

MOLECULAR ANALYSIS BY SSRs MARKERS IN A COLLECTION OF DURUM WHEAT (*Triticum durum* Desf.) SELECTED IN ALGERIA

Yasmina SEMIANI*, Sabrina OUMATA*, Abdelkader BENBELKACEM*, Mohammed SEMIANI**

* National Agronomic Research Institute of Algeria (INRAA), Biotechnology and plant breeding Division, Laboratory of molecular biology, Baraki, Algeries, Algeria.

** National Agronomic Research Institute of Algeria (INRAA), Biotechnology Division, Laboratory of plant physiology, Baraki, Algeries, Algeria
Correspondence author: Semiani Yasmina, National Agronomic Research Institute of Algeria (INRAA), Biotechnology and plant breeding Division, Laboratory of molecular biology, Baraki, Algeries, Algeria, e-mail: semiani_y@yahoo.fr

Abstract. This work aimed to test differentiation among genotypes and search markers that present a detectable polymorphism within 17 genotypes of durum wheat (*Triticum durum* Desf.) selected in Algeria by the use of SSRs.

All of the primer pairs for SSRs markers revealed a total of 88 alleles for all the genotypes, the size varied from 100 to 345 bp, with a number of alleles per microsatellite primers varied between 3 to 10 with an average of 7.16 alleles / locus. Two main groups were identified, group 1: is composed of 5 sub-groups, the first sub-group is composed of TAR and ALTA genotypes, the second is composed only of MIN/COM/DUC genotype, the third sub-group is composed only of SORA genotype, the fourth sub-group is composed of LDN7 and SIG genotypes, as for the fifth sub-group, it is composed only of VT genotype. Group 2: is composed of 7 sub-groups; the first one is composed only of the BM genotype, the second is also composed only of the SH-3 genotype, the third is also composed of a single PLATA genotype. The fourth subgroup is composed of the LLAR and MIN/PLA genotypes, for the fifth group, it is composed only of BCR genotype, the sixth group is composed of two genotypes WH and MIN/COM, the last group is composed of CND and LD357. We note a large differentiation between the genotypes which could be explained by the genetic of wheat (polyploidy) has a very important role in the evolution of plants and creation of large genetic diversity. The relationships between pedigrees and estimates of genetic similarity are not all significant. Genetic distances can be explained by selection pressure and also the parental of ancestors with no known pedigree.

Key words: Durum wheat (*Triticum durum* Desf.); diversity; polymorphism; microsatellites; alleles.

INTRODUCTION

Cereals constitute an important part of the food resources of humans and animals [20]. In addition, most of the food is supplied by grain feed, 96% of which is produced by cereal crops such as wheat, barley, rye, rice, corn and triticale. Durum wheat (*Triticum durum* Desf.) is one of the oldest and most cultivated cereals in the world with more than 17 million hectares and represents the largest import market for the Mediterranean basin. This is due to the great Mediterranean consumption of durum wheat derivatives [15].

In Algeria, durum wheat (*Triticum durum* Desf.) occupies an important social, agronomic and economic place, it takes a very privileged place in households, unfortunately its grain yield remains the lowest in the Mediterranean basin. Bouzerzour [8] explain this low production by the large intra and inter-annual variations in rainfall and temperatures, which negatively affect yields. However, recent studies reveal that low winter and spring temperatures handicap this speculation much more [1].

The water deficit occurs at different stages of the development of wheat and disturbs its growth process. In cereal growing areas, more particularly in highland areas, the water deficit is generally accompanied by winter and / or spring frosts. These stresses exhibit great spatial variability in their severity and duration [12, 19]. In addition to this constraint, there are those related to varieties, which are unstable and inefficient.

Algeria has developed agricultural strategies, among strategies plant improvement which consists in creating a new genotypes. To meet this objective, wild genotypes constitute an important resource of genes

useful for the amelioration of cultivated genotypes and adaptation to environmental constraints.

