INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 5, No. 4, p. 108-116, 2014

RESEARCH PAPER OPEN ACCESS

Evaluation of genetic diversity of wild-type barley (*Hordeum vulgare* L. sub sp. *Spontaneum*) based on storage proteins polymorphism

Monireh Rahimi[1*], Islam Majidi Harvan[1], Mostafa Valizadeh[2], Farokh Darvish Kajori[1], Farshad Ebrahimpour[3]

[1]*Department of Agronomy and Plant Breeding, College of Agriculture, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Agronomy and Plant Breeding, Tabriz University,Tabriz,Iran.*

[3]*Department of Agriculture, Payame Noor University, Tehran, Iran*

Key words: Barley, domestication, genetic diversity, Fertile Crescent, Hordein polymorphism.

http://dx.doi.org/10.12692/ijb/5.4.108-116 Article published on August 18, 2014

Abstract

Today, most scientists have accepted that cultivated barley was originated from wild-type barley, *Hordeum spontaneum*. By the way, this is unclear whether domestication processes just occurred in Fertile Crescent or simultaneously in different areas all over the world. Additionally, there are disagreements in relation to center or main diversity centers of this plant. In the present study, in order to evaluate genetic and geographic diversity and also determining of barley populations phylogenic relations, 266 wild-type, *H. spantaneum*, from areas located in or out of Fertile Crescent and also 44 samples of cultivated barley from different world areas were studied. Barley samples storage proteins were analyzed by SDS-PAGE method and were assessed in three hordein groups including D, C and B. Genetic parameters, such as patterns number and genetic diversity index showed that by getting away from Fertile Crescent, barley genetic diversity was reduced, However, North Africa and East Asia could be considered as a secondary centers of diversity of the plant. The study of genetic distances showed that wild barley genotypes of Iraq and Turkey could be the nearest populations to modern cultivated barley samples. Based on these results, Fertile Crescent especially Iran, Iraq, South East of Turkey and Jordan could be main centers of diversity of wild barley H. Spontaneum.

* **Corresponding Author:** Monireh Rahimi ✉ Breeding60@yahoo.com

Introduction

Barely (*Hordeum vulgare L. subsp. Vulgare*) is one of the five important crops that commonly used as human and animal feed and also malt production. In year 2009, barley world production was over than 150 million tones that were harvested from 54.13 million hectares (FAOSTAT, 2010). Today's, although scientists are convinced that cultivated barley evolved from wild-type two-rowed barley, debates about center or domestication centers of this plant still continue. The results of archeological searches in different parts of the Fertile Crescent (from Palestine and Jordan to south of Turkey and Iran's south-west areas, Figure1) indicate that barley domestication processing and also beginning of agriculture; around 10000 years ago; occurred with simultaneously primary emmer and einkorn wheat in these areas (Zohary and Hopf, 1993). In despite, there are some evidences about domestication of this plant in east areas of Iran, Afghanistan, Tajikistan, India and Himalaya and also some areas of Europe (Zohary and Hopf, 2000). In other researches, north of Africa including Ethiopia and Morocco also were introduced as centers of domestication (Aberg, 1938; Bekele, 1983; Molina- Cano *et al*., 1987).

Due to advances in genetics science, different kinds of biochemical and molecular markers have been developed to study of plant species phylogenetic relations that can be used for fairly accurately determining the origins of plants. Application of storage proteins polymorphism in barley (Hordein) to determine the structure of diversity, genotype identification and phylogenetic studies have been well documented (Åberg, 1940; Atienza *et al*., 2000; Bekele.,1983; Bushuk and Zillma.,1978). Based on electrophoretic mobility and amino acid composition Hordeins are divided to three main groups D, C and B. These proteins are controlled by *hor3, hor1* and *hor2* genes that located in chromosome 5, respectively .(Lue *et al.,* 2009). Hordeins has been widely used to study genetic diversity (Leistrumaite and Paplauskiene, 2007).Therefore, it promoted us to utilize Hordeins aiming at the detection of genetic diversity and marker–trait associations in barley.

