www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 3/2 (2010) 1-11

RAPD markers and morpho-physiological characterization of some Tunisian Barley ecotypes

Kadri KARİM [*1,2], Abdellawi RAWDA [2], Cheikh mhamed HATEM [2], Ben naceur M'BAREK [2]

[1] Centre Régional de la Recherche en Agriculture Oasienne, Laboratoire de Biotechnologie et de culture des Tissus végétales, Degach 2260, Tunisie.
[2] Institut National de la recherche Agronomique de Tunisie, Laboratoire de Biotechnologie et Physiologie Végétale, Rue Hédi karray 2049, Ariana, Tunisie

Abstract

The genetic variation and relationships among 12 local barleys and the varieties Martin, Rihane and Manel were evaluated using Random Amplified Polymorphic DNA (RAPD) markers and morpho-physiological traits. To fulfil this purpose, some ecotypes were collected from different bioclimatic regions and studied at morpho-physiological and molecular levels. 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 adaptation to environmental conditions and acquired over time. Also, accessions that were grown mixed together in the same area and having similar physiological behaviour were found different using the RAPD markers method. This result showed an important degree of genetic variability, which indicating the Tunisian germplasm richness.

Key words: Barley; Morpho-physiological traits; RAPD markers; Genetic diversity

1. Introduction

Cultivated barley (*Hordeum vulgare* L.) is one of the oldest cultivated plants. It was believed formerly that barley was originating from the desert areas of Southwest Asia, more than 10.000 years ago. However, recent researches attribute two origins for barley: mountainous areas of Ethiopia and Southeast Asia (Badr et al.,2000)

North Africa is considered as one of the main secondary cereal centers (Boeuf, 1931). Indeed, Tunisia constitutes an area of great cereal diversity. The local landraces are very adapted to stress conditions (drought and self), since they groom 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.,2001). However, replacing native germplasm by an improved and introduced materiel could lead to local phytogenetic resources erosion. Therefore, more importance should be given to local resource conservation.

Prospection, collection and assessment of genetic resources in the three Maghreb countries (Tunisie, Algeria and Morocco) started long time ago (Badr et al.,2000; Erroux, 1958) but the most recent, date back to 1982 (El Falah, 1998), 1990 (Benlaghlid *et a.*,, 1990) and 1994 (Ban Naceur *et al.*,1998). These studies were focused especially on the morphological variability of the vegetative part, ears and seeds (Benlaghlid et al.,1990) or on reserve protein diversifies (Bettaïeb and Attias, 1992; Bettaïeb 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). At present the most reliable methods are the molecular marker techniques (Nuel et *al.*, 2000). Recent studies based on molecular variability have been carried out. (Lalaoui-Kamel and Assali, 1997) used RFLP to distinguish the genetic polymorphism on *Medicago* genus, Snoussi *et al*, (2004), used microsatellites to analyze the genetic diversity among grape varieties and Ben Naceur and Rouaïssi

* *Corresponding author* / Haberleşmeden sorumlu yazar: kadrikarim2001@yahoo.fr

(2003), analyzed varietal polymorphism in wheat by AFLP method.

In Tunisia, attention is now given to study genetic variability among barley germplasm at both molecular and morphological level. The aim of this study is to characterize some barley ecotypes collected from parts of the country at the morpho-physiological and molecular level. Morpho-physiological traits and molecular RAPD markers polymorphisms were compared and checked whether we still have great genetic barley variability.

2. Materials and methods

2.1. Plant material

Twelve local winter barley ecotypes *(Hordeum vulgare, L.)* of diverse geographic origins were used in this study. These ecotypes were obtained after prospecting different Tunisian bioclimatic regions (Fig. 1). Once collected, these ecotypes were named according to their collection region. They were: Tozeur 1, Tozeur 2, Kébilli 1, Kébilli 2, Kébilli 3, Kasserine, Sidi Bouzid, Jendouba 1, Jendouba 2, Kalaâ, Kélibia 1 and Kélibia 2. Martin, Rihane and Manel varieties traditionally grown in Tunisia were also 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 ofthe country. They are characterized by a sub-humid bioclimatic sector where the annual rainfall is 600 mm.
- The Kasserine and Sidi Bouzid district is in the Tunisian West-center and belonging to the aridsuperior bioclimatic region where the average annual rainfall is 300 mm.
- 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.

2.2. Morpho-physiological traits:

The morpho-physiological criteria used were heading date, ear density and length, plant height and the response of the ecotypes to saline stress (length of epicotyl and chlorophyll content). Three different salt concentrations were applied (0, 6 and 12 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 12 g /L of NaCl). Germination was achieved in obscurity at 25±1°'C using an incubator. The length of epicotyl was determined after 7 days.

The chlorophyll content, which represents photosynthetic potential of the plant, was also determined. It was measured during the heading stage. Four replications were carried out for each ecotypes and each treatment a Spadmètre instrument (KONICA MINOLTA) was used to determine the amount of chlorophyll. In fact Chlorophyll Meter SPAD is an instrument which measures the content chlorophyl directly on the leaf of the plants and indicates values SPAD. Measurements are instantaneous on the plant without having to cut sheets, simply by projecting light through the measured sheet. The chlorophyl concentration of the plants is strongly correlated with the state of nutrition nitrogenized of those.

