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Phenotypic diversity of barley (*Hordeum vulgare* L. subsp. *vulgare*) landraces from the Center and the South of Tunisia and identification of potential area of on-farm conservation

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Abstract-Landraces represent a crucial reservoir of biodiversity and source of novel gene alleles for breeding programs and for sustainable agriculture towards climate change. In this context, a morphological characterization of 882 barley accessions collected from the Center (Mahdia) and the South (Gabes) of Tunisia was conducted using 24 qualitative and 3 quantitative traits related to the spike and grain characteristics. The phenotypic diversity was determined by the Shannon-Weaver diversity Index (*H'*)and revealed that spike length (*H'* = 0.83), aleurone color (*H'* = 0.73) and intensity of the anthocyanin coloration of awn tips (*H'* = 0.71) werethe most polymorphic traits. The genetic diversity within populations (H_S= 0.25) represented the major proportion of the total genetic diversity (H_T= 0.28). Data showed considerable gene flow (N_m = 4.17) and low genetic differentiation (G_{ST} = 0.11). This suggests that Tunisian barley landraces are closely related but have an intra-population variability.Regarding the surveyed areas, Mahdia showed a slightly higher phenotypic diversity compared to Gabes. In particular, the locality of Menzel Habib showing the highest polymorphismwas proposed as suitable place for the *in situ*/on-farm conservation of local germplasm. The factorial correspondence analysis showed that morphological markers play a relevant role in discriminating accessions than geographic regions. Row type, spike shape and density, rachilla hair type, and grain and aleurone color were the discriminatorytraitsofTunisian barley landraces.

Keywords: Barley,*in situ* conservation,landraces, morphological traits, Shannon-Weaver diversity index (*H'*),.

1. Introduction

In Tunisia, barley (*Hordeum vulgare* L. subsp. *vulgare*) is the second cereal, cultivated in all the regions of the country from the Northern to the Southern zones. However, this species is more abundant in the semi-arid and arid regions of Central and Southern Tunisia(El Felah and Medimagh 2005). It is mainly used as livestock feed (85%) and secondarily as human food (15%). In semi-arid areas, barley is mainly cultivated by farmers and mown one or two times depending on the availability of forage and pastoral resources. In intermediate zones, this species is cultivated for grain production. However, in areas where oats are not available, barley is coupled with forage legumes for hay production (El Felah and Medimagh 2005). The national production of barley is only 50% of the current demand of this commodity (El Felah2011). A greater dependence of the cropping areas on climatic conditions and on the use of cultivars characterized by a low performance, unstable and sensitive to the main diseases represent a major constraint of this crop.

Since 1931, a large number of new barley cultivars was introduced in Tunisia. Around the same time, genetic crosses were based on conventional improvement aiming the productivity and the technological quality (Deghaïs et al. 1999; Deghaïs et al. 2007). The substitution of barley landraces by the improved varieties had led farmers to practice the monoculture in uncountable farmlands. Currently, the improved varieties, Rihane and Manel, cover more than 50% of the barley-cultivated

area. However, barley landraces are cultivated by few poor resourced farmers in marginal environments (El Felah2011; Gharbi and El Felah 2013). The most important are distributed on 8 geographical areas; Djebali (North of Tunisia), Frigui (West South of Tunisia), Beldi (Sahel), Sahli (Moknine), Sfira (Gabes), Ardhaoui (Gabes and Medenine), Djerbi (Djerba), and Aarbi (common barley) (El Felah2011). Although less productive, these landraces are genetically more diversified and better adapted to the local environmental conditions. They constitute a valuable genetic reservoir that can be useful in future breeding programs (El Felahet al. 1991).

To preserve this native material, programs of genetic resources management (i.e. collection, characterization, evaluation and conservation of genetic resources) deserve more attention. The characterization of the existing biodiversity is a main component of such program. Several authors indicated that morphological markers including spike and seed qualitativeand quantitative traitscould be used for an effective characterization of wheat or barley diversity(Al Khanjari 2008; Hadado 2009).

