Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 6 (2): 261-268, 2007
ISSN 1682-3974

Morpho-Physiological and Molecular Characterization of Some Tunisian Barley Ecotypes

[1,2]Raoudha Abdellaoui, [1]Hatem Cheik M'Hamed, [1]M'barek Ben Naceur,
[2]Leîla Bettaïeb-Kaab and [2]Jeannette Ben Hamida
[1]Laboratoire de Biotechnologie et Physiologie Végétale,
Institut National de la Recherche Agronomique de Tunis, Rue Hédi Karray, 2049 Ariana, Tunisie
[2]Département de Biologie, Faculte des Sciences de Tunis, 2092 Campus Universitaire, El Manar II, Tunisie

Abstract: This research aim is to study whether we still have genetic diversity of barley all around the country, or there has been genetic erosion leading to a reduction in landraces barley cultivars. To fulfill this purpose, some ecotypes were collected from few frequented various bioclimatic regions and morpho-physiological and molecular level were studied. Our results showed differences among the ecotypes studied based on the morpho-physiological criteria such as heading date, density and ear length and response to saline stress. The molecular analysis showed the limits of the morpho-physiological approach. In fact, identical ecotypes were found grown in different parts of the country and the morpho-physiological differences observed could be due to environmental conditions' adaptation acquired over time. Also, ecotypes that were grown mixed together in the same area and having similar physiological behavior were found different using the RAPD markers method. Important local barley genetic variability was found, concluding the Tunisian germplasm richness.

Key words: Barley, morpho-physiological traits, polymorphism, RAPD markers

INTRODUCTION

North Africa is considered as one of the main secondary cereal centers (Boeuf, 1931). In deed, Tunisia constitutes an area of great cereal diversity. The local landraces are very adapted to stress conditions (drought and salt), since they grow and produce a good feeding quality under harsh conditions (Hamza *et al*., 2004). They also contribute to genetic diversity and to new variety creations (Ben Naceur *et al*., 1998). However, replacing native germplasm by an improved and introduced material could lead to local phytogenetic resources erosion. Therefore, local resource conservation should be given more importance.

For a long time, improving or creating new barley varieties was underestimated, since barley was considered as a secondary cereal, grown specially for animal feed (85%) and occasionally (10-15%) for human food (El Falah *et al*., 2004). On the contrary, attention has been focused on wheat improvement. Thus, from 1922-1923 till now, 46 durum wheat and 28 bread varieties were created against only 15 barley varieties of which (Martin variety) was introduced from Algeria since 1931 (Deghaïs *et al*., 1996; Deghaïs *et al*., 1999). For a long time, Tunisian farmers sow few adapted improved varieties such as Martin, *Caudebec*: Australian barley 552... or local ecotypes (Ardhaoui, white Cap Bon barley, white Ras jbel barley) which are more adopted to Tunisian environmental conditions (Seguela and Jacquard, 1953). Since 1985 some improved barley varieties have been developed and used (El Falah, 1998).

Prospection, collection and assessment of genetic resources in the three Maghreb countries (Tunisia, Algeria and Morocco) started long time ago (Boeuf, 1931; Erroux, 1958) but the most recent, date back to 1982 (El Falah, 1998), 1990 (Benlaghlid *et al*., 1990) and 1994 (Ben Naceur *et al*., 1997). These studies were focused especially on the morphological variability of the vegetative part, ears and seeds (Benlaghlid *et al*., 1990) or on reserve protein diversities (Bettaïeb-Ben Kaâb and Attias, 1992; Bettaïeb-Ben Kaâb *et al*., 2005). These studies were lacking precision and sometimes were contradictory since morphological traits and protein diversity; related to the differential genes expression in response to the plant environment; vary according to environmental conditions (Liang and Pardee, 1992; Gibson and Somerville, 1993).

Recent studies based on molecular variability have been carried out. Lalaoui-Kamel and Assali (1997) used RFLP to distinguish the genetic polymorphism on

Corresponding Author: Raoudha Abdellaoui, Laboratoire de Biotechnologie et Physiologie Végétale,
Institut National de la Recherche Agronomique de Tunis, (INRAT) Rue Hédi Karray, 2049 Ariana, Tunisie
Tel: 00216 71 230 024 Fax: 00216 71 230 667

Medicago genus, Snoussi *et al.* (2004) used microsatellites to analyze the genetic diversity among grape varieties and Ben Naceur and Rouaïssi (2003) analyzed varietal polymorphism in wheat by AFLP method.