2.3. DNA extraction

The DNA was extracted and purified from leaves, using a CTAB (Cetyl trimethyl ammonium Bromide) method (Webb and Knapp, 17). 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:

$C(\mu g\ \mu L^{-1}) = OD_{260} \times 10$. The OD_{260} / OD_{280} ratio was also calculated to determine DNA purity.

2.4. PCR amplification

Eighty Operon primers were tested on DNA samples. DNA amplification was carried out in a final volume of 25 µL containing 2.5 Mm $MgCl_2$, 200 µM dNTP, 20 pmol of Operon primer, 20 ng of DNA, 5X Taq buffer, 0.5 U of Taq polymerase and adjusted with distilled water. The program of amplification; using a thermocycler (Biometra UNO II); consisted of a pre-denaturation cycle of 4 min at 94°C, 40 cycles of a denaturation for 30 sec at 94°C, an annealing for 60 sec at 38°C, an extension for 2 min at 72°C followed by a post-extension cycle for 10 min at 72°C. The amplification products of each primer were electrophoresed at 80V for 2 h in horizontal l.8 % agarose gel prepared in 1x TAE (TRIS Acetate EDTA) buffer containing 0.01 % of ethidium bromide. For ach sample, 10 µL of the amplified product were mixed with 2 µL of loading dye (6X) and loaded in agarose gel (1.8%). Bands were visualized under UV light and photographed by using Polaroid camera system.

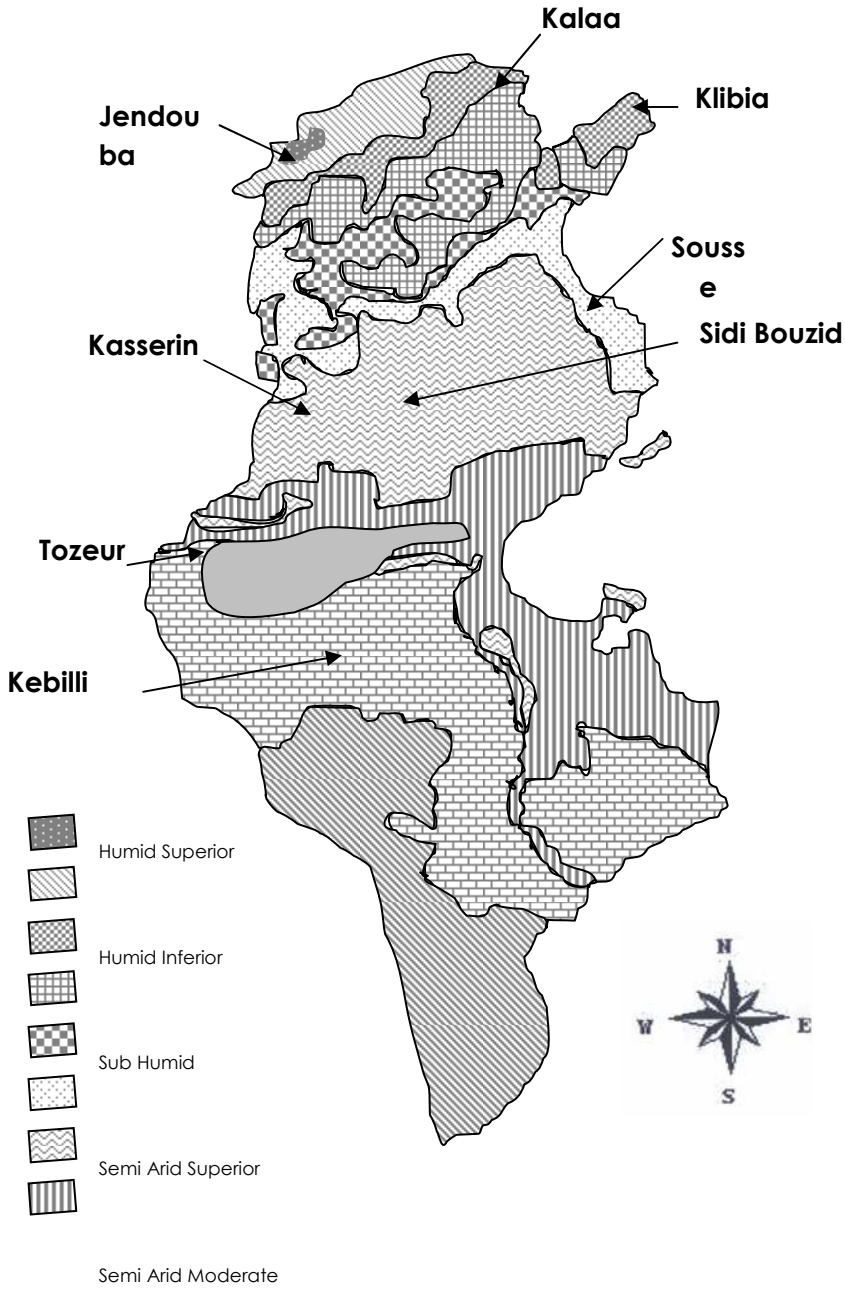

Figure 1. The origin of bioclimatic ecotypes

2.5. Data and Statistical Analysis

Eighty primers were used but only fefteen primers showed clear, reproducible and polymorphic bands. These primers were considered to make binary matrix, study similarity and discuss polymorphism between ecotypes. Data obtained were scored in a binary form as presence (1) or absence (0) of bands for each accession and entered into a data matrix (Hou et al.,2005). Genetic Similarity (GS) between ecotypes was calculated according to Nei and Li,

(1979) formula. Based on the similarity 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 NTSYS software. 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.

