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GENETIC DIVERSITY IN SOME TUNISIAN BARLEY LANDRACES BASED ON RAPD MARKERS

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Abstract

The genetic diversity analysis of 15 barley landraces was carried out using RAPD markers. These landraces were collected from various bioclimatic Tunisian zones. The amplification products varied from 4 to 11 bands ranging between 250 pb and 3000 pb. On 698 fragments counted, 578 are polymorphic showing a high level of polymorphism (82.8%). The relationship between the studied landraces was evaluated according to (UPGMA) method that classified barley landraces in 4 homogeneous groups. Among which, the group D included the majority of the landraces with the introduced variety 'Martin'. The genetic distance between these landraces is reduced, may be because of the presence of a common ancestor which led to a narrow genetic diversity.

Introduction

Tunisia, one of the principal secondary centers of cereal in the world, is very rich in both landraces and wild relatives of barley (Bœuf, 1931). The local germoplasm, well adapted to harsh environmental conditions, has been considered to be a large reservoir of genetic diversity and of great importance to varieties' improvement. Among cereals, barley (*Hordeum vulgare* L.) is one of the principal cereal crops in Tunisia since it is cultivated in different zones from northern to southern Tunisia and occupies about the 1/3 of Tunisian cereals' cultivated area.

Barley landraces have many useful characters, drought avoidince, important biomass and yield for feed and food alimentation *etc*…. Thus the need for collection, conservation and use of these precious resources increased. Thus, many prospections have been conducted (Bettaieb-Ben Kaâb & Elfalah, 1982; Ben Naceur, 1994; *etc*…) in all the country to collect barley local germoplasm. Assessment of the genetic variability within barley landraces is fundamental for barley breeding (Bettaieb & Attias, 1992), for the conservation of genetic resources and also for the protection of the copyright in seeds sectors (Forster *et al*., 2000)

Molecular markers have been proved to be powerful tools in the characterization and evaluation of genetic diversity within and between species and populations (Russel *et al*., 1997). They were used in wheat (Bohn & Melchinger, 1999; Ben Naceur, 1998), maize (Hahn *et al*., 1995), barley (Russel *et al*., 1997), in cruciferous species (Thormann *et al*.,1994), in sorghum (Yang *et al*.,1996), in tomato (Tam *et al*., 2006) and in many other species for detecting genetic diversity (Fernández *et al*., 2002). Of these techniques, RAPD markers have proven to be a reliable marker system for genetic fingerprinting and also for determining the genetic relationships among germplasm collections. RAPD

markers have the advantages of simplicity and the ability to detect relatively small amounts of genetic variation and also need no prior information on the (Williams *et al*., 1991). One frequently reported disadvantage associated with RAPD is unreproducibility that may arise if experimental conditions are not standardized carefully (Prenner *et al*., 1993). Despite this fact, RAPD markers have provided informative data consistent with other markers especially at the intraspecific level (Dos santos *et al*., 1994; Lerceteau *et al*., 1997) and are effective for large-scale population genetic analysis.

We used this technique to survey the genetic diversity between the selected Tunisian barley landraces.

Materials and methods

Plant material: A total of 15 barley landraces from 8 locations of different bioclimatic zones were used in this study (Table 1). The choice of prospecting zones was described by Abdellaoui *et al*. (2007).

DNA extraction: To extract DNA from barley leaves many protocols have been described in the literature (Saghai-Maroof *et al*., 1984; Clarrke *et al*., 1989; Lodhi *et al*., 1994; Ben Naceur & Rouaissi, 2003). However, in this study, genomic DNA was extracted from leaves according to Ben Naceur & Rouaissi (2003) method, which combines the three methods characterized by high CTAB (Cetyl Trimetyl Ammonium Bromide) concentration to overcome polysaccharides' contamination.

Amplification protocol: A total of sixty arbitrary 10-mer oligonucleotides (Operon Technology) were used (Table 2). The PCR (Polymerisation Chain Reaction) reaction mixture contained 40 ng genomic DNA, 1×PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM primer and 1U *Taq* polymerase (Promega) in a 25µL volume. The amplification protocol was 94ºC for 5 min to pre-denature; followed by 35 cycles of 1 min. AT94ºC , 1 min. at 37ºC and 1 min. at 72ºC for, with a final extension at 72ºC for 10 min. Amplification products were loaded on 1.8% agarose gel.

