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Full Length Research Paper

Population genetic structure analysis in endangered *Hordeum vulgare* landraces from Tunisia: Conservation strategies

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Genetic markers have been employed in combination with morphological characters to identify patterns of population structure in 13 barley landrace populations from Tunisia. These endangered barley populations are grown by few local farmers in low-input farming systems. Based on 117 random amplified polymorphic DNA markers and 34 morphological traits, variance analyses indicated that most of the variation is partitioned within rather than between populations. Inbreeding index, gene flow values and cluster analysis revealed also significant differentiation between all populations. Gene flow decreased rapidly as the geographic distance increased. This may imply that seed exchange between farmers was limited to a regional scale. The lower correlation between the Euclidean distance matrices based on morphological and molecular data suggests that both data are comparably important to generate an unbiased picture of differentiation trends. Our findings support the required setting up of conservation strategies for *Hordeum vulgare* L. landraces from Tunisia.

Key words: Barley landrace germplasm, RAPD, morphology, population differentiation analysis, conservation.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most economically important crops in the world, ranking fifth in the world production that is used for animal feed, brewing malts and human consumption (Hayes et al., 2002). *Hordeum* L. is a widely distributed genus of the tribe Triticeae of the Poaceae (Graminae) family found throughout the temperate zones of both northern and southern hemispheres (Morrell et al., 2003). Barley landraces constitute the evolutionary link (Jaradat et al., 2004) between wild barley (*Hordeum spontaneum* K. Koch), the only recognized wild progenitor of cultivated barley (*H. vulgare*) and modern barley cultivars.

In Tunisia, some poor resourced farmers still grow traditional barley landraces in marginal, low-input, drought-stressed environments for both grain and straw for animal and human feed. These landraces, which may represent valuable reservoirs of interesting genes useful in crop improvement for adaptation to biotic and abiotic stresses (Brush, 1995), are represented by small populations with a high risk of local extinction due to a progressive substitution by improved modern barley varieties. These latter are extensively used by most farmers replacing the old cultivars.

The conservation of genetic diversity within species of economic importance such as barley is important to ensure the potentialities of barley landrace populations' use. These landraces should be collected and thoroughly evaluated for future breeding programs (Soulé and Mills, 1992). Information on genetic variation within and between populations and about existing adaptability can therefore be valuable in prioritizing populations for conservation and for developing sustainable management practices (Storfer, 1996). The detection of within-population diversity structure, evolutionary history of populations and phylogeography is made easy by the high polymorphism of DNA molecular markers. For instance, with the advances in plant molecular biology, a number of molecular markers have been developed and

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Abbreviations: RAPD, Random Amplified Polymorphic DNA **.**

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Table 1. Origin, sample size and geographical characteristics for the sampled populations of Tunisian *H. vulgare* L. landraces.

Numeric code	Population locality	Sample size	Region of sampling	Latitude	Longitude
1	Beja	4	North-west	36°44'0" N	9°11'0"E
2	Kef	10	North-west	36°11'56"N	8°42'53"E
3	Bizerte	6	North	37°16'28"N	9°52'26"E
4	Siliana	5	North-west	36°5'0"N	9°22'0"E
5	Zaghouan	5	North	36°24'0"N	10°9'0"E
6	Kasserine	3	Centre-West	35°10'2"N	8°49'44"E
7	Kairouan	3	Centre-West	35°40'28"N	10°6'6"E
8	Sidi Bouzid	2	Centre-West	35°2'25"N	9°29'37"E
9	Sousse	2	Centre-East	35°49'32"N	10°38'28"E
10	Mahdia	6	Centre-East	35°30'17"N	11°3'44"E
11	Sfax	9	South-East	34°44'26"N	10°45'37"E
12	Gabès	4	South-East	33°53'0"N	10°7'0"E
13	Medenine	8	South-East	33°21'17"N	10°30'19"E

used extensively in diversity studies and germplasm management (Karp et al., 1996; Iqbal et al., 2009). In particular, RAPD can provide valuable data about genetic variations within and among populations of a species (Lynch and Milligan, 1994). Studies on *H. vulgare* landraces by means of morphological and molecular markers showed that most genetic variation was found within populations rather than between populations (Parzies et al., 2000; Koebner et al., 2002).

