RESEARCH NOTE

Genetic diversity in barley landraces (*Hordeum vulgare* L. subsp. *vulgare*) originated from Crescent Fertile region as detected by seed storage proteins

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Introduction

Barley is of renewed interest throughout the world because of its nutritional value and low glycemic index (Ullrich 2011). Lebanon belongs to the near east Crescent Fertile region which is considered as one of the most important centres of diversity in the world and where barley domestication started 10,000 years ago (Zohary and Hopf 1993). In recent years, the diversity in this region and the crop production are seriously threatened by the climate change and various anthropogenic pressures. Landraces are a useful source of genetic variability that offer an interesting tool to mitigate climatic change effects. However, little attention has been attributed to assess their variability and potential in breeding programmes. Thereafter, a thorough investigation of the existing variability will be of major importance for both evaluating and managing barely landraces. For these reasons, various tools have been used for studying barley genetic diversity. Phenological and morphological quantitative traits have been largely used, but these markers are not enough to verify genetic diversity in barley (Buck-Sorlin 2002; Hübner *et al.* 2013). In recent years, it has been suggested that biochemical accomplished with molecular markers can be used to solve population diversity of barley.

Hordeins are the storage proteins of the wild and cultivated barleys. Numerous studies have reported (Eshghi *et al.* 2012; Hajmansoor *et al.* 2013; Rahimi *et al.* 2014) that it is possible to use hordeins for diversifying barley species because of its considerable variation. In fact, the hordeins can be divided into three main groups based on differences in size and sulphur content: sulphur-rich (B-hordeins and A-hordeins), sulphur-poor (C-hordeins) and high molecular weight (HMW, D-hordeins) (Shewry *et al.* 1995). B-hordeins and C-hordeins are the two major groups of polymeric storage proteins. B-hordeins are larger component with a fractions account for 70–80% of the total hordein in the barley endosperm than C-hordeins (10–12%) (Molina-Cano *et al.* 2001). There is scarcely any research work carried on hordein polymorphism in near-east and north Africa. To the best of our knowledge, there is only one paper on hordein polymorphism for studying the genetic structure of Jordanian barley landraces in the literature (Baloch *et al.* 2014). The aim of this work was to explore the genetic diversity of Lebanese barley landraces (*Hordeum vulgare* L. subsp. *vulgare*) using biochemical markers as hordeins (loci *Hord1* and *Hord2*) as a first step towards their further utilization in breeding programme in the near east region.

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Keywords. landraces; genetic diversity; hordein polymorphism; storage proteins, *Hordeum vulgare* L.

Materials and methods

Samples collection

Fifty-three Lebanese landraces of barley were used in this study including four sets of materials: (i) 35 landraces collected in 2011 from different agroclimatic areas in Lebanon (LR1–LR35); (ii) 15 landraces recovered from the GenBank of ICARDA-Aleppo and initially collected in the nineties from different agroclimatic areas in Lebanon (LR36–LR50); (iii) two improved barley varieties released by ICARDA (International Centre for Agricultural Research in the dry areas), namely Rihane-3 and Manel (6 rows); (iv) a sample of *H. spontaneum* widely growing in Lebanon. The geographical data of barley accessions under study are shown in figure 1 and table 1.

Extraction of seed storage proteins

For each landrace, five single seed progenies were analysed. Three seeds per landrace were skinned and crushed into a fine powder and then the endosperm storage proteins were extracted. The procedures of extraction were carried out in accordance with Doll and Andersen (1981).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed to separate the extracted proteins by using 12% separating and 5% stacking gels with a Mini Protean System from Bio-Rad according to Laemmli modified method (Laemmli 1970). In this study, we adopted constant current electrophoresis instead of constant voltage. The loading of sample was 15 μL. Starting current was 30 mA, and then shifted to 50 mA when the bromophenol blue marker got to the borderline of stacking and separation gels. After electrophoresis, protein bands were stained for about 4 h overnight using staining solution: 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250 (Sigma), and

