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ARTICLE

Genetic diversity analysis of North Africa's barley using SSR markers

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Geographical origin

Abstract It was demonstrated that some North Africa barley accessions have diverse tolerance sources for abiotic stresses and a good nutritional quality, but the studies done were incomplete since they were realized separately in each country apart.

To implement a more complete analysis, 31 barley accessions originated from North Africa (Algeria, Tunisia and Egypt) were analyzed using 11 SSR markers selected from the seven barley linkage groups for studying the genetic diversity among these chosen barley accessions.

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Over the 11 SSR markers, a total of 478 reproducible bands were scored with an average of 2.13 alleles/primer and the average polymorphism information content of 0.5.

Genetic distance analysis of the 31 accessions showed a large genetic diversity and high number of different groups. The most accessions are clustered according to their eco-geographical origin, according to their pedigree and agronomic characters or according to the caryopsis character (hulled or naked caryopsis). This high number of obtained groups is an invaluable aid in crop improvement strategies and confirms the opinion suggesting that North Africa could be a secondary center of origin of barley. The various growing conditions and the multiple uses of barley in each country may be the cause of the large variability of the barley germplasm in each region.

1. Introduction

Barley is one of the oldest cultivated crops in the world. It is the fourth most important cereal crop. It is used as feed of livestock, as human food and as malts or cosmetic product.

The genetic variation which exists in barley germplasm worldwide is a consequence of many evolutionary pressures which have modified the barley gene pool. The domestication of wild barley in various geographical areas could explain the highly diverse forms of barley which are subjected, for a long time, to a new environmental pressures and leads to development of geographical races [26].

According to Badr et al. [5], the wild progenitor of Barley (*Hordeum spontaneum*) is still colonizing its primary habitats in the Fertile Crescent, central Asia including Afghanistan and the Himalayan region. This barley is also reported in Greece, Egypt, southwestern Asia, Ethiopia, Somalia, Eritrea and North Africa (Morocco, Algeria, and Tunisia) [12,13].

The variation of growing conditions of barley worldwide and its multiple uses have resulted in a large variability of the local barley germplasm.

In Tunisia, as well as in the other North Africa countries, barley is one of the most important cereal crop cultivated and occupies between 34% and 38% of the cereal cultivated area.

According to Abbas et al. [1], knowledge about any germplasm diversity and genetic relationships could be an invaluable aid in crop improvement strategies. A number of methods are currently available for analysis of genetic diversity in germplasm accessions [1].

Thus, criteria for genetic diversity estimation can be different: pedigree records, morphological traits, biochemical markers and molecular markers [19]. Diversity in barley breeding program based on morphological traits and pedigree information was implemented by many authors in the world [2,15]. They showed that grain yield is an ultimate product of the action and interaction of number of components such as number of tillers, number of grains per spike, 1000-grain weight, plant height, harvest index and so on.

Biochemical markers also are key tools in the evaluation of genetic variability in both natural populations and germplasm accessions. As example, storage protein (Hordein and glutenin) has a great inter-genotypic variation, and has been used as marker in cultivar identification, genetic diversity studies, determination of phylogenic origins [11] and in covered and hulless barley [28,18].

In the other hand, molecular markers have been used as a valuable tool in the characterization and evaluation of genetic diversity within and between species and population. The advent of the polymerase chain reaction (PCR) favoured the development of different molecular techniques such as RAPD, simple sequence repeats (SSR), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP) and inter-simple sequence repeat polymorphic DNA (ISSR), and so on. These molecular markers had been used in genotype identification, genetic mapping and in genes differentially expressed [27,20,25,31].

Among different types of molecular markers available for barley, microsatellite or simple sequence repeats (SSRs) have proven to be the markers of choice for marker-assisted selection (MAS) in breeding and genetic diversity studies. The value of microsatellite markers for both genetic diversity studies and for barley breeding was demonstrated as early as 1994 [34,37,29,39,23].

Studying North Africa barley accessions on the morphological and molecular level will be helpful for understanding their genetic diversity, for managing their conservation and their effective utilization in breeding programs.

