Genetic Diversity among Ancient Rainfed Lebanese Barley (*Hordeum vulgare* L.) Landrace *Baladi* by Using Morphological Traits and Microsatellites Markers

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Original Research Article

ABSTRACT

Lebanese barley landrace *Baladi* is not sufficiently analyzed morphologically and genetically. This investigation was carried out to evaluate *Baladi* in its center of diversification by using morphological traits and microsatellite markers (SSR). The genetic diversity of 237 accessions of Lebanese barley landrace *Baladi* was evaluated by using 19 morphological traits. Principal components analysis showed that plant height, awn color, number of rows, length of rachilla hairs, number of spikelets per spike, number of seeds per spike and shape of lemma tip for sterile spikelets were the most significant traits. Regarding the relationship between plants, dendrogram constructed according to Jaccard distance, clearly separated the studied spikelets into 19 distinguished morphological profiles. A total of 55 genomic DNA extracted from selected accessions were analyzed using seven

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microsatellite markers (SSRs). A high level of polymorphism information content (PIC; average = 0.66) was observed. The polymorphic primers revealed 16 alleles with an average of 5.3 alleles per polymorphic primer. The highest number of polymorphic bands was developed by the primer pairs MGB402 (7 alleles). In the dendrogram constructed based on the SSR data, the evaluated lines were classified into two major clusters corresponding to the number of rows per spike (two-rows or six-rows). Based on this study, it can be concluded that there is a high level of genetic diversity within *Baladi* barley landrace in Lebanon. These results will be useful for barley germplasm management in terms of biodiversity protection and as a valuable source of gene pool for new useful alleles for crop improvement.

1. INTRODUCTION

Barley (*Hordeum vulgare* L.) landraces are of particular interest in the Fertile Crescent where barley was first domesticated from its wild progenitor *Hordeum spontaneum* [1,2]. Barley landraces are dynamic populations that have evolved across thousands of years, by natural selection, under local environmental condition and farming systems. Therefore, they represent a large reservoir of useful genes for adaptation to biotic and abiotic stresses [3].

Lebanon belongs to the near east Fertile Crescent region which is considered as one of the most important centers of diversity for barley landraces and several wild relatives [2,4]. Barley is the second most widely cultivated cereal in the country (12 586 ha) [5].

The majority of barley cultivated varieties and landraces are located in the plain region Bekaa accounting for 88% of the total barley cultivated area [5]. *Barley* is mainly used as a major source of fodder for livestock feed and for malt production. It is one of the best adapted cereals due to its tolerance to salinity, low temperatures and low water demand.

In a landrace, the diversity is structured between and within populations (at the field/farmer level). *Baladi*, the most widespread landrace in Lebanon, has been shown to have a large variability. *Baladi* means 'local', that is, something autochthonous. Hence, the name *Baladi* has been used by farmers to indicate barley landraces that were believed to be Lebanese. Farmers were cultivating *Baladi* landraces over centuries. These landraces became adapted to traditional farming systems and water deficit. *Baladi* landraces persisted due to these agronomic traits which usually lacks in the genetic diversity of improved barley varieties.

Baladi genetic diversity is progressively being lost in farmers fields and in nature. This threat is the consequence of several factors and is processing at an alarming rate. The most crucial factor includes the displacement of indigenous landraces by new genetically uniform crop cultivars, as well as the habitat conversion and degradation. In addition, the climate change and the drought that prevailed particularly in the regions of North East Lebanon has directly or indirectly caused considerable genetic erosion.

There is a lack of information regarding the morphological variability and the genetic diversity present in barley landraces in Lebanon, a country experiencing loss of biodiversity of barley and several wild relatives. Therefore, studying *Baladi* landrace on the morphological and molecular level will be helpful for understanding its genetic diversity, for managing its conservation and its effective utilization in breeding programs. Previously, few studies were conducted to assess the genetic relationships among *Baladi* genotypes using morphological and molecular characterization [6]. This study was undertaken with an aim to assess the level of variability for barley landrace *Baladi* in its center of diversification by using morphological traits and molecular markers (simple sequence repeat SSR). The objectives were to investigate the phenotypic variations within *Baladi* populations and to identify the major characters contributing to the germplasm diversity in order to initiate breeding programs.