Genetic conservation is also considered as major element of any strategy of protection of genetic diversity. Both the *in situ* and *ex situ* conservation are a complementary techniques required to counteract the genetic erosion. In particular, the *in situ* conservation is an appropriate technique for the traditional varieties. In fact, the genetic and ecological evolution of this material synchronized with wild relatives is maintained, making a dynamic and balanced gene transfer (Engelmann and Engels2002). In opposite, the *ex situ* conservation might lead to the loss of genetic diversity due to genetic drift after rejuvenation and seed increase cycles (Jaradat2004). On-farm conservation is one approach to *in situ* conservation of genetic resources. Negri et al. (2009) defined this type of conservation as "the management of genetic diversity of locally developed crop varieties (landraces) by farmers within their own agricultural, horticultural or agri-silvicultural systems". Given their expertise, incorporation of farmers and local communities into the program of genetic resources management is judicious. In fact, in a low-input context in marginal systems, farmers still save a proportion of seed of their crops for sowing and cultivation, and these cycles had been repeated for millennia. In addition, farmers are likely to know the nature and extent of local crop resources better than anyone through their daily interactions with the diversity in their fields.

In this work, we aimed to (i) assess the phenotypic diversity of barley landraces collected from the Center (Mahdia) and the South (Gabes) of Tunisia using a large number of international morphological descriptors (ii) identify the discriminatory traits of barley landraces, and (iii) determine a potential area for the *in situ*/on-farm conservation.

2. Materials and methods

2.1. Plant collection

A total eight hundred eighty two (882) accessions of barley were used in this study. The barley populations, relative to different collection sites (9 sites), were especially collected in Mahdia (Tunisian Center) and Gabes provinces (Tunisian South) (Table 1).

Table 1. Locations and numbers of collected barley accessions.

Site	Province	Locality	Latitude	Longitude	No. of Accessions
10	Gabes	Menzel Habib	34°11'55.9''N	9°42'32.7''N	117
12	Gabes	Menzel Habib	34°11'36.7''N	9°42'00.4''N	9
13	Gabes	Menzel Habib	34°11'36.7''N	9°42'32.7''N	20
18	Gabes	El Hamma	33°53'03.8''N	9°44'05''N	60
21	Gabes	El Hamma	33°41'48.4''N	9°59'12.2''N	198
70	Mahdia	Kassass	35°29'51.6''N	11°01'29.9''N	209
73	Mahdia	Bou Merdes	35°27'17.54''N	10°44'6.65''N	71
74	Mahdia	Bou Merdes	35°27'16.68''N	10°44'6.31''N	85
78	Mahdia	El Hekayma	35°28'49.50''N	10°54'31.6''N	112
					Total: 882

2.2. Morphological characterization

The description of barley collection was conducted using international standards of 'Bioversity International' (IPGRI1994) and 'International Union for the Protection of New Varieties of Plants' (UPOV1994). Morphological data of each accession were based on 24 qualitative and 3 quantitative traits related to spike and grain characteristics (Table 2).

Table 2. List of the studied traits.

Traits	Source	Type	States
Spike shape	UPOV	C	Tapering, parallel, fusiform
Spike density	IPGRI	C	Very lax, lax, medium, dense, very dense
Spike attitude	UPOV	C	Erect, semi-erect, horizontal, semi-recurved, recurved
Number of rows	IPGRI	C	Two rowed, six rowed, other
Attitude of sterile spikelet	UPOV	C	Parallel, parallel to weakly divergent, divergent
Number of spikelet groups (triplets) per spike	IPGRI	Q	Very weak, weak, medium, strong, very strong
Spike length (excluding awns)	UPOV	Q	Very short, short, medium, long, very long
Lemma awn/hood	IPGRI	C	Awnless, awnleted, awned, sessile hoods, elevated hoods
Awn color	IPGRI	C	White, yellow, brown, reddish, black, other
Anthocyanin coloration of tip awns	UPOV	C	Absent, present
Intensity of anthocyanin coloration of tip awns	UPOV	C	Very weak, weak, medium, strong, very strong
Awn length (compared to spike)	UPOV	C	Short, medium, long
Length of glume and its awn relative to grain	IPGRI	C	Shorter, equal, longer, glume plus awn nearly twice as long as kernel, lemma-like
Curvature of first segment of rachis	UPOV	C	Absent or very weak, weak, medium, strong, very strong
Length of first segment of rachis	UPOV	Q	Short, medium, long
Lemma awn barbs	IPGRI	C	Smooth, intermediate, rough
Rachilla hair type	IPGRI	C	Short, long
Husk	IPGRI	C	Absent, present
Glume color	IPGRI	C	White, yellow, brown, black
Lemma color	IPGRI	C	Amber, tan/red, purple, black, other
Anthocyanin coloration of nerves of lemma	UPOV	C	Absent or very weak, weak, medium, strong, very strong
Spiculation of inner lateral nerves of dorsal side of lemma	UPOV	C	Absent or very weak, weak, medium, strong, very strong
Kernelcovering	IPGRI	C	Naked grain, semi-covered grain, covered grain
Grain (pericarp) color	IPGRI	C	Amber, tan/red, purple, black/grey, other
Color of aleurone layer	UPOV	C	Whitish, weakly colored, strongly colored
Disposition of lodicules	UPOV	C	Frontal, clasping
Hairiness ofventral furrow	UPOV	C	Absent, present