The main aim of this study was determine of diversity and primary center or centers of barley domestication based on studying of the storage proteins polymorphism.

Material and methods

Plant materials

In this study, 266 barley genotypes including 22 samples from south-west of Iran, 20 samples from south-east of Turkey, 20 samples of north of Iraq, 22 samples from Syria, 22 samples from Jordon, 19 samples from Palestine, 17 samples from Morocco, 20 samples from west of Libya, 19 samples from east of Afghanistan, 20 samples from north of Tajikistan, 22 samples from north of Kazakhstan, 22 samples from Himalaya and 44 cultivated barley (*Hordeum vulgare L. sub sp. Vulgare*) samples with regard to polymorphism in hordeins were assessed. These materials were obtained from Seed and Plant Improvement Institute, Karaj, Iran.

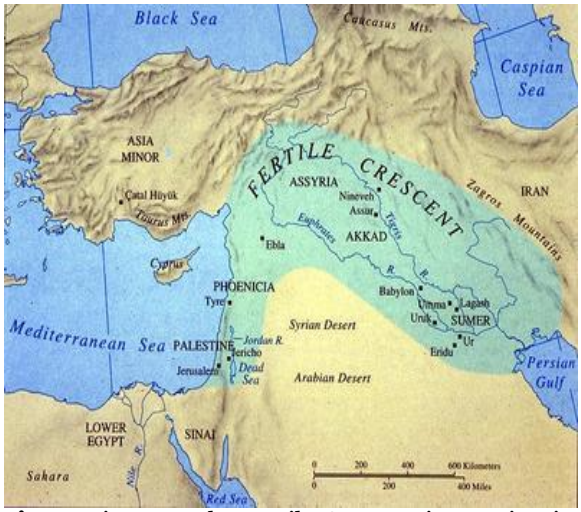

Fig. 1. Figure 1. The Fertile Crescent is a region in Western Asia. It includes the comparatively fertile regions ofMesopotamia and the Levant, delimited by the dry climate of the Syrian Desert to the south and the Anatolian highlands to the north. The region is often considered the cradle of civilization, saw the development of many of the earliest human civilizations, and is the birthplace of writing and the wheel.

Protein Extraction

Hordeins were extracted from seed as described by Osman-Abdalla (2003). Flours (2 g) from each barley

genotype were incubated with 6 ml 0.1M NaCl. The soluble albumins and globulins were first removed by centrifugation, and the insoluble hordein fraction was extracted with 5 ml 70% hot (60 _C) ethanol containing 0.5% DTT.

Hordein Electrophoresis

Hordein polymorphism was studied by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Lallemand and Briand (1990). The stacking gel layers contained 4% (w/v) acrylamide and 0.11% (w/v) N,No-methylenebisacrylamide (BIS); the separating gels contained 12% (w/v) acrylamide and 0.024% (w/v) BIS.

Staining and storage of gels

Gels were stained in methanol: acetic acid: water (25:9:65, v/v/v) containing 0.025% (w/v) Coomassie Brilliant Blue R250 for 45 minimums at 45 _C according to Lallemand and Briand (1990). After staining, gels were stored in 10% acetic acid.

Data analysis

Hordein bands were coded by 1 or 0 for their presence or absence in each genotype. Cluster analysis for Hordein based on Jaccard's coefficient, was performed using the NTSYS-pc Version 2.1 Rohlf (2000) software and clustered with unweighted pair group method with arithmetic average (UPGMA). Bands molecular weights were assessed by using ladder marker (Molecular weight marker, SM0431) applied from Fermentas Company.