In this study, we assessed genetic diversity among barley landrace populations from Tunisia using RAPD markers and morphological traits. The objectives were to investigate the extent and distribution of RAPD and phenotypic variations within and between local landrace populations and provide baseline information for the development of strategies for the conservation.

MATERIALS AND METHODS

Plant material

Thirteen (13) barley (*H. vulgare* L.) landrace populations, comprising two to ten accessions each (Table 1), were used in this study. The accessions (67) were collected from various growing locations in Tunisia (Table 1).

RAPD analysis

Molecular genotyping was carried out using 10 RAPD markers (Table 2) following established methods (Zoghlami et al., 2007).

Phenotypic measurements

Morphological data were based on a set of 34 characters, corresponding to a subset of standard UPOV 'notes' (Table 5), including vegetative, inflorescence and grain descriptors. The accessions were randomised and seeded in trials (30 to 50 cm, three rows of 30 plants) with three replications in a sand peat mixture (2/3:1/3) under controlled conditions (16 h photoperiod and a day/night temperature regime of 18°C).

Data analysis

For RAPD analysis, bands were scored as 1 denoting presence or 0 denoting absence and a matrix of RAPD phenotypes was then, assembled across all individuals and populations. For each primer, the number of bands and the polymorphic ones were calculated.

The index of phenotypic diversity (H_o), the average diversity over all populations (H_{pop}) and the mean diversity at species level (H_{sp}) were estimated as described by Yeh et al. (1995). The component of within-population diversity was estimated as H_{pop}/H_{sp}, and that of between-population diversity as $1 - H_{pop}/H_{sp}$. All the stated calculations were undertaken by POPGENE 1.32 (Yeh et al., 1995).

A pair-wise Euclidean distance matrix was generated with the computer package AMOVA-PREP 1.01 (Miller, 1998) and was then, used as input for WINAMOVA 1.55 for AMOVA analysis (Excoffier et al., 1992) to test whether populations had differing amounts of RAPD variation. The gene flow (N_m, number of migrants per generation) (Whitlock and McCauley, 1999) was approximated as: $N_m = (1/4)\,[(1/F_{st}) - 1]$, where, F_{st} (inbreeding index) values were available from a matrix of pair-wise combinations produced by WINAMOVA. Log (Nm) was plotted against log (km) according to Slatkin (1993), to illustrate the relationship between gene flow and geographic distance.

A dendrogram among the populations was constructed with software Darwin (Version 5.0.148) (http://darwin.cirad.fr/darwin) by using the matrix of pair-wise F_{st} values and the unweighted pair-group method with arithmetical averages (UPGMA) algorithm (Sneath and Sokal, 1973).

For morphological data, nested ANOVA analysis was performed for each of the traits measured to test the significance of variation among populations, using the PROC GLM procedure of SAS software (SAS Institute Inc., 1996). Between-population (σ^2_{GB}) and within-population (σ^2_{GW}) variance components were estimated for all populations using the REML algorithm of PROC VARCOMP in SAS (SAS Institute Inc. 1996). The correlation between the Euclidean distance matrices based on morphological and RAPD data was also tested for significance using a Mantel test by 1000

Table 2. RAPD primers assayed in 13 Tunisian barley landrace populations with the number of bands per primer, number of polymorphic bands, index of phenotypic diversity per population (H0), average diversity over all populations (Hpop), diversity at species level (Hsp), and within (Hpop/Hsp) and between (1-Hpop/Hsp) populations' diversity indices. Population codes are given in Table 1.