C - qualitative trait; Q - quantitative trait

2.3. Statistical analysis

2.3.1. Relative phenotypic diversity index (*H'*)

The Shannon-Weaver diversity index (*H'*) (Shannon and Weaver 1949), as described by Hutchenson(1970), was used to assess the phenotypic variability of each character within province, locality and collection site:

$$H' = -\sum_{i=1}^{n} Pi\mathrm{Ln}Pi$$

where *n* is the number of phenotypic classes of each trait and P_i is the relative genotype frequency in the i^{th} class of the j^{th} trait. In this case, quantitative traits (e.g. number of spikelets per spike, spike length and length of first segment of rachis) were converted into qualitative traits with different phenotypic classes. The index (*H*) was standardized by converting it to a relative phenotypic diversity index (*H'*) after dividing it by H_{max} (Ln (*n*)).

$$H' = -\sum_{i=1}^{n} Pi\mathrm{Ln}Pi \,/H_{max}$$

The minimum value of the index (*H'*) is zero for monomorphic trait. However, the value of this index increases with the rise of polymorphism and reaches the maximum value (1) when all phenotypic

classes have equal frequencies. Experimental data were subjected to one way analysis of variance using the SAS package (SAS V9.1) with the Least Significance Difference (LSD) test. Differences were considered significant at $P<0.05$. For genetic diversity analysis, total genetic diversity (H_T), genetic diversity within populations (H_S) and genetic differentiation (G_{ST}) and the gene flow(N_m) were calculated according to Nei(1978). Statistical analysis was conducted using POPGENE 1.32 software.

2.3.2. Factorial correspondence analysis (FCA)

The FCA, a multivariable analysis to visualize genetic distances between barley accessions based on similarities in trait interrelationships and adjustments, was performed in SAS software (version 9.1). Factorial axes were ordered according to their eigenvalues, larger values explaining more of the general structure in the dataset.

3. Results and discussion

3.1. Estimation of the relative diversity index of traits

The average relative diversity index revealed that the Tunisian collection of barley landraces was characterized by a relatively low polymorphism ($\overline{H'}$ = 0.28) compared to those of Ethiopia ($\overline{H'}$ = 0.68) (Negassa1985), ($\overline{H'}$ = 0.71) (Demissie and Bjornstad 1996), ($\overline{H'}$ = 0.53) (Fekadu et al. 2018)and Oman ($\overline{H'}$ = 0.50) (Jaradat 2004)(Table 3). Spike length (H' = 0.83), aleurone color (H'= 0.73) and intensity of the anthocyanin coloration of awn tips (H' = 0.71) were the most polymorphic traits. Similar findings were reported by Negassa(1985),Al-Nashash et al.(2007)and Hagenblad et al. (2019)showing that spike length was the most polymorphic trait in Ethiopian and Jordanian barley populations. However, Demissie and Bjornstad(1996) found that rachilla hair type (H' = 0.92) and aleurone color (H' = 0.90) were the most polymorphic qualitative traits in Ethiopian barley populations.It is worth noting that morphological markers were used to assess the genetic diversity of barley landraces but also to determine their differences and similarities. Indeed, frequencies of phenotypic classes and the relative phenotypic diversity index revealed some similarity between the studied populations. For the qualitative traits, these populations had awns longer than the spike (100%) with moderate pilosity (100%) (Figure 1). These results are in accordance with those observed by Medimagh (2000) showing that Tunisian barley landraces only presented long and rough awns. In fact, smooth awned types are accompanied by greater floret sterility and are slightly lower-yielding than the rough awned ones. On the other hand, smooth awns are preferred and used in barley breeding programs to avoid irritation caused by the rough awns (Harlan1940). The collection was also monomorphic for the kernel covering with 100% covered grain. The naked barley is very rare in Tunisia and was only represented by one variety, named “prophet barley” or “Moknine barley” (Bel Hadj Salem1999). In fact, previous reports have demonstrated that naked gene has multiple effects on many traits including yield reduction and lower seed weight (Barabashi 2012; Choo 2001) which can explain the rarity of this morphotype. All the glumes had yellowish color, the lodicules had clasping tendency and the ventral furrow was always glabrous (Figure 2). The six-row barleys were the most prevalent in the studied collection (97%) (Figure 1). This type of barley represent the main species of cultivated barley in Tunisia, whereas the four-row barleys are absent in local populations (El Felah and Medimagh2005). The predominance of the six-row barleys was also noticed in Ethiopia (Negassa 1985; Demissie and Bjornstad 1996), in Oman (Jaradat2004)and in Canary Islands (Hagenblad et al. 2019)revealing the farmers’ preference of this barley type. Based on general spike shape, the barley landraces were mainly characterized by spikes with parallel edges (95%) and intermediate density (92%) (Figure 1). The same result was reported by Medimagh(2000) showing that farmers selected rachis with distant points of insertion to favor a better grain filling. The attitude of spike was a polymorphic trait as shown in Ethiopian barley germoplasm (Mekonnon et al. 2015). Accessions with yellow awns (99%), amber lemma color (99%) and long rachilla hair (91%) were the most frequent (Figures 1, 2). These accessions were also characterized by a very strong speculation of inner lateral nerves of lemma dorsal side (98%) and an equal length of glume and its awn to the grain size (89%). These results could suggest the presence of a conscious or unconscious selection for these traits developed by barley during its domestication in Tunisia. However, polymorphic traits e.g. spike attitude, anthocyanin coloration of tip awns and anthocyanin coloration of nerves of lemma showed no evident relevance in the empirical choice of the local germplasm (Figures 1, 2).