2. MATERIALS AND METHODS

2.1 Field Survey

A field survey was conducted in the Bekaa region including 20 potential sites under semi-arid climatic conditions, as an attempt to select the best sites of barley landraces. This region is

experiencing a quick loss in its agrobiodiversity due to habitat conversion, adoption of new varieties, land reclamation and overgrazing. Five non irrigated plots characterized by a high diversity of local barley landraces were selected in five sites: two sites in Aarsal (Aarsal 1 and Aarsal 2), Younine, Mazraat-Beit Mcheik and Bar-Elias (Table 1). These stands were spread from latitude 33.7747 (Bar Elias) to 34.17925 (Aarsal 1), longitude 35.9042 (Bar Elias) to 36,41955 (Aarsal 1). They are characterized by a rainfall ranging between 153 mm (Aarsal 1 and Aarsal 2) to 725 mm (Bar Elias) and altitude 900 m (Bar Elias) to 1500 m (Aarsal 1).

2.2 Morphological Traits

Samples of 35 to 50 spikes were collected from each stands. A total of 237 spikes of barley landrace *Baladi* distributed in five stands were collected in order to study the morphological traits based on barley descriptors which were developed by the International Center for Agricultural Research in the Dry Areas (ICARDA) and the International Plant Genetic Resources Institute (IPGRI) [7,8]. The morphological traits included seven qualitative traits (awn color, shape of lemma tip for sterile spiklets, attitude of sterile spiklets, spike density, inner lateral nerve of lemma specules, seed color, tillering capacity), and twelve quantitative traits (length of rachilla hairs, number of fertile tillers per plant, plant height, number of internodes, length of last internode, average internode, spike length, spike width, awn length, number of rows, number of spiklets/spike, number of seeds per spike).

2.3 Molecular Analysis

Molecular characterization was performed on 55 genomic DNA extracted from accessions of the different groups of the dendrogram based on morphological traits. In addition, two released barley varieties commonly used in Lebanon,

Litaneh and Rihane, were used as checks. Seeds of the distinguished spikes were sown in small plastic pots. After two weeks, the produced seedlings were cut and packed in plastic bags and stored at -20°C for DNA extraction. DNA extraction of barley landraces was performed according to William et al. (1990) [9]. Seven primer pairs of microsatellites were selected and used in this study based on their good results for amplification, simple locus and high power of discrimination on barley [10,11].

Microsatellite amplifications were performed in a total volume of 25 µl with 2.5 µl PCR buffer (10mM Tris-HCl, 50 mM KCl, 1,5 mM Mg^{2+}), 200 µM of each dNTP, 0.4 µM of each primer, 1U of Taq DNA polymerase and 50 ng of barley genomic DNA. The amplification program consisted of 4 min at 94°C, 45 cycles of 60 s at 94°C, 65 s at 50 to 52°C and 90 s at 72°C, followed by a 10 min extension at 72°C. The PCR amplification products were separated on a 6% denatured polyacrylamide gel and visualized by silver staining.

2.4 Data Analysis

For qualitative traits, scores were attributed according to ICARDA and IPGRI barley descriptors [8]. A phenotypic diversity index, h_{sj} (Shanon index) was calculated for each site to describe the phenotypic diversity of barley [12]. The following formula was used for calculating h_{sj} for each trait with n categories $h_{sj}=-\Sigma\ P_i \ln P_i$ where P_i is the relative frequency in the *i*th category for the *j*th trait. The average diversity (H) over k traits of each site was estimated as: H $=\Sigma\ h_{sj}/k$. Traits evaluation was performed by using the Principal Component Analysis (PCA). The relationships between spikes based on their quantitative and qualitative traits were studied using cluster system for STATISTICA program and reported as a dendrogram with Jaccard distances [13].

