Pak. J. Bot., 46(4): 1397-1402, 2014.

GENETIC DIVERSITY AND STRUCTURE ANALYSIS BASED ON HORDEIN PROTEIN POLYMORPHISM IN BARLEY LANDRACE POPULATIONS FROM JORDAN

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Abstract

Jordan is unanimously considered to be one of the centers of genetic diversity for barley, where wild and landraces of barley has been grown under different climatic conditions. The genetic diversity and genetic structure based on hordein polymorphism was assessed in 90 different accessions collected from four different sites of Jordan. A-PAGE was used to reveal hordein polymorphism among the genotypes. A total of 29 distinct bands were identified, out of them 9 bands were distinguished for D, 11 for C, and 9 for the B hordein regions. The observed genetic similarity was an exceptionally high between the populations than expected, which is probably due to high gene flow estimated between them. The genetic diversity parameters were not differ largely among the populations, indicating that local selection of a particular site did not play a key role in shaping genetic diversity. Analysis of molecular variance (AMOVA) revealed significant population structure when accessions were structured according to population site. There was 94% of hordein variation resided within the populations and only 8% present among the populations. Both Bayesian and Principale Coordinate Analysis (PCoA) concordantly demonstrated admixture genotypes of the landraces barley populations. Consequently, none of the population found to be clustered separately according to its population site. It is concluded that this approach can be useful to explore the germplasm for genetic diversity but perhaps is not suitable for determining phylogenic relations in barley.

Introduction

Barley (*Hordeum vulgare* L.) has been cultivated since earlier times of human civilization, which makes it one of the earliest domesticated plants (Salamini *et al*., 2002). The barley crop is best known for its ability of adaptation to wide range of environments and thus can be cultivated from polar to dry climates (Persson, 1997). Jordan is believed to be one of the countries of barley domestication where wild and landraces is widely distributed. Barley landraces (*H. vulgare* L.) have been evolved across thousands of years in numerous environments and local farming systems which caused abundant variations and would represent a large unutilized reservoir of useful genes for adaptation to arid and semi-arid regions (Jaradat *et al*., 1987). In general, barley landraces are described as being very heterogeneous (Leino & Hagenblad, 2010). Landraces consist the valuable genetic resource of cultivated barley in countries like Syria (Ceccarelli *et al*., 1987), Ethiopia (Muhe & Assefa, 2011), Iran (Brown & Munday, 1982), Oman (Al- Dakheel *et al*., 2012), Turkey (Brush, 1995) and Jordan (Jaradat, 1989).

Hordein, the major storage proteins of the barley seeds, which account for 35-50% of the total protein in the grain (Jaradat, 1991) and display high genetic variation between the genotypes and have also been used as bio-chemical markers for cultivar identification, genetic diversity analysis and in determining phylogenic origin (Doll & Brown, 1979; Shewry *et al*., 1983). Previous work has shown that hordeins are highly tolerant to mutations and selected neutrally (Nevo *et al*., 1983). They represent a multiple gene family, and their variability is higher when compared with allozyme loci (Echart-Almeida & Cavalli-Molina, 2000). Grain storage protein can be explained by three groups on the basis of their chemical composition and electrophoresis mobility called as B, C and D. Genetic studies (Doll & Brown 1979; Kreis *et al*., 1984) reported that B and C are encoded by single structural genes *Hor-2 and Hor-1*, respectively, and is located on the short arm of chromosome 5. D Hordein, which contains the highest molecular weight than B and C hordeins, is synthesized by the *Hor-3* locus located on the long arm of Chromosome 5.

Jordan, the center of diversity experiences the loss of biodiversity due to the replacement of landraces with improved cultivars, land fragmentation and frequent drought periods since couple of decades. The indigenous crop genetic reservoirs of Jordan are at the risk of extinction and could be lost before they are sufficiently collected and thoroughly evaluated (Aida *et al*., 2007). Efforts are needed to investigate the genetic diversity of barley from this primary center of diversity. Therefore, the present research work was undertaken to explore the genetic diversity of hordein protein within and among populations of barley landraces native to Jordan.