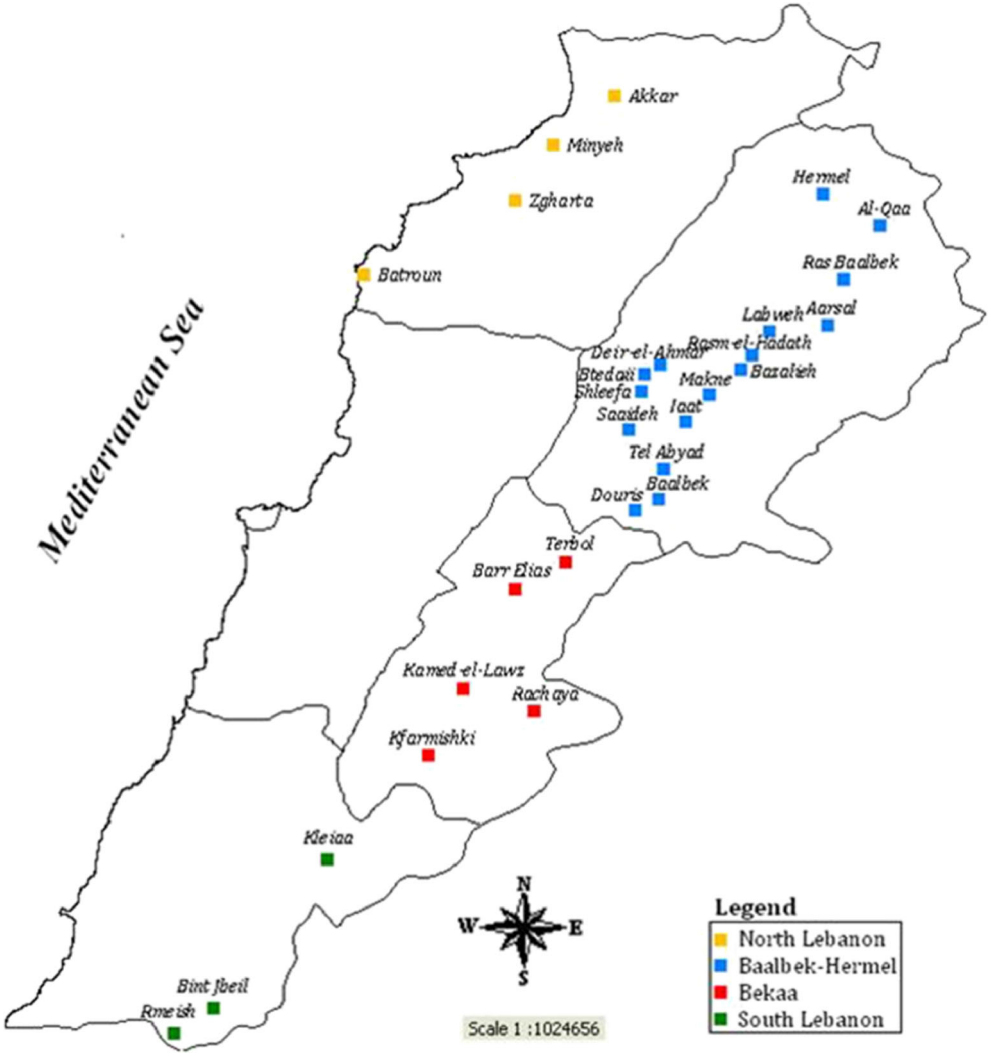

Figure 1. Collection site and geographic distribution of barley landraces collected in this study. Landraces presented in red colour are from Bekaa Caza; blue, Baalbek-Hermel; green, south Lebanon; dark yellow, north Lebanon.

Table 1. Climatic and geographic distribution of the locations surveyed in Lebanon for collecting barley landraces.