Table 1. Geographic and climatic data of barley studied sites in Bekaa region

Location Site	Longitude °/ E	Latitude °/N	Elevation (m)	Annual Rainfall (mm)	Mean of Temperature (°C)
Aarsal 1	36. 41955	34. 17925	1500	153.40	16.1
Aarsal 2	36.22314	34.12324	1200	153.40	16.1
Younine	36. 27489	34. 07848	1250	287.26	12.8
Mazraat Beit Mcheik	36.02517	34.0358	1250	450	16.3
Bar Elias	35.9042	33.7747	900	725	14

To assess the information given by microsatellite markers, the following parameters were studied: number of alleles per locus, percentage of observed heterozygosity (Ho), Polymorphism Information Content (PIC=1- Σ $(P_{ij})^2$, where Pij is the frequency of the *j*th SSRs pattern for marker *i* and the summation covers n patterns). The data matrix was used to compute the diversity for each SSRs marker. F-statistics (Fst) and P value were assessed for significance of heterozygote deficiency using Weir and Cockerham method [14]. The discrimination power was calculated PD= 1 $-\Sigma$ p_i $(Np_i-1)/(N-1)$, where p_i was the frequency of the *i*-th molecular pattern revealed by primer or locus *j* and N was the number of genotypes [15]. Genetic distances were calculated according to Jaccard [16]. Trees were produced by clustering the data with the unweighted pair-group method (UPGMA) with SAHN–clustering and tree programs of NTSYS.

3. RESULTS AND DISCUSSION

3.1 Morphological Characterization

The characterization of the collected 237 *Baladi* accessions using different morphological traits showed high level of variation among the accessions, traits and their interactions. The Principal Component Analysis (PCA) revealed that the first 3 components explained 47% of the total variation, based on 19 morphological traits (Table 2). The first component represented 26% of the variability and accounted primarily for plant height, awn color, number of rows and length of rachilla hairs. The second component contributed to 14% of the variation derived from number of spikelets per spike and number of seeds per spike. The third component accounted 7% of the variation and was related to the shape of lemma tip for sterile spikelets. The high frequency of these traits could be the result of natural and farmers' selections privileging certain phenotypes adapted to the prevailing climatic and edaphic conditions. Farmers usually select barley lines for their agronomic traits that are associated with yield performance (plant height, number of spiklets/spike, grain yield) [17-19].

The average of genetic diversity based on the most discriminant traits for the studied plants ranged from 0.13 for Aarsal 1 to 0.33 for Bar Elias (Table 3). The sites of Bar Elias and Aarsal 2 (0.31) had a significantly higher diversity index than Aarsal 1.

The amount of variation in morphological traits was site dependent. Lemma tip's shape for sterile spikelets (rounded, square and pointed), length of rachilla hairs (short to long) and plant height (42 cm to 81.5 cm) showed the highest variation between the landraces collected from Aarsal 2. The variation in awn color (yellow and black) was the highest between the accessions of Bar Elias. The variation in number of spikelets and grains per spike (ranging between 5 to 20) was the highest between the accessions of Beit Mcheik (Table 3). In other hand, Aarsal 1 presented the lowest variation in awn color, shape of lemma tip for sterile spikelets and length of rachilla hairs (Table 3). These results showed that the genetic variation is high within populations or accessions collected from the same site than between populations. Several studies on barley landraces indicated that the total genetic variation captured is higher within landraces (50 to 60%) than among landraces (40 to 50%) [20,21].