In the last few years, studies on agronomic traits [7], isozymes [11] and molecular level ([8,21,14] showed that North Africa barley accessions has diverse tolerance sources for abiotic stresses and a good nutritional quality [16]. Unfortunately, these studies have been performed but in a fragmentary level.

Microsatellites or simple sequence repeats (SSRs) is able to exhibit high level of polymorphisms within and between species and populations. In this context, a total of 31 accessions of North Africa barley gathered from Algeria, Tunisia and Egypt using 11 SSR markers have been analyzed in this study. Barley germplasm was also characterized for morphological and agronomic traits and relationship between genetic similarities based on SSR markers and agronomic traits was developed.

2. Material and methods

2.1. Plant materials

Thirty one six rows barely accessions supplied by the National Agronomic Research Institute of Tunisia (INRAT), the National Agronomic Research Institute of Algeria (INRAA) and the National Research Centre of Cairo-Egypt (NRC), were used in this study (Table 1).

Table 1 Description of plant material used in this study.

No.	Genotype	Description of agronomic characters
V1	Tozeur-1	Six rows, Tunisian local barley accession, collected in 2000, precocious, productive in the favorable conditions and tolerant to salinity and fungi diseases
V2	Tozeur-2	Six rows, Tunisian local barley accession, collected in 2000, less precocious and less productive than Tozeur-1 but more tolerant to salinity and fungi diseases
V3	Djerba	Six rows, Tunisian local barley accession, collected in 1983, precocious, productive and tolerant to fungi diseases
V4	Kairouan	Six rows, Tunisian local barley accession, collected in 1983, moderately precocious, moderately productive and tolerant to fungi diseases
V5	Manel	Six rows, Tunisian barley improved variety, registered in 1983, moderately precocious, productive and tolerant to fungi diseases
V6	Gabès	Six rows, Tunisian local barley accession, collected in 1983, moderately precocious, moderately productive and tolerant to fungi diseases
V7	Rihane	Six rows, Tunisian barley improved variety, registered in 1987, moderately precocious and tolerant to drought and fungi diseases.
V8	Sidi-Bouzid	Six rows, Tunisian local barley accession, collected in 2000, late and moderately productive in the favorable conditions
V9	Kébilli-2	Six rows, Tunisian local barley accession, collected in 2000, highly tolerant to salinity but moderately sensitive to fungi diseases
V10	*Tombari	Six rows, Tunisian naked barley accession, collected in 2000, late and moderately productive in the favorable conditions
V11	Témacine	Six rows, Algerian local barley accession, collected in Tougourt desert, late and moderately productive in the favorable conditions
V12	Ksar Megrine	Six rows, Algerian local barley accession, collected in Tougourt desert, late, moderately productive in the favorable conditions and tolerant to fungi diseases
V13	Rihane-3	Six rows, Algerian barley improved variety, moderately tolerant to drought, issued from the cross: AS 46//AVT 11 ATHS 2L-1AP-3AP-OAP, realized at ICARDA [4]
V15	Techedrett	Six rows, Algerian barley improved variety, moderately tolerant to drought, issued from the cross: C95203S F4N°1998/99, realized at Technique Institute of Grande Culture (ITGC). This variety is late, tolerant to drought and frost but sensitive to fungi diseases
V16	Azrir	Six rows, Algerian local barley accession, collected in Adrar/Touat desert, late, productive in the favorable conditions and sensitive to fungi diseases
V17	Saida	Six rows, Algerian local barley accession, collected in the Adrar/Touat desert, late, fairly productive in the favorable conditions and tolerant to fungi diseases
V18	Sidi Mehdi	Six rows, Algerian local barley accession, collected in Adrar/Touat desert, late, productive in the favorable conditions but sensitive to fungi diseases
V19	Ras El Mouche	Six rows, Algerian local barley accession, collected in Adrar/Touat desert, moderately precocious, fairly productive in the favorable conditions and sensitive to fungi diseases
V20	Neïlia	Six rows, Algerian barley improved variety, issued from the cross: CMB 72-189-3Y-IB-2Y-1BX1Y-OB, realized at ICARDA, precocious, tolerant to drought and to fungi diseases but sensitive to frost
V21	Giza 123	Six rows, Egyptian barley variety, precocious, moderately productive in the favorable conditions and tolerant to salinity and fungi diseases. It is issued from the cross of: Giza 117 /FAO86 [35]
V22	Giza 127	Six rows, Egyptian barley accession, precocious, moderately productive in the favorable conditions and tolerant to fungi diseases
V23	*Giza 130	Six rows, Egyptian naked barley accession, precocious, moderately productive in the favorable conditions and tolerant to drought and fungi diseases. It has been selected from the crosses "Comp.cross" 229//Bco.Mr./DZ02391/3/ Deir Alla 106 using the bulk method [17]
V24	El Arich	Six rows, Egyptian barley accession collected in North Sinai (Egypt) in 2005, moderately precocious, productive in the favorable conditions and tolerant to fungi diseases
V25	Ksar	Six rows, Egyptian barley accession, precocious, productive in the favorable conditions and tolerant to drought and fungi diseases
V26	Giza 2000	Six rows, Egyptian barley variety, late, productive in the favorable conditions, tolerant to salinity and to fungi diseases. It is issued from the following cross: Giza 117/Bahteem52//Giza118/FAO 86* Giza 121 [4]
V27	*Giza 129	Six rows, Egyptian naked barley accession, late, moderately productive in the favorable conditions and tolerant to drought and fungi diseases
V28	Giza 126	Six rows, Egyptian barley accession, late, productive in the favorable conditions and tolerant to drought and fungi diseases. It is issued from the following cross: Baladi Bahteem/SD 729 Por 12769-BC [3,35]
V29	Giza 125	Six rows, Egyptian barley accession, late, productive in the favorable conditions and tolerant to drought and fungi diseases
V30	Giza 131	Six rows, Egyptian barley accession, moderately precocious, productive in the favorable conditions and tolerant to drought and fungi diseases. It is issued from the following cross: CM67-B/CENTENO//CAMB/3/ ROW906.73/4/ GLORIAEAR/COM E -B/5/FALCON-BAR/6/LINO [41].
V31	Early 1	Six rows, Egyptian barley accession, extremely precocious, productive in the favorable conditions and tolerant to fungi diseases and to drought since it finishes his development cycle quickly before the arrival of drought of end cycle
V32	Early 2	Six rows, Egyptian barley accession, extremely precocious, productive in the favorable conditions and tolerant to fungi diseases and drought since it finishes his development cycle quickly before the arrival of drought of end cycle