No big information is available in genetic variability among Tunisian barley germoplasm at both molecular and morphological level. This study aim is to characterize at the morpho-physiological and molecular level some barley ecotypes collected from parts of the country where there is few barley seed exchange with the outside. So, farmers produce their own seeds and keep them from one harvest to the next sowing. Morpho-physiological traits and molecular RAPD markers polymorphisms were compared and checked whether we still have great genetic barley variability.

MATERIALS AND METHODS

Plant material and collection areas: Thirteen local winter barley ecotypes (*Hordeum vulgare*, L.) of diverse geographic origins were used in this study. These ecotypes were obtained after prospection carried out in different Tunisian bioclimatic regions (Fig. 1) that were fewly frequented. Once collected, these ecotypes were named according to their collect region. They were: Tozeur 1, Tozeur 2, Kébilli 1, Kébilli 2, Kébilli 3, Kasserine, Sidi Bouzid, Jendouba 1, Jendouba 2, Souihli, Kalaâ, Kélibia 1 and Kélibia 2. Martin variety traditionally grown in Tunisia was added.

- The Jendouba district is in the West-North of Tunisia, belonging to the humid inferior bioclimatic stage where the annual rainfall is 800 mm and the average annual temperature is 18°C (Monthly Bulletin of the National Meteorological Institute from 1975 to 2004).
- The Kélibia and Kalaâ districts belong to the East-North of the country. They are characterized by a sub-humid bioclimatic sector where the annual rainfall is 600 mm.
- Souihli, a traditional cultivar grown in most regions, originally belonging to semi-arid inferior climate stage characterized by an annual rainfall of 400 mm.
- The Kasserine and Sidi Bouzid district is in the Tunisian West-center and belonging to the arid-superior bioclimatic region where the average annual rainfall is 300 mm.

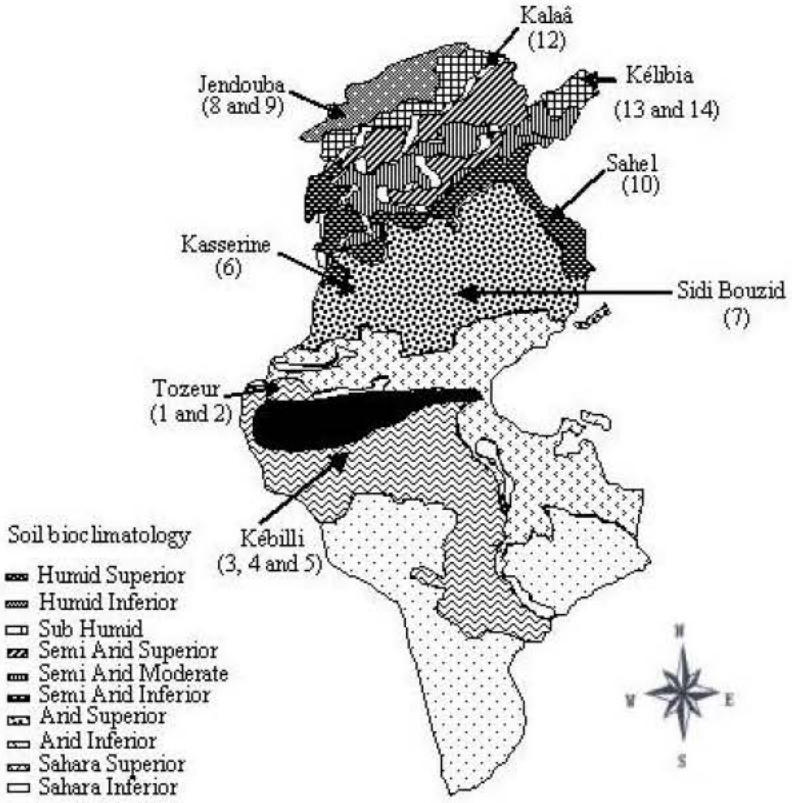

Fig. 1: Bioclimatic ecotypes' origin. 1 = Tozeur 1, 2 = Tozeur 2, 3 = Kebilli 1, 4 = Kebilli 2, 5 = Kebilli 3, 6 = Kassenine, 7 = Sidi bouzid, 8 = Jendouba 1, 9 = Jendouba 2, 10 = Souihli, 12 = Kalaa, 13 = Kelibia 1 and 14 = Kelibia 2

- The Tozeur and Kébilli districts originated from Tunisia southern and belonging to the desert bioclimatic zone where the average annual rainfall is less than 150 mm.

Morpho-physiological traits: The morpho-physiological criteria used were heading date, ear density and length, plant height and the ecotypes' reaction to saline stress (epicotyl's length and chlorophyll (a) content).