Result and discussion

Genetic diversity and distribution of genetic diversity

By determining the molecular weight of storage polypeptide profiles, observed hordein bands in this experiment were divided into three groups D, C and B, according to Shewry *et al* .,1980 respectively(Figure2). These bands were ranged from 34 to 119 KD. It should be noted that in any genotype from 1 to 4 bands with a molecular weight less than 20 KD was also seen which they can be considered as part of A-hordein. This hordein without protein structures, is not considered as component of storage proteins by most of researchers (Salcedo *et al*., 1980). Therefore, their polymorphic bands were not used in the genetical analysis of populations .Bands were observed in the region of D-hordein had 98 to 119 KD molecular weight. For these proteins in any genotype only one band and totally 12 different patterns (Table1) were observed. Different results have been reported for these hordeins. Although Eshghi and Akhundova ., 2009 and Peltonen *et al*.,1994 reported no polymorphism for this region bands, Leistrumaite and Paplaskiene ., 2007 and Atanassov *et al* ., 2001 observed 3 and 4 different patterns for this region, respectively. Additionally, Haj-mansoor *et al*., 2009 reported a profile with two bands for these proteins. Among studied samples, D-hordein showed (Table2) the lowest polymorphic information content (PIC) in comparison with B and C-hordeins (0.674 versus 0.834 and 0.841 respectively). As *hor3* is single copy gene that encodes this hordein and in turn being multiple copy for *hor1* and *hor2* genes that encode hordeins B and C, these results seems to be logical. Eshghi *et al*., 2012 observed 31 polymorphic bands in hordein analysis. The number of bands in each genotype ranged from 7 to19 with an average of 5.12. In any area for D, C and B-hordeins, 5.14 and 12 bands were detected and bands ranged from 1.7 to 76.7 percent.The molecular weight of observed bands in C-hordein was 50 and 90 KD. Totally 21 different bands was observed for C-hordein proteins. In these proteins band range was between 2 to 9. Additionally, in C-hordein region 92 bands with different patterns were observed**.** Haj-Mansoor *et al*., 2009 observed weights from 47 to75 KD in this region. Polypeptide bands in genotypes ranged from 2 to 8 and 21 polymorphic bands were observed. In this study, B-hordein molecular weights ranged between 34 to 46 KD. Totally 20 different bands were observed in this region**.** We observed 4 to 10 bands for studied samples. Totally, 91different patterns were observed for these proteins (Table 1), While Haj- Mansoor *et al*., 2009 observed polypeptide bands ranged from 4 to 9 and 22 polymorphic bands. Our results were consistent with the findings of Haj-Mansoor *et al*.,

2009 and Shewry and Miflin ., 1982. Based on the number of individual counted protein bands, it was found that the most prevalent was B-Hordein. B-Hordein also had the highest polymorphism among other hordeins and C and D- hordeins allocated to the next level, respectively. The results of this study showed a high polymorphism among all studied samples (Table2). Analysis of seed varieties showed different patterns of bands in hordein electrophoresis and was Similar to the results obtained by Haj-Mansoor *et al.*, 2009 and Heisel *et al.*,1986.

Table 1. D,C and B-Hordein patterns observed in wild and cultivated barley populations.