Table 3. Relative diversity index *(H')* of the different studied traits of barley collection according to regions and localities.

Traits	Relative diversity index (*H'*) of the collection	Relative diversity index (*H'*) according to provinces		Relative diversity index (*H'*) according to localities				
		Gabes	Mahdia	Bou Merdes	El Hekayma	El Hamma	Kassass	Menzel Habib
Spike shape	0.28	0.12	0.38	0.00	0.00	0.00	0.66	0.27
Spike density	0.26	0.34	0.22	0.00	0.07	0.04	0.46	0.74
Spike attitude	0.60	0.60	0.75	0.81	0.62	0.63	0.68	0.57
Number of rows	0.19	0.35	0.00	0.00	0.00	0.13	0.00	0.62
Attitude of sterile spikelet	0.12	0.23	0.00	0.00	0.00	0.09	0.00	0.62
Number of spikelet groups (triplets) per spike	0.58	0.35	0.68	0.79	0.75	0.09	0.61	0.73
Spike length (excluding awns)	0.83	0.82	0.80	0.79	0.52	0.64	0.76	0.70
Lemma awn/hood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Awn color	0.01	0.00	0.02	0.06	0.00	0.00	0.00	0.00
Anthocyanin coloration of tip awns	0.57	0.35	0.70	0.99	0.18	0.36	0.08	0.33
Intensity of anthocyanin coloration of tip awns	0.71	0.85	0.68	0.82	0.82	0.84	0.66	0.93
Awn length (compared to spike)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Length of glume and its awn relative to grain	0.32	0.25	0.39	0.42	0.40	0.09	0.46	0.45
Curvature of first segment of rachis	0.49	0.43	0.60	0.52	0.58	0.05	0.67	0.68
Length of first segment of rachis	0.48	0.44	0.52	0.49	0.20	0.25	0.60	0.64
Lemma awn barbs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rachilla hair type	0.43	0.26	0.54	0.37	0.22	0.17	0.74	0.39
Husk	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glume color	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lemma color	0.04	0.00	0.06	0.15	0.00	0.00	0.00	0.00
Anthocyanin coloration of nerves of lemma	0.66	0.64	0.66	0.75	0.61	0.65	0.71	0.79
Spiculation of inner lateral nerves of dorsal side of lemma	0.09	0.00	0.14	0.27	0.00	0.00	0.11	0.00
Kernel covering	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grain (pericarp) color	0.31	0.28	0.32	0.17	0.13	0.20	0.47	0.58
Color of aleurone layer	0.73	0.70	0.72	0.43	0.57	0.58	0.93	0.80
Disposition of lodicules	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hairiness ofventral furrow	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$\overline{H'}$	0.28±0.05	0.26±0.05[a]	0.30±0.06[a]	0.28±0.06[a]	0.20±0.05[a]	0.17±0.05[a]	0.31±0.06[a]	0.35±0.06[a]

$\overline{H'}$ - values are means ± standard error and different letters indicate significant differences at P<0.05 (LSD test) between provinces and localities.