Primer	Total band	Polymorphic band	H0 for each population																
			1	2	3	4	5	6	7	8	9	10	11	12	13	Hpop	Hsp	Hpop /Hsp	1-Hpop /Hsp
UBC-235	5	3	0	0.288	0	0	0.218	0.162	0	0	0.138	0.299	0.38	0.077	0.166	0.132	0.248	0.532	0.468
UBC-212	10	10	0	0.117	0.279	0.037	0.183	0.03	0	0	0	0.26	0.192	0.229	0.147	0.113	0.18	0.627	0.373
UBC-248	8	8	0.109	0.216	0.057	0.0854	0.261	0.098	0.074	0	0.207	0.315	0.225	0.32	0.121	0.16	0.276	0.579	0.421
UBC-246	19	18	0.197	0.24	0.23	0.218	0.346	0.148	0.097	0.161	0.092	0.339	0.229	0.218	0.143	0.204	0.336	0.607	0.393
UBC-245	21	13	0.228	0.439	0.303	0.215	0.322	0.144	0.219	0.254	0.191	0.319	0.248	0.092	0.219	0.245	0.3	0.186	0.814
UBC-238	24	24	0.191	0.214	0.223	0.165	0.271	0.212	0.061	0	0.138	0.237	0.178	0.198	0.117	0.169	0.259	0.652	0.348
UBC-264	10	10	0.096	0.249	0.191	0.18	0.382	0.029	0.029	0.124	0.041	0.257	0.214	0.353	0.186	0.179	0.337	0.531	0.469
UBC-232	7	3	0.166	0.144	0.291	0.179	0.27	0	0.099	0	0.138	0.291	0.141	0.442	0.406	0.197	0.287	0.686	0.314
UBC-226	12	11	0.066	0.241	0.294	0.202	0.347	0.16	0.081	0	0.188	0.266	0.262	0.208	0.167	0.19	0.262	0.725	0.275
UBC-241	17	17	0.024	0.147	0.158	0.099	0.209	0.167	0.063	0	0.121	0.214	0.145	0.184	0.106	0.125	0.199	0.628	0.372
Me	13.3	11.7	0.1077	0.2295	0.2026	0.13804	0.2809	0.115	0.0723	0.0539	0.1254	0.2797	0.2214	0.2321	0.1778	0.172	0.268	0.641	0.359

Table 3. AMOVA analysis for the thirteen barley populations using 117 RAPD bands *** P < 0.001.

Source of variation	df	Sum of square	Variance component	Variation (%)
Between populations	12	325.581	2.455***	14.32
Within populations	54	793.583	14.695***	85.68
Total	66	1119.164		

permutations.

RESULTS

A total of 133 discernible and reproducible RAPD bands were generated with 10 selected primers across the 67 individuals of 13 populations of local barley landraces, out of which 117 (87.96%) were polymorphic (Table 2). Primers varied in their ability to detect variation both within and between populations. The within populations H_0 varied from 27.5% for UBC-226 primer to 81.4% for UBC-245. On average over all primers, the population with the smallest sample size (population 8) exhibited the lowest level of within-population genetic diversity (mean H_0 0.0539), while the other populations displayed mean H_0 ranging between 0.0723 and 0.2797 (Table 2). Population 6 was the most variable (mean H_0 0.2797) although, its population size (6 individuals) was less than that of population 2 (10 individuals and mean H_0 0.2295) (Tables 1 and 2).

The variance components of within and between populations detected with AMOVA were 85.68 and 14.32% of the total variance, respectively, which were both significant ($p < 0.001$) (Table 3). This was in approximate agreement with results derived from H_0 index, in which within and between population variations were 64.1 and 35.9%, respectively. It seems clear that while most of the variation is partitioned within populations, there is still considerable variation between populations.