Table 2 Barley simple sequence repeat (SSR) markers, the number of amplified fragments and the polymorphic information content (PIC).

	Oligo name	Seq	Length	T_m		Total amplified fragment	Polymorphic fragment	PIC
1	MGB391:F	AGCTCCTTTCCTCCCTTCC	19	53.6	2(2H) (http://wheat.pw.usda.gov/ggpages/bgn/34/KP1_6.htm)	2	0.58	0.5
	MGB391:R	CCAACATCTCCTCCTCCTGA	20	53.6				
2	EBmac624:F	AAAAGCATTCAACTTCATAAGA	22	47.9	6(6H) (http://wheat.pw.usda.gov/ggpages/bgn/34/KP1_6.htm)	2	0.5	0.48
	EBmac624:R	CAACGCCATCACGTAATA	18	47.0				
3	MGB357:F	GCTCCAGGGCTCCTCTTC	18	53.3	7(5H) (http://wheat.pw.usda.gov/ggpages/bgn/34/KP1_6.htm)	2	0.5	0.27
	MGB357:R	AGCTCTCTCTGCACGTCCTT	20	52.9				
4	MGB402:F	GCTCCAGGGCTCCTCTTC	18	60	5(1H) (http://wheat.pw.usda.gov/ggpages/bgn/34/KP1_6.htm)	4	0.25	0.58
	MGB402:R	AGCTCTCTCTGCACGTCCTT	20	62				
5	VITR1:F	CCACTTGCCAAACACTAGACCC	22	57.2	3(3H) [32]	3	0.3	0.6
	VITR1:R	TTCATGCAGATCGGGCCAC	19	58.8				
6	MS1:F	CTGACCCTTTGCTTAACATGC	21	53.8	7(5H) [32]	3	0.33	0.49
	MS1:R	TCAGCGTGACAAACAATAAAGG	22	54.3				
7	MGB371:F	ATTCGGTTTCTAGAGGAAGAA	21	62	6(6H) (http://wheat.pw.usda.gov/ggpages/bgn/34/KP1_6.htm)	5	0.46	0.62
	MGB371:R	CACCAAGTTCACCTCGTCCT	20	58				
8	HV13GEIII:F	AGGAACCCTACGCCTTACGAG	21	54	3(3H) [32]	2	0.45	0.5
	HV13GEIII:R	AGGACCGAGAGTGGTGGTGG	20	66				
9	MGB318:F	CGGCTCAAGGTCTCTTCTTC	20	52.9	7(5H) (http://wheat.pw.usda.gov/ggpages/bgn/34/KP1_6.htm)	2	0.5	0.35
	MGB318:R	TATCTCAGATGCCCCTTTCC	20	52.9				
10	Bmag0013:F	AAGGGGAATCAAAATGGGAG	20	54.2	3(3H) [32]	3	0.3	0.5
	Bmag0013:R	TCGAATAGGTCTCCGAAGAAA	21	53.0				
11	HVB23D:F	GGTAGCAGACCGATGGATGT	20	53.5	4(4H) [32]	3	0.33	0.49
	HVB23D:R	ACTCTGACACGCACGAACAC	20	53.0				