Considering the grain characteristics, the studied accessions showed a predominance of grey grains (91%) and highly colored aleurones (66%) (Figure 2). In opposite, El Felah and Medimagh(2005) found that yellow grains are the most frequent in local populations collected in three provinces (Mahdia, Monastir and Kairouan). The predominance of these colors might depend on the regional farmer's preference and the subsequent use of these types of barley.

For quantitative traits, spikes with very short (23%), short (31%) and medium (37%) length were the most frequent in this collection. These morphotypes could be considered as a form of adaptation to the environmental conditions of the Center and the South of Tunisia. Most accessions were also characterized by a very low (66%) or low (22%) number of spikelets per spike. In fact, this characterdepend on spike length and density, and on grain size (Medimagh 2000; Madić 2012).

3.2. Variation of the relative diversity index according to provinces

Regardingthe surveyed areas (Table 3), the region of Mahdia ($\overline{H'}$ = 0.30) showed higher phenotypic diversity compared to the region of Gabes ($\overline{H'}$ = 0.26), but the difference was not significant (P<0.05). The region of Mahdia had wide distribution among the different phenotypic classes forthe majority of traits. Therefore, the cultivation conditions in the Center region of Tunisia seem to favor a better diversification oflocal barley.This could be attributed to the habits of local communities,the main actors of the conservation and the evolution of thegenetic diversity.

Our study showed also the absence of a morphological specificity of Tunisian barley landraces according to provinces, except the presence of two-row barleys in the region of Gabes.In opposite, a close association between phenotypic classes andwell-determined regions was recorded in Ethiopian barley landraces (Demissie and Bjornstad 1996; Kebebew 2001; Derbew 2013).

3.3. Variation of the relative diversity index according to localities

The present research showed a large morphological variation of several traits between localities (Table 3). For instance, Bou Merdes was the only locality showing a variability in awn color (H' = 0.06), lemma color (H' = 0.15) and spiculation of inner lateral nerves of lemma dorsal side (H' = 0.28). On the other hand, bothtypes of barley (two-row and six-row barley) were only found in the localities El Hamma (H' = 0.13) andMenzel Habib (H' = 0.62). However, traits including intensity of anthocyanin coloration of tip awns, spike length, anthocyanin coloration of lemma nerves presented approximately a similar diversityfor all the localities.The average relative diversity index was not significant (P<0.05) between the localities. Menzel Habib was the most polymorphicregion ($\overline{H'}$ = 0.35), whereas El Hamma was the lowest polymorphic region ($\overline{H'}$ = 0.17). In fact, Menzel Habib is known as an airflow zone(Personal communication), which could promote the intercrossing.

Currently, there is an increasing interest in the identification of a suitable area for the *in situ*/on-farm conservation. This type of conservation might maintain the genetic integrity of the local populations and preservetheir evolutionary potential (Rao 2004). Consequently, the great diversityobserved in Menzel Habib makes this site an exceptionally suitable placefor this dynamic conservation.

3.4. Variation of the relative diversity index according to populations

Traits includingspike shape and density showed a large variation of the diversity index between populations. In opposite, spike attitude, spike length and curvature of the first segment of rachis showed the same pattern of morphological diversity (Table 4). The highest and the lowest $\overline{H'}$-value was recorded in population 70 (0.32) and population21 (0.18), respectively.

Figure 1. Macroscopic characteristics of spike of collected barley accessions.