Several adaptive traits, phenological, morphological and physiological nature, are useful for improvement of tolerance to abiotic stresses [6]. Several tools as morpho-physiological criteria and molecular markers are used to achieve these objectives [7, 9].

In this context, our study was oriented to find polymorphic SSR markers associated with drought resistance for discrimination of 17 cultivars of durum wheat selected from international trials from CIMMYT by the use of 12 primer pairs SSRs.

MATERIALS AND METHODS

Plant material

The plant material used in our study consists of 17 varieties and lines of durum wheat (*Triticum durum* Desf.), from CIMMYT (Mexico), selected in Algeria, 10 individuals/cultivar and the samples were mixed (Table 1).

Microsatellites markers

Our study was carried out at the National Institute of Agronomic Research of Algeria (INRAA). Twelve microsatellite primer pairs were chosen for SSR analysis. The primer characteristics are shown in table 2. The markers chosen are of 3 types; Xgwm or WMS identified according to Roider [10], WMC (Wheat Microsatellite Consortium) selected from the database www.graingenes.org and Xcfa from <http://wheat.pw.usda.gov>.

Table 1. varieties and lines, abbreviations, pedigrees and origins

Code	Varieties and lines	Abbreviations	Pedigrees	Origins
1	LD357E/2*TC60//JO69/3/FGO/4/GTA/5/SRN_1/6/...	LD357	CDSS04Y00755T-0TOPB-12Y-0M-06Y-1M-1Y-0B	Rép1-11
2	SORA/2*PLATA_12/3/SORA/2*PLATA_12//SOMAT_3/4/AJAIA_13/...	SORA	CDSS02B00849T-0TOPB-0Y-0M-7Y-2M-04Y-0B	Rép1-15
3	CND/VEE//CELTA/3/PATA_2/6/ARAM_7//CREX/ALLA/5/ENTE/...	CND	CDSS02B00429S-0M-9Y-06Y-1M-1Y-0B	Rép1-21
4	MINIMUS/COMB DUCK_2//CHAM_3/3/RCOL*2/4/...	MIN/COM	CDSS02B01108T-0TOPB-0Y-0M-5Y-4M-04Y-0B	Rép1-09
5	Beni Mestina	BM	Beni Mestina BM Lahn/Ch1.2003	Témoin
6	MINIMUS_6/PLATA_16//IMMER/3/SOOTY_9/...	MIN/PLA	CDSS02B00396S-0M-4Y-06Y-4M-1Y-0B	Rép1-03
7	PLATA_7//LBOR_1//SOMAT_3/3/CABECA_2/PATKA_4//ZHONG ZUO/...	PLATA	CDSS04Y00053S-13Y-0M-06Y-4M-1Y-0B	Rép1-16
8	MINIMUS/COMB DUCK_2//CHAM_3/3/RCOL*2/4/SOMAT_4/INTER_8	MIN/COM/DUC	CDSS02B01108T-0TOPB-0Y-0M-5Y-4M-04Y-0B	Rép1-19
9	BCRIS/BICUM//LLARETA INIA/3/DUKEM_12/2*RASCON_21/4/...	BCR	CDSS04Y00362S-27Y-0M-06Y-4M-1Y-0B	Rép1-13
10	Sigus	SIG	Ter-1/3/Stj3// Bcr /LKs4	Témoin
11	LDN7D(7A)/3*ASCONCHI/3/SORA/2*PLATA_2*PLATA_12//...	LDN7	CDSS04Y01137T-0TOPB-24Y-0M-06Y-1M-1Y-0B	Rép1-24
12	ALTAR84/STINT//SILVER_45/3/GUANAY/4GREEN_14//YAV_10/...	ALTA	CDSS04Y00341S-11Y-0M-06Y-3M-1Y-0B	Rép1-01
13	TARRO_1/2*YUAN_1//AJAIA_13/YAZI/3/SOMAT_3/PHAX_1//...	TAR	CDSS02B01143T-0TOPB-0Y-0M-7Y-4M-04Y-0B	Rép1-18
14	LLARETA INIA/4/SKEST//HU/TUB/3/SILVER/5/LHNKE/RASCON//...	LLAR	CDSS02B00574S-0M-12Y-06Y-2M-1Y-0B	Rép1-14
15	Waha	WH	Plc/Ruff//Gta/3/ Ritte	Témoin
16	Hoggar	VT	Vitron	Témoin
17	SHAM-3= KORIFLA	SH-3	Témoin	Témoin