2.2. Genomic DNA extraction

The trials were conducted in field under favorable water conditions. Each accession was shown on two rows of 1.5 m long and 0.20 m wide, with three replications. One month later, approximately 1 g of leaf tissues of each accession was harvested, and total genomic DNA was extracted following the protocol described by Ben Naceur et al. [9]. DNA quality was examined and estimated using agarose-gel electrophoresis. DNA samples were diluted to about 50 ng/µl using dH_2O and stored at −20 °C.

2.3. PCR amplification and electrophoresis running

To cover the whole genome of barley, eleven SSR markers were selected from different locations of each linkage group (Table 2). PCR reactions were performed in a volume of 25 µl in a Biometra Thermocycler (Germany). The reaction mixture contained 50 ng DNA, 5 µl of 5× Green GoTaq Reaction Buffer (Promega), one unit of GoTaq DNA polymerase (Promega), 0.2 mM dNTPs and 0.25 µM of each primer. The cycling parameters were: 1 cycle of 94 °C for 3 min; 35 cycles of 1 min denaturing step at 94 °C, 1 min annealing temperatures between 52 and 60 °C depending on the different primer combinations and 2 min extension at 72 °C, followed by 5 min at 72 °C (post-extension). Amplified PCR products were separated by electrophoresis using 2% agarose gel (1× TBE buffer), stained by ethidium bromide (0.5 mg/ml) and visualized under UV light.

2.4. Data analysis

Data obtained from SSR analysis were scored as presence (1) or absence (0) of fragments for each barley genotype and entered into a matrix [22]. Genetic dissimilarity (GD) between accessions was calculated according to the formula of Nei and Li [30]. Based on the matrix of (GD) values, the UPGMA (unweighted pair-group method with arithmetic averages) clustering method was used to obtain the dendrogram, depicting genetic relatedness of the cultivars (Treecon 1.3b software).

The polymorphism information content (PIC) was calculated for each SSR according to the method of Smith et al., [36] as follows:

$$PIC = 1 - \sum (Pij)^2$$

where Pij is the frequency of the ièm band revealed by the jèm primer.

P(ij) is summed through all the bands revealed by the primers.

3. Results

3.1. Genetic variability within the North African barley genotypes based on morphological traits

A wide variation among genotypes is shown for the morphological traits (Table 3).

Table 3 Agronomic character of the barley accessions used.