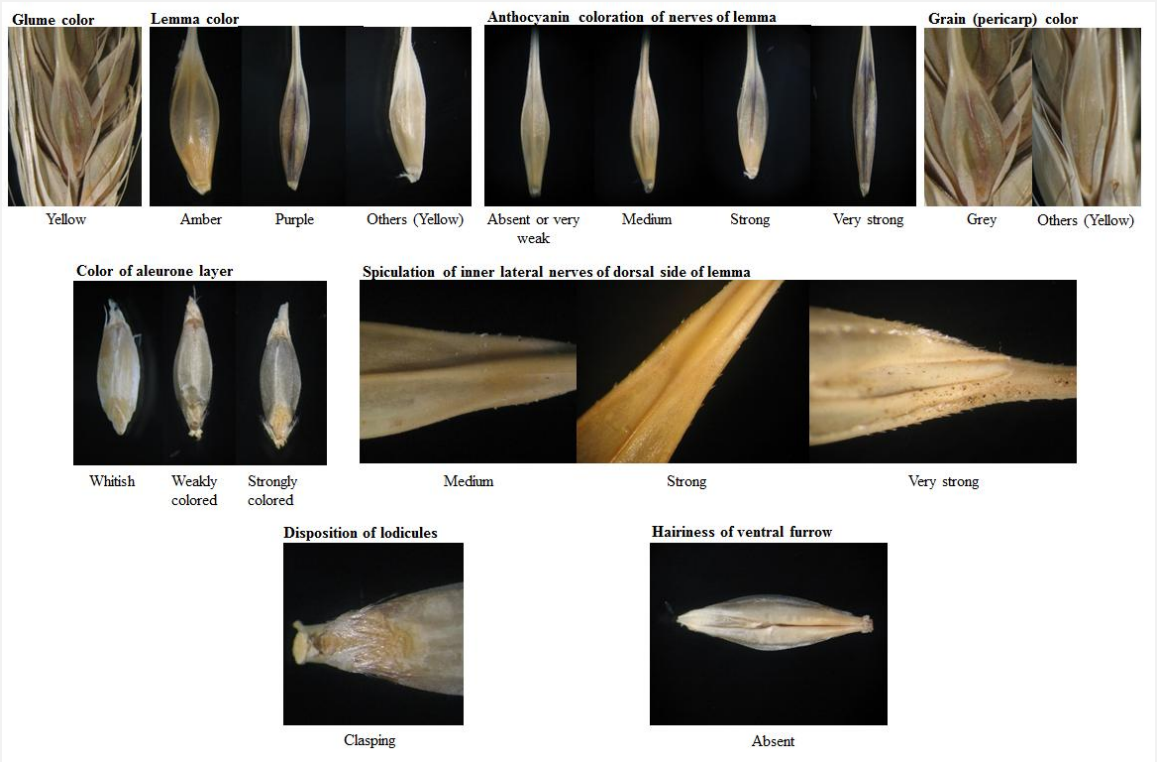

Figure 2. Macroscopic characteristics of glume, lemma and grain of collected barley accessions.

Table 4. Relative diversity index (H′) of the different populations relative to the collection sites.

Traits	010	012	013	018	021	070	073	074	078
Spike shape	0.00	0.59	0.00	0.00	0.00	0.66	0.00	0.00	0.00
Spike density	0.00	0.00	0.00	0.18	0.00	0.46	0.00	0.00	0.32
Spike attitude	0.54	0.59	0.72	0.63	0.76	0.68	0.82	0.76	0.62
Number of rows	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00	0.00
Attitude of sterile spikelet	0.00	0.00	0.00	0.37	0.00	0.00	0.00	0.00	0.00
Number of spikelet groups (triplets) per spike	0.54	0.91	0.86	0.39	0.00	0.61	0.81	0.89	0.75
Spike length (excluding awns)	0.69	0.87	0.47	0.86	0.61	0.76	0.96	0.76	0.52
Lemma awn/hood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Awn color	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.00
Anthocyanin coloration of tip awns	0.00	0.00	0.29	0.52	0.33	0.08	0.87	0.82	0.18
Intensity of anthocyanin coloration of tip awns	0.72	0.00	0.00	0.81	0.70	0.66	0.82	0.81	0.82
Awn length (compared to spike)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Length of glume and its awn relative to grain	0.27	0.00	0.95	0.18	0.08	0.46	0.47	0.37	0.40
Curvature of first segment of rachis	0.49	0.59	0.72	0.49	0.50	0.67	0.81	0.50	0.58
Length of first segment of rachis	0.64	0.86	0.61	0.26	0.24	0.60	0.54	0.45	0.20
Lemma awn barbs	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Rachilla hair type	0.19	0.00	0.00	0.18	0.17	0.74	0.26	0.45	0.22
Husk	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glume color	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lemma color	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00
Anthocyanin coloration of nerves of lemma	0.80	0.92	0.81	0.41	0.64	0.71	0.63	0.82	0.61
Spiculation of inner lateral nerves of dorsal side of lemma	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.41	0.00
Kernel covering	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Grain (pericarp) color	0.49	0.00	0.00	0.65	0.12	0.47	0.19	0.20	0.13
Color of aleurone layer	0.83	0.00	0.99	0.31	0.62	0.93	0.47	0.62	0.57
Disposition of lodicules	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hairiness ofventral furrow	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$\overline{H'}$	0.23±0.06[a]	0.20±0.07[a]	0.24±0.07[a]	0.25±0.05[a]	0.18±0.05[a]	0.32±0.06[a]	0.29±0.07[a]	0.30±0.06[a]	0.22±0.05[a]

$\overline{H'}$ - values are means ± standard error and different letters indicate significant differences at P<0.05 (LSD test).