3. Results and discussion

3.1. 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 25 days difference between them. However, for the other ecotypes, this criterion is intermediary and varied from 2 to 15 days. Furthermore, plant length showed that Kébilli 3 and Sidi Bouzid 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 1). 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.

The study of the morphological traits showed that Sidi Bouzid, Jendouba 1 and Jendouba 2 shared the same criteria and have some common traits with the variety Martin which could be due to a common ancestor. Same remark is given for the accessions Tozeur 1 and Kébilli 1. They also shared some morphological traits with the other southern accessions (Tozeur 2, Kébilli 2 and Kébilli 3). The northern accessions also presented some common morphological traits like ear attitude and sterile spikelet attitude (Table 1).

Table 1. Morphological traits of ecotypes

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	Growth habit	Ear: attitude	Ear: density	Sterile spikelet: attitude (in mid-third of ear)
Tozeur 1	110.5	8.43	Very loose	133 days	140 days	Prostrate	Semi erect	very dense	divergent
Tozeur 2	111.0	6.00	Compact	135 days	143days	Erect	Semi erect	Dense	Parallel to weakly divergent
Kébilli 1	115.0	5.00	Compact	138 days	145 days	Prostrate	Semi erect	very dense	divergent
Kébilli 2	115.0	4.50	Very compact	147 days	154 days	Semi erect	Semi erect to horizontal	medium	parallel
Kébilli 3	122.0	7.00	Half-loose to half compact	153 days	160 days	Erect	Semi erect	very dense	parallel
Kasserine	111.5	7.50	Loose	153 days	160 days	Semi erect	erect	lax	Parallel to weakly divergent
Sidi Bouzid	134.0	6.95	Loose	147 days	154 days	Semi prostrate	Semi erect	lax	Parallel to weakly divergent
Jendouba 1	134.0	6.95	Loose	153 days	160 days	Semi protrate	Semi erect	lax	Parallel to weakly divergent
Jendouba 2	144.7	10.00	Loose	153 days	160 days	Semi protrate	erect	lax	Parallel to weakly
Rihane	111.7	8.00	Loose	147 days	154 days	intermediate	Semi erect	Very dense	parallel
Martin	132.0	7.75	Compact	147 days	154 days	intermediate	erect	dense	parallel
Kalaâ	105.0	6.50	Very compact	147 days	154 days	Erect	erect	intermediate	Parallel to weakly divergent
Kélibia 1	125.0	7.00	Compact	153 days	160 days	Dense	dense	erect	parallel
Kélibia 2	128.0	8.25	Compact	153 days	160 days	intermediate	Semi erect	lax	parallel
Manel	130.5	5.25	Compact	147 days	154 days	Dense	erect	intermediate	Parallel to weakly

We used the percent similarity and median joining method to draw morphological distances (Fig. 2). The Comparison of morphological characters using similarity percentage gave five accession groups. The first group consisted of 'Tozeur l', `Tozeur 2' and 'Kébilli l' with a percentage similarity that varies between 53% and 83 %. `Kébilli 2', 'Kébilli 3' and 'Kasserine' form the second with a similarity that oscillates between 73% and 91%. 'Sidi Bouzid' and 'Jendouba l', showing a very high similarity (92.5%), occupy the third position. The accessions 'Jendouba 2', `Rihane' and `Martin' constituted the fourth group; they are assembled with a percentage spread out between 81% and 86%. Nevertheless, 'Kalaâ', 'Kélibia l', 'Kélibia 2' and 'Manel' share the latest group with a high similarity situated between 75% and 90%. Referring to the dendrogram, we remark that accessions collected from the same origin have high similarity rates as examples, 'Kébilli 2/ Kébilli 3' with 93 %.