A matrix of pair-wise *Fst* values, effective number of migrants (N_m) between populations and geographical distances are presented in Table 4. Values of *Fst* ranged from 0.0059 (between populations 10 and 12) to 0.362 (between

Table 4. Pair-wise *Nm* values (above diagonal) versus geographical distances (below diagonal) between the 13 Tunisian barley landraces populations. Population codes are given in Table 1.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1		1.332	0.841	1.079	0.856	1.22	0.575	0.642	1.138	2.327	2.914	2.691	0.77
2	105		0.722	16.41	1.17	7.102	0.987	1.868	3.373	3.271	1.942	1.51	1.094
3	107	212		0.707	2.108	0.704	0.474	0.766	1.058	5.069	0.876	1.195	0.44
4	183	106	192		0.975	7.102	1.006	1.658	2.762	2.762	2.623	1.833	1.025
5	117	154	122	70		1.302	0.827	1.322	8.083	3.916	1.116	1.332	0.575
6	225	120	332	167	264		0.689	1.535	3.426	11.11	3.271	3.916	1.498
7	180	175	218	139	119	145		1.45	9.75	1.799	4.135	1.601	1.072
8	285	173	348	172	237	87	130		12.907	5.305	4.466	3.848	1.322
9	190	232	208	196	89	202	57	187		4.557	7.325	7.562	3.271
10	252	294	270	258	151	264	119	249	62		4.594	42.12	1.766
11	317	278	335	275	216	192	136	121	127	104		7.325	2.558
12	453	447	471	411	352	252	272	244	263	240	136		2.59
13	529	523	547	355	428	328	348	218	339	316	212	76	