Caza	Location	Altitude (m)	Rainfall (mm)	T_{min^*} (°C)	Latitude (°N)	Longitude (°E)	Landraces collected
Bekaa	Terbol	887	253.2	−0.2	33.8181	35.9831	LR1 (6 row)**
	Kfarmishki	1195	1089	3.38	33.5153	35.7667	LR2 (2 row)
	Kamed Lawz	1222	847	−0.06	33.6203	35.8214	LR3 (6 row)
	Barr Elias	879	318	−0.06	33.7747	35.9042	LR4 (2 row), LR5 (6 row)
	Rachaya	1480	1123	−5.72	33.5833	35.9333	LR50 (2 row)
South Lebanon	Bint Jbeil	724	937.2	6.27	33.12	35.43	LR6 (6 row), LR7 (6 row), LR8 (2 row)
	Kleiaa	714	1000	6.56	33.3311	35.6204	LR9 (6 row)
	Rmeish	562	308	4.64	33.08	35.37	LR10 (2 row), LR11 (6 row)
Baalbek-Hermel	Deir-el-Ahmar	986	1200	−7	34.1253	36.1311	LR12 (6 row)
	Shleefa	990	427	−0.88	34.0853	36.1003	LR13 (6 row)
	Iaat	1083	253	0	34.0369	36.1714	LR14 (2 row)
	Btedaii	998	432	−0.83	34.1122	36.1061	LR15 (6 row)
	Saaideh	1018	185	0	34.0258	36.0817	LR16 (6 row)
	Douris	1134	423.6	−1.57	33.8978	36.0919	LR17 (6 row), LR18 (2 row)
	Bazalieh	1041	350	0	34.1197	36.2584	LR19 (2 row), LR20 (6 row)
	Tel Abyad	1025	423.6	−1.57	33.95	36.15	LR21 (2 row)
	Makne	1068	350	−0.06	34.0797	36.2067	LR22 (2 row)
	Rasm Al-Hadath	958	300	0	34.1414	36.2756	LR23 (6 row)
	Ras Baalbek	1214	300	−1.76	34.2589	36.4192	LR24 (2 row), LR25 (2 row), LR26 (6 row)
	Hermel	696	208.4	1.19	34.3942	36.3847	LR27 (6 row), LR28 (2 row)
	Al-Qaa	648	225.8	0.35	34.3442	36.4744	LR29 (2 row), LR30 (6 row), LR31 (2 row), LR32 (6 row)
	Aarsal	1228	432	−0.74	34.1886	36.3923	LR33 (6 row), LR34 (2 row)
	Labweh	1007	350	0	34.1789	36.3021	LR35 (2 row)
	Baalbek	1050	383.6	−5.81	33.9333	36.1002	LR36 (2 row), LR37 (2 row), LR38 (2 row)
North Lebanon	Batroun	150	900	6.78	34.2667	35.6667	LR39 (6 row), LR40 (6 row), LR43 (6 row), LR48 (6 row), LR49 (6 row)
	Zgharta	426	1148	6.17	34.440	35.903	LR41 (6 row), LR42 (6 row)
	Minyeh	150	837.9	7.45	34.4333	36.05	LR47 (6 row)
	Akkar	100	539.2	7.20	34.5258	36.0119	LR44 (6 row), LR45 (6 row), LR46 (6 row)

*T_{min} is calculated from the average of the coldest month, **LR1–LR35, landraces collected in 2011 from different agroclimatic areas in Lebanon; LR36–LR50, landraces recovered from ICARDA GenBank (Aleppo) and initially collected in the nineties from different agroclimatic areas in Lebanon.

finally destained in solution containing 9% methanol and 2% acetic acid. Relative electrophoresis was performed at 4°C until the blue marker reached the bottom of the gels. The gels were visualized by transilluminator and scanned by Bio-Rad versa DOC3000 gel imaging system. 'Broad range protein marker' was brought from BIOMATIK.

Analysis of banding patterns

Gels were photographed while identification and analysis of protein bands were performed by the software of Quantity one 4.5.1 (Bio-Rad). Molecular weight of protein bands were estimated by their relative mobility. Monomeric prolamins were classified as present (represented by 1) or absent (represented by 0) of all the examined bands. A data matrix was prepared for the analysis and their frequencies were calculated.

Statistical analysis

The data matrix was converted into a matrix of similarity (S) values using Jaccard coefficient (Jaccard 1908). For a pair of two accessions, i and j, this coefficient is calculated as:

$$S_{ij} = \frac{n_{ij}}{n_{ij} + n_i + n_j},$$

where n_i is the number of bands present in accession i and absent in accession j, n_j is the number of bands present in j and absent in i, and n_{ij} is the number of bands shared by the two accessions i and j.

A tree is then inferred using the unweighed pair group method by unweighed pair group method with arithmetic means (UPGMA) clustering algorithm and Jaccard similarity coefficients. All analyses were done using NTSys Pc program ver. 2.1 (Rohlf 1999). To find out the relation

between the obtained linkage groups and parameters of row numbers, agroclimatic conditions and geographic origin, a chi-square test was conducted (Pearson 1897).