Pattern	Hordein Region	Wild barley in Fertile Crescent
1,3,4,5,7,8,9,10,11	D-Hordein	
11,15,30,34,35,45,61,67,72,79,90,92	C-Hordein	Iran
1,21,36,38,39,44,45,49,56,64,65,72	B-Hordein	
1,2,3,4,5,6,7,8,9,10,11,12	D-Hordein	
16,17,19,22,29,38,40,43,52,66,69	C-Hordein	Turkey
4,9,10,16,19,26,31,37,40,41,47,69	B-Hordein	
1,2,3,4,5,6,7,8,9,10,11,12	D-Hordein	
4,6,11,14,19,49,63,68,70,73,78,80	C-Hordein	Iraq
2,3,11,12,14,17,19,35,56,58,64,68,70	B-Hordein	
1,3,4,5,6,7,8,9,11	D-Hordein	
5,8,9,12,26,41,46,50,55,65,76,91	C-Hordein	Syria
10,14,20,27,28,30,41,57,70,74,75,76,77,78,79,80	B-Hordein	
1,2,3,4,5,7,8,9,10,11	D-Hordein	
1,17,18,26,44,53,56,59,74,81	C-Hordein	Jordan
6,18,27,29,48,52,55,57,63,74,75,76	B-Hordein	
1,2,3,4,5,7,9,10	D-Hordein	
2,9,18,25,27,28,54,74,75,77	C-Hordein	Lebanon
6,8,15,27,46,48,51,52,63,73	B-Hordein	
1,2,3,4,5,6,7	D-Hordein	
20,21,39,42,51,57,60,62,71,84,88	C-Hordein	Palestine
23,25,32,33,42,53,54,59,60,66,67	B-Hordein	
Pattern	Hordein Region	North of Africa
3,5	D-Hordein	
10,13,42,60,83,85,89	C-Hordein	Morocco
22,24,32,34,43,50,60	B-Hordein	
7,11	D-Hordein	
10,13,20,51,57,81,83,85,87,89,91	C-Hordein	Lybia
7,22,23,43,53,61,66	B-Hordein	
Pattern	Hordein Region	Central Asia
2,11	D-Hordein	
3,24,32,48,51,64	C-Hordein	Afghanistan
5,9,13,14,38,49,81,84,85,86,91	B-Hordein	
1 ‹3 ‹9	D-Hordein	
6,23,37,58,60,82,86,87	C-Hordein	Tajikistan
5,9,38,54,82,87,88,89,91	B-Hordein	
1 ‹3 ‹4 ‹7 ‹8 ‹9 ‹11	D-Hordein	
6 ‹23 ‹31 ‹47 ‹51 ‹55 ‹82 ‹86	C-Hordein	Kazakhstan
6 ‹36 ‹41 ‹54 ‹75 ‹81 ‹82 ‹83 ‹87 ‹90	B-Hordein	
3 ‹7 ‹8	D-Hordein	
17 ‹33 ‹36 ‹51 ‹60	C-Hordein	Himalaya
13 ‹14 ‹30 ‹71	B-Hordein	
Pattern	Hordein Region	Cultivated barley
2 ‹3 ‹5 ‹7 ‹8 ‹9 ‹12	D-Hordein	
4 ‹6 ‹9 ‹11 ‹14 ‹16 ‹17 ‹19 ‹22 ‹25 ‹29 ‹30 ‹31 ‹32 ‹34 ‹37 ‹ 38 ‹40 ‹41 ‹44 ‹47 ‹48 ‹54 ‹58 ‹63 ‹67 ‹69 ‹70 ‹73 ‹80	C-Hordein	Cultivated barley
4 ‹7 ‹9 ‹10 ‹11 ‹12 ‹14 ‹17 ‹19 ‹21 ‹35 ‹37 ‹47 ‹48 ‹56 ‹58 ‹ 59 ‹62 ‹68 ‹69 ‹70 ‹81 ‹82 ‹83	B-Hordein	

D-Hordein:98-119k.Da, C-Hordein:50-79k.Da, B-Hordein:34-46KD.

Table 2. Genetic diversity index values and the number of pattern(in wild and cultivated barley populations).