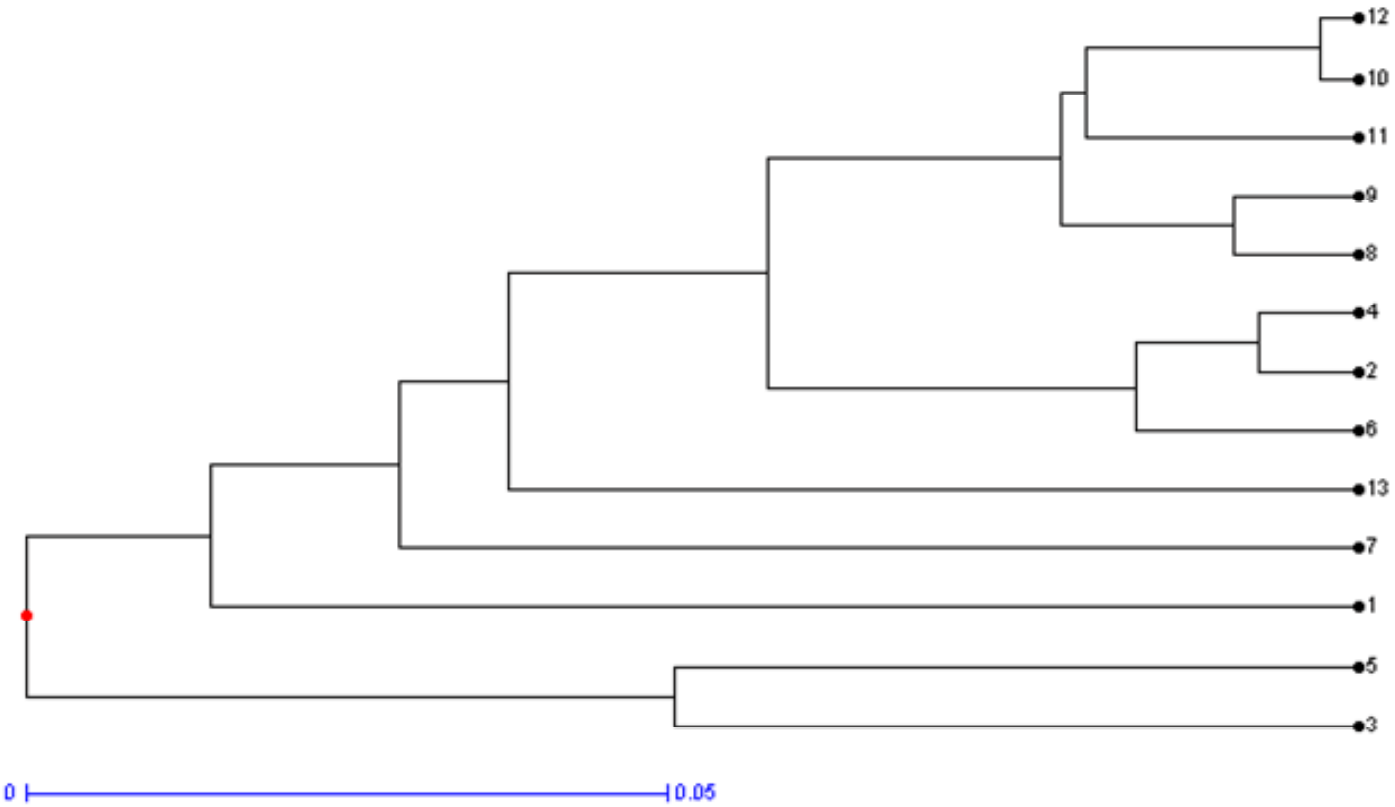

Figure 1. UPGMA derived dendrogram of the 13 local barley landrace populations based on the pair-wise F_{ST} values (Population names are given in Table 1).

populations 3 and 13) and were all significant at $p < 0.001$. This indicates that all populations may be considered different from each other, with population 13 being the most different from the others and populations 10 and 12 being the most similar. The overall F_{ST} across all the populations was 0.137.

The significant differentiation between populations was also revealed in the clustering analysis (Figure 1) and reflected in the estimates of gene flow (N_m) (Table 4). Values of N_m ranged from a moderate value of 0.44 to a high value of 42.12, averaging 3.413, but the majority of the values were between 0.44 and 1.942, which indicated

moderate gene flow between the thirteen studied barley populations.

To illustrate the relationship between gene flow and geographic distance, log (*Nm*) was plotted against log (Km) according to Slatkin (1993). Despite from the low coefficient of determination (R^2 = 0.078), the regression line shows a significant relationship between gene flow and geographic distance, that is, increasing exchange of genes between populations grow in close proximity in a radius of 100 km and little gene flow between geographically distant populations, as proposed for natural populations by the isolation-by-distance model (Slatkin, 1993).The gene flow between populations 10 and 12 (about 240 km) was unexpectedly high.

For morphological traits, there was a high level of variation among the populations and families studied (Table 5). ANOVA analysis revealed, significant differences among populations and among samples within population in almost all the traits measured ($P < 0.001$ or 0.05, Table 5) except traits 1, 2, 15, 17, 22, 23, 25, 26, 31 and 32 (Table 5).

As indicated by variance component analysis (Table 5), the extent of differentiation between tested populations showed that 68% of the total variation is partitioned within samples within populations, with a minimum of 55% for lemma type to a maximum of 100% for flag leaf (growth habit and auricles anthocyanin coloration), lower leaves (sheaths hairiness), Rachis (importance of zig-zag), spiktlets (glume, glume awn and glume color) and grain (aleurone color and anthocyanin coloration of palea inner nerves) traits.

The correlation between the Euclidean distance matrices based on morphological and RAPD data was low and non-significant ($r = –0.0189$, $P = 0.3103$).

DISCUSSION

Indigenous farming communities in developing countries contributed for millennia, to the evolution, enrichment and *in situ* conservation of many crop landraces, such as barley (Ceccarelli et al., 1987). However, little has been done to understand the intra-specific diversity in their subsistence agricultural ecosystems (Busso et al., 2000). Most likely, Tunisian barley landraces evolved over many generations through the long process of natural selection under harsh conditions. It is highly likely that desirable plant traits, conducive to development and survival under severe climatic conditions (drought and salt) are available with high frequencies in these landraces.

In this study, the marker technology has been employed in combination with morphological characters to detect genetic variation and population structure of *H. vulgare* landraces from Tunisia and has once again demonstrated its usefulness in gaining information quickly and usefully in conservation programs. RAPD markers, along with appropriate statistical procedures are suitable for genetic variation analyses at both intra- and inter-population levels (Chalmers et al., 1992; Martin et al., 1997).

The level of genetic diversity at RAPD markers detected here (*Hsp* = 0.268) is comparable to or higher than, diversity levels reported for barley landraces when using biochemical and molecular markers (Donini et al., 2000; Koebner et al., 2002).