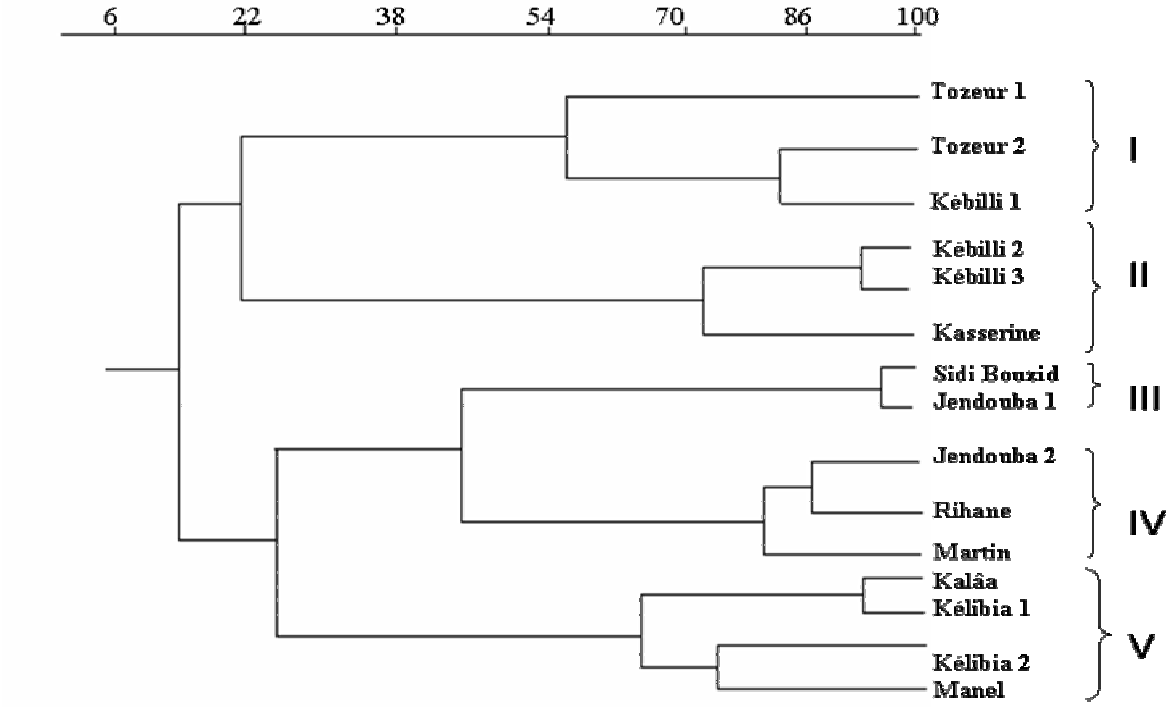

Figure 2. Morphological classification of the accessions based on median constrained distance using MVSP 3.131.

3.2.Physiological parameters:

3.2.1.Epicotyl's length at the germination level

Table 2 showed that even moderate saline stress of 100 mM (6 g NaCI L^{-}') could affect seriously the epicotyl's length. In deed, the ecotypes Jendouba 2, Martin, Kalaâ and Kélibia 1 showed low epicotyl's length percentage of 38%, 40%, 42% and 36%. 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. (1998) for wheat.

For more severe stress of 200 mM (12 g NaCI L^{-}'), the aerial part length was more affected for Jendouba 2 (-80% of the control), Kélibia 2(-82%) and Kalaâ (-80%). However, Kébilli 1, Tozeur 2, Kébilli 3, Sidi Bouzid were able to keep more than 50% of their control length and particulary Kébilli 2 with 60%. 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 behavioural differences between and within the ecotypes collected from the same-origin confirming the results from morphological traits.

3.2.2. Chlorophyll content variation

Salt-induced restriction in water supply can cause stomata closure, which will in turn lead to decreased absorption of CO_2 and eventually result in reduction of photosynthesis (Delfine et *al.*, 1998; Sultana et *al.*,1999). Chlorophyll content is associated directly with light harvesting potential and is normally considered as one of the important components in photosynthetic capacity *(*Delfine et *al.*, 1998). In the current study, salt stress caused a significant reduction in the contents of chlorophyll compared to control plants (fig 3). However, this response varied according to stress intensity and to the ecotypes. Similar results showing a decrease in leaf chlorophyll content under salt stress were 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), for Thellungiella halophila. They recorded that high salt

Table 2. Percentage of epicotyl's length under salt stress intensity compared to control

Ecotype	6 g Nacl L^{-1}	12 g Nacl L^{-1}
Tozeur 1	67,90	43,27
Tozeur 2	69,17	57,84
Kébilli 1	70,75	48,94
Kébilli 2	69,65	57,33
Kébilli 3	72,03	48,67
Kasserine	74,10	45,29
Sidi Bouzid	66,87	30,38
Jendouba 1	63,02	20,24
Jendouba 2	37,42	20,23
Martin	39,86	33,55
Kalaâ	42,93	22,02
Kélibia 1	35,61	20,63
Kélibia 2	51,37	18,37
Rihane	56,73	20,90
Manel	51,67	20,69

concentrations disturbed plant growth, which exhibited anthocyanine production and chlorophyll degradation. When the stress was 12 g NaCI l^{-1}, the chlorophyll content was affected, especially for Martin, Kalaâ, Rihane and Manel, 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 chlorophyll concentration of leaves (Lutts *et al*, 30). Therefore, slight decline observed in leaf chlorophyll concentration at Tozeur 2, Kébilli 1, Kébilli 2 and Sidi bouzid ecotypes (≤ 15% of the controls) could explain their better tolerance to salt and could contribute to their photosynthesis and plant growth stability.