3.2 Classification of Accessions Based on Morphological Attributes

The accessions could be separated into groups based on the seven most discriminant traits. This analysis may help in finding contrasting barley accession groups or profiles for future breeding programs. The resulted dendrogram separated *Baladi* accessions into 19 distinguished profiles at zero Jaccard distance and classified them in 5 groups at 0.22 Jaccard distance (Fig. 1). The first group was constituted by 26 accessions of two rows from Aarsal 1, Beit Mcheik and Younine sites which are characterized by their yellow awn color and pointed shape of lemma tip for sterile spikelets. The second group clustered only two accessions of 6 rows from Bar Elias site which are characterized by their black awn color, rounded shape of lemma tip for sterile spiklets, long length of rachilla hairs and low number of spiklets/spike. This barley profile was specific to the site of Bar Elias. The third group and largest one clustered together 152 accessions from the five studied stand. The majority of these accessions are two rows type with yellow awn color, short length of rachilla hairs and an intermediate number of spiklets/spike. The fourth group contains 45 accessions from Aarsal 2 and Bar Elias sites. These accessions have mainly black awn, long length of rachilla hairs and rounded shape of lemma tip for sterile spikelets. Finally, the fifth group contains 12 accessions from Aarsal 2 and Bar Elias. Barley accessions of this last group are characterized by a high number of spikelets/spike and plant height.

Results of *Baladi* accessions classification revealed that accessions within each cluster belonged to different regions which suggested that there was no clear relationship between accessions and geographical diversity. Such results have been reported in different crops by several studies, e.g. on durum wheat [22,23]. This variability could be attributed to free exchange of materials within the studied Bekaa region.

Table 2. Principal component analysis and percentage of total variance for 19 morphological traits of barley landrace cultivated in Lebanon. The first three components of the principal component analysis are referred to as PC1, PC2 and PC3

| Traits	Components		
	PC1	PC2	PC3
Tillering capacity	-0.64	0.53	0.22
Number of fertile tillers per plant	-0.64	0.53	0.22
Plant height	0.75	0.22	0.14
Number of internodes	0.00	-0.05	0.13
Length of last internode	0.22	0.51	0.18
Average internode	0.31	0.06	-0.17
Spike length	-0.49	0.66	-0.11
Spike width	0.38	0.44	0.00
Awn color	0.77	-0.05	0.14
Awn length	0.20	-0.42	-0.2
Number of rows	0.92	-0.09	0.05
Shape of lemma tip for sterile spikelets	0.03	0.08	0.69
Attitude of sterile spikelets	0.01	-0.03	-0.62
Spike density	0.13	0.24	-0.1
Number of spikelets/spike	0.44	0.69	-0.27
Number of seeds per spike	0.44	0.69	-0.27
Inner lateral nerve of lemma specules	0.62	0.09	0.01
Length of rachilla hairs	0.80	0.07	0.2
Seed color	-0.24	0.09	-0.37
Percentage of total variance	26%	14%	7%
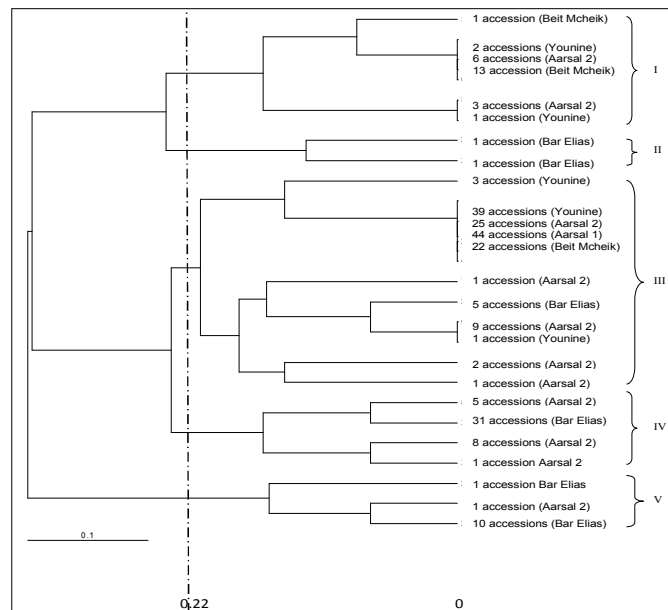
Fig. 1. Dendrogram of 237 plants of Baladi barley landrace based on 7 discriminate morphological traits, using Jaccard distances