	Genotypes	Plant height (cm)	Days to heading	Number of grain/spike	Spike weight	1000-kernel weight
V1	Tozeur-1	90.9	126	56.2	1.87	39.5
V2	Tozeur-2	112	138	19.4	0.64	35.75
V3	Djerba	123	135	49.7	1.79	36.4
V4	Kairouan	109.5	139	35.2	1.76	48.57
V5	Menal	94.95	126	49.2	2.1	30.65
V6	Gabès	121.1	126	43.6	1.82	40.92
V7	Rihane	114.2	131	46.7	1.95	42.42
V8	Sidi Bouzid	122.8	145	36.6	1.33	37.13
V9	Kébilli-2	110.1	131	59.2	1.24	24.35
V10	Tombari	108.6	143	57.8	0.89	15.42
V11	Temacine	119.7	134	53.4	1.7	32.87
V12	Ksar Megrine	134.2	139	36.9	1.7	41.92
V13	Rihane-3	126.7	136	52	1.59	25.82
V15	Techedrett	109	141	44.5	2.47	53.87
V16	Azrir	111.2	139	36.6	0.78	21.47
V17	Saida	112.9	143	47.6	2.26	52.35
V18	Sidi Mehdi	133.7	142	34.2	1.44	43.3
V19	Ras El Mouche	99.7	124	51.5	1.42	31.25
V20	Naïlia	102.9	127	59.2	2.7	51.67
V21	Giza 123	119.3	119	64.9	3.014	40.1
V22	Giza 127	109.1	126	68.5	2.65	39.05
V23	Giza 130	89.1	126	52.6	1.95	37.42
V24	El Arich	92	118	45.5	1.87	37.62
V25	Ksar	108.3	118	48.8	1.87	37.65
V26	Giza 2000	120.2	121	57	2.75	49.02
V27	Giza 129	114.3	121	56	2.21	40.1
V28	Giza 126	108.9	121	62.8	3.40	44.02
V29	Giza 125	110.9	124	43.9	2.60	59
V30	Giza 131	118	121	67.6	3.24	48.6
V31	Early 1	78.8	114	56.8	2.62	37.8
V32	Early 2	83.3	117	46.1	1.65	30.3

*(V14 is missing).

3.1.1. Plant height

Table 3 showed that the genotype V12 (Ksar Megrine from Algeria) is the highest plant (134.2 cm); however, the genotypes V31 and V32 (Early 1 and Early 2 from Egypt) and V1 (Tozeur 1 from Tunisia) recorded the lowest one, 78.8, 83.3 and 90.9 cm, respectively.

3.1.2. Number of days from sowing to heading stage

The most early genotypes are V31 (Early 1 from Egypt), V32 (Early 2 from Egypt) and the most late ones are V8 and V10 (Sidi Bouzid and Tombari from Tunisia) and V17 (Saida from Algeria) (Table 3).

3.1.3. Main weight of the spike

The spike weight shown a huge variability illustrated in Table 3. It revealed the superiority of the Egyptian genotypes on the other ones (V21; V26; V28; V30 corresponding to Giza 123, Giza 2000; Giza 126 and Giza 131, respectively) and also the Algerian genotypes V20 (Naïlia).

3.2. Genetic diversity based on molecular characterization

To study 31 North African barley accessions, many primer pairs were used (30). But only eleven polymorphic profiles were generated, with 66.5% of polymorphism. A total of 478 reproducible bands were scored with an average of 2.13 alleles/primer. The number of alleles per pair primer is usually affected by accession, primer sequences and protocol conditions (Table 2).

The obtained profiles were transformed on binary matrix and treated with Treecon for Windows program. The result showed a great genetic dissimilarity between North Africa barley accessions.

To reveal genetic distance among the 31 North Africa's accessions, a dendrogram was constructed (Fig. 1). The 31 accessions were clustered into nine groups, indicating a wide genetic diversity.

The first group gathered the accessions Giza 123, Giza 125, Giza 126, Giza 130, Giza 131, Giza 2000, El Arich, Ras El Mouche, Tombari, Ksar, Early 1, Early 2 and Sidi Mehdi. Except for Ras El Mouche (Algerian) and Tombari (Tunisian) all the other accessions are from Egypt.