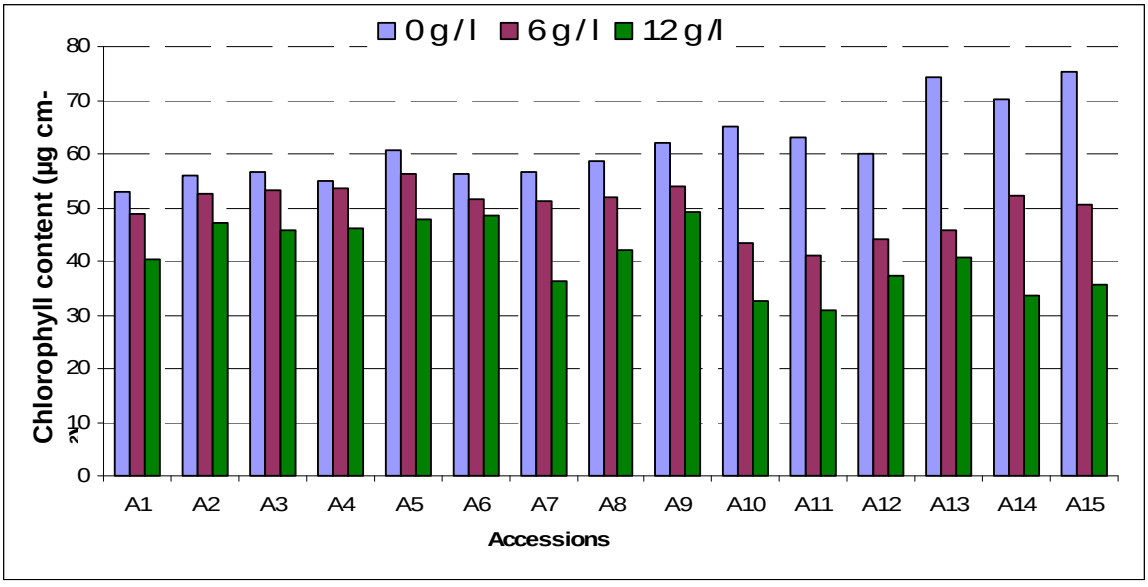

Figure 3. Chlorophyll content of barley accessions under different salt concentrations.
A1: Tozeur 1; A2: Tozeur 2; A3: Kebilli 1; A4: Kebilli 2; A5: Kebilli 3; A6: Kasserine; A7: Sidi Bouzid; A8: Jendouba 1; A9: Jendouba 2; A10: Martin; A11: Kalâa; A12: Klibia 1; A13: Klibia 2; A14 : Manel et A15: Rihane

3.3. Molecular study:

Electrophoresis of the amplified DNA products for the 80 primers tested, showed only 15 primers, which were able to generate visible and reproducible band profile (fig.4). The other primers 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 $MgC1_2$ (Pomper et al., 1998). A total of 93 bands were detected, among which 69 bands were polymorphic with the mean of 4.6 per primer (Table 3). For each primer, the bands number

ranged from 4 to 10, with an average of 6.2. Band size varied from 3 to 0.25 Kb, but we only took account of those that were clearly visible. All clear bands generated from 15 RAPD primers were subjected to calculate the Genetic Similarity (GS) among the 15 barley ecotypes. The dendrogram (Fig. 4) based on Similarity matrix was implemented according to the NTSYS software's UPGMA cluster (Unweighted PairGroup Method using Arithmetic Average), which separated studied ecotypes into 5 groups.

The first group is composed of three accessions: 'Kébilli 3', 'Martin' and 'Manel', the similarity percentage between accessions of the first group, varies between 60 % and 65 %. Indeed, within this group, same characters of similarity (biological and morphological) were observed such as the length of the stem, (134 cm), a very early heading date (120 -125 days after sowing) (Cheik-Mhamed, 2004), in the same way for the size and the shape of ears which is short and pyramidal.

Table 3. Number of bands and fragments generated by the RAPD primers.

Primers	**Primer's sequence 5'—3'**	**Band's number /gel**	**Number of total bands/ primer**	**Number of polymorphic bands/primer**
OPD02	GGACCCAACC	60	8	6
OPD10	GGTCTACACC	32	6	5
OPD18	GAGAGCCAAC	25	4	2
OPD20	ACCCGGTCAC	47	9	7
OPG12	CAGCTCACGA	45	8	6
OPG14	GGATGAGACC	30	5	2
OPG10	AGGGCCGTCT	40	8	3
OPJ10	AAGCCCGAGG	27	4	2
OPF03	CCTGATCACC	72	10	8
OPH13	GACGCCACAC	67	9	7
OPE03	CCAGATGCAC	30	4	3
OPE07	AGATGCAGCC	38	5	3
OPE12	TTATCGCCCC	45	8	5
OPB05	TGCGCCCTTC	47	7	6
OPB18	CCACAGCAGT	42	6	4
Total		647	93	69
Average		43.13	6.2	4.6

The second is subdivided in three sub-group ('Tozeur 2' and 'Kébilli 1'); ('Kébilli 2') and ('Rihane'). Their similarities vary from 68 % to 78 %. From morphological point of view, the accessions of the second group present features of similarity at the levels of the size of ears (long size) and the fitting of the grains on the rachis (parallel) also the length of the barbs (long beards) and a late heading date (143-145 days after sowing). Six accession composed the third group which were subdivided on three sub-group ('Kasserine','Sidi Bouzid' and 'Jendouba 1); ('Kalâa' and 'Kilibia 2') and ('Jendouba 2'). The similarity percentage between accessions of the first sub-group varies between 77 % and 83 %; but that of the second sub-group and the last sub-group was ranged between 67 % and 75%.