Results and discussion

Polymorphism of hordein bands

The SDS-PAGE is considered as a reliable technology, simple and mostly employed for identification of genetic diversity and analysis of genetic structure of germplasm. In this study, hordein polymorphism analysis was carried out with the SDS-PAGE method. The full compositions of storage proteins were separated by 12% separation gel and divided into four groups (A, B, C and D hordeins). The major part of hordeins (80%) is formed by B-hordeins and C-hordeins, while A-hordeins are not considered to be a storage fraction and the D-hordeins are less variable than B-hordeins and C-hordeins (Salcedo *et al.* 1980). Two zones, A and D, were not taken into account to make long migrations and obtain a better resolution for zones B and C. All possible bands of the slow moving *Hor-1* and the fast migrating *Hor-2* were identified. A total of 37 bands were observed ranging from 20 to 87 KDa. Electrophoresis of barley storage protein revealed 53 types of patterns with different electrophoresis motilities as shown in figure 2a. This result indicated an important set of polymorphism. While examining the results of C-hordeins for all accessions, 17 patterns (C1–C17) and nine different bands ranged from 56–87 kDa were observed, of which pattern C4 was the most common occurring in 19.16% of the accessions. The diagrammatic representation of hordein band patterns is illustrated in figure 2b. As of the Lebanese landraces, all bands were polymorphic, except one that was monomorphic and found in all accessions. In the zone B, 36 patterns (B1–B36) and 28 bands ranging from 20 to 53 kDa were observed; the most common pattern was B3 which was found in three accessions, followed by B1 recorded in two accessions. The electrophoregram revealed a total of 37 bands and 53 different patterns among the studied accessions in areas B and C with an average number of bands of 12.28 and a maximum of 17 bands per landrace (figure 2b). Accessions designated as LR26 and LR16 as well as Manel and Rihane-3 varieties had the least number of eight bands, while accessions LR34 and LR19 had the maximum number of 17 bands.

These results are in line with those of Eshghi *et al.* (2012) obtained in naked barley accessions, where an average band number of 14.65 and 32 different patterns were found for B-hordeins and C-hordeins. Cooke (1995) reported up to 105 bands among 706 barley cultivars, while Sipahi *et al.* (2010) obtained 15 bands in 34 cultivars. Recently, Baloch *et al.* (2014) observed 20 hordein band patterns in 90 accessions collected from four different sites of Jordan. In fact, this large variability can be explained by the duplication and the divergence of an ancestral wild gene that generates a multigenic family (Shewry 1995). This later family provides additional opportunities for increasing polymorphism resulting from its neutral nature at evolution level and its high tolerance to mutations. One cause of this variability may be the linkage of the *hordein* loci to the *Mla* locus, which specifies the resistance to different races of powdery mildew (Oram *et al.* 1975).

Genetic similarities and cluster analysis

To elucidate the genetic relationships among the 53 barley accessions studied, a dendrogram was produced using UPGMA cluster analysis and Jaccard similarity coefficients based on the polymorphism data generated by B-hordeins and C-hordeins. Four groups were differentiated at a distance of 0.66 (figure 3). Group 1 is the largest one, constituted of 27 landraces of different row numbers and various agroclimatic areas. The second group contains only nine landraces LR33, LR17, LR32, LR25, LR28, LR6, LR13, LR26 and LR18. The third group is formed by 14 landraces LR14, LR27, LR11, LR29, LR24, LR31, LR4, LR12, LR22, LR41, LR20, LR40, LR2, LR16, in addition to the wild *H. spontaneum*. Finally, the two commercial varieties, Manel and Rihane-3, are found as singleton groups. It is worth noting that landraces sampled from the different agroclimatic areas of Lebanon do not cluster separately. As to the chi-square test (table 2), the genetic variability revealed in this study was not correlated to the geographic origin of samples provenance ($P = 0.095$; chi-square = 14.87) or to the landrace row numbers ($P = 0.694$; chi-square = 1.45).

According to the genetic relationships among the 53 accessions based on the B-hordein and C-hordein markers, the landraces were clustered into three different groups separately from the commercial varieties. In addition, landraces included in the third group are less domesticated than the other studied samples. Further, the accessions designated as LR45, LR46, LR39, LR5, LR35, LR49, LR50, LR47, LR38 and LR37 are genetically more divergent than the commercial varieties. However, the two landraces LR36 and LR3 are very similar (GS = 1). This later result can therefore be explained by two assumptions; LR36 and LR3 accessions are identical, but they are duplicated under two different designations, or these two landraces have a common ancestor that is very close in the time.