Three doses of salt were applied (0, 6 and 9 g of NaCl L^{-1}), at the germination level. Each treatment was repeated five times.

Twenty seeds were placed in a Petri dish on a filter paper soaked with 10 mL distilled water (control) or 10 mL saline solution (6 or 9 g L^{-1} of NaCl). Germination was achieved in obscurity at 25±1°C using an incubator. The epicotyl's length was determined at the end of the experiment that lasted 7 days.

The chlorophyll content, which represents the plant's photosynthetic potential, was also determined. It was measured during the heading stage. Four replications were carried out for each ecotypes and each treatment. A modified Arnon's principle (1949) was used to determine the amount of chlorophyll (a). Chlorophyll (a) content was calculated according to the Arnon's formula.

Extraction, purification and quantification of the DNA: The DNA was extracted and purified from seed embryos, using a CTAB (Cetyl trimethyl ammonium Bromide) method (Webb and Knapp, 1990) as modified by Ben Naceur *et al.* (1998). DNA was then quantified at 260 nm using a spectrophotometer (standard CECIL CE2501 series 2000/3000): 5 μL DNA samples was diluted in 995 μL of Tris-EDTA (TE) buffer and compared with a control containing 1000 μL of TE. The DNA concentration (C) was calculated as follows: C (μg μL^{-1}) = $DO_{260}\times10$.

DO_{260}/DO_{280} ratio was also calculated to determine DNA purity.

PCR amplification and amplified product electrophoresis: Forty Operon's primers were tested on DNA samples. DNA amplification was carried out in a final volume of 25 μL containing 12.5 μL of ready mix (buffer with $MgCl_2$, dNTP and Taq polymerase), 20 μM of Operon primer, 20 ng μL^{-1} of DNA and adjusted with distilled water. The program of amplification; using a thermocycler (Biometra UNO II); consisted of a pre-denaturation cycle of 2 min at 94°C, 35 cycles of a denaturation for 30 sec at 94°C, an hybridization for 30 sec at 50°C, an extension for 30 sec at 72°C followed by a post-extension cycle for 5 min at 72°C.

The amplification products of each primer were electrophoresed at 80V for 1 h in horizontal 1% agarose gel prepared in 1xTAE (TRIS Acetate EDTA) buffer containing 0.01% of ethidium bromide. For each sample, 7 μL of the amplified product were mixed with 2 μL of loading dye and loaded in well gel. The migration of these mixtures was done according to Ben Naceur *et al.* (1998) protocol. Bands were visualized under UV waves on a Polaroid camera system.

Table 1: Primers' sequence used to perform Random Amplified Polymorphic DNA (RAPD)

Primers	Primers' sequence
Op E-03	5'-CCA-GAT-GCA-C-3'
Op E-07	5'-AGA-TGC-AGC-C-3'
Op E-12	5'-TTA-TCG-CCC-C-3'
Op E-15	5'-ACG-CAC-AAC-C-3'
Op E-20	5'-AAC-GGT-GAC-C-3'
Op B-01	5'-GTT-TCG-CTC-C-3'
Op B-05	5'-TGC-GCC-CTT-C-3'
Op B-08	5'-GTC-CAC-ACG-G-3'
Op B-10	5'-CTG-CTG-GGA-C-3'
Op B-18	5'-CCA-CAG-CAG-T-3'

The primers used and the data analysis: Forty primers were used but only ten primers showing clear, reproducible and polymorphic bands. These primers were considered to make binary matrix, study dissimilarity and discus polymorphism between ecotypes (Table 1).

Data obtained were scored in a binary form as presence (1) or absence (0) of bands for each ecotype and entered into a data matrix (Hou *et al.*, 2005). Genetic Dissimilarity (GD) between ecotypes was calculated according to Nei and Li (1979) formula. Based on the dissimilarity matrix, a dendrogram showing the genetic relationships between ecotypes was constructed using the Unweighted Pairgroup Method with Arithmetic Average (UPGMA) (Sneath and Sokal, 1973) by means of Treecon 2.2 software.

Statistic analysis: All measurements were replicated at least five times. The data presented are the mean values of the repetitions. Data were subjected to Analysis of Variance (ANOVA) by STATITCF software package at the 5% level.