Table 2. Characteristics of primers [7, 13, 17, 25]

SSRs locus	Forward primer	Reverse primer	Marker size (pb)	locus	Repeated unit	Temperature (°C)	References
Xgwm251	CAA CTG GTT GCT ACA CAA GCA	GGG ATG TCT GTT CCA TCT TAG	109-110	4B	(CA)28	55	7
Xgwm408	TCG ATT TAT TTG GGC CAC TG	GTA TAA TTC GTT CAC AGC ACG C	148-182	5B	(CA)22(TA)(CA)7(TA)9	55	13
Xgwm11	GGA TAG TCA GAC AAT TCT TGT G	GTG AAT TGT GTC TTG TAT GCT TCC	202-213	1B	(TA)6CATA(CA)19(TA)6	50	13
Xgwm148	GTG AGG CAG CAA GAG AGA AA	CAA AGC TTG ACT CAG ACC AAA	165-167	2B	(CA)22	60	7
Xgwm135	TGT CAA CAT CGT TTT GAA AAG G	ACA CTG TCA ACC TGG CAA TG	153 -176	1A	(GA)20	60	25
Xgwm6	CGT ATC ACC TCC TAG CTA AAC TAG	AGC CTT ATC ATG ACC CTA CCT T	207 – 196	4B	(GA)40	55	17
WMC177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	184	2A	(CGCA)10	55	17
WMC54	TATTGTGCAATCGCAGCATCTC	TGCGACATTGGCAACCACTTCT	142	3B	(GT)8	60	7
WMC63	GTGCTCTGGAACCTTCTACGA	CAGTAGTTTAGCCTTGGTGTGA	192	2A	(GA)12	60	7
WMC167	AGTGGAATGAGGTGAAAGAAG	TCGGTCGTATATGCATGTAAAG	185	2B	(CA)22 87 to 130, (CA)8 535 to 550, (CA)8 553 to 568	55	25
XWMC166	ATAAAGCTGTCTCTTTAGTTG	GTTTAAACACATATGCATACCT	305	7B	(GA)8 347 to 362, (GT)8 297 to 312, (GT)8 327 to 342	50	25
Xcfa2114	ATTGGAAGCCACGATACAC	CCCGTCGGGTTTATCTAGC	209	6A	(CA)32	55	25

DNA extraction and quantification

For DNA extraction, we used the PURELINK PLANT DNA PURIF KIT, K183001, it was carried out on 100 mg of fresh plant material, taken from 12 days old plants. DNA samples were stored at -20 °C.

The extracted DNA samples are placed in wells on agarose gels (1%) in order to check the DNA quality after ultraviolet exposure. For the quantification of DNA is performed by spectrophotometry.

Amplification of microsatellite markers SSRs

DNA was amplified using microsatellites primers. The protocol used for the amplification of SSRs is based on the use of the PCR technique which allows amplifying the nucleic acid sequence in vitro to many thousand copies using a thermal cycler (Gene Amp PCR system 9700, Applied Biosystems). Which be denatured and separated on acrylamide gels 6%. Staining was done by silver nitrate coloration

PCR reactions were performed in a total volume of 25 µL, which consisted of 22.5 µL of MIX PCR, 1 µL of each primer, 1.0U Taq polymerase and 20 ng genomic DNA.

The PCR program used was initiated by 94°C for 5 minutes followed by 35 cycles (94°C, 60°C, 72°C) and ended with the final extension at 72°C for 7 minutes.

Electrophoretic migration and silver nitrate revelation

PCR products were separated on acrylamide gels 6%. At the end of the electrophoretic migration, the revelation of DNA strands was done by silver nitrate coloration (a fixing solution, a silver nitrate solution and two developing solutions).