The genetic diversity within populations (H_S= 0.25) presented 89.3% of the total genetic diversity (H_T= 0.28) (Table 3), whereas the diversity attributed to differences between populations is minority (Table 5).Indeed, G_{ST} value near to 1.0 indicates a majority of genetic variability among populations. Conversely, a lower G_{ST} value as obtained in our study (G_{ST} = 0.11) suggests a majority of genetic variability within populations. This result suggests that the Tunisian barley landraces are closely related, but present an intra-population variability.These findings are in agreement with those of Harrabi et al. (1988)who assumed that all local cultivated barleyfrom the North to the South of Tunisia are both closely related and veryvariables. Abdellaoui et al. (2007) reported that it is difficult todistinguish between the different barley ecotypes based on themorphological markers. The intra-population variability might be attributed to the farmer's preferences and some climatic conditions. As reported by Negri et al. (2009), farmers tend to suit the intra-farm multiple eco-agricultural conditions by growing a diverse range of landraces. Farmers select different types, characterized by different ripening times, destination uses, local taste preferences and other characteristics, so that more than one landrace is often developed on their farms.On the other hand, similarity in Tunisian barley landraces might be attributed to migration of genetic information between populations referred to gene flow (Allendorf and Luikart2007). Gene flow is a critical determinant of population genetic structure. Considering this barley germoplasm, the N_m value (4.17) is indicative of considerable gene flow between natural populations (Table 5). This value is above the level (N_m= 1) needed to consider that genetic drift is the main factor of genetic differentiation attributed, however, to gene flow (Slatkin 1987). As barley is predominantly a self-pollinating species (Kasha2007), the seed exchange between farmers or anticipate admixtures may be the main vectors of such migration. Thereafter, the various designations used by farmers ('Sahli', 'Beldi','Ardhaoui', 'Frigui', etc.) only indicate origins but not different forms of barley (Bettaïb-Ben Kaab1989). For example, the 'Frigui' name can have as origin the historical name of Tunisia 'Ifriquia', while the 'Ardhaoui' name might be linked to Berber poems and folk dance (El Felah and Medimagh 2005).These different provenances might be, however, the origin of the diversification of agro-physiological behaviors (El Felahet al. 1991), particularly related to their adaptation to local edaphic and climatic conditions.

Table 5. Genetic differentiation and gene flow among and within the barley populations.

Total geographical populations	H_T	H_S	G_{ST}	N_m
9	0.28±0.05	0.25±0.02	0.11	4.17

H_T - Total genetic diversity; H_S - Genetic diversity within populations; G_{ST} - Coefficient of gene differentiation; N_m - Gene fl among populations.

3.5. Factorial correspondence analysis (FCA)

In the FCA, the first six components (having eigenvalues > 1) explained about 63% of the variation. Thedistribution of barley accessions was performed on the main planformed by the first two axes, which contributed to35.69% of the total variation (Table 6, Figure 3). The axis 1 (22.47%) was positively correlated with spike shape, grain color, rachilla hair type and aleurone color. The same axis was negatively correlated with spike density. The axis 2 (13.23%) was positively correlated with number of spikelet groups per spike and spike length. It was negatively correlated with row number and anthocyanin coloration of tip awns.

Table 6. Eigenvectors, eigenvalues, total variance and cumulative variance of the first two factorial axes.

Traits	Axis 1	Axis 2
Spike shape	**0.916**	-0.029
Spike density	**-0.806**	0.414
Spike attitude	0.261	0.219
Number of rows	0.100	**-0.675**
Number of spikelet groups (triplets) per spike	0.048	**0.846**
Spike length (excluding awns)	0.334	**0.695**
Awn color	0.040	0.129
Anthocyanin coloration of tips awns	-0.168	**-0.517**
Length of glume and its awn relative to grain	-0.056	0.227
Curvature of first segment of rachis	0.325	0.046
Length of first segment of rachis	0.527	0.129
Rachilla hair type	**0.750**	-0.047
Lemma color	0.024	-0.001
Anthocyanin coloration of nerves of lemma	0.173	-0.069
Spiculation of inner lateral nerves of dorsal side of lemma	0.006	-0.039
Grain (pericarp) color	**0.797**	-0.074
Color of aleurone layer	**0.704**	0.060
Eigenvalue	3.81	2.24
Total variance (%)	22.47	13.23
Cumulative variance (%)	22.47	35.69