Wild populations in Fertile Crescent or neighboring areas								
Mean		B-Hordein		C-Hordein		D-Hordein		
0.897	(11)	0.909	(12)	0.905	(12)	0.876	(9)	Iran
0.893	(11.67)	0.9	(12)	0.88	(11)	0.9	(12)	Turkey
0.9	(12.33)	0.91	(13)	0.88	(12)	0.91	(12)	Iraq
0.883	(12.33)	0.926	(16)	0.897	(12)	0.826	(9)	Syria
0.894	(10.67)	0.909	(12)	0.888	(10)	0.884	(10)	Jordan
0.867	(9.33)	0.889	(10)	0.875	(10)	0.839	(8)	Lebanon
0.866	(9.67)	0.892	(11)	0.887	(11)	0.82	(7)	Palestine
0.886	(11)	0.905	(12.28)	0.887	(11.14)	0.865	(9.57)	Mean
Wild populations in north of Africa								
Mean		B-Hordein		C-Hordein		D-Hordein		
0.611	(5.33)	0.671	(7)	0.747	(7)	0.415	(2)	Morocco
0.596	(6.67)	0.755	(7)	0.855	(11)	0.18	(2)	Libya
0.603	(6)	0.713	(7)	0.801	(9)	0.297	(2)	Mean
Wild populations in central Asia								
Mean		B-Hordein		C-Hordein		D-Hordein		
0.72	(6.33)	0.847	(11)	0.814	(6)	0.499	(2)	Afghanistan
0.693	(6.67)	0.79	(9)	0.785	(8)	0.505	(3)	Tajikistan
0.648	(8.33)	0.715	(10)	0.665	(8)	0.566	(7)	Kazakhstan
0.608	(4)	0.739	(4)	0.655	(5)	0.421	(3)	Himalaya
0.646	(6.22)	0.753	(8)	0.755	(7.5)	0.431	(3.16)	Mean
Cultivated barley's Population								
Mean		B-Hordein		C-Hordein		D-Hordein		
0.886	(20.33)	0.924	(24)	0.938	(30)	0.798	(7)	Cultivated barley
0.783	(9.61)	0.841	(11.29)	0.834	(10.92)	0.674	(6.64)	Total Mean

High Polymorphisms in the barley hordein polypeptides were first reported in 1977 (Shewry *et al*., 1978b; Marchylo and Laberge, 1987; McCausland and Wrigley, 1977). Haj- Mansoor *et al*., 2009 observed high polymorphism in the storage proteins in comparison with the quantitative traits. Eshghi *et al* ., 2012 by studying genetic parameters such as number of patterns and levels of genetic diversity index in the studied populations showed the maximum assessed parameters for samples of central Syria, west Iran and South West Turkey (which are located in the Fertile Crescent) and reducing in the studied parameters by getting far away from the fertile Crescent (the Kazakhstan samples that had the farthest location to the fertile Crescent showed the lowest number of patterns and diversity index in this study). So it can be concluded that fertile Crescent is the most likely location domestication region of barley plants. There was similar results reported by Badr *et al*.,2000; Blattner *et al*., 2001 and Morrell and Clegg , 2007; that have been studied barley populations from different parts of the world through DNA markers.

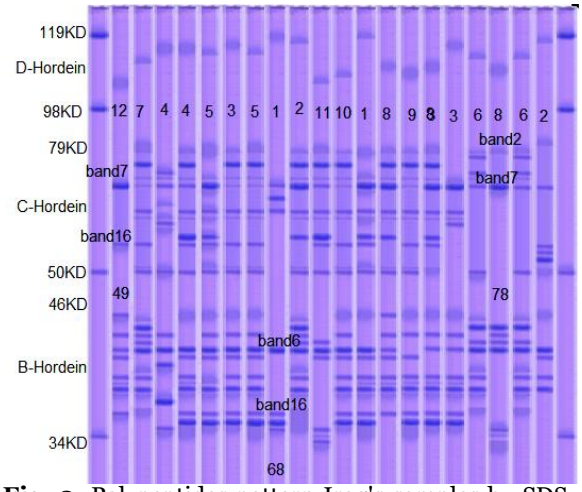

Fig. 2. Polypeptides-pattern Iraq's samples by SDS-PAGE and the name of some patterns and bands.

This research results showed existence of specific patterns in all analyzed populations that just were

observed in the particular area and not repeated in the other area genotypes (Table 1). For example in the all samples of Turkey's east genotypes, all 12 D-Hordein patterns were observed pattern No.12 just was observed in cultivated and wild-type samples of Iraq's north and Turkey's south-east samples (Table1) can be substantial for understanding of barley domestication. This result is similar the same study results reported by Atanassov *et al.*, 2001 on 35 samples of barley applied from ICARDA (International Center for Agricultural Research in Dry), 9 samples from GIS (Genetic Institute of Sofia) and 5 samples applied from TAAM (Timiriazev Agricultural Academy of Moscow). In this research hordein polypeptide frequency was studied and found that the frequency of some polypeptides specific for a specific region and separated based on it (Atanassov *et al.*,2001).