In this group, the highest genetic dissimilarity coefficient (GD) is shown between Tombari and Giza 130 (33%); Ksar and Giza 130 (28%) or Ksar and Giza 126 (26%) and, in the other hand, between Tombari and Giza 123, Tombari and Giza 126; Tombari and Arich (24%) (Table 4). Nevertheless, the lowest one is observed between Giza 125 and Giza 2000; Giza 125 and Early 1; Giza 131 and Early 1 (3%) or between Early 2 and Giza 125 or Early 2 and Sidi Mehdi (4%). Furthermore all the Giza accessions have GD varying between 7% and 15%, which means that these accessions may have a common parent (Giza 117: Baladi 16/Palestine 10 or this genotype

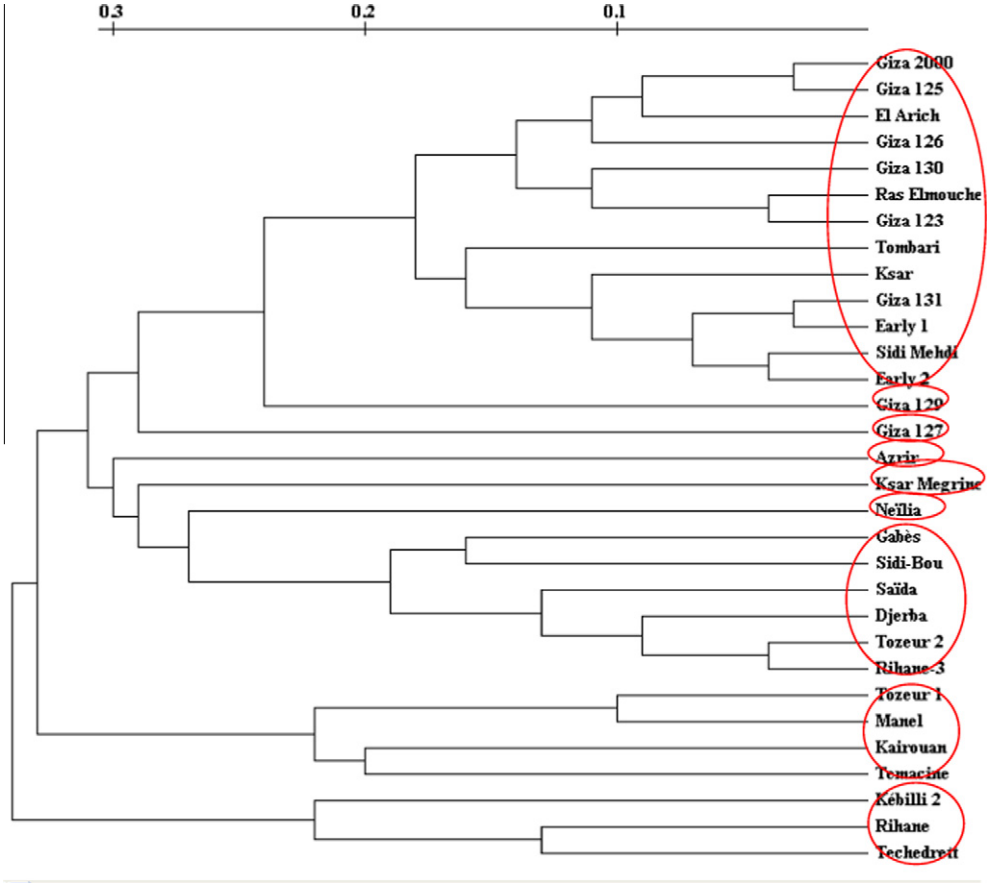

Figure 1 Dendrogram resulting from an UPGMA cluster analysis of 31 North Africa barley accessions and based on data of 11 microsatellite primer pairs.