Materials and Methods

Plant materials and protein extraction: A total of four populations consisted of 90 different accessions were used in this experiment collected from different sites of Jordan (Table 1), all four populations were collected and provided by Prof. Hartwig H. Geiger, Institute of Plant Breeding, Seed Science, and Populations Genetics, University of Hohenheim, Stuttgart, Germany. For hordein protein analysis, seeds were individually grounded and seed storage protein was extracted with 0.65 ml solution containing 25% of 2-cholro ethanol and 0.05% of methyl green (Tang *et al*., 2002). The mixture was initially centrifuged at 400 rpm for 2 hours at 25^{o}C and subsequently mixture was allowed to extract protein for overnight at 25^{o}C. Before electrophoresis, the final centrifugation was done at 13000 rpm for 10 minutes. For electrophoresis and identifications of hordein bands, the method was used as described by Zhang *et al*., (1997).

Table 1. Geographical data of barley landraces populations collected from Jordan.

Population	Sample size	Latitude (decimals)	Longitude (decimals)	Altitude (meters)	Annual rain (mm)
Mafraq Manisha	20	32.25	36.20	695	164
Mafraq Balwaneh	27	32.25	36.20	695	164
Madaba Team	25	31.68	35.83	785	358
Showbak Gair	18	30.30	35.30	1460	315

Geographical data is based on Ghani *et al.,* (2004)

Table 2. Allele frequencies patterns of D, C and B Hordein subunits in landraces barley populations.

Allele	Mafraq Manisha	Mafraq Balwaneh	Madaba team	Showbak Gair
D-1	0.051	0.000	0.000	0.000
D-2	0.258	0.139	0.175	0.183
D-3	0.258	0.183	0.083	0.150
D-4	0.225	0.279	0.047	0.422
D-5	0.776	0.666	0.717	0.666
D-6	0.105	0.306	0.200	0.183
D-7	0.105	0.279	0.225	0.292
D-8	0.367	0.569	0.600	0.764
D-9	0.367	0.279	0.400	0.333
C-1	0.000	0.018	0.000	0.150
C-2	0.051	0.000	0.000	0.057
C-3	0.683	0.528	1.000	0.666
C-4	0.367	0.361	0.105	0.218
C-5	0.134	0.206	0.336	0.254
C-6	0.048	0.097	0.278	0.057
C-7	0.612	0.490	0.307	0.473
C-8	0.000	0.000	0.040	0.057
C-9	0.329	0.000	0.000	0.218
C-10	0.163	0.666	0.307	0.292
C-11	0.000	0.037	0.040	0.000
B-1	0.025	0.037	0.040	0.000
B-2	0.258	0.254	0.251	0.254
B-3	0.000	0.000	0.020	0.000
B-4	0.078	0.037	0.040	0.000
B-5	0.051	0.097	0.083	0.183
B-6	0.193	0.206	0.128	0.150
B-7	0.193	0.077	0.040	0.000
B-8	0.025	0.118	0.200	0.118
B-9	0.025	0.057	0.040	0.057

Data analysis: Bands of hordein protein were counted as present (1) or absent (0) and the data generated was used for statistical analysis. The software POPGENE version 1.32 (Yeh *et al*., 2000) was used to calculate genetic diversity of each population, the amount of each population was estimated by computing the mean number of allele per locus (N_a), the effective number of alleles (N_e), the percentage of polymorphic loci (P%) and mean gene diversity (H_e) (Nei, 1973). Apart of these, the gene flow (N_m) was also observed among the populations. GenA1Ex 6.5 software (Peakall & Smouse, 2006) was also used to conduct principale coordinate analysis (PCoA) and analysis of molecular variance (AMOVA). AMOVA was used to partition the total genetic variation among individuals within the populations and between populations within a habitat. The significance of this F-statistic analogue was tested with 999 random per mutation. Furthermore we used the model-based (Bayesian clustering) method implemented in STRUCTURE (Pritchard *et al*., 2000) to detect the population structure and to assign all the accessions to their pre-defined populations, using the admixture model by assuming four populations with a burn-in of 5000 iterations and a run-length of 5000 replications.

Results

Distribution of alleles: In the present study, A-PAGE gel was used to separate hordein polypeptides in the barley grains and found a total of 9, 11 and 9 different banding patterns between the D, C and B hordein subunits, respectively. All hordein subunits migrated as separate groups each with distinct molecular weight regions (Fig. 1). The allele frequencies of each electrophoretic pattern in the four barley populations are given in Table 2. Patterns D-2, D-5, D-6, D-7, D-8 and D-9 at *Hor-3*, patterns C-3, C-4, C-5, C-7 and C-10 at *Hor-1* and patterns B-2 and B-6 at *Hor-2* were common frequencies which exceeded than 0.100, while the majority of the banding frequencies patterns were below than 0.100.