Lebanon is a small country where seeds are easily exchanged between farmers' communities in the different parts of the country. This may explain the absence of correlation between the genetic variability and the geographic origin of sample provenance as shown in the clustering of landraces. Similar results were previously reported for barley landraces growing in Jordan (Baloch *et al.* 2014). According to Nevo (1998), gene flow is expected in the Fertile Crescent region since populations of cultivated barley have long been growing in close proximity. Similar results were previously reported by Atanassov *et al.* (2001). Contrarily, Rahimi *et al.* (2014) pointed out the relation between the polymorphism of storage proteins and geographical provenances of barley accessions. It is worthy to note that such correlation is

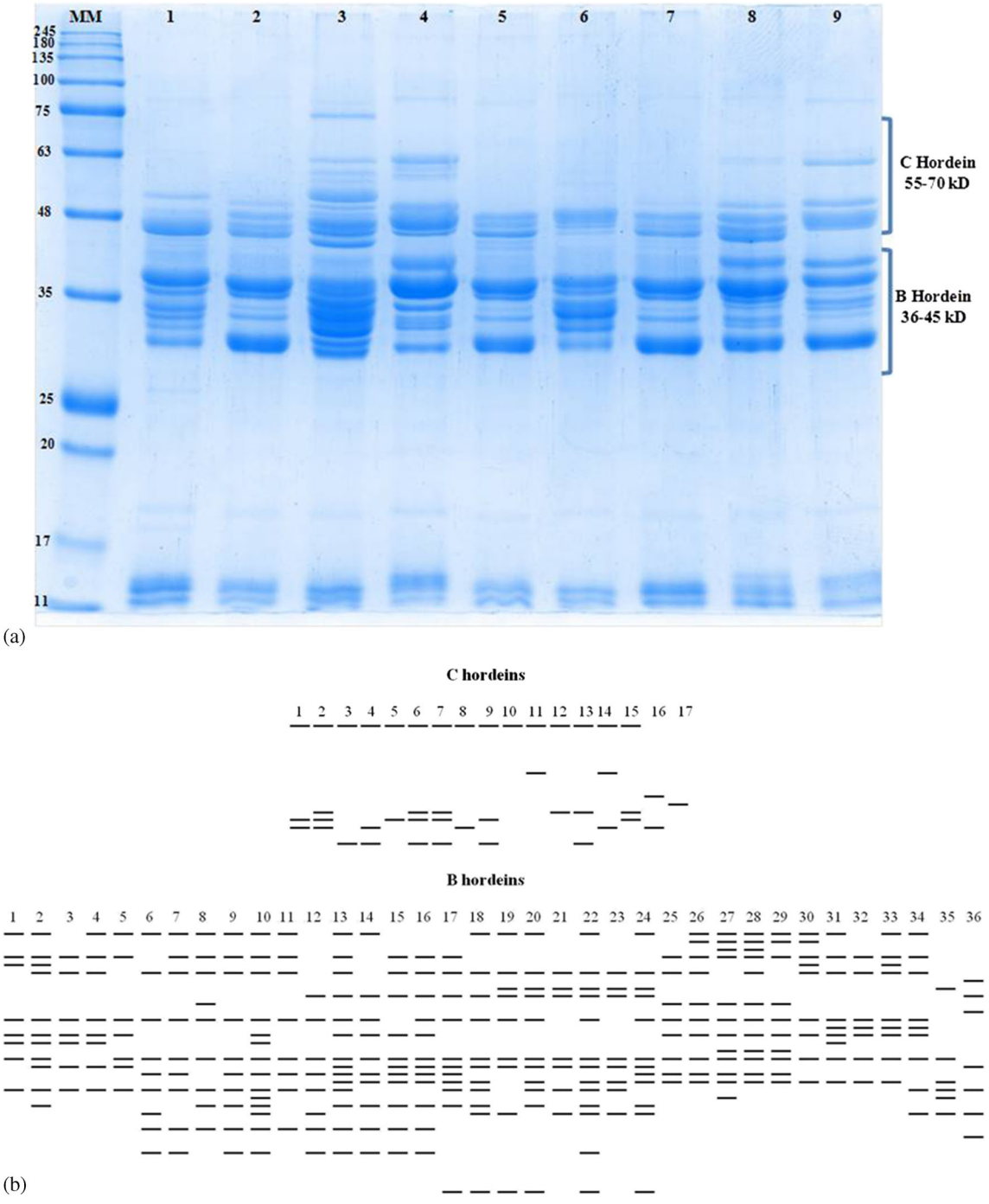

Figure 2. SDS-PAGE analysis of B- and C-hordein proteins in different barley landraces. (a) Electrophoretic profile of some barley landraces separated by SDS-electrophoresis from single seeds. PM indicates protein molecular weight marker 'broad range protein marker' from Biomatik. (1), (2), (3), (4), (5), (6), (7), (8) and (9) represent different barley landraces. (b) Diagrammatic representation of all SDS-PAGE patterns of B-hordein and C-hordein observed in the materiel study. The thickness of the line represents the most common intensity of the band of each accession.

strongly influenced by the number of samples considered in the study.