Data analysis

Gels treatment

Gels analysis was carried out at two levels. The first level consisted of identifying the presence or absence of the desired amplicon in all genotypes. For positive results, the allele dosage was scored for each individual and each marker, each allele being present in 1-4 doses. In the case where a sample has four alleles, each one was coded by single dose, when we have only one allele, this one was in four dose. If we have two alleles, each one was coded by double doses.

Polymorphism analysis and genetic diversity

After the binary coding of the gels (1:0) presence or absence of the desired amplicons, a database was recorded and the allele frequency was calculated with "GENE 4X", version 1.0, 1997 software [22, 24].

Genetics similarity between cultivars

Genetic similarities, between each pair of genotypes, were calculated using Darwin 6 (simple matching) version 6.1.

A dendrogram was constructed with Mega7 program, that defines groups of related genotypes.

RESULTS

Diversity of genotypes

SSRs markers shows polymorphism for some microsatellite markers and absence for others.

All of the 12 SSRs primers revealed in total the presence of 88 alleles for all the genotypes, the size varied from 100 to 345 bp, with a number of alleles per microsatellite locus varied between 3 to 10 with an average of 7.16 alleles / locus (Table 3).

The number of alleles for all the loci for each genotype varied from 15 alleles for genotype CND to 25 alleles for genotypes MIN / COM / DUC and SH-3. Number of individuals corresponding to each allele and each marker is represented in the Table 3.

Allele composition of each genotype is shown in Fig. 1. For a given genotype each color corresponds to an allelic level. The most frequent allele for each locus is shown in red color; the other alleles are represented by other colors as shown in Fig.1. The null allele is represented without color. In general, an allelic homogeneity is noted for SORA, SIG, LDN7 and VT cultivars. For the other genotypes (LD357, CND, MIN / COM, BM, MIN / PLA, PLATA, MIN / COM / DUC, BCR, ALTA, TAR, LLAR, WH and SH-3), the colors are much more variable (Fig. 1).

Genetic similarity

A genetic matrix similarity was calculated, according to the method of Nei and Li [16], between each pair of the 17 genotypes for all the microsatellite markers. The minimum similarity value was noted between the MIN/PLA and LLAR lines (0.29) while the maximum (0.96) was observed between 2 pairs of

Table 3. Number of alleles detected, size of the different alleles and number of individuals corresponding to each allele

Loci	Number of alleles	Size (pb)	Alleles									
			A	B	C	D	E	F	G	H	I	J
WMC167	8	130-240	1	2	10	1	9	4	1	2		
WMC177	7	170-240	1	1	5	10	1	10	6			
Xgwm11	7	170-260	4	4	1	1	5	3	2			
Xgwm6	10	270-150	2	1	5	1	3	6	5	2	1	1
WMC63	9	150-235	1	1	17	2	17	1	1	1	1	
Xgwm408	8	135-250	1	8	1	1	2	9	2	6		
Xgwm135	6	115-175	8	2	1	1	2	11				
WMC54	10	130-270	1	1	1	3	5	1	8	2	2	3
Xgwm251	6	100-195	1	1	1	2	8	2				
Xgwm148	6	130-180	9	3	3	1	8	7				
Xcfa2114	3	200-260	13	3	7							
WMC166	8	240-345	1	7	1	2	7	4	5			