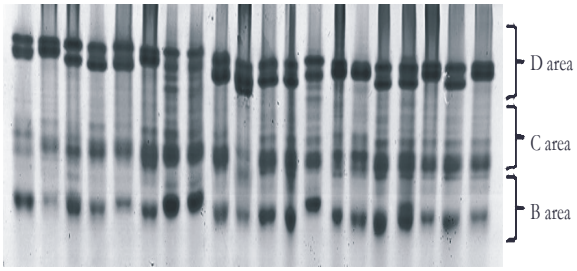

Fig. 1. Hordein polypeptides patterns in different accessions of barley landraces.

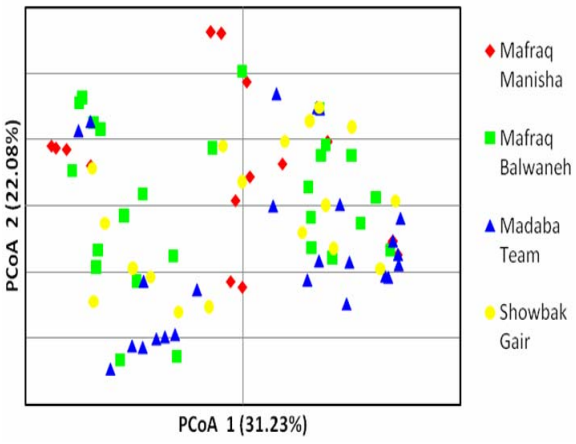

Fig. 2. Principale coordinates analysis showing diversity among the accessions but could not separate the genotypes according to their respective population sites.

Extent and structure of hordein polymorphism: The coefficient of hordein genetic similarity (Nei's genetic similarity index, 1973) of all loci between landrace barley populations was calculated. An exceptionally high genetic identity (on average, 0.976) was estimated between landrace barley populations (Table 3). The genetic diversity parameters varied from 1.793, 1.358, 79.31, 0.224 to 1.862, 1.405, 86.21, and 0.250 for N_a, N_e, P%, H_e, respectively, whereas very high gene flow (8.982) was also estimated among the landraces populations (Table 4). Utilizing PCoA based on standardized covariance input, the first two dimensions explained 53.31% of total genetic variation (31.23 and 22.08, respectively; Fig. 2). AMOVA (Table 5) revealed low but significant population differentiation, suggesting that accessions used in the current study are genetically diverse from each other. Based on AMOVA results, the larger genetic variance was recorded within the populations, which is accounted for 94%, while among populations variance accounted for 6%. On the basis of hordein data, we further investigated the genetic structure of genotypes from each population by structure software. The optimal number of populations was inferred to be four for the sample of 90 accessions. Structure analysis showed that accessions were clustered inconsistently to their respective populations (Fig. 3a and b). The irrespective allocation of individuals indicated that the genotypes of the populations are highly admixture.