RESULTS AND DISCUSSION

Morphological traits: The heading date, which represents the difference between sowing date and inflorescence emergence period, showed that Tozeur is the earliest and Jendouba is the latest one with 20 days difference between them. However, for the other ecotypes, this criteria is intermediary and varied from 2 to 15 days. Furthermore, plant length showed that Jendouba 2, Jendouba 1, Sidi Bouzid and Martin were the longest. However, Kalaâ was the shortest. The other ecotypes were medium. Both density and ear length showed clear

differences among and within collected ecotypes from the same geographic region. The most distinctive morphological trait is ear density which varies from very loose to very compact (Table 2). In fact, collected ecotypes from Kébilli region showed different ear structure; the same remark observed for Tozeur's ecotypes. However, a similar ear density was observed for collected ecotypes from different regions.

Physiological parameters:

Epicotyl's length at the germination level: Table 3 showed that even moderate saline stress (6 g NaCl L^{-1}) could affect seriously the epicotyl's length. In deed, the ecotypes Kasserine, Kélibia 1, Martin, Kébilli 3, Kélibia 2 and Kalaâ showed low epicotyl's length percentage of 22.68, 31.68, 35.58, 36.03, 40.96 and 47.64. The other ecotypes showed percentage more than 50% compared to their control. Similar observations were, reported by Touraine and Ammar (1985) for triticale and barley and by Ben Naceur *et al.* (2001) for wheat.

For more severe stress (9 g NaCl L^{-1}), the aerial part length was more affected for [Kélibia 1 (~5% of the control) Kélibia 2 (~18%)] and particularly Kalaâ (0%). However, Kébilli 1, Kébilli 2, Kébilli 3, Sidi Bouzid and Tozeur were able to keep more than 50% of their control length. Our results were similar to those of Garcia-Legaz *et al.* (1993) and Mwai *et al.* (2004) that showed a variable stress effect on the aerial part growth of many plant species.

This result showed behavior differences between and within the same-origin collected ecotypes confirming what was found by morphological traits.

Chlorophyll content (a) variation: Under salt stress, ecotypes' chlorophyll content (a) (Fig. 2) decreased compared to control plants. However, this response varied according to stress intensity and to the ecotypes. Similar results showig leaf chlorophyll content decreased under salt stress intensity are reported for tomato by (El-Khlil *et al.*, 2002) and for wheat by (Kingsbury *et al.*, 1983). The same observations were, also made by Wang *et al.* (2004). They recorded that high salt concentrations disturbed plant growth, which exhibited anthocyan production and chlorophyll degradation.

When the stress was 6 g NaCl L^{-1}, the chlorophyll (a) content was affected, especially for Souihli, Martin and Kalaâ, where the reduction percentage was ≥ 50% compared to control.

The plant photosynthetic capacity is determined by several factors including photosynthetic pigment composition (chlorophyll content), CO_2 fixation capacity, light intensity and various enzyme activities (Mwai *et al.*, 2004). Furthermore, light-capture efficiency is directly correlated to leaves' chlorophyll concentration (Lutts *et al.*, 1995). Therefore, slight decline observed in leaf chlorophyll concentration at Tozeur 2, Kasserine and Kébilli 2 ecotypes (≤15% of the controls) could explain their better tolerance to salt and could contribute to their photosynthesis and plant growth stability.

Molecular study: Electrophoresis of the amplified DNA product for the 40 primers tested, showed only 10 primers, which were able to generate visible and reproducible band profile (Fig. 3).

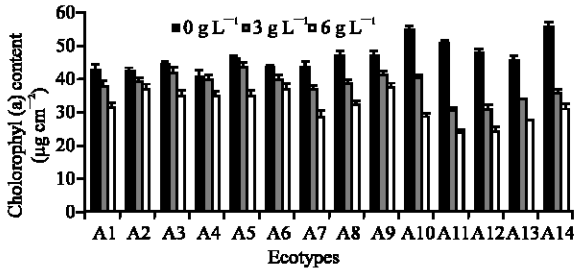

Fig. 2: Ecotypes cholorophyll (a) content under different salt stress concentration

Table 2: Ecotypes' morphological traits

Ecotypes	Plant length (cm)	Ear length (cm)	Ear density	Beginning of inflorescence emergence related to sowing date	End of inflorescence emergence related to sowing date
Tozeur 1	110.5	8.43	Very loose	133 days	140 days
Tozeur 2	111.0	6.00	Compact	135 days	143 days
Kébilli 1	115.0	5.00	Compact	138 days	145 days
Kébilli 2	115.0	4.50	Very compact	147 days	154 days
Kébilli 3	122.0	7.00	Half-loose to half compact	153 days	160 days
Kasserine	111.5	7.50	Loose	153 days	160 days
Sidi Bouzid	134.0	6.95	Loose	147 days	154 days
Jendouba 1	134.0	6.95	Loose	153 days	160 days
Jendouba 2	144.7	10.00	Loose	153 days	160 days
Souihli	111.7	8.00	Loose	147 days	154 days
Martin	132.0	7.75	Compact	147 days	154 days
Kalaâ	105.0	6.50	Very compact	147 days	154 days
Kélibia 1	125.0	7.00	Compact	153 days	160 days
Kélibia 2	128.0	8.25	Compact	153 days	160 days