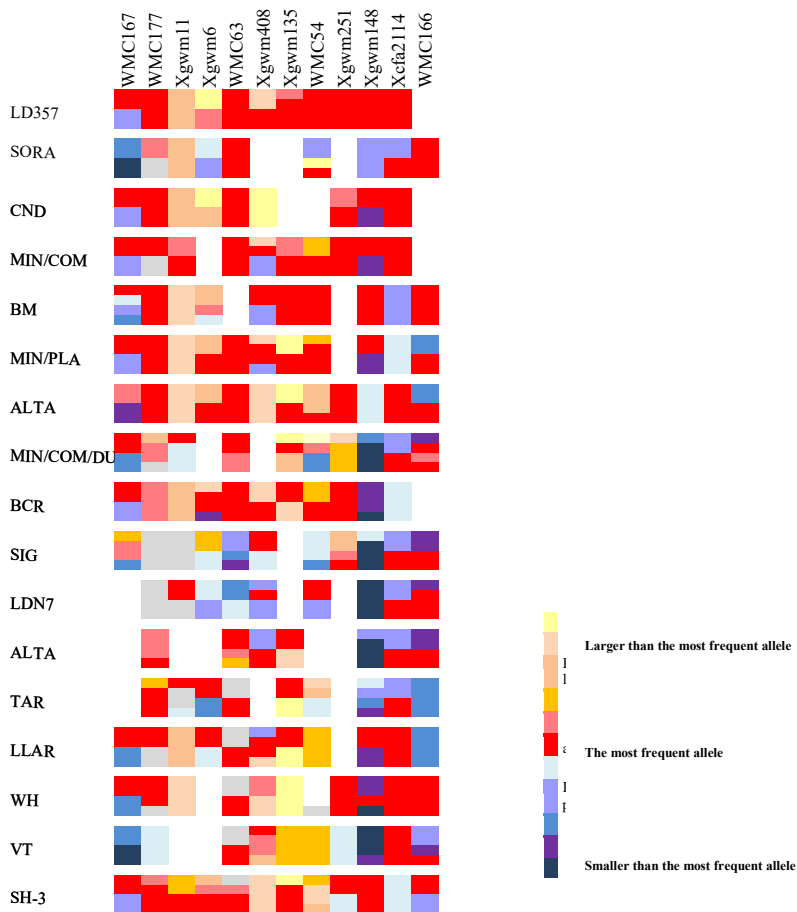


Figure 1. Allele composition of 17 wheat genotypes

genotypes; the MIN/PLA genotype with the SIG genotype and the BM genotype with VT genotype (Table 4).

Genetic structure of the cultivars and cluster analysis

From the genetic similarity matrix, we performed a cluster analysis according to the UPGMA algorithm for the 17 genotypes. The dendrogram resulting from this analysis shows the presence of genetic diversity in our studied germplasm (Fig. 2).

By dividing this dendrogram at 0.37, we have identified two main groups. This measuring point was chosen subjectively.

Group 1: is composed of 5 sub-groups, the first sub-group is composed of TAR and ALTA cultivars, the second is composed only of MIN/COM/DUC cultivar, the third sub-group is composed only of SORA cultivar, the fourth sub-group group is composed of LDN7 and SIG cultivars, as for the fifth subgroup, it is composed only of VT cultivar.

Table 4. Genetic distance matrix between each pair of the genotypes

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
2	0.81	1														
3	0.42	0.71	1													
4	0.4	0.85	0.48	1												
5	0.56	0.81	0.77	0.69	1											
6	0.65	0.81	0.65	0.46	0.46	1										
7	0.6	0.85	0.67	0.56	0.69	0.48	1									
8	0.9	0.65	0.88	0.73	0.85	0.85	0.85	1								
9	0.46	0.77	0.58	0.42	0.81	0.46	0.65	0.81	1							
10	0.9	0.67	0.83	0.92	0.88	0.96	0.88	0.73	0.92	1						
11	0.9	0.5	0.88	0.83	0.75	0.83	0.92	0.75	0.92	0.5	1					
12	0.81	0.69	0.81	0.67	0.71	0.69	0.79	0.63	0.75	0.77	0.58	1				
13	0.81	0.65	0.79	0.73	0.77	0.63	0.65	0.71	0.79	0.81	0.67	0.6	1			
14	0.5	0.65	0.52	0.42	0.63	0.29	0.58	0.81	0.48	0.85	0.77	0.67	0.54	1		
15	0.58	0.81	0.5	0.4	0.77	0.56	0.52	0.73	0.6	0.88	0.9	0.65	0.77	0.56	1	
16	0.85	0.73	0.79	0.65	0.96	0.79	0.83	0.73	0.77	0.79	0.81	0.69	0.83	0.67	0.65	1
17	0.6	0.9	0.71	0.58	0.65	0.46	0.6	0.79	0.56	0.98	0.92	0.85	0.75	0.6	0.69	0.81