The genetic variation was maintained within populations (14.32%) as detected with AMOVA. Similarly, Koebner et al. (2002) found that in barley 15.1 and 84.9% of the total variation was partitioned among and within varieties, respectively. Given the breeding system of barley (Hamrick and Godt, 1997), genetic variation is expected to be higher among than within populations. For instance, Lakew et al. (1997) noticed that variation for genotypic markers among plant populations can be from nine to 40 times higher than the variation within populations. These findings also indicated significant genetic subdivision of the barley landrace populations according to F_{st} values (F_{st} = 0.137, $P < 0.001$). The F_{st} estimate is considered to be more biased than Φ_{ST} for evaluation of differentiation coefficient for dominant marker data and may suggest that the *H. vulgare* landrace populations analyzed are moderately differentiated according to Wright's interpretation of F_{st} values (Wright, 1978). Therefore, our results are in general accordance with what is expected for barley landraces, as these crops are genetically heterogeneous populations comprising of inbreeding lines and hybrid segregates generated by a low level of random out-crossing in each generation (Nevo, 1992).

Gene flow is the migration of genetic information between populations through vectors such as pollen and seeds. In a predominantly inbreeding species such as barley, the migrations of pollen and natural seed dispersal are expected to be small or non-existent (Parzies et al., 2004). Consequently, gene flow found between local barley populations may be due to seed exchange between farmers or to anticipate admixtures and only to an inconsiderable extent to out-crossing with foreign pollen.

In our case, gene flow (*Nm*= 3.413) was on average 19 times higher than that reported for natural inbreeding species (Hamrick and Godt, 1990). This difference may be explained by the influence of human activity. The coefficient of determination of the regression line in Figure 2 may confirm the general suggestion that exchange of seeds between farmers is mainly limited to local areas and decreases exponentially with geographic distance. Exceptions to this trend may be caused by increasing mobility of farmers. These findings suggest that farmers in Tunisia are very concerned about seed quality of their barley landraces, but when their own seed supplies are insufficient, they tend to buy seeds from well recognized skilled farmers.

On the other hand, although, not all of the 34 qualitative

Table 5. Anova analyses for the 34 morphological traits measured in Tunisian barley landraces and variance components calculated within and between populations.

