ASSESSING GENETIC DIVERSITY OF SOME BARLEY (*Hordeum vulgare* L.) CULTIVATED IN ALGERIA BY MICROSATELLITE MARKERS

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Abstract. The study consists to evaluate the genetic variation of eight barley genotypes (*Hordeum vulgare* L.) from the breeding program of the Setif research station of the Technical Institute of Field Crops, using 11 SSR microsatellite markers. Genotypes used in this study were the subject of an agronomic evaluation and a study on their performance and their adaptation to diverse locations in Algeria. Thirty-one reproducible bands were detected of which 29 bands (93.5%) were polymorphic. The number of bands varies between 2 and 5, with an average number of 2.81 bands per primer. Polymorphic information content (PIC) values range from 0.21 to 0.71 with an average value of 0.46. The index of genetic dissimilarity between the studied genotypes varies between 0.313 and 0.818, with an average of 0.604. The greatest genetic similarity was recorded between Fouara and Rihane (0.313), while the lowest genetic similarity was observed between Dahbia and Tichedrett (0.818). SSR markers used showed a large differentiation among the different barley genotypes. This confirms the high capacity of SSR markers in genetic analysis to determine the genetic similarity of barley genotypes.

Key words: dissimilarity; barley (*Hordeum vulgare* L.); genetic variation; SSR markers.

INTRODUCTION

Barley (*Hordeum vulgare* L.) has a long history as a domesticated crop, as one of the first to be adopted for cultivation. The migration of people together with their seed crop led to a major diversification and adaptation to new area, and the crop is now found worldwide [5]. Barley possesses some special properties that enable it to adapt desirably to different unfavorable climatic conditions compared to other crops, ranging from dry land conditions to arctic regions of the earth with longer winter period and reduced sunlight on different continents [28]. It is a short-season, early maturing annual grain crop with some degree of tolerance to drought and salinity [8]. In Algeria, its cultivation is part of extensive systems associating cereal farming with sheep farming where it plays an important role in the precarious balance in the economy of small farms [7].

Greater allelic diversity has been detected in the wild than in cultivated barley [19]. Breeding of new barley lines with high yield potential and more stable production involves the use of adapted plant material, using traits that are strongly related to yield and stability. Certain local varieties or wild species represent a large source of variation for morpho-physiological characteristics likely to contribute to the stability of the yield [11].

Traditionally, morphological traits, cytological characters, biochemical tests, and pedigree information are used to assess genetic diversity and classify barley germplasm [26]. Various molecular marker techniques have been developed into powerful tools for diversity analysis and establishing relationships between cultivars [25]. These molecular markers are more interesting in selection since they are relatively little influenced by environmental fluctuations [12]. Among these markers, microsatellite markers (SSR-Simple Sequence Repeats) are often used for genetic mapping and population studies [16]. These are sequences of a few nucleotides (1 to 6) repeated in tandem [2]. They are locus-specific, multi-allelic, follow a codominant mode of inheritance and are a robust marker system, which can be easily exchanged between laboratories [14].

The objective of this study was to assess the genetic variations in eight genotypes of barley (*Hordeum vulgare* L.) grown in Algeria using SSR molecular markers.

MATERIALS AND METHODS

Plant materials

Eight barley genotypes from the Setif Agricultural Research Station breeding program of the Field Crops Technical Institute were included in this study to evaluate polymorphism degree. These genotypes have been subject to an agronomic evaluation in order to identify high-yielding and stable genotypes with good or specific adaptation [17].

Acsad$_{176}$. Six-row barley, selected by Tiaret Station of Field Crops Technical Institute (Algeria), comes from the Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD, Syria).

Hamra (Barberousse). Six-row barley variety comes from INRA France, introduced in Algeria in 1985 by the Ministry of Agriculture and Rural Development. It is sensitive to the photoperiod and whose vegetation does not become very active until late.

Dahbia (Jaidor). Six-row barley variety comes from INRA France, introduced in Algeria by the Ministry of Agriculture and Rural Development. Dahbia is sensitive to spring frost. Issued from the cross: Rika/ Baladi$_{16}$/Robur.