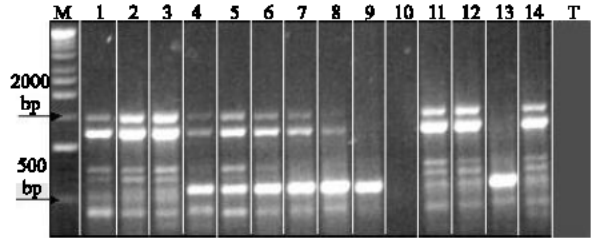

Fig. 3: Genetic fingerprints of barley ecotypes DNA using RAPD makers and OP E 20 primer (M = 1 Kb ladder (Promage), 1 = Tozeur 1, 2 = Tozeur 2, 3 = Kébbili 1, 4 = Kébilli 2, 5 = Kébilli 3, 6 = Kasserine, 7 - Sidi Bouzid, 8 = Jenduba 1, 9 = Jendouba 9, 10 = Souihli, 11 = Martin, 12 = Kalla â, 13 = Kélebia 1, 14 = Kélebia 2 and T = negative control)

Table 3: Percentage ecotypes' epicotyl's length compared to control under salt stress intensity

Ecotypes	6 g NaCl L^{-1}	9 g NaCl L^{-1}
Tozeur 1	50.85	40.71
Tozeur 2	61.61	50.82
Kébilli 1	95.41	50.51
Kébilli 2	84.83	69.85
Kébilli 3	36.03	43.96
Kasserine	22.68	20.75
Sidi Bouzid	78.36	53.24
Jendouba 1	51.86	30.18
Jendouba 2	56.25	36.44
Souihli	50.99	30.86
Martin	35.58	21.06
Kalaâ	47.64	0.00
Kélibia 1	31.68	18.14
Kélibia 2	40.96	4.87

Table 4: Number of bands and fragments generated by the used primers

Primers	Primers' sequence	Bands' number/gel	Fragment number
Op E-03	5'-CCA-GAT-GCA-C-3'	20.0	4
Op E-07	5'-AGA-TGC-AGC-C-3'	34.0	5
Op E-12	5'-TTA-TCG-CCC-C-3'	37.0	6
Op E-15	5'-ACG-CAC-AAC-C-3'	58.0	8
Op E-20	5'-AAC-GGT-GAC-C-3'	26.0	4
Op B-01	5'-GTT-TCG-CTC-C-3'	20.0	4
Op B-05	5'-TGC-GCC-CTT-C-3'	43.0	6
Op B-08	5'-GTC-CAC-ACG-G-3'	23.0	3
Op B-10	5'-CTG-CTG-GGA-C-3'	29.0	4
Op B-18	5'-CCA-CAG-CAG-T-3'	49.0	6
Total		339.0	50
Average		33.9	5

The other primers used generated scarcely visible bands and/or monomorphic patterns, which resulted from amplification where the annealing temperature should be optimized or where the cocktail would require a higher concentration of $MgCl_2$ (Pomper *et al*., 1998) Band size varied from 0.25 to 0.2 Kb, but we only took account of those that were clearly visible.

A total of 339 bands were detected, among which 109 bands were polymorohic with the mean of 10.9 per primer (Table 4). For each primer, the bands number ranged from 3 to 8, with an average of 5.

All the bands generated from 10 RAPD primers, were subjected to calculate the Genetic Dissimilarity (GD) among the 14 ecotypes. The dendrogram (Fig. 4) based on dissimilarity matrix was implemented according to the Treecon software's UPGMA cluster (Unweighted Pair-Group Method using Arithmetic Average), which separated studied ecotypes on 4 groups.

The first group was, constituted by Souihli, which exhibited a very late heading date (heading date = 154 days after sowing), very loose ear density and high sensitivity to salt stress. These traits make it genetically very different from the other cultivars genetic dissimilarity (GD) = 79.08% (Table 5).

The second group was made of Kélibia 1 (GD = 52%). This ecotypes was genetically different from Kélibia 2 (GD = 23%). Thus, in Kélibia region, farmers cultivate a mixture of two different ecotypes as they have identical morpho-physiological traits (heading date = 160 days, identical response to salt stress) but they presented structurally different ears. This last parameter probably explains their genetic difference.