Data analysis: The positions of scorable RAPD bands were transformed into a binary character matrix ('1' for the presence and '0' for the absence of a band at a particular position).The genetic dissimilarity (GD) was calculated using Nei & Li (1979) method. Based on the dissimilarity matrix, a dendrogram showing the genetic relationships between landraces was constructed using the unweighted pairgroup method with arithmetic average (UPGMA) (Sneath & Sokal, 1973) using the software Treecon for Windows version 1.3b. Polymorphic information content (PIC) values were calculated for each RAPD primer according to the formula: $PIC = 1-\Sigma(P_{ij})^2$, where P_{ij} is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Botstein *et al*., 1980).

Results

Because of the reported sensitivity of the RAPD technique, the protocol was carefully optimized for various experimental parameters. Of the 60 primers screened to evaluate the genetic diversity among the 15 Tunisian barley landraces, only 10 primers generated polymorphic and reproducible bands. The other 50 primers generated variable and poor amplification products and/or non-repeatable banding patterns.

Table 1. Landraces' origin, bioclimatic stage and rainfall (Monthly Bulletin of the National Meteorological Institute).

Landraces	Origin	Bioclimatic stage	Rainfall
Tozeur 1	Tozeur	Sahara	150
Tozeur 2	Tozeur	Sahara	150
Kebilli 1	Kebilli	Sahara	150
Kebilli 2	Kebilli	Sahara	150
Kebilli 3	Kebilli	Sahara	150
Kasserine	Kasserine	Arid sup	300
Sidi Bouzid	Sidi Bouzid	Arid sup	300
Jendouba 1	Jendouba	Humid inf	800
Jendouba 2	Jendouba	Humid inf	800
Martin			
Kalaa	Kalaa	Sub humid	600
Klibia 1	Klibia	Sub humid	600
Klibia 2	Klibia	Sub humid	600
Manel	ICB81-607 realized in ICARDA and introduced in Tunisia since 1981 as F2. Descendants 527 / 5 / As54 / Tra // 2*Cer / Tol I / 3 / Avt / Tol I ICB 81-607 – 1Kf-1BJ- 12Bj- 11Bj- 1Bj- 1Bj-0Bj		
Rihane	Atlas 46 / Arivat // Athénaïs ICB76-2L-1AP-0AP Realized by ICARDA since 1976 and introduced in Tunisia since 1981/1982 and officially subscribed in Tunisian catalogue since 1987.		

PCR amplification using the efficient 10 primers produced useful marker patterns and analysis gave strong evidence of major genetic differences among barley landraces. On a total of 698 scored DNA fragments 578 bands were polymorphic (82.8%) with an average of 57.8 bands per primer (Table 2). The size of the amplified bands ranged from 250 to 3000 bp. Each primer generated 4 to 11 bands. The lowest number of bands (4 fragments) is generated by OPH-13, while the highest (11 fragments) is generated by OPD-20 of which 10 are polymorphic (Table 2 and Fig. 1).The polymorphic information content (PIC) ranged from 0.74 to 0.96 with an average of 0.87, . The lowest and the highest PIC values were recorded for primers OPH-13 and OPJ- 10 respectively.

All the 698 bands, generated from 10 RAPD primers, were used to calculate the Genetic Dissimilarity index (RAPD-GD) among the 15 barley landraces (Table 3). The RAPD-GD value ranged from 0.243 to 0.55, with an average of 0.40. The highest genetic dissimilarity was found between the following landraces' groups (Tozeur 1–Tozeur 2*:* 0.547); (Rihane – Manel: 0.506) and (Tozeur 1–Sidi Bouzid: 0.483); (Tozeur 1–Jendouba 2: 0.488), while the lowest genetic dissimilarity was observed between (Kebilli 3 – Kasserine: 0.243) and (Kebilli 3 – Jendouba 2: 0.250).

'Martin' is a known variety introduced in Tunisia since 1931 and, because of its high yield seeds it continues to be cultivated in different Tunisian areas. It has been used by Tunisian breeders as an ancestor in their cereal improvement programs. That's why it is clustered with some landraces in the same group (Sidi-Bouzid and Jendouba 1).

The relationships within and between groups were estimated by a UPGMA cluster analysis of GD matrices (Table 3). It indicated that all 15 barley landraces could be distinguished by RAPD markers.