Fouara. Six-row barley variety, is a selection of Setif station of Field Crops Technical Institute (Algeria), issued from plant material of International

Center for Agricultural Research in the Dry Areas (ICARDA, Syria). Issued from the cross: Deir alla 106/Strain 205//Gerbel. ICB85. 1376. 0AP. 1AP. 2AP.

Rihane. Six-row barley, Tunisian improved variety, issued from the breeding program of International Center for Agricultural Research in the Dry Areas (Syria). It is moderately tolerant to drought. Issued from the cross: AS46/AVT11 ATHS21-1AP-3AP-0AP.

Saida 183. Six-row barley variety, strain resulting from genealogical selection made within Algerian barley populations by Khroub station of the Field Crops Technical Institute (Algeria). Saïda 183 is very suitable for the west of the Algerian highlands.

Tichedrett. Six-row barley, local improved variety, selected from Algerian populations by Setif station of the Field Crops Technical Institute (Algeria). This variety is well adapted to the east of the Algerian highlands. Tichedrett is of the half-winter type, tolerant of winter cold and responding to vernalization. Issued from the cross: Rebelle C5 95203 SF4 N°21 1998/99.

Soufara. Tow-row barley, local improved variety, issued from the breeding program of International Center for Agricultural Research in the Dry Areas (ICARDA, Syria).

DNA extraction and amplification

DNA of eight genotypes was extracted and purified from young leaves of three weeks old plants in full growth according to the method of Cetyl Trimethyl Ammonium Bromide (CTAB) [24]. The DNA was purified by RNAse treatment to achieve a good quality DNA yield. DNA quantity and quality were verified on 0.8% agarose gel. DNA samples were stored at -20° C.

DNA was amplified using 11 microsatellite primers described by Ramsay *et al.* [22] (Table 1). PCR reactions were performed a total volume of 10 µl and consisted of 20 ng genomic DNA, 1 X PCR buffer, 0.3 µl Taq polymerase, 0.3 µM of forward and reverse primers, 200 µM dNTPs and 0.5 µCi of (α-32P) of dCTP. After PCR equal volumes of electrophoresis charge buffer containing 95% formamide were added to the samples, which were then denatured at 95 °C, then cooled and subjected to electrophoresis in 6% of the polyacrylamide gel.

Data Analysis

For dissimilarity analysis, each variant fragment of the microsatellite was considered an allele. Allele presence is represented by (1), while the absence by (0). The genetic dissimilarity between genotypes was calculated from the Jaccard coefficient. Based on the dissimilarity matrix, a dendrogram showing the genetic relationships between genotypes was constructed by the un-weighted pair group method using arithmetic average (UPGMA) using Darwin 6.0 software. In addition, a principal coordinate analysis (PCoA) was carried out in order to group the genotypes studied. Polymorphism information content (PIC) according to Nei (1973) [20]: $PIC = 1 - \Sigma P_i^2$ (Pi is the frequency of the i^{th} SSR allele) was calculated.

RESULTS

In this study, we have evaluated 11 SSR markers in eight barley genotypes. A total of 31 bands were obtained of which 29 bands (93.5%) were polymorphic. The null allele was not observed. The number of alleles varied from 2 to 5 with a mean of 2.81 per locus. The size of the alleles varies between 127 and 208 base pairs.

The PIC values ranged from 0.21 (HVM4) to 0.71 (Bmac0316) with an average value of 0.46. According to the criteria proposed by Botstein *et al.* (1980) [6], six markers were highly informative (PIC > 0.5), two were reasonably informative (0.5 > PIC > 0.25) and three were only slightly informative (Table 2).

Table 1. Primer sequences, expected fragment size and chromosomal location [22]