Discussion

Efficient and effective utilization of germplasm for crop improvement program depends on the extent of genetic diversity either existing or created (Naz *et al*., 2006; Akbar *et al*., 2011). Genetic diversity in germplasm collection is the base of exploiting desirable genes for genetic improvement of various economically important traits and thus we need first hand information about genetic variation for such traits (Fayyaz *et al*., 2014). One of the major reasons of eradication of plant genetic resources has been the adaptation of narrowly based cultivars for intensive cultivation practices. Landraces offer great amount of variability for broadening the crop gene pool. Landraces are available genetic resources which can be utilized as basic material for breeding programs as well as direct use of variety after selection. Landraces can also be exploited for their useful genes with visible or measurable effects on important traits, like protein content, grain size, lodging resistance etc. (Hadjichristodoulou, 1995). Extensive hordein polymorphisms in barley have already been reported by several researchers, including (Doll & Brown, 1979; Nevo *et al*., 1983; Jaradat, 1991; Yin *et al*., 2003). However, hordein polymorphism in barley landraces from Middle East has not been reported yet. In the present study, all four populations did not much differ in amount of hordein variations as estimated by mean number of alleles per locus, effective number of alleles, percentage of polymorphic loci and genetic diversity (N_a, N_e, P%, H_e). We interpret the observed pattern of hordein polymorphism as due to the combination of gene flow and genetic drift despite local selection. Large numbers of hordein bands were found in landrace barley populations, however, there were many common alleles present among four populations with similar molecular weight. On the other hand, only few unique alleles were also found but these alleles could not distinguish the genotypes of the populations from each other. The value of coefficient of genetic similarity among landrace populations was very high; this indicates that the barley populations are genetically uniform. The high genetic affinity is consistent with high number of constantly encountered alleles in high frequencies, many minor widespread or common alleles in relatively low frequencies and a small amount of unique alleles (Nevo *et al*., 1986). Lower genetic differentiation may be consequences of relatively higher gene flow observed in these sampled populations, with concomitant founder events and genetic bottlenecking. However, it does not imply that the differences between accessions are not significant for germplasm enhancement purposes. This may be due to limited inherent in the use of hordein protein electrophoresis in investing genetic diversity at molecular level. In general, self pollinating diploid plant such as *Triticum* species show low genetic diversity (Nevo & Cleve 1978), but *Hordeum* species which is also a self pollinated diploid grass however demonstrates more gene differentiation than expected (Brown *et al*., 1978). In the current study, fairly high gene flow could be the reason for high genetic similarity. In the Fertile Crescent region, the gene flow is expected because populations of

cultivated barely have long been growing in close proximity (Nevo, 1998). Wright (1943) reported that gene flow even at low rate is enough for reducing the genetic differentiation between the populations. The proportion of gene diversity within the populations was substantially greater than among the populations. These results are in agreement with those of Malysheva-Otto *et al*., (2007), Backes *et al*., (2009) and Shakhatreh *et al*., (2010). These results suggest that further collections of landraces in Jordan should give more emphasis on collection and evaluation of more samples within the population than between the populations. Principale coordinate analysis revealed uneven distribution of genotypes and none of the population clustered into single group according to their geographical origin where as genotypes of different populations overlapped to each other. We further carried out structure analysis, which also demonstrated highly admixture of genotypes among the populations. The similar results were also obtained by Jaradat (1991) while studying wild barley populations collected from 12 sites of Jordan for hordein variations. According to Murphy *et al*., (1996), those enzymatic and non-enzymatic proteins do not always produce sufficient variability for population structure, breeding biology and other inter-population applications. Thus, it indicates that the efficiency of biochemical markers in respect to distinct the phylogenic relations in the populations according to their geographically origin, is still in question. Barley landraces belong to the primary gene pool of cultivated barley. Thus, possess an important amount of genetic variation, which can play a key role for the improvement of modern cultivars with domestication and widening the narrow genetic background. However, hordein polymorphism showed by different accessions in the present study would be considered for breeding programs to improve the barley cultivars.

