hamam 1	ICW92-0455-1AP-1AP-2AP-3AP-0AP	ICARDA (Syria)
V5	5119		SERASEN E(France)
V6	Milan/S872 30/babax	CMSS97MO-3687T6040Y-03M-020Y-030M-015Y-38M	CIMMYT (Mexico)
V7	Angi-4	ICW92-0326-12AP-1AP-2AP-3AP-0AP	ICARDA (Syria)
V8	Cham 6	CM39992-8M-7Y-OM-0AP	CIMMYT (Mexico)
V9	Attila	CM85-836-50Y-0M-OY-3M-0Y	CIMMYT (Mexico)
V10	Attila/2 Pastor	CGSS97Y00042M-099T0PB-058Y-099M-099Y-099B	CIMMYT (Mexico)

2.2. DNA extraction and SSR analysis

Total genomic DNA was extracted from frozen young leaves following a CTAB method modified and described by Ben Naceur [5], 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 mM Tris- HCl, 1 mM EDTA, pH 8.0).

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 II), 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.

2.3. 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 II.

TABLE II
DESCRIPTION OF TESTED SSR PRIMERS

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

2.4. Diversity 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 [6].

Estimates of genetic similarity (GS) among all genotypes were also calculated using NEI and LI [7] 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. 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 [8].

2.5. Marker Polymorphism

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

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

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.

3. Results and Discussion

A total of 16 SSR primers were tested, although only 11(WMC14, WMC15, WMC17, WMC20, WMC21, WMC24, WMC25, WMC27, WMC48, WMC50, WMC283) produced polymorphic bands (Table III).

TABLE III
PIC VALUES, ALLELES AND ANNEALING TEMPERATURE GENERATED BY POLYMORPHIC PRIMERS

Primer	PIC	Ta (°C	Nb Allele	Primer	PIC	Ta (°C)	Nb Allel
WMC 14	0.66	58	5	***WMC 25***	0.61	52	3
WMC 15	0.37	55	2	***WMC 27***	0.26	55	2
WMC 17	0.33	54	5	***WMC 48***	0.48	64	2
WMC 20	0.49	54	2	***WMC 50***	0.70	60	5
WMC 21	0.13	55	2	***WMC 283***	0.56	60.4	3
*WMC **24***	0.42	52	2				

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, WMC 48 to 5 for WMC 14 and WMC 50 with an average number of 3.2 alleles per locus. The maximum number of alleles was observed at WMC 14 in Fig.I and WMC 50 and their size ranged from 219 to 239 bp.

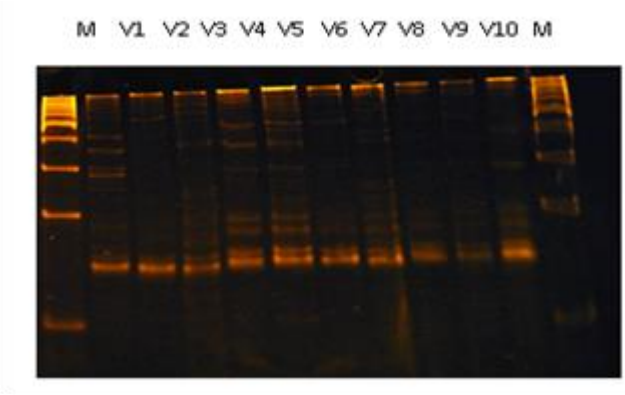

Fig. 1 Typical examples of SSR profile (*WMC 14*) obtained using genomic DNA template on polyacrylamide gel of ten genotypes of bread wheat. (M: marker 100-bp DNA ladder. V1: Ain Abid; V2: Arz; V3: Hidhab; V4: Hamam1; V5: 5119; V6: Milan / S8 7230 / babax ; V7: Angi-4; V8: Cham6; V9: Attila; V10: Attila/2 Pastor)

A similar pattern of allelic variation was also detected at other loci [4]. 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 ten bread wheat varieties is also lower than that reported by LEISOVA et al. [9].The Polymorphism Information Content (PIC) ranged from 0.13 to 0.70 respectively for the primer WMC 24 and WMC 50 with an average of 0.48 and 0.49 per primer pair (Table III).