The third group was, formed by Tozeur 1 (GD = 47%). The observation already made above is valuable for Tozeur 1 and Tozeur 2 ecotypes (GD = 11%) and the difference between them was 34%. Also, both ecotypes were relatively similar at morpho-physiological level, but they differed in ear structure which is a dominant character easily revealed by dominant markers like the RAPD (Demarly and Sibi, 1996).

The fourth group was itself subdivided into three subgroups. The first subgroup included Tozeur 2, Kébilli 3, Kalaâ, Martin and Kélibia 2, where the GD was only 21%.

The second subgroup included Sidi Bouzid, Jendouba 1 and Jendouba 2, which were genetically closely related to each other. This subgroup was particularly late (heading date varied from 154 to 160 days) and differed by ear structure.

The third subgroup was made up of Kébilli 1, Kébilli 2 and Kasserine. This subgroup presents a heading date varying between 154 and 160 days. Kébilli 1 and Kébilli 2 ecotypes were morphologically similar, but they differed from that of Kasserine considering ear structure and color.

It should be noticed that the best results were obtained with the Op-E-20 (8 bands), Op-B-8 (6 bands) and Op-B-1 (6 bands) primers, in accordance with what Monna *et al*. (1994) and Jenderek *et al*. (1997) showed. These primers contained 70% of bases in the form of G+C, the proportion of G was relatively equilibrated with that of C and the primer ended in 3' by G or C (Table 5). But the

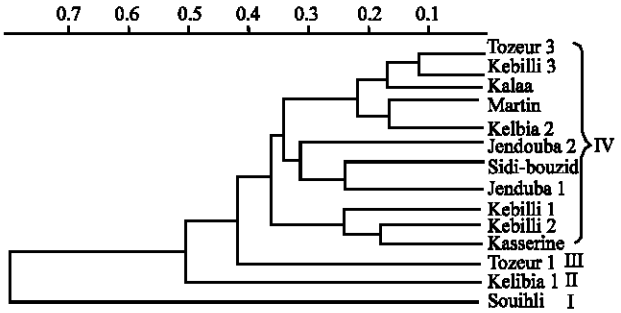

Fig. 4: Genetic diversity distance calculated using UPGMA procedure according to Nie's and Lee method

Table 5: Dissimilarity matrix

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
V1	0.00													
V2	34.55	0.00												
V3	37.93	11.48	0.00											
V4	44.68	36.00	28.30	0.00										
V5	40.43	20.00	24.53	19.05	0.00									
V6	43.48	42.86	38.46	17.07	26.83	0.00								
V7	45.10	33.33	29.83	34.78	26.09	42.22	0.00							
V8	37.26	37.04	26.32	30.44	30.44	37.78	24.00	0.00						
V9	41.18	33.33	33.33	30.44	30.44	42.22	28.00	32.00	0.00					
V10	93.33	75.76	77.78	76.00	76.00	75.00	79.31	79.31	79.31	0.00				
V11	38.18	17.24	24.59	36.00	32.00	46.94	33.33	33.33	37.04	75.76	0.00			
V12	52.73	17.24	14.75	36.00	28.00	46.94	37.04	40.74	40.74	81.82	27.59	0.00		
V13	65.22	51.02	53.85	41.46	41.46	55.00	42.22	55.56	42.22	75.00	55.10	46.94	0.00	
V14	38.98	22.58	13.85	33.33	33.33	43.40	27.59	24.14	34.48	78.38	16.13	22.58	54.72	0.00

V1: Tozeur 1; V2: Tozeur 2; V3: Kébilli 1; V4: Kébilli 2; V5: Kébilli 3; V6: Kasserine; V7: Sidi Bouzid; V8: Jendouba 1; V9: Jendouba 2; V10: Souihli; V11: Martin; V12: Kalaâ; V13: Kélibia 1 and V14: Kélibia 2

primers that did not generate visible polymorphic bands were those whose G and C bases were highly imbalanced and ended in 3' by A or T, in accordance with what Monna *et al.* (1994) showed.

CONCLUSIONS

The morphological traits analysis showed clear differences among ecotypes (plant height, ear compacity, heading date,...). The heterogeneity observed between ecotypes collected from different fields may be related to the fact that Tunisia is a geographically based cereal population. However some ecotypes share the same morphological traits so it's difficult to distinguish them on this base.

Analysis of genetic distances between the 14 ecotypes clustered them in only 4 groups. The first group was made up of Souihli, which was genetically very distant from the others. The second group was made of Kélibia 1, which was very distant from Kélibia 2 on the base of molecular analysis and on the base of morphological traits. This result means that in Kélibia region, farmers used a mixture of two different ecotypes. The third group made up of Tozeur 1, which is genetically distant (34%) from Tozeur 2 but the two accessions have similar morpho-physiological traits. This similarity could be related to the environmental adaptation.