Table 4 Dissimilarity matrix.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28	V29	V30	V31	V32
Toz1	0																														
Toz2	39	0																													
Djerb	30	8	0																												
kairo	27	28	20	0																											
Manel	10	30	22	18	0																										
Gabès	42	11	19	23	33	0																									
Rihane	24	42	33	39	33	36	0																								
S Bou	41	20	27	38	41	16	29	0																							
Kébil2	25	56	48	39	33	50	20	36	0																						
Tomba	46	31	24	36	46	27	26	21	33	0																					
Témaci	22	23	15	20	22	33	33	33	33	31	0																				
K. Meg	47	27	36	62	47	39	30	31	48	44	36	0																			
Riha-3	42	4	11	31	33	14	36	16	50	27	18	22	0																		
Teched	36	36	28	42	36	38	13	24	23	29	28	24	31	0																	
Azrir	40	30	22	25	30	33	33	33	50	39	30	37	25	36	0																
Saïda	55	12	20	42	45	23	39	24	54	29	28	14	8	33	27	0															
S Mehd	50	26	19	31	42	21	28	23	43	13	26	30	21	23	25	15	0														
R Elmo	36	28	20	25	27	39	39	38	39	29	12	33	23	33	27	17	15	0													
Neïlia	33	25	17	30	33	28	27	36	44	33	25	30	28	22	33	30	20	30	0												
Giz 123	39	31	23	20	30	33	33	33	33	24	15	36	26	28	30	20	11	4	25	0											
Giz127	39	39	31	28	30	33	50	27	41	38	31	54	41	36	39	44	26	28	33	23	0										
Giz 130	33	42	33	22	24	36	27	36	28	33	25	40	36	30	24	30	20	13	36	8	25	0									
Arich	30	39	31	28	30	41	25	33	26	24	23	36	33	28	39	28	18	12	25	8	31	17	0								
Ksar	42	26	19	38	42	36	28	29	43	20	26	30	21	31	25	15	14	15	28	19	41	28	19	0							
Giz 2000	36	36	29	26	36	31	31	25	31	16	21	42	31	33	36	26	10	11	31	7	21	15	7	17	0						
Giz 129	40	48	39	27	30	45	33	48	33	39	30	47	42	36	40	36	25	18	33	13	30	14	13	33	20	0					
Giz 126	39	46	39	36	39	41	33	27	33	24	31	45	41	36	39	36	18	20	42	15	23	17	15	26	7	30	0				
Giz 125	33	41	33	31	42	36	28	29	29	20	18	39	36	31	42	31	14	15	28	11	26	20	11	21	3	25	11	0			
Giz 131	42	26	19	31	42	29	20	23	36	13	26	30	21	23	25	15	7	15	20	11	33	20	11	7	10	25	19	14	0		
Early 1	44	29	21	33	44	31	23	19	31	16	29	33	24	18	28	18	10	19	23	14	29	23	14	10	13	28	21	17	3	0	
Early 2	48	23	15	28	39	26	25	27	41	17	23	27	19	20	22	12	4	12	17	8	31	17	15	11	14	22	23	19	8	7	0

V1 = Tozeur-1; V2 = Tozeur-2; V3 = Jerba; V4 = Kairouan; V5 = Manel; V6 = Gabes; V7 = Rihane; V8 = Sidi-bouzid; V9 = Kebilli-2; V10 = Tombari; V11 = Temacine; V12 = Ksar-Megrine; V13 = Rihane-3; V15 = Techedrett; V16 = Azrir; V17 = Saida; V18 = Sidi-Mehdi; V19 = Ras El Mouch; V20 = Neilia; V21 = Giza123; V22 = Giza127; V23 = Giza130; V24 = El Arich; V25 = Ksar; V26 = Giza2000; V27 = Giza129; V28 = Giza126; V29 = Giza125; V30 = Giza131; V31 = Early 1 et V32 = Early 2.

is common parent of Giza 123, Giza 124, Giza 125 and Giza 126) as they share some of the morphological traits (Table 3).

This group could be subdivided into three subgroups, where the naked one (Tombari and Giza 130) are slightly separated from the others.

It is to notify that Tombari is Tunisian naked local barley with a low phytic acid value and high nutritional quality as well as Giza 130 and Giza 129 which are also naked Egyptian barley.

From the second to the fiftieth group, each one contains only one accession that differs from the other by the heading date, the morphological traits or by having naked or covered grain (Giza 129, Giza 127, Azrir, Ksar Megrine and Neïlia).

The sixtieth group is formed by Gabes, Sidi Bouzid, Saïda, Djerba, Tozeur-2 and Rihane-3. All these accessions are originated from Tunisia or having their relatives from Tunisia (case of Rihane-3). Indeed there are many landraces locally called ''Djebeli barley'' which are collected by the Australian scientists in the beginning of the 19th century and named them ''Atlas'' (synonym of Djebeli in Arabic). These landraces were used in their barley improvement program. So Rihane-3 might have a Tunisian origin. In this group the highest GD is shown between Djerba and Sidi Bouzid (27%) and the lowest one is observed between Rihane-3 and Tozeur-2 (4%) or between Rihane-3 and Saïda (8%) and Tozeur-2 and Djerba (8%).