In fact, cultivars of this group represent morpho-physiological similarities with known length of the barbs (long), the size of ears (short) as well as the precocity of heading (130-133 days after sowing). Finally, the accessions 'Tozeur l' (62%), and 'Kélibia l'(64%) form, respectively, groups IV and V. On the morphological level the accession Tozeur 1 (62%) is characterized by the short size of its ears and its barbs that comparable with the accession Klibia 1 (64%) which, on the contrary, present vigorous ears with long beards. Although they present genetic similarities, these two cultivars are morphological different and cultivated in two geographically distant areas (figure 1). That can be due to the forms of climatic adaptation especially that these two accessions belong to two differents bioclimatic regions (El Falah et *al*.,2004). It should be noticed that the best results were obtained with 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, in accordance with what Monna et al,(1994) and Jenderek et al,(1997) showed in their study. But the 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, which is in accordance with the results of Monna et al,(1994).

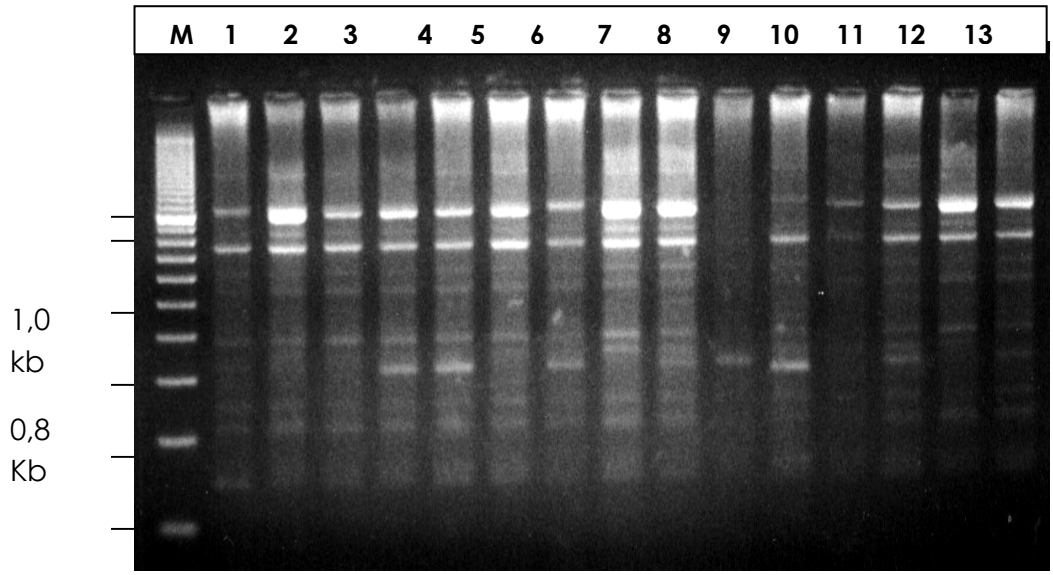

Figure 4. Typical examples of amplification products obtained by OPD20 RAPD primer using genomic DNA template of Tunisian barley accessions. M: marker (100 bp PCR Molecular Ruler, Biorad). Numbered wells correspond to the studied accessions. 1= Tozeur1, 2 = Tozeur2, 3 = Kebilli1, 4 = Kebilli2, 5 = Kebilli3, 6 = Sidi Bouzid, 7 = Kasserine, 8 = Jendouba1, 9 = Jendouba2, 10 = Martin, 11 = Kalâa, 12 = Klibia1, 13 = Klibia2, 14 = Rihane, 15 = Manel.

Dendrogram corresponding to morpho-physiological parameters showed no correlation between accessions originating front the same geographic area, which is in contradict with what Julier et *al*, *36* have shown. In fact, they found that the geographic origin of the collected material was sufficient to obtain a reasonable structuration in groups.