The fourth group was itself subdivided into three subgroups. These ecotypes have different origins and belong to different climate stage. They could have the same parents as they shared some morpho-physiological traits.

Molecular study of the genetic fingerprints by RAPD markers allowed us to discover differences among barley ecotypes. This molecular tool could be used to supplement and clarify ambiguities in morpho-physiological studies. The good choice of primers to be used in this kind of study would enhance the method's efficiency.

REFERENCES

Arnon, D.I., 1949. Cooper enzymes in isolated chloroplastes. Plant Physiol., 24: 1-15.

Benlaghlid, M., N. Bouattoura, P. Monneveux and C. Borries, 1990. Les blés des oasis: Etude de la diversité génétique et de la physiologie de l'adaptation au milieu. Options Méditerranéennes. Série A/n°11, 1990, pp: 171-194.

Ben Naceur, M., R. Paul and Ben H. Salah, 1997. Screening of six barley varieties for drought resistance by using leaf water potential, membrane stability and phosphatasic activity parameters. Medit., 4: 51-54.

Ben Naceur, M., M. Ben Salem, C. Rahmoune, A. Chorfi, S. El-Jaafari and R. Paul, 1998. Etude comparée du comportement de quelques variétés anciennes et quelques variétés nouvelles de blé dur (*Triticum durum* Desf.), sous contrainte hydrique. Ann. de l'INRAT (Tunisie), 78: 251-277.

Ben Naceur, M., C. Rahmoune, H. Sdiri, M.L. Meddahi and M. Selmi, 2001. Effet du stress salin sur la germination, la croissance et la production en grains de quelques variétés Maghrébines de blé. Sécheresse, 12: 167-174.

Ben Naceur, M. and M. Rouaïssi, 2003. Etude de la diversité génétique chez 6 variétés de blé moyennant des marqueurs AFLP. Annales de l'INRAT (Tunisie), 76: 35-51.

Bettaïeb-Ben Kaab, L. and J. Attias, 1992. Electrophoretic heterogeneity of the hordiens of five barley (*Hordium vulgare* L.) cultivars from Tunisia. C.R. Acad. Sci. Paris t 314, Série III, pp: 141-146.

Bettaïeb-Ben Kaab, L., M. El Felah and M. Lauriere, 2005. Relationships between some hordein components and quality properties in two tunisian barley varieties as influenced by nitrogen fertilisation. Czeh J. Plant Breed., 41: 11-16.

Boeuf, F., 1931. Le blé en Tunisie: La plante, le milieu physico-chimique. Ann. du Service Bota. et Agro., 1: 200.

Deghaïs, M., 1996. Le blé tendre en Tunisie un siècle de recherche variétale. Ann. de l'INRAT., numéro spécial, pp: 286.

Deghaïs, M., El M. Falah, M.S. Gharbi, T. Zarkouna and M. Chakroun, 1999. Les acquis de l'amélioration génétique des céréales en Tunisie. Article de synthèse. Ann. de l'INRAT., 72: 3-30.

Demarly, Y. and M. Sibi, 1996. Vitrovariations ou variations somaclonales. Amélioration des plantes et biotechnologies, éd-John Libbey, pp: 59-84.

El Falah, M., 1998. Evaluation de 423 écotypes d'orge collectés en Tunisie (Centre et Sud). Utilisation en amélioration variétale pour la sélection d'idiotypes adaptés à différentes régions bioclimatiques. Thèse de Doctorat de Biologie. Faculté des Sciences de Tunis, pp: 300.

El Falah, M., L. Bettaeib-Ben Kaab and N. Chelbi, 2004. Tunisian insular Barley landraces: A significant Hordeins drift. 9th International Barley Genetics Symposium Proceeding, Brno, Czech Republic, 20-26 June, pp: 39-42.

El-Lklil, Y., M. Karrou, R. Rachid Mrabet and M. Benichou, 2002. Effet du stress salin sur la variation de certains métabolites chez *Lycopersicon esculentum* et *Lycopersicon sheesmanii*. Can. J. Plant Sci., 82: 177-183.

Erroux, J., 1958. Note sur les blés des oasis du Sahara algériens. In « Bull. Soc. Hist. Nat. Afrique du Nord., 49: 56.

Garcia-Legaz, M.F., J.M. Ortiz, A. Garcia-Lidon and A. Cerda, 1993. Effect of salinity on growth, ion content and CO_2 assimilation rate in lemon varieties on different rootstocks. Physiol. Plant., 89: 427-432.