Table 2. Primer sequences, amplified bands, polymorphic bands and PIC values in RAPD analysis.

	Primers	Sequences 5'—3'	Number of total bands	Polymorphic bands	PIC
1.	OPD02	GGACCCAACC	8	6	0.874
2.	OPD10	GGTCTACACC	6	5	0.896
3.	OPD18	GAGAGCCAAC	4	2	0.840
4.	OPD20	ACCCGGTCAC	9	7	0.899
5.	OPG12	CAGCTCACGA	8	6	0.792
6.	OPG14	GGATGAGACC	5	2	0.849
7.	OPG10	AGGGCCGTCT	8	3	0.827
8.	OPJ10	AAGCCCGAGG	4	2	0.846
9.	OPF03	CCTGATCACC	10	8	0.863
10.	OPH13	GACGCCACAC	9	7	0.741
Total			**71**	**48**	**Average : 0.8427**

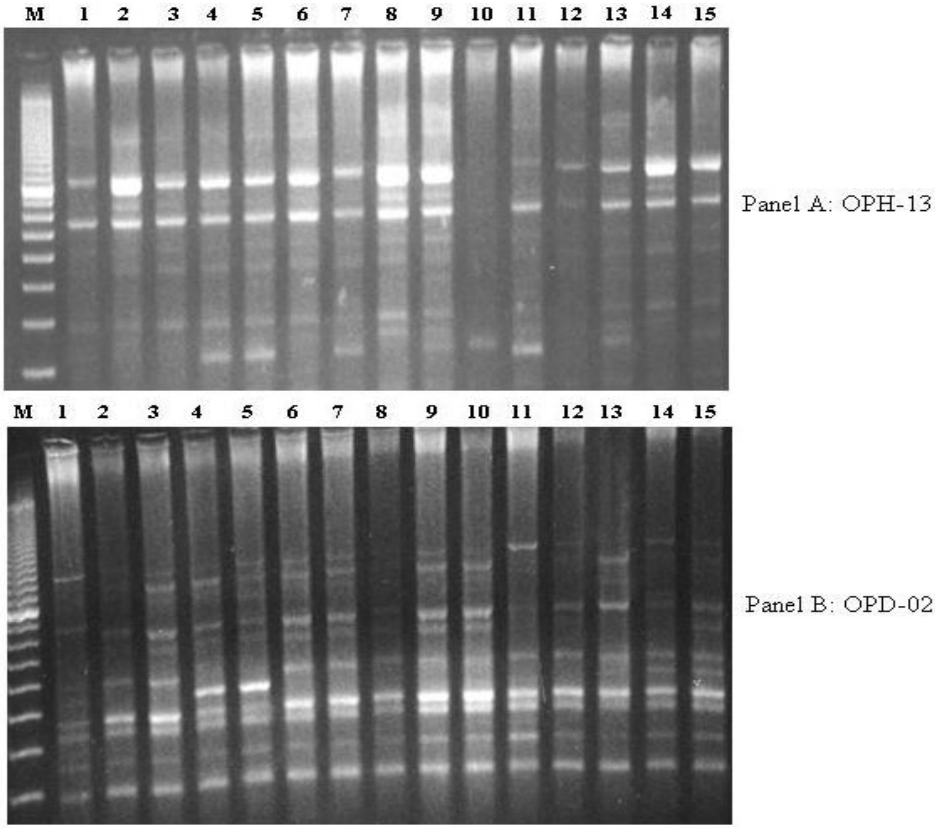

Fig. 1. Typical examples of RAPD profile obtained using genomic DNA template of Tunisian barley landraces by OPH-13 (Panel A) and OPD-02 (Panel B). M: marker (100 pb PCR Molecular Ruler for the panels A and B). Numbered wells correspond to the studied genotypes. 1= Tozeur 1; 2 = Tozeur 2; 3 = Kebilli 1; 4 = Kebilli 2; 5 = Kebilli 3; 6 = Sidi Bouzid; 7 = Kasserine; 8 = Jendouba 1; 9 = Jendouba 2; 10 = Martin; 11 = Kalaa; 12 = Klibia 1; 13 = Klibia 2; 14 = Rihane and 15 = Manel.