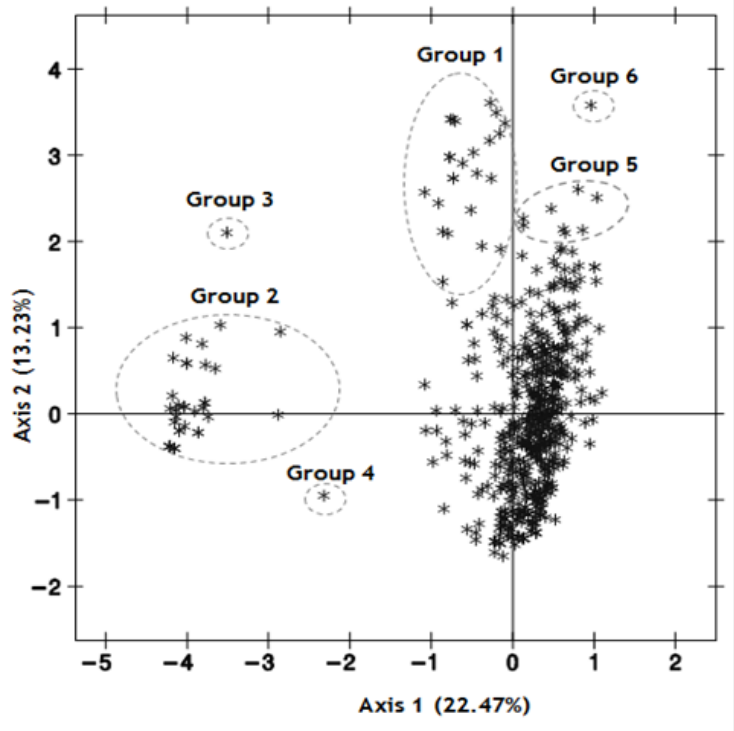

Figure 3. Two-dimensional plot of the factorial correspondence analysis (FCA) of 882 barley accessions.

The results showed the presence of an agglomeration of accessions in the center of the graph and several accessions were detachedfrom the whole (Figure 3). Based on their distribution, six groups were identified (Table 7). The FCA showed a clear distinction between two-row (Group 1) and six-row barleys (Groups 2, 3, 4, 5 and 6).This discrimination based on the number of rows was alsoobserved in other barley germplasms(Lasa 2001; Žáková and Benková 2004; Al-Nashash 2007; Muñoz-Amatriaín 2014; Reda et al. 2018). The groups formed by thesix-rowed accessions were

segregated based onthe spike density and shape (e.g. Group 3 and Group 4). Similarly,characters relative to spike (i.e. spike shape, spike density, spike length,etc.) were highly polymorphic and contributed to the discrimination ofseveral sub-populations of Omani barley (Jaradat2004). The spike length, density or attitude were often used by farmers to distinguish between their landraces(Negassa1985). Rachilla hair type, grainand aleurone color have also contributed to the distinction of barley accessions (e.g. Group 4 and Group 5). Consequently, the segregation of the different accessions was not based on the collection sites, but only on the discriminatory characters as reported by Al-Nashash et al.(2007) and Abebe et al.(2010).

Table 7. Characteristics of the six groups of barley accessions identified from the factorial correspondence analysis (FCA).

Group	No. of accessions	Collection site	Characteristics
1	22	13 -18	Two-rowed barley - parallel-shaped spike - long rachilla hair - very low intensity of the anthocyanin coloration of tip awns -strongly colored grain
2	25	70	Six-rowed barley - pyramid-shaped spike - dense spike - very weak curvature of the first segment of rachis - short rachilla hair – amber colored grains - whitish colored aleurone
3	1	70	Six-rowed barley - pyramid-shaped spike - very dense spike - very weak curvature of the first segment of rachis - short rachilla hair - grey colored grains - weakly colored aleurone
4	1	70	Six-rowed barley - parallel shaped spike - intermediate density of spike- very weak curvature of the first segment of rachis - short rachilla hair – amber colored grains - whitish colored aleurone
5	8	70 - 73 - 74 - 78	Six-rowed barley - very long spike – parallel-shaped spike - intermediate density of spike - grey colored grains - high number of spikelets per spike - long rachilla hair - highly colored aleurone
6	1	73	Six-rowed barley - very long spike – parallel-shaped spike - intermediate density of spike – grey colored grains – very high number of spikelets per spike - long rachilla hair - highly colored aleurone

4. Conclusion

Our investigation suggested that Tunisian barley landraces are closely related and the observed diversity is mainly within populations. Morphological markers showed a relevant role in discriminating accessions than geographic regions. This could be considered for future collections of barley germplasm and the choice of *in situ* conservation places.

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