		Population			Sample within population			Error
S/N	Morphological character	df	MS	F	df	MS	F	F
1	Flag leaf: growth habit	12	5.2	2.34 (NS)	54	4.38	2.61 (***)	2.21
2	Flag leaf: anthocyanin colouration of auricles	12	0.096	1.37 (NS)	54	6.05	2.19 (***)	0.07
3	Flag leaf: glaucosity of sheath	12	16.42	7.79 (***)	54	13.09	6.59 (***)	2.1
4	Awn: anthocyanin colouration of tips	12	122.46	25.42 (***)	54	0.3	10.8 (***)	4.81
5	Ear: glaucosity	12	20.16	11.71 (***)	54	8.02	4.66 (***)	1.72
6	Ear: growth habit	12	22.52	5.93 (***)	54	14.91	3.93 (***)	3.79
7	Plant: height	12	2667.43	22.29 (***)	54	1792.45	14.98 (***)	119.62
8	Ear: density	12	10.36	16.97 (***)	54	4.82	7.89 (***)	0.61
9	Ear: length	12	50.41	53.34 (***)	54	15.77	16.69 (***)	0.94
10	Awn: length compared to ear	12	128.59	9.73 (***)	54	47.64	3.6 (***)	13.21
11	Plant: growth habit	12	8.62	6.008 (***)	54	5.3	3.69 (***)	1.43
12	Lower leaves: hairiness of sheaths	12	4.38	3.17 (***)	54	4.9	3.54 (***)	1.38
13	Ear: shape in dorsal view	12	6.05	17.76 (***)	54	2.19	6.42 (***)	0.34
14	Lemma awn barbs	12	13.09	11.79 (***)	54	5.2	4.68 (***)	1.11
15	Rachis: length of first segment	12	0.3	0.96 (NS)	54	0.35	1.14 (NS)	0.31
16	Rachis: incurvation of first segment	12	2.22	12.63 (***)	54	1.47	8.34 (***)	0.17
17	Rachis:" importance" of zig-zag (6 rows)	12	0.36	2.21 (NS)	54	0.69	4.18 (***)	0.16
18	Spiklets median: Glume and glume awn (outer glumes)	12	1.28	6.66 (***)	54	1.67	8.67 (***)	0.19
19	Lemma type	12	4.07	13.59 (***)	54	0.97	3.26 (***)	0.29
20	Lemma awn/hood	12	0.003	3.66 (***)	54	0.0016	1.7 (*)	0.0009
21	Glume colour	12	1.46	7.23 (***)	54	1.68	8.35 (***)	0.2
22	Steril spikelets: growth habit	12	0	.	54	0	.	0
23	Grain: paleas	12	0	.	54	0	.	0
24	Grain: rachilla hair type	12	5.2	110.24 (***)	54	13.45	44.18 (***)	0.3
25	Grain : presence of teeth inner lateral nerves	12	0.096	.	54	0	.	0
26	Grain: ventral furrow presence of hairs	12	16.42	.	54	0	.	0
27	Grain: layout of lodicules	12	122.46	34.05 (***)	54	0.17	8.5 (***)	0.02
28	Aleurone colour	12	20.16	26.34 (***)	54	6.23	28.39 (***)	0.21
29	Grain : anthocyanin colouration inner nerves of palea	12	22.52	13.79 (***)	54	0.14	14.01 (***)	0.01
30	Grain (pericarp) colour	12	2667.43	14.39 (***)	54	9.08	13.24 (***)	0.68
31	Ear: number of rows	12	10.36	.	54	0	.	0
32	Seasonal type	12	50.41	.	54	0	.	0
33	Time of ear emergence (first spikelet visible on 50% of ears)	12	128.59	18.93 (***)	54	13.33	8.58 (***)	1.55
34	Yield	12	8.62	5827.81 (***)	54	15.03	3762.39 (***)	0.003

***, Significant at P<0.001; *, significant at P<0.05; NS, non significant.

and quantitative morphological traits used in this study are of direct agronomic importance, most are typically used in barley germplasm characterization (Lakew et al., 1997; Koebner et al., 2002).

Characterization by means of morphological markers has been previously used for germplasm collections of landraces in the barley primary (Brown and Munday, 1982; Jaradat et al., 1987; Parzies et al., 2000) and secondary (Demissie and Bjornstad, 1996) centers of genetic diversity. This diversity was found to be higher when compared with that detected with biochemical (Jaradat et al., 1987) and molecular markers (Koebner et al., 2002) in cultivated barley. These differences were attributed to the multi-genic nature of most individual phenotypic markers and hence, variation at more than one locus is being analyzed.