According to NEI'S [7], the similarity coefficient between cultivars ranged from 0.33 and 0.90 with an average of 0.63 (Table IV).

TABLE IV
SIMILARITY MATRIX FOR THE SEVEN BREAD WHEAT VARIETIES BASED ON THEIR MICROSATELLITE MARKERS

	AinAbid	Arz	Hidhab	Hammam1	5119	Milan babax	Angi4	Cham6	Attila	Attila2Pastor
Ain Abid	1,000									
Arz	0,609	1,000								
Hidhab	0,560	0,455	1,000							
Hammam1	0,560	0,636	0,667	1,000						
5119	0,640	0,455	0,500	0,667	1,000					
MilanS87230babax	0,500	0,381	0,696	0,522	0,609	1,000				
Angi4	0,583	0,476	0,696	0,609	0,696	0,909	1,000			
Cham6	0,348	0,400	0,364	0,455	0,545	0,476	0,571	1,000		
Attila	0,385	0,435	0,480	0,640	0,560	0,667	0,750	0,696	1,000	
Attila2Pastor	0,720	0,545	0,500	0,333	0,667	0,609	0,696	0,545	0,480	1,000

Most of genotypes showed a high degree of genetic similarity. The lowest genetic distance value of 0.33 has been scored between Hammam1 and Attila/2Pastor. Milan/S87230/babax and Angi4 were the most similar (0.90). The UPGMA among 10 genotypes of bread wheat was generated (Fig. 2).

The consensus tree showed that it divided the wheat genotypes into four main clusters, the first included wheat varieties Ain Abid, Attila/2 Pastor and 5119. The second main cluster was divided into two sub-clusters. The first sub-cluster included wheat varieties Hidhab and Hamam 1. The second one included cultivars

Milan/S87230/babax, Angi-4 and Attila. The third group is formed by chem-6 and the last group is composed by a local genotype Arz.

The dendrogram presented in Fig. 2 demonstrate the ability of microsatellites to detect large amount of genetic diversity in genotypes with expected narrow genetic pool.

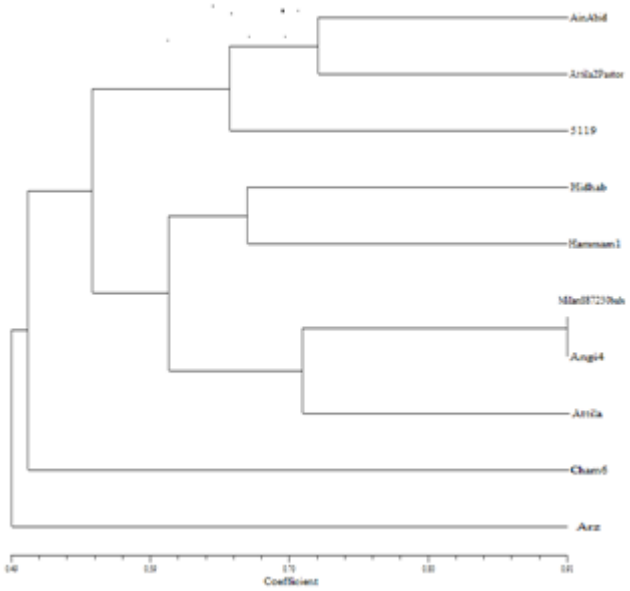

Fig. 2 Dendrogram based on data of 11 microsatellite primers from 10 bread wheat genotypes constructed by UPGMA

The cophenetic coefficient was r = 0.68, indicating that there is good fit between dendrogram clusters and the similarity matrix.

4. Conclusion

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. 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. These findings clearly demonstrate the reliability, usefulness and efficiency of SSRs in analyzing genomic diversity. 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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