SSR locus	Primer sequence (5'- 3')	chromosome	Repeat motif	Expected product size (bp)
Bmac0113	TCAAAAGCCGGTCTAATGCT GTGCAAAGAAAATGCACAGATAG	5H	(AT)7(AC)18	187
Bmac0306	CCTTGTGTGAGTGTGTGTG ACATGCACATGAACTAATCAA	5H	(AC)10-(AC)5	127
Bmac0316	ATGGTAGAGGTCCCAACTG ATCACTGCTGTGCCTAGC	6H	(AC)19	135
Bmag021	ATTTTTATCAACGTCTCTC CTAACTTCTCTCTCCCTCTCC	7H	(CA)10AA(GA°28	143
Bmag0211	ATTCATCGATCTTGTATTAGTCC ACATCATGTCGATCAAAGC	1H	(CT)16	174
Bmag0219	ATATTTATGAAACGGTGAAGC GGGTTTATCCTCTGGTCC	6H	(AG)5GG(AG)14	181
Bmag0579	CCTAGATAAGGAACATAGCCA CAAAGACCCTAACTCATGTTC	1H	(AC)6(AG)15	126
EBmac0906	CAAATCAATCAAGAGGCC TTTGAAGTGAGACATTTCCA	4H	(GC)5GGG(GT)16	153
HVM4	AGAGCAACTACCAGTCCAATGGCA GTCGAAGGAGAAGCGGCCCTGGTA	7H	(AT)9	198
HVM14	CGATCAAGGACATTTGGGTAAT AACTCTTCGGGTTCAACCAATA	6H	(CA)11	158
HVM30	AGTGGGGAATGAGAGAATGG TGCTTGTGGGTCATCACAC'	5H	(AC)8	150

Table 2. Description of eleven barley microsatellite, the size range of alleles, total number of bands, polymorphic bands and polymorphic information content (PIC)

SSR	Allele size range	Total number of bands	Polymorphic Bands	PIC
Bmac0113	183-190	2	2	0.42
Bmac0306	127-144	3	3	0.67
Bmac0316	145-160	5	5	0.71
Bmag021	146-152	2	2	0.35
Bmag0211	175-179	2	2	0.22
Bmag0219	172-185	2	2	0.21
Bmag0579	160-199	3	2	0.56
EBmac0906	163-172	4	4	0.65
HVM4	194-220	2	2	0.22
HVM14	171-180	2	2	0.50
HVM30	165-175	4	3	0.64
Total		**31**	**29**	
Mean		2.81	2.63	0.46

Table 3. Genetic dissimilarity among barley genotypes based on the Jaccard index

Genotype	Acsad	Dahbia	Fouara	Hamra	Rihane	Saida	Soufara	Tichedrett
Acsad 176	0							
Dahbia	0.800	0						
Fouara	0.412	0.783	0					
Hamra	0.588	0.810	0.538	0				
Rihane	0.375	0.714	0.313	0.400	0			
Saida	0.684	0.700	0.556	0.357	0.629	0		
Soufara	0.722	0.688	0.667	0.688	0.663	0.625	0	
Tichedrett	0.750	0.818	0.632	0.357	0.633	0.380	0.722	0

A significant genetic variation was found among studied barley genotypes. The dissimilarity matrix obtained shows that the genetic dissimilarity coefficients vary from 0.313 to 0.818 with a mean of 0.604. The highest genetic similarity was recorded between Fouara and Rihane (0.313), and the lowest genetic similarity was between Dahbia and Tichedrett (0.818) (Table 3).

In order to assess the clustering of barley genotypes, a dendrogram was developed from a dissimilarity matrix. Dendrogram obtained discriminated all the genotypes and they were grouped separately into two principal groups (Fig. 1). The first group gathered the genotypes Hamra, Saida and Tichedret. The second group was formed by Acsad176, Fouara and Rihane genotypes. The two first axes obtained by principal coordinate analysis (Fig. 2) absorb 53.85 % of the total variance, with 37.15 % of the information for the first axis and 21.64 % for the second axis. The analysis revealed distinct grouping for genotypes from the different geographical origins. The PCoA results were in agreement with those of the dendrogram, where the first group included the three genotypes Acsad 176, Fouara and Rihane and the second group consisted of the three genotypes Hamra, Saida and Tichedret. However, the two Dahbia and Soufara are widely dispersed.