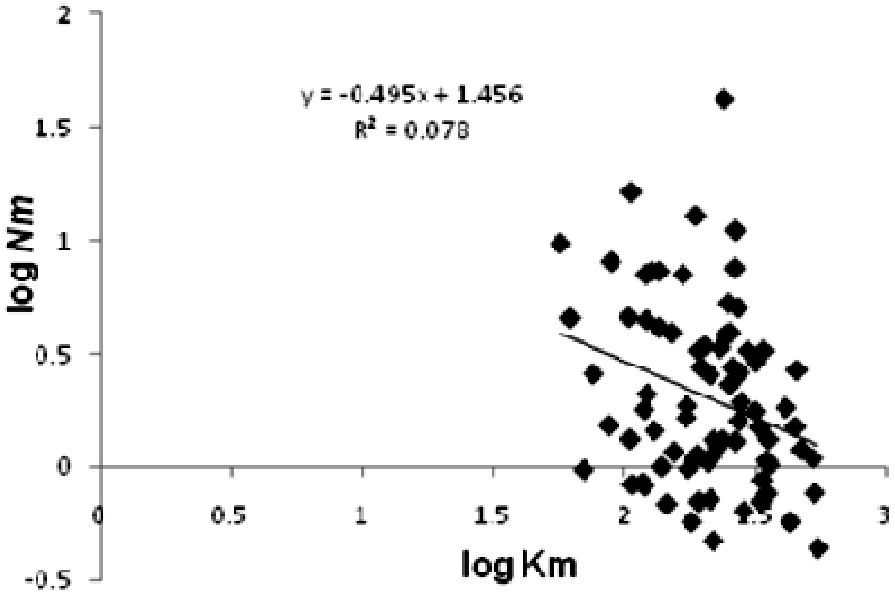

Figure 2. Gene flow (log (Nm)) versus geographic distance (log (km)) of 13 Tunisian barley landraces populations.

The phenotypic and statistical evidences reported in this study indicated that variation for phenotypic markers among populations was higher than the variation within populations. That is, 68% of the total variation is partitioned within samples within populations. These findings corroborate with those published for barley landraces (Brown and Munday, 1982; Parzies et al., 2000; Koebner et al., 2002).

As expected, variance components due to differences among samples within populations point to the high levels of variation (100%) for the following traits in Tunisian barley landraces: flag leaf (growth habit and auricles anthocyanin coloration), lower leaves (sheaths hairiness), Rachis (importance of zig-zag), spiktlets (length of glume and awn compared with kernel and glume color) and grain (aleurone color and anthocyanin coloration of palea inner nerves) traits. As reported by Kebebew et al. (2001), the aforementioned morphological traits rank in the panel of agrononomic traits that are continuously selected by farmers, while distinguishing between barley cultivars of which grain color is the most selective criterion (Ceccarelli et al., 1987; Demessie and Biornstad, 1996; Parzies et al., 2000; Kebebew et al., 2001).

Similar to the findings of Lund (2002) in barley, the weak correlation observed between the Euclidean distance matrices drawn from molecular and morphological data, probably implies differences in degree of genomic coverage between molecular markers and morphological characters (Veteläinen et al., 2005). Therefore, both data are comparably important in population diversity studies in Tunisian barley landraces and are likely suitable to generate an unbiased picture of diversity trends in barley.

The exceptionally high within-landrace variation levels detected here by means of both molecular and morphological traits is maintained, because farmers have to keep seed of their barley crop for the next year. Similar to the findings of Swanson (1996), these results indicate that the best protection against the loss of within landrace variation would be conservation following these strategies: Firstly, the information available warrants the *in-situ* conservation (on-farm conservation) strategy that would define core areas completely free from perturbation, at least for the most diverse populations (namely populations 6 and 13). This would guarantee the maintenance of most of the species genetic variation.

Nevertheless, F_{st} values suggest that all populations are important for conservation. Secondly, sampling for *ex-situ* conservation programs (seed banks) should include representatives from all of the sampled populations given the high levels of within population diversity and population differentiation, as suggested by Mattner et al. (2002). Hamrick (1993) suggested that five strategically placed populations should maintain 99% of their total genetic diversity when more than 80% of the total genetic diversity resides within populations. In our case, 85.68% of the genetic variation is observed within populations in AMOVA analysis, which may imply the need to conserve all populations to maintain genetic diversity within this species.

In all, for barley preservation, both of the *in situ* and *ex situ* conservation techniques are required to counteract genetic erosion by preserving a stock of genetic diversity that would be relevant to commercial and public breeding programs. According to Gonzales (2000), none of the suggested conservation strategies is favored over the other: the *in situ* conservation strategy complements *ex*

situ maintenance and helps to maintain not only key elements that are missed by *ex situ* methods, but also aids in generating new material for areas that are often bypassed by crop improvement programs connected to ex situ facilities (Brush, 1995).

In conclusion, the information gathered in this study indicated the usefulness of both molecular and morphological approaches for studying history of populations, monitoring gene flow and identifying patterns of genetic variation and guided various plans for the conservation of the Tunisian barley landrace genetic resources.

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