The seventieth group contains Tozeur 1, Manel, Kairouan and Temacine. The genetic distance varies from 10% to 20%. Except for Temacine (from Algeria), all the other genotypes are from Tunisia and share the same vigor and the same heading date (Table 3).

The eightieth group is formed by Kebilli 2, which is the most tolerant to salt stress [7].

The ninetieth group is formed by two improved varieties: Rihane (As 46/Avt/Aths Sel, 1AP-3AP-0AP-0Kf, from Tunisia) and Techedrett (C95203S F4N 1998/99, cross realized at Technique Institute of Cereal in Algeria). These two varieties are both late, well adapted to drought and having large seeds but sensitive to fungi. Their presence in the same group could be explained by the traits cited and by their eco-geographic adaptation.

4. Discussion

The genetic diversity and differentiation among all North Africa's barley accessions using SSR markers were estimated (Table 4).

The variation of genetic diversity and allele distribution were strongly dependent on the loci that were analyzed. The genetic distance among the 31 barley accessions based on SSR data obtained in this study showed that these accessions are hugely different since we count nine different groups where four of them contain two or three sub-groups. This result means that there are many different barley genotypes widely distributed in this region and it is in agreement with Boeuf's suggestions [12], for which North Africa is the secondary center of cereal in the world.

Compared to previous studies using SSR markers, the mean values of genetic diversity and the total number of alleles (0.502 and 2.9, respectively) are between those of the two-rowed wild barley from Tibet (0.49 and 3.9) [42] and those described by Russell et al. [33] (0.566 and 5.38) but similar to that find by Hamza et al. [21] (0.53 and 3.2) and Belghouthi [10] (0.5 and 3) working on other barley accessions from Tunisia.

The distribution of our studied barley accessions on different groups showed also that each group obtained in this study share, at least, the same agronomic character and sometimes the accessions were clustered according to their geographic origin but with some exceptions: indeed Giza 2000, Giza 123, Giza 125, Giza 126, Giza 130, El Arich, from Egypt were closely related to each other and their allocation supports the idea of Bahieldin et al. [6] who demonstrated that Egyptian barley genotypes are genetically very close and originated from closely related genotypes.

In the other hand, Gabès and Sidi Bouzid or Tozeur-2 and Manel, from Tunisia were grouped together. This was a little bit comparable to that found by Ivandic et al. [24], Wang et al. [40] and by Bchini et al. [7] where their germoplasms were clustered according to the same eco-geographical region. This relative relationship observed between SSR markers and the geographic origin of the North Africa barley accessions may be explained by the long term adaptive conditions under the specific regions or sub-regions of each country. These particular conditions may influence the cultivar behavior and lead to some treats of adaptation such as earliness to avoid water deficit or small spike that will be rapidly filed and so on.

It is also clear that, whatever their origin (Tombari from Tunisia or Giza 129 and Giza 130 from Egypt) the naked barley is situated in groups or subgroups different from that of hulled accessions. Most studies on the history of barley domestication were focused on the row type characters but little attention has been done to the hulled or naked caryopsis character. Nevertheless, Taketa et al. [38] studying a collection of barley (wild, hulled domesticated, and naked domesticated lines), found four alleles in which only the allele IV in wild barley and in naked domesticated lines, but also in a single accession from southwestern Iran. They conclude that naked barley has a monophyletic origin, probably in southwestern Iran. Then it was spread throughout Europe, Asia and North Africa to become perhaps among the most important cereal crop on these regions.

This present finding strengthens previous reports on the correlation between eco-geographical distribution and SSR markers [20,7]. It shows also that SSR markers can be used effectively to estimate genetic distances among genotypes and distinguish between naked and hulled barley accessions. However, it is suggested that more molecular data is required to distinguish accessions coming from the same region and consequently more efficient utilization of existing variability for improvement of barley in North Africa.

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