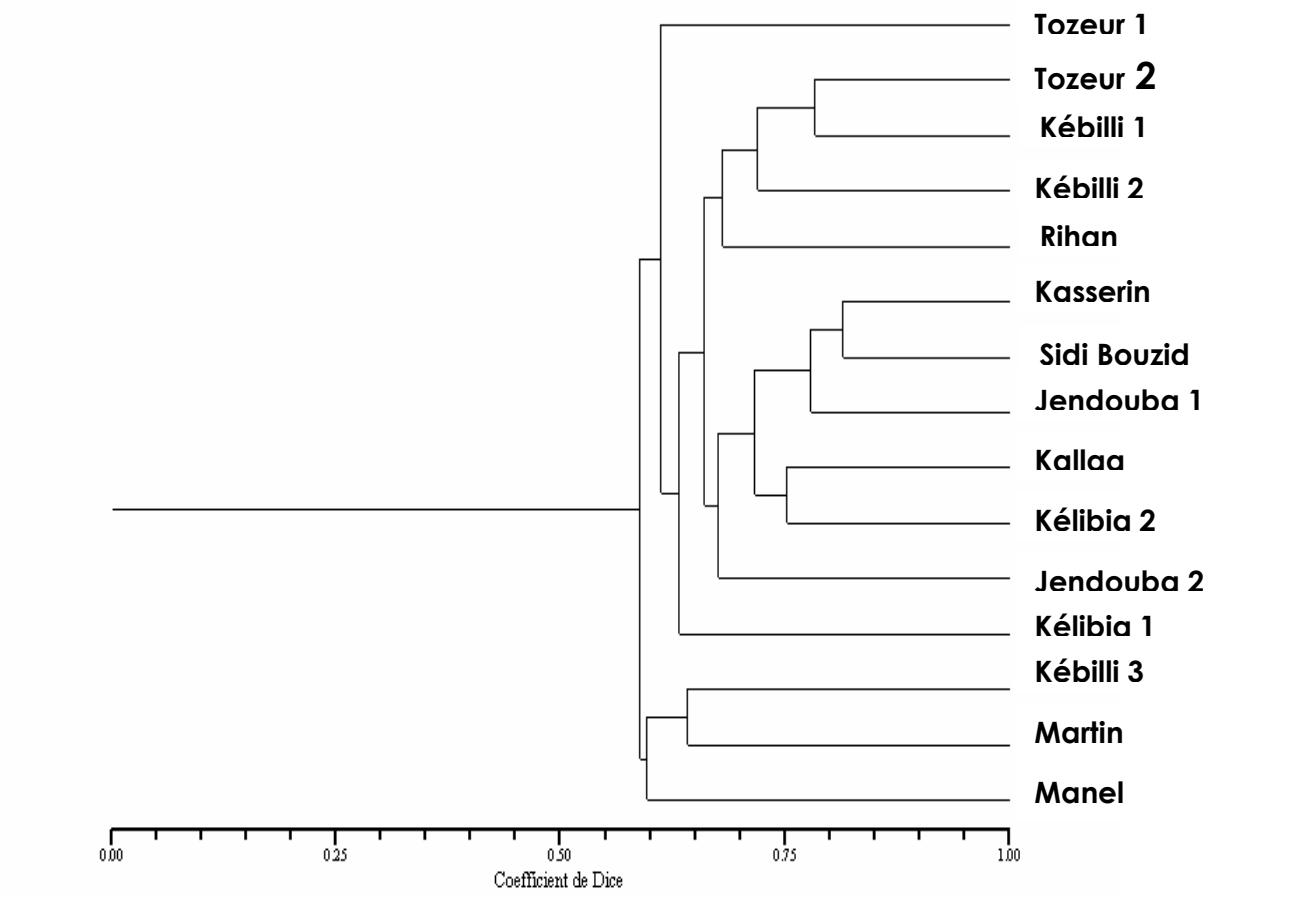

Figure 5. Dendrogram of the studied barley accessions, established by UPGMA method using the similarity matrix.

RAPD markers seemed to be effective to discriminate local barleys defined as accessions or populations geographically based (Yong et *al*., 2005). It is also a valuable tool for assessing genetic diversity levels. In our study, dendrogram obtained by RAPD markers classified the studied barley accessions according to, climatic stage and some morphological traits especially ear attitude, ear density and sterile spikelet attitude, which could be inherited independently from the environmental conditions.

4. Conclusion

In this study, distance matrices derived from RAPD markers and morpho-physiological data showed a low correlation (r = 0.14). This result is in agreement with those of Chia-Szu and Hsiao, (1999) working on *Lilium longiflorum* and reporting very low correlation *(r* = 0.035) between RAPD markers and morphological characters. However this result is in disagreement with those of Duarte et al,(1999) who found a correlation of 0.89 between the genetic distances obtained with RAPD and the distances of Mahalanobis indicating that the markers provide similar estimates of genetic divergence to those obtained using morpho-agronomical data on bean cultivars. This low correlation shows that there is a week association between molecular and physiological traits in these accessions. In other studies, Roldân-Ruiz et *al* ,(2001) working on perennial ryegrass found a correlation coefficient of 0.42 between STS markers and morphological traits methods. Mariç et *al*, (2004) studying hexaploid wheat cultivars, did not obtained a significant correlation between RAPD markers, morphological traits and coefficients of parentage. In the same way, Spooner et al,(2005*)* have obtained low correlation coefficient between potato genotypes by means of AFLP and morphological characters. On the other hand, Crochemore et al,(1998) working on 26 alfalfa population genetic structures found a global correlation coefficient of 0.51.

Hence, important consideration should be given to collection and conservation of local material for breeding, in order to maintain and preserve local barley germplasm from genetic erosion. 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 efficiency of RAPD method.