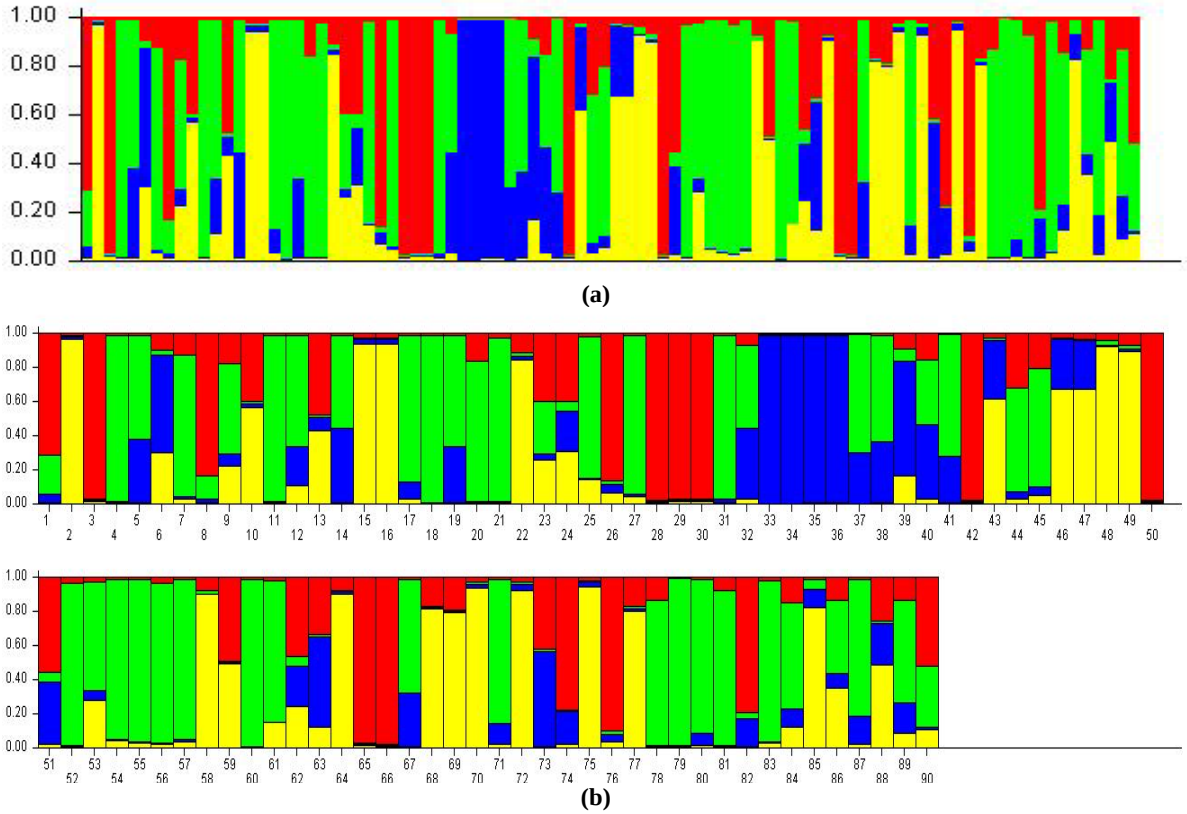

Fig. 3. Bayesian model-based inference (structure 2.3.4) of population structure demonstrating levels and patterns of admixture/shared among accessions of four barley landrace populations. (a) Showing highly mixed clusters (b) showing accessions with their corresponding codes i.e., from 1-20 (Mafraq Manisha), 21-47 (Mafraq Balwaneh), 48-72 (Madaba Team) and 73-90 (Showbak Gair).

Table 3. Coefficients of Nei's genetic similarity (above diagonal) and genetic distance (below diagonal) based on three hordein loci between landraces barley populations.

Population ID	Mafraq Manisha	Mafraq Balwaneh	Madaba Team	Showbak Gair
Mafraq Manisha	**	0.973	0.967	0.979
Mafraq Balwaneh	0.027	**	0.973	0.983
Madaba Team	0.032	0.027	**	0.984
Showbak Gair	0.020	0.016	0.015	**

Table 4. Within population genetic diversity estimates based on hordein protein for landraces barley populations.

Population ID	Mean number of alleles per locus (N_a)	Effective number of alleles (N_e)	Percentage of polymorphic loci *(P %)*	Genetic diversity (H_e)	Gene flow ($N_{m)}$
Mafraq Manisha	1.862	1.374	86.21	0.235	
Mafraq Balwaneh	1.827	1.405	82.76	0.245	8.982
Madaba Team	1.827	1.358	82.76	0.224	
Showbak Gair	1.793	1.401	79.31	0.250	

Gene flow (N_m) is calculated as 0.5 (1-G_{st})/G_{st}

Table 5. Analysis of molecular variance (AMOVA) based on 29 hordein subunits of barley landraces.

Source of variation	Degree of freedom	Sum of squares	Mean of squares	Variance components	Percentage of variation	Probability level
Among populations	3	29.494	9.831	0.252	6	<0.001
Within populations	86	363.028	4.222	4.221	94	<0.001
Total	89	392.522		4.473	100	

References

Aida, A.N., M. Hussein, A.S. Mohamad, S. Hani and M. Sameer. 2007. Assessment of phenotypic diversity among Jordanian barley landraces (*Hordeum vulgare* L.). *Biotechnology*, 6: 232-238.

Akbar, F., M.A. Rabbani, M.S. Masood and Z.K. Shinwari. 2011. Genetic diversity of sesame (*Sesamum indicum* L.) germplasm from pakistan using RAPD markers. *Pak. J.Bot*., 43(4): 2153-2160.