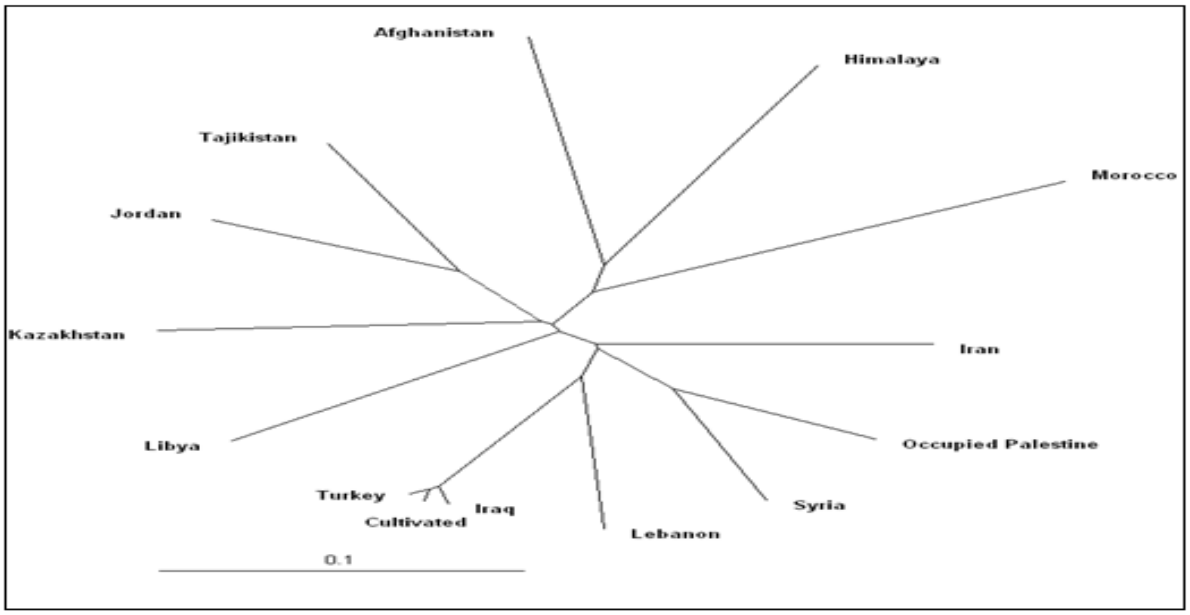

Fig. 3. Dendrogram showing the genetic distance among studied barley populations based on hordein polymorphism.

As shown in the Figure (3) displaying genetic distances in the analyzed cultivated and wild-type samples, a lot of each area samples located near each other in the same group. Also there were patterns that particularly existed in the geographically neighbor areas. Based on these results it may be concluded that storage proteins polymorphism depends on geographical diversity. This dependency may be due to a kind of compatibility between special geographical areas and hordeins encoding genes or may be due to the other genes linked with.

Based on storage proteins polymorphism, average of genetic diversity index for Fertile Crescent populations was equal H= 0.886 and average for populations located out of Fertile Crescent was equal H= 0.646(Table2). These substantial differences are clear especially in the D-hordein indices. Additionally, the average of pattern frequency for population located in Fertile Crescent was 11 and for other populations was 6.22(Table2). The minimum pattern frequency and also the average of diversity index belonged to samples from west of Libya, Himalaya and south of Morocco. In the other word, by faring from Fertile Crescent, genetic diversity reduces. Also in Fertile Crescent, Iran south-west, Iraq north, Turkey's south-east and Jordan's populations had higher diversity in comparison with Palestine and Lebanon populations. It may be conclude that based on hordein polymorphism analysis, Fertile Crescent area specially Iran south- west, north of Iraq, Turkey's south- east and Jordan areas can be considered as main diversity centers of *H. spantaneum* wild-type barley. As mentioned above for *hor1* and *hor2* genes,

some of distinct patterns were observed in the samples from out of Fertile Crescent.