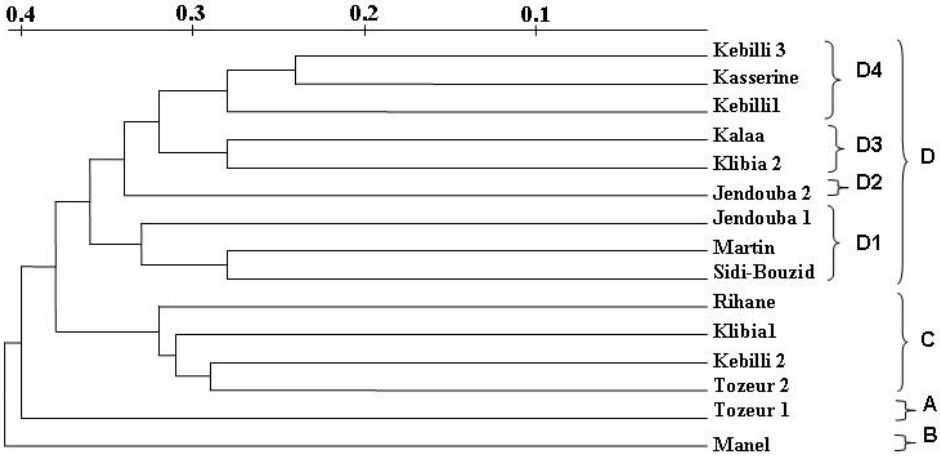

Fig. 2. Dendrogram of 15 barley landraces constructed from RAPD-GD.

In the dendrogram (Fig. 2) 4 different groups were observed: A, B, C and D. The first group (A) is composed by Tozeur 1 which was less related with other groups: (more than 41% of dissimilarity). The group B is only composed by Manel. The third group (C) gathered the landraces Rihane, Klibia 1, Tozeur 2 and Kebilli 2 (34%). In the last group D, four subgroups were evident: D1 (Martin, Sidi Bouzid and Jendouba 1), D2 (Jendouba 2), D3 (Kalaa and Klibia 2) and D4 (Kebilli 3, Kasserine and Kebilli 1). These landraces were more closely related with each other since their dissimilarity percentage is of 28%.

Discussion

Due to its worldwide distribution, the assessment of the genetic diversity among barley germplasm from different countries was performed (Ben Hmida-Ben Salem, 2000; Fernandez *et al*., 2002; Feng *et al*., 2003). In fact, it has been found that the average genetic diversity based on RAPD analysis of 18 barley accessions from Netherlands, France, Great Britain, Germany and Italy was 0.521 (Russel *et al*.,1997). Also, analysis of 31 Tunisian barley cultivars (Ben Hmida-Ben Salem, 2000) by RAPD markers showed an average genetic diversity of 0.560. In other conducted work on 30 barley American cultivars (Pillen *et al*., 2000), the average genetic diversity was 0.682. Whereas, the genetic diversity between tea populations (Kaundun & Park, 2002) was 0.18. In the present study, the average genetic dissimilarity of barley landraces was 0.42. These results suggest that the selected barley landraces have narrow genetic diversity, despite the high PIC value of primers used.

Correlation between RAPD markers and the geographic origin is low: in deed Jendouba 1 from the north of Tunisia (sub humid region) was closely related to Sidi-Bouzid landrace which is from an arid region of center Tunisia. Also, the landraces Tozeur 1 and Tozeur 2 collected from the same region showed an important GD. However, Kalaa and Klibia 2 belonging to the same bioclimatic stage showed a low GD. This study corrobore with those found in Korean tea populations (Kaundun & Park, 2002), where no geographical trends were observed among the populations. This relatively poor relationship observed between RAPD markers and the geographic origin of Tunisian barley landraces in this study may be explained by the neutrality of molecular

markers compared with adaptive traits under harsh conditions; even if these conditions may influence the cultivar behavior and leads to some adaptation traits such as earliness to avoid water deficit or small ears that will be rapidly filled *etc*….

This study revealed the efficacy of RAPD markers in detecting the polymorphism among barley landraces and establishing relationship among them. It demonstrated the potential efficiency of molecular markers in landraces classification, and indicated the feasibility of a comprehensive effort to determine the relationships among barley landraces using molecular markers. Further collection, evaluation, and utilization of local germplasm, is clearly a high priority in barley improvement.

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