References

Badr,A.,Muller K., Schafer-Pregl,R., El Rabey,H., Efegen,S., Ibrahim,H., Pozzi,C., Rohde,W.,lamini,F.,2000. On the origin and domestication history of barley *Hordeum vulgare*. Molecular Biology Evolution. **17.** 499-510

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

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

Ben Naceur, M., Rahmoune, C., Sdiri, H., Meddahi, M.L., Selmi, M.,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 .

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

BenNaceur, M., Paul, R., Ben Hamadi, S.,1997. Screening of six barley varieties for drought resistance by using leaf water potential, membrane stability and phosphatasic activity parameters. Medit., **4**. 51-54.

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

Bettaïeb-Ben Kaab, L., El Felah, M., Lauriere, M.,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., 188 - 200.

Cheikh- M'hamed, H. 2004. Evaluation de la tolérance de certaines accessions d'orge vis à vis du stress salin. Mastère de l'Institut National Agronomique de Tunisie INAT, Tunisie. 160 -170.

Chia-szu, W,. Hsiao, J.Y. 1999. Genetic differentiation of *Lilium longiflorm* Thunb. var. Scabrum Masam (Liliacece) in Taiwan using Randon Amplified polymorphic DNAs and morphological characters. Botanical Bulletion of Academia Sinica., **40**. 65-71.

Crochemore, M.L., Huyghe, C., Écalle, C., Julier, B. 1998. Structuration of alafalfa. genetic diversity using agonimic and morphological chatacteristics. Relationship with RAPD markers. Agronomie., **1.** 79- 90.

Delfine , S., **Al**vino, A., Zacchini, M., Lor**et**o, F. 1998. Consequences of s**al**t stress on diffusive conductances, Rubisco characteristics and anatomy of spinach leaves. Aust J Plant Physiol. 25. 395-402

Duarte, J.M., Dos Santos, J.B., Cunh, M.L.1999. Genetic divergence among common bean cultivars from different

races based on RAPD markers. Genet. Mol. Biol., **3.**. 419- 426.

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., 250-270.

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

El-Lklil, Y., Karrou, M., Mrabet, R., Benichou, M. 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 c Bull. Soc. Hist. Nat. Afrique du Nord., 49 -56.

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

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

Hamza, S., BenHamida, W., Rebat, A., Harrabi, M., 2004. SSR6-based Genetic Diversity Assessment among Tunisian Winter Barley and Relationship with Morphological Traits. Euphytica., **135**. 1076 -1079.

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

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

Julier, B., Porcheron, A., Écalle, C. Guy, P.1995. Genetic variability for morphlogy, growth and forage yield among perennial diploid and tetraploid Luceme populations *(Medicago sativa L.).* Agronomie., **15** , 295/304.

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

Lalaoui-Kamel, M.,Assali, N.E., 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 6" 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.,Pardee,B., 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science,volume. **257**. 967-971.

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

Marié, S., Bolarié, S.J., Martincic, I.P., Kozumplik, V.2004.Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. Plant Breeding.**4**. 366-369.

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

Mwai, G.N., Onyango J.C., and Onyango, M. 2004. Effect of salinity on growth and leaf yield of spiderplant *(Cleome gynandra L.). Afr.J.Food Agric.Nut.Develop*., **4**, 20 /25. Delfine, S., Alvino, A., Villani, M.C., Loreto, F. 1999. Restrictions to CO_2 conductance and photosynthesis in spinach leaves recovering from salt stress. Plant Physiol.**119**. 1101- 1106.

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

Nuel G, Baril, C.P., Robin, S., 2000.Varietal distinctness assisted by molecular markers: a methodological approach. Acta Hort., **546**. 520-527

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

Roldân-Ruiz, I., Van Eeuwijk, F.A., Gilliland, T.J., Dubreuil, P., Dillmann, C., Lallemand, J., De Loose, M., and Baril, C.P. 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass *(Lolium perenne L.)* varieties Theor. Appl. Genet.**103**. 1138-1150.

Sneath, P.H.A., Soka1, R.R., 1973. Numerical Taxonomy. Freeman, San Francisco. 190-201.

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

Spooner, D.M., McLean, K., Ramsay, G., Waugh, A., and Bryan, G.J. 2005. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *PNAS*.**41**. 14694 - 4699.

Sultana, N., Armstrong, J.A., Peter, G.W. 1999.Benzopyran derivatives from the aerial parts of *Eriostemon rhomboideus .Phytochemistry***.,** **52** /5, 895- 900.

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

395.
Wang, Z., Li, P., Fredricksen, M. Z., Gong, C.S., Kim, C., Zhang, H.J., Bohnert, J.K., Bressan, R.A., and Hasegawa, P.M. 2004. Expressed sequence tags from Thellungiella halophila, a new model to study plant salt-tolerance. Plant Sci. **3**. 61- 71.
Webb, D.M., Knapp,S.J., 1990. DNA extraction from a previously recalcitrant plant genus. Plant Mol. Biol. Rep., **8**. 18-185.
Yong-Cui, H., Ze-Hong Y., Yu-Ming, W., You-Liang, Zh.2005. Genetic diversity in barley from west China based on RAPD and ISSR analysis. Barley Genetics Newsletter., 35. 9 -22.

(Received for publication 26 April 2009; The date of publication 01 August 2010)