Conclusion

As mentioned there were 12 different patterns for D-Hordein that especially were observed in Iraq's north and Turkey's south-east populations. Also pattern number 12 for D-hordein with 27% frequency for cultivated samples just was observed in Iraq and Turkey samples. These results also indicate genetic enrichment of these populations and also can propose that two populations set genes geographically close to each other and may have the maximum role in domestication of cultivated barley samples. Figure (3) indicate also a maximum similarity between cultivated samples and wild-type samples from Iraq and Turkey for genetic distance based on hordein polymorphism. Finally, based on our result it can concluded that the most possible domestication center for barley plant could be Iraq's north and Turkey's south- east areas.

References

Åberg E. 1938. *Hordeum Agriocrithon* Nova Sp: A Wild Six rowed Barley, Almqvist&WilksellsBoktryckeri-AB.

Åberg E. 1940. "The taxonomy and phylogeny of *Hordeum* L. Sect.Cerealia Ands. With special reference to Thibetan barleys."The taxonomy and phylogeny of *Hordeum* L. Sect.Cerealia Ands. With special reference to Thibetan barleys.

Atanassov P, Borries C, Zaharieva M, Monneveux P. 2001. Hordein polymorphism and variation of agromorphological traits in a collection of naked barley. Genetic Resources and Crop Evolution, **48(4),** 353-360. http://dx.doi.org/10.1023/A:1012085714353.

Atienza SG, Gimenez MJ, Martin A, Martin L M. 2000. Variability in monomeric prolamins in Hordeum chilense. Theoretical and Applied Genetics, **101(5-6),** 970-976. http://dx.doi.org/10.1007/s001220051569.

Badr A, Sch R, El Rabey H, Effgen S, Ibrahim HH, Pozzi C, Rohde W, Salamini F. 2000. On the origin and domestication history of barley (Hordeum vulgare). Molecular Biology and Evolution, **17(4),** 499-510.

Bekele E. 1983. "A differential rate of regional distribution of barley flavonoid patterns in Ethiopia, and a view on the center of origin of barley. Hereditas **98(2),** 269-280. http://dx.doi.org/10.1111/j.16015223.1983.tb00605.x

Blattner FR, Méndez AGB. 2001. "RAPD data do not support a second centre of barley domestication in Morocco. Genetic Resources and Crop Evolution . **48(1),** 13-19. http://dx.doi.org/10.1023/A:1011299021969

Bushuk W, Zillman R. 1978. "Wheat cultivar identification by gliadin electrophoregrams. I. Apparatus, method and nomenclature. Canadian Journal of Plant Science **58(2),** 505-515. http://dx.doi.org/10.4141/cjps78-076.

Eshghi R, Akhundova E. 2009. "Genetic diversity of the monomeric prolamins and hordein in hulless barley genotypes and their relation with agronomical traits. African Journal of Biotechnology **8(9).** http://dx.doi.org/10.5897/AJAR09.363.

Eshghi R, Ebrahimpour F, Ojaghi J, Salayeva S, Baraty M, Rahimi M. 2012. Evaluation of genetic variability in naked barley (Hordeum vulgare L.).International Journal of Agriculture and Crop Sciences **4,** 1166-1179.

FAO. (Food and Agriculture Organization of the United Nations). 2010. FAOSTAT. http://faostat.fao.org/

Hajmansoor S, Bihamta MR, Nabipour A, Mohammadi A, Pirseyedi SM, Nikkhah HR. 2009. Genetic Diversity in Barley Genotypes: I. Seed Storage Proteins (Hordeins) and Agronomic Traits.

Seed and Plant Improvment Journal **25(4),** 585-604.

Heisel SE, Peterson DM, Jones BL. 1986. "Identification of United States barley cultivars by sodium dodecyl sulfate polyacrylamide gel electrophoresis of hordeins." Cereal. Chemistry **. 63(6),** 500-505.