Gibson, S. and C. Somerville, 1993. Isolating plant genes. Tibtech., 11: 306-313.

Hamza, S., W. Ben Hamida, A. Rebaï and M. Harrabi, 2004. SSR6-based Genetic Diversity Assessment among Tunisian Winter Barley and Relationship with Morphological Traits. Euphytica, 135/1076118.

Hou, Y.C., Z.H. Yan, Y.M. Wei and Y.L. Zheng, 2005. Genetic diversity in barley from west china based on RAPD and ISSR analysis. Barley Genet. Newslett., 35: 9-22.

Jenderek, M.M, K.A. Schierenbeck and A.J. Olney, 1997. Development of Randomly Amplified Polymorphic DNA Markers. Characteristic of *Hibiscus rosa-sinensis* and *H. syriacus*. Center for Irrigation Technology. CATI Publication 970902, pp: 1-8.

Kingsbury R.W., E. Epstein and R.W. Pearly, 1983. Physiological responses to salinity in selected lines of wheat. Plant Physiol., 74: 417-423.

Lalaoui-Kamel, M. and N.E. Assali, 1997. Utilisation des marqueurs moléculaires RFLP pour la caractérisation de la diversité génétique: 355-360. In: Biotechnologies, Amélioration des plantes et sécurité alimentaire. Actes des 6èmes Journées Scientifiques du Réseau AUPELF-UREF Biotechnologies Végétales; Génie génétique des plantes Université de Paris Sud XI Centre Universitaire d'Orsay du 30 Juin au3 Juillet. Editions ESTEM.

Liang, P. and B. Pardee, 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science, 257: 967-971.

Lutts, S., J.M. Kinet and J. Bouharmont, 1995. Changes in Plant Response to NaCl During Development of Rice (*Oryza sativa* L.) Varieties Differing in Salinity Resistance. J. Exp. Bot., 46: 1843-1852.

Monna, L., A. Miyao, T. Inoue, S. Fukuoka, M. Yamazaki, H.S. Zhong, T. Sasaki and Y. Minobe, 1994. Determination of RAPD Markers in Rice and their conversion into Sequence Tagged Sites (STSs) and STS-Specific Primers Rice. Genome Research Program, National Institute of Agrobiological Resources, 1-2 Kannondai 2-chome. Tsukuba, Ibaraki 305, Japan/Institute of Society for Techno-Innovation of Agriculture, Forestry and Fisheries, 446-1 Ippaizuka, Kamiyokoba, Tsukuba, Ibaraki 305, Japan. DNA Res., 1: 139-148.

Mwai, G.N., J.C. Onyango and M. Onyango, 2004. Effect of salinity on growth and leaf yield of spiderplant (*Cleome gynandra* L.). Afr. J. Food Agric. Nut. Develop., 4: 2.

Nei, M. and W.H. Li, 1979. Mathematical models for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA., 76: 5269-5273.

Pomper, K.W., A.N. Azarenko, N. Bassil, J.W. Davis and S.A. Mehlenbacher, 1998. Identification of random amplified polymorphic DNA (RAPD) markers for self-incompatibility alleles in *Corylus avellana* L. Theor. Applied Genet., 97: 479-487.

Seguela, J.M. and P. Jacquard, 1953. Importance du facteur variétal dans la céréaliculture tunisienne. Extrait du Bulletin Economique et Social du mois de Décembre 1953, No. 8.

Sneath, P.H.A. and R.R. Sokal, 1973. Numerical Taxonomy. Freeman, San Francisco.

Snoussi, H., M. Ben Slimane-Harbi, L. Ruiz-Garcia, J.M. Martinez-Zapater and R. Arroyo-Garcia, 2004. Genetic relationship among cultivated and wild grapevine accessions from Tunisia. Genome, 47: 1211-1219.

Touraine, B. and M. Ammar, 1985. Etude comparée de la sensibilité aux sels d'un triticale et d'une orge. Agronomie, 5: 391-395.

Wang, Z., P. Li, M. Fredricksen, Z. Gong, C.S. Kim, C. Zhang, H.J. Bohnert, J.K. Zhu, R.A. Bressan and P.M. Hasegawa, 2004. Expressed sequence tags from Thellungiella halophila, a new model to study plant salt-tolerance. Plant Sci., 3: 61-71.

Webb, D.M. and S.J. Knapp, 1990. DNA extraction from a previously recalcitrant plant genus. Plant Mol. Biol. Rep., 8: 180-185.