In conclusion, our paper provided the first assessment of genetic diversity for a set of 50 Lebanese barley landraces in comparison to the wild *Hordeum spontaneum* and two commercial varieties. Although preliminary, the clustering of landraces described here is of high priority to be used through appropriate breeding methods. Our findings confirm the effectiveness of hordein markers in studying the polymorphism and assessing the phylogenetic relationship among accessions collected from various agroclimatic areas. Nevertheless, this technique remains limited by the low number of seed storage protein markers in any particular species that may generate an insufficient polymorphism

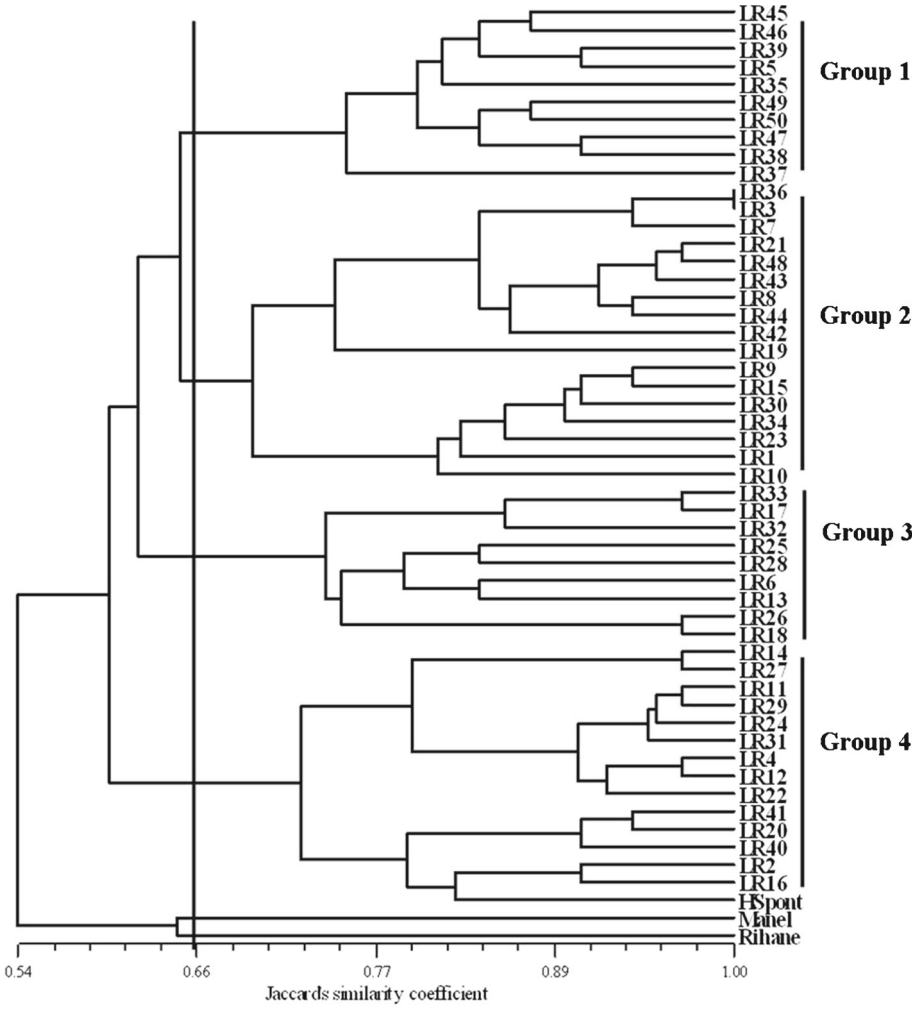

Figure 3. Dendrogram of 53 barely accessions generated by unweighed pair group method using an arithmetic average cluster analysis using Jaccard similarity coefficients from B-hordein and C-hordein protein markers.

Table 2. Probability value for a chi-square test from four barely linkage groups.

	Rows number (2 or 6)	Region*	Altitude**	Rainfall**
Chi-square	1.45	15.70	4.63	19.36
P value	0.694	0.073	0.592	0.022

* All studied landraces were selected from four Lebanon regions (Bekaa, south, Balbek and north), see table 1 for more details.
** Based on the data in table 1 and the box plot performed by SPSS software, the altitude and rainfall were classified into three and four groups, respectively.

among closely-related genotypes (Bonfitto *et al.* 1999). For this reason, this first evaluation of barley genetic resources in Lebanon should be further completed by a DNA-based markers analysis such as microsatellites.

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