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Table 3. Diversity indices of 7 discriminant traits and minimal/maximal values of quantitative traits of barley accessions from the five studied sites. Numbers in brackets indicate diversity indices

Traits	Aarsal 1	Beit Mcheik	Aarsal 2	Younine	Bar Elias
Awn colour	Yellow (0)	Yellow (0)	Yellow-Black (0.39)	Yellow (0)	Black-yellow (0.5)
Shape of lemma tip for sterile spiklets	Rounded (0)	Rounded (0)	Rounded, square (0.25)	Rounded pointed (0.24)	Rounded (0)
Length of Rachilla hairs	Short (0)	Short to long (0.1)	Short to long (0.68)	Short to long (0.1)	Long (0.09)
Plant height (cm)	28-48.5 (0.2)	24.5-52 (0.27)	42-81.5 (0.67)	22-64.5 (0.1)	59-97 (0.4)
Number of spiklets/spike	10-26 (0.36)	5-20 (0.68)	14-49 (0.09)	10-24 (0.24)	7-46 (0.68)
Number of grains/spike	10-26 (0.36)	5-20 (0.68)	14-49 (0.09)	10-24 (0.24)	7-46 (0.68)
Row number	Two (0)	Two (0)	Two (0)	Two (0)	Six (0)
Mean of diversity indices	0.13 b	0.25 ab	0.31 a	0.13 b	0.33 a

Means followed by the same letter in the same row are not significantly different (P= .05)

3.3 Relationships among Morphological Traits

The study of correlative links between quantitative traits of landrace accessions is of major interest since selecting for a certain trait may be positively or negatively influenced by the expression of other traits. In our study, the coefficient of correlation revealed that number of rows had positive association with awn color and length of rachilla hairs. Indeed, all six rowed accessions presented black awns while two rowed accessions had yellow awns. Six rowed spikes had longer rachilla hairs than those of two rowed. Besides number of rows had positive association with plant height and number of spikelets per spike. Barley accessions with six rows presented higher plants (59-97 cm) than those with two rows (22-81.5 cm (Table 3). On the other hand, number of rows had a negative correlation with number of fertile tillers per plant. Two rowed barley showed a higher tiller production. These observations are in consonance with the findings of Kirby and Riggs [24]. Briggs explain these results by the fact that six rowed cultivars have usually more spikelets per spike as a compensatory effect of yield components which lead to similar levels of yield potential [25].

3.4 Molecular Characterization

Molecular characterization was performed on 55 genomic DNA extracted from accessions of different groups of the dendrogram based on morphological traits (one accession from each of the 19 groups and 3 to 5 similarities within groups) and two checks (Litaneh and Rihane varieties). Among seven primer pairs used, three were polymorphic (MGB402, MGB318 and MGB357), one was monomorphic (MGB391) and three did not generate any band (Bmag13, MGB371 and Ec624). The polymorphic primers revealed 16 alleles with an average of 5.3 alleles per polymorphic primer and the size ranged from 93 to 262 bp (Table 4). The highest number of polymorphic bands was developed by the primer pairs MGB402 (7 alleles).

The PIC (Polymorphism Information Content) value of each SSRs marker is indicator of locus diversity. In this study, the PIC value ranged from 0.61 for the primer MGB318 to 0.76 for the primer MGB357; therefore, these markers are moderately to highly informative according to the criteria proposed by Botstein et al. [26]. It should be noted that these values obtained in the present study for a single landrace are comparable to studies done for different landraces collected from different geographical regions. These results revealed that *Baladi* landrace has high genetic variation and presents a high level of polymorphic alleles. This is an expected and desirable property of landraces being genetically heterogeneous. Moreover, the SSR marker used were relatively of high efficiency for barley genetic analysis and could reveal the genetic differences of barley

germplasms as described in previous studies [27,28].