Lallemand J, Briand F. 1990. "Identification variétale des orges par électrophorèse. Description de 280 variétés. Agronomie. **6,** 447-450. http://dx.doi.org/10.1051/agro:19900602.

Leistrumaitė A, Paplauskienė V. 2007. "Genetic resources of spring barley: analysis of Hordein polymorphism." Biologija. **53(3)**.

Luo MC, Deal KR, Akhunov ED, Akhunova A R, Anderson OD, Anderson JA, Dvorak J. 2009. "Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae. Proceedings of the National Academy of Sciences. **106(37),** 15780-15785. http://dx.doi.org/10.1073/pnas.0908195106.

Marchylo B, Laberge D. 1981. "Barley Cultivar Identification by Electrophoretic Analysis of Hordein Proteins II. Catalogue of Electrophoregram Formulae for Canadian-Grown Barley Cultivars. Canadian Journal of Plant Science. **61(4),** 859-870. http://dx.doi.org/10.4141/cjps81-129.

McCausland J, Wrigley C. 1977. "Identification of Australian barley cultivars by laboratory methods: gel electrophoresis and gel isoelectric focusing of the endosperm proteins." Animal Production Science. **17(89),** 1020-1027. http://dx.doi.org/10.1071/EA9771020.

Molina-Cano JL, Fra-Mon P, Salcedo G, Aragoncillo C, De Togores FR, García-Olmedo F. 1987. "Morocco as a possible domestication center for barley: biochemical and agromorphological evidence. Theoretical and Applied Genetics. **73(4),** 531-536. http://dx.doi.org/10.1007/BF00289190.

Morrell PL, Clegg MT. 2007. "Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent. Proceedings of the national academy of sciences **104(9),** 3289-3294. http://dx.doi.org/10.1073/pnas.0611377104.

Osman AM. 2003. "Barley and malt proteins and proteinases: I. Highly degradable barley protein fraction (HDBPF), a suitable substrate for malt endoprotease assay. Journal of the Institute of Brewing. **109(2),** 135-141. http://dx.doi.org/10.1002/j.20500416.2003.tb00143.x.

Peltonen J, Rita H, Aikasalo R, Home S. 1994. "Hordein and malting quality in northern barleys." Hereditas. **120(3),** 231-239. http://dx.doi.org/10.1111/j.1601-5223.1994.00231.x

Rohlf F, NTSYS-pc NT. 2000. "Multivariate Analysis System, Version 2.10 e, Appl. Bio Stat." Inc., New York.

Salcedo G, Sanchez-Monge R, Argamenteria A, Aragonsillo C. 1980. "The A-hordeins as a group of salt soluble hydrophobic proteins. Plant Science Letters **19(2),** 109-119. http://dx.doi.org/10.1016/0304-4211(80)90086-3.

Shewry PR, Faulks AJ, Parmar S, Miflin BJ. 1980. "Hordein polypeptide pattern in relation to malting quality and the varietal identification of malted barley grain. Journal of the Institute of Brewing. **86(3),** 138-141. http://dx.doi.org/10.1002/j.20500416.1980.tb03974.x.

Shewry PR, Hill JM, Pratt HM, Leggatt MM, Miflin BJ. 1978. An evaluation of techniques for the extraction of hordein and glutelin from barley seed and a comparison of the protein composition of Bomi

and Risø 1508. Journal of Experimental Botany, **29(3),** 677-692. http://dx.doi.org/10.1093/jxb/29.3.677.

Shewry PR, Miflin BJ. 1982. "Genes for the storage proteins of barley. Plant Foods For Human Nutrition. **31(3),** 251-267. http://dx.doi.org/10.1007/BF01108634.

Zohary D, Hopf M. 1993. Domestication of plants in the world. The origin and spread of cultivated plants in west Asia, Europe and the Nile valley, Clarendon Press, Oxford.

Zohary D, Hopf M. 2000 . Domestication of Plants in the Old World: The origin and spread of domesticated plants in Southwest Asia, Europe, and the Mediterranean Basin, Oxford University Press.