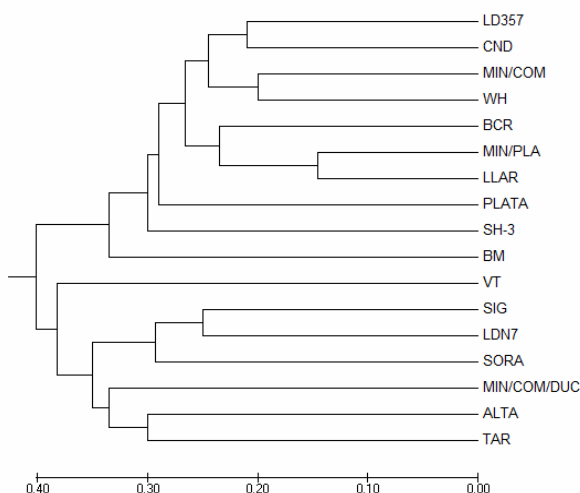


Figure 2. Dendrogram obtained from a UPGMA cluster analysis based on SSRs markers

Group 2: is composed of 7 sub-groups; the first is composed only of the BM cultivar, the second is also composed only of the SH-3 cultivar, the third is also composed of a single PLATA genotype. The fourth subgroup is composed of the LLAR and MIN/PLA genotypes, for the fifth group, it is composed only of BCR cultivar, the sixth group is composed of two genotypes WH and MIN/COM, the last group is composed of CND and LD357.

DISCUSSION

Estimation of genetic diversity present in gene pools is an important determination for breeding programs. We note the presence of a differentiation between the cultivars which could be explained by the number of valuable genes introduced into our germplasm during the genetic improvement which was carried out on these cultivars to improve and establish the desired character which is in our study tolerance to drought. This situation could also be explained by the genetic structure of wheat (tetraploid) which promotes the maintenance of large diversity [10-12, 23].

The gathering of the majority of cultivars in the first group as shown in Fig.2, could be explained by the presence of several physiological and morphological characters in common [5].

In addition some cultivars are related by their pedigrees, cultivars with a family relationship are clustered together [14]. A similarity of 81% between the two cultivars MIN / PLA and SORA is explained by the fact that they have the same first parent of the genetic cross. BCR is a direct parent of SIG, which explains a 92% similarity between cultivars SIG and BCR.

Sora and CND cultivars have a similarity rate of 71%. A similarity rate of 81% is observed between cultivars LD357 and ALTA, which can be explained by the parental origin.

The relationships between pedigrees and genetic distances are not all significant. However, weak

correlations between the parent link and genetic similarity based on molecular markers have also been reported by other authors [3, 4, 14, 18]). These estimates can be explained by selection pressure and also the parental of ancestors with no known pedigree.

Bohn [13] pointed out that the bias generated in the wheat segregation process is based on the phenotypic similarity to the ancestors parents. However, DNA markers have the advantage of directly detecting sequence variation between cultivars and this is not influenced by assumptions inherent in pedigree analysis [21].

The information obtained at the phenotypic level is often difficult to interpret, because it is continuous variations where many genes can be involved. Genetic markers whose expression is independent of the environment were used to characterize populations and evaluates their genetic diversity at the intra- and inter-populations levels.

The wheat breeding program is therefore based on the selection of parental genotypes before crossing according to their genetic distances estimated by variations in molecular markers. Crossbreeding between genotypes genetically distant, increases genetic diversity in a germplasm. This is important in a crossing table especially for the improvement of a multigenic character.

Genetic improvement will be facilitated in wheat if knowledge is acquired on the genetic determinism of traits of agronomic interest. Among the new methodologies making it possible to study such traits, the use of phenotype / genotype association methods at the population level, would be an interesting way for the exploitation of the physical link between markers and localizations of potential QTL involved in agronomic traits [2].

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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