The dendrogram based on microsatellites data clustered 28 distinguished spikes at zero Jaccard distances (Fig. 2) from which 26 for *Baladi* landrace and one accession for each Litaneh (56) and Rihane (57) varieties. These primers classified the evaluated lines in two major clusters at 0.43 Jaccard distance, corresponding to the number of rows per spike. The first cluster regrouped lines of two-row spike type (36 accessions). All 6 row-type plants were clustered in the second group (plants 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34 and 57) with the 2 improved varieties (Litani "plant 56" and Rihane

"plant 57") and few plants of 2 row-type from different studied sites. In general, the clustering patterns of accessions were not correlated to the geographical site from where they were collected. Other researchers have reported that classifications of barley accessions based on SSRs did not reflect their geographic origin [27,29]. However, Karim *et al.* reported that the dendrogram generated by the SSR matrix classified local Tunisian barley accessions based on their geographic origin and according to some morphological traits [30]. In this study, some lines (plants 16 and 20; 25 and 34; 38 and 39; 41 and 42; 48, 51 and 52) were not distinguishable neither for the genetic markers nor for the morphological traits.

Table 4. Annealing temperature, number of alleles, molecular weight, observed heterozygosity (Ho), Polymorphism information content (PIC), F-statistics (Fis), probability (P) and discrimination power (Dp) of seven microsatellites studied in barley

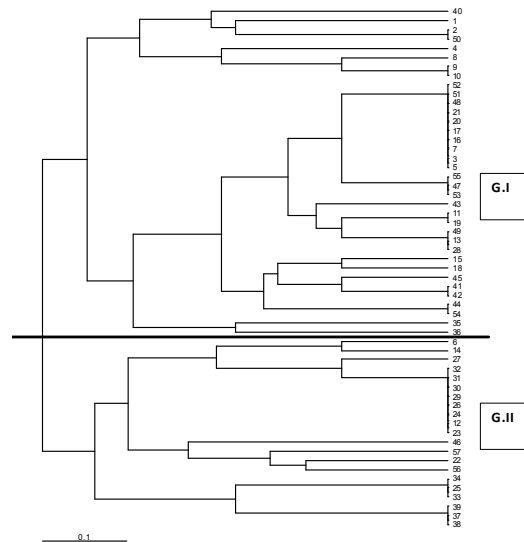| Locus	Annealing temperature	Number of alleles	Molecular weight	Ho	PIC	FIS	P	Dp
MGB402	54	7	222-262pb	0.33	0.62	0.8*	0.00	0.62
MGB318	54	3	93-110	0.99	0.61	-0.60*	0.00	0.47
MGB357	52	6	95-255pb	0.95	0.76	-0.23*	0.00	0.61
MGB391	54	1	250 pb	-	-	-	-	-
MGB371	52	-	-	-	-	-	-	-
Bmag13	52	-	-	-	-	-	-	-
Ec624	52	-	-	-	-	-	-	-

Fig. 2. Dendrogram constructed from single-locus SSR, using SimQual program, Jaccard distance and UPGMA clustering of 55 barley Baladi accessions and two checks (Litaneh (56) and Rihane (57) varieties). G.I refers to the first cluster group containing two-rows spike type, G.II refers to the second group including all six-rows spike type. Accessions from 1 to 14 were taken from Arsal 1, those from 15 to 22 were taken from Beit Mcheik, 23 to 34 from Bar Elias, 35 to 45 from Younine and 36 to 55 from Arsal 2

4. CONCLUSION

The results of this study revealed a large morphological diversity and a high genetic variation among the Lebanese barley landrace *Baladi*. The dendrogram based on SSR markers was not in accordance with the one generated by the morphological traits. It separated the studied lines into two major clusters according to the number of rows per spike (two-rows or six-rows). Morphological and molecular clusters have distinguished different lines of *Baladi* landrace which may help in the selection of the most diverse profile and expand genetic variation for barley improvement. Seeds of each distinguished *Baladi* lines were provided to the Lebanese national gene bank in order to multiply and preserve them. *Baladi* seeds constitute a potential wealth of genetic diversity which can be used in future barley breeding program.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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