DISCUSSION

Evaluation of the genetic diversity of barley accessions can be an important source of information to discover and exploit novel alleles that could be used in breeding programs Ferreira *et al.*[10]. Thirty-one alleles were detected with 11 SSR loci. The average allele number varied from 2 to 5 with a mean of 2.81 per locus. In 13 local barley accessions using 15 SSR markers, Abdellaoui *et al.* [1] recorded 43 bands with an average of 2.87 bands per marker. In barley from North Africa, 478 bands were obtained with an average of 2.13 bands per marker [3]. In the others studies, the number of alleles was higher, Yahiaoui *et al.* [27]

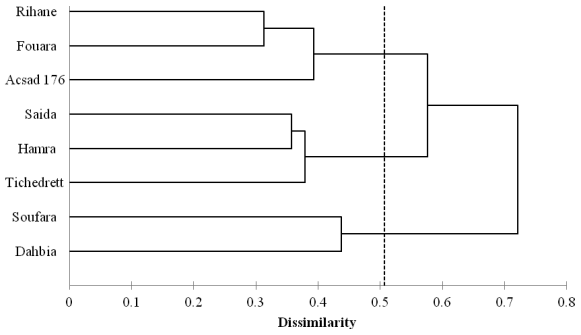

Figure 1. Dendrogram based on the UPGMA method, showing the relationships among eight barley genotypes.

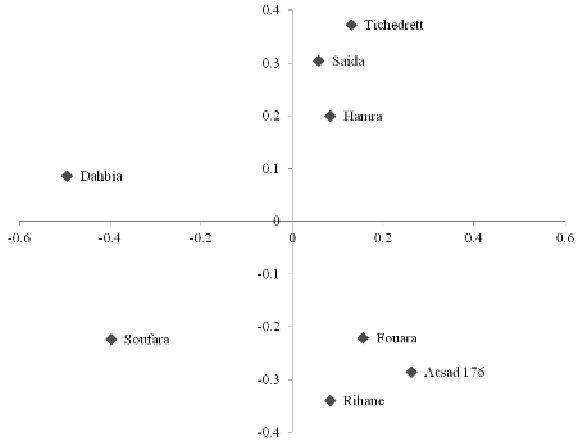

Figure 2. Principal coordinate analysis (PCoA) based on the genetic distance of eight barley genotypes.

obtained 669 alleles with an average of 10.5 alleles in Spanish barley genotypes. According to Nevimova *et al.* [21], the total number of alleles and the average number by locus depend on the number of genotypes, including the diversity of their origin, as well as the number of SSR markers.

Wide variation was found among the polymorphic loci. PIC values ranged from 0.21 to 0.71 with an average value of 0.46. In previous studies, different PIC values were obtained in barley, Monawekh *et al.* [18] reported PIC values ranging from 0.28 to 0.96 with a mean value of 0.65. Yahiaoui *et al.* [27] indicated that in Spanish barley genotypes, the mean PIC value was 0.62 in six-row barley and 0.54 in two-row barley. Ferreira *et al.*[10] have detected lower PIC among barley genotypes bred in Brazil compared with foreign and wild genotypes.

In this study, barley genotypes were grouped according to their geographical origin. Our results are in agreement with those obtained by Bchinin *et al*. [4] and El-Awady *et al.* [9], who observed a clear differentioation between groupes of barley cultivars according to their geographic origine. In other studies, Gougerdchi *et al*. [13], Monawekh *et al.* [18] and Rustgi *et al.* [23] reported in their study that barley genotypes were grouped according to their pedigree, but the Tunisian barley accessions were classified according to climatic stage and some morphological traits especially ear attitude, ear density and sterile spikelet attitude [15]. In the present study, clustering of Saida and Tichedret genotypes in the same group would probably be due to geographic origin from the selection of Algerian populations which allow them an expression of adaptive properties towards local environmental conditions. However, Hamra (Barbarossa) was also clustered with the local genotypes Saida and Tichedret, this may have been due to it good adaptation to Mediterranean conditions. The other group gathered genotypes originating from Syria. Acsad 176 comes from ACSAD, while Fouara and Rihane are issued from the breeding program of ICARDA.

In conclusion, the results obtained showed a large genetic variation among the barley genotypes includes in this study. High genetic similarity was observed in Saida, Tichedrett and Hamra genotypes, as well as in Acsad 176, Fouara and Rihane genotypes. This confirms the great capacity of SSR markers to determine the genetic similarity and they are more efficiente to distinguish between the genetically related genotypes in barley. In order to understand the similarities and dissimilarities existing among different local barley genotypes, studies will be carried out by combining morphological characters and genetic markers in local barley genotypes in order to exploit the existing genetic variability in future breeding programs for barley improvement in Algeria.

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

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