Al-Dakheel, A.J., M.B. Fraj, G.M. Shabbir and A.Q.M. Al-Gailani. 2012. Evaluation of Batini barley landraces from Oman and breeding lines under various irrigation salinity levels *Agri. Sci. Res. Jour*., 2(1): 42-50.

Backes, G., J. Orabi, A. Wolday, A. Yahyaoui and A. Jahoor. 2009. High genetic diversity revealed in barley (*Hordeum vulgare*) collected from small-scale farmer's fields in Eritrea. *Genet. Resour. Crop Evol*., 56: 85-97.

Brown, A.H.D. and J. Munday. 1982. Population genetic structure and optimal sampling of landraces of barley from Iran. *Genetica*, 58: 85-96.

Brush, S.B. 1995. In situ conservation of landraces in center of crop diversity. *Crop Sci*., 35: 346-354.

Brwon, A.H.D., E. Nevo, D. Zohary and O. Dagan. 1978. Genetic variations in natural populations of wild barley *(Hordeum spontaneum). Genetica,* 49: 97-108.

Ceccarelli, S., S. Grando, J.A.G. Van Leur. 1987. Genetic diversity in barley landraces from Syria and Jordan. *Euphytica*, 36:389-405.

Doll, H. and A.H.D. Brown. 1979. Hordein variation in wild (*Hordeum spontaneum*) and cultivated (*Hordeurn vulgare* L.) barley. *Can. J. Genet. Cytol*., 21:391-404.

Echart-Almeida C. and S. Cavalli-Molina. 2000. Hordein variation in Brazilian barley varieties (*Hordeum vulgare* L.) and wild barley (*H. euclaston* Steud. and *H. stenostachys* Godr.). *Genet. Mol. Biol*., 23: 425-433.

Fayyaz, L., Farhatullah, M.A. Rabbani, S. Iqbal, M. Kanwal and I. Nawaz. 2014. Genetic diversity analysis of *Brassica napus/Brassica campestris* progenies using microsatellite markers. *Pak. J. Bot*., 46(3): 779-787.

Ghani, A.H.A., K. Heiko, A.O. Parzies and H.H. Geiger. 2004. Estimating the out-crossing rate of barley landraces and wild barley populations collected from ecologically different regions of Jordan. *Theor. Appl. Genet*., 09: 588-595.

Hadjichristodoulou, A. 1995. Evaluation of barley landraces and selections from natural outcross of *H.vulgare ssp.spontaneum* with *ssp. vulgare* for breeding in semi-arid areas. *Genet. Resour. Crop Evol.,* 42: 83-89.

Jaradat, A.A. 1989. Diversity within and between populations of two sympatrically distributed *Hordeum* species in Jordan. *Theor. Appl. Genet*., 78: 653-656.

Jaradat, A.A. 1991. Grain protein variability among population of wild barley (*Hordeum spontaneum* C. Koch) from Jordan. *Theor. Appl. Genet*., 83:164-168.

Jaradat, A.A., T.T. Jaradat, S. Jana and J.P. Srivastava. 1987. Diversity for quantitative characters in Jordanian landraces of barley. In: *Barley genetics.* (Eds.): Yasuda, S and T. Konishi. V. Proc. 5th Int. Barley Genet. Symp. Okayama, pp. 109-117.

Kreis, M., P.R. Shewry, B.G. Forde, S. Rahman, M.B. Bahramian and B.J. Miflin. 1984. Molecular analysis of mutations conferring the high-lysine phenotype on the grain of barley (*Hordeum vulgare* L.). *Cell*, 34: 161-167.

Leino, M.W. and J. Hagenblad. 2010. Nineteenth century seeds reveal the population genetics of landrace barley (*Hordeum vulgare*). *Mol. Biol. Evol*., 27(4): 964-973.

Malysheva Otto, L., M.W. Ganal. J.R. Law, J.C. Reeves and M.S. Roder. 2007. Temporal trends of genetic diversity in European barley cultivars (*Hordeum vulgare* L.). *Mol. Breeding*, 20: 309-322.

Muhe, K. and A. Assefa. 2011. Diversity and agronomic potential of barley (*Hordeum vulgare* L.) landraces in variable production system, Ethiopia. *World J. Agric. Sci*., 7 (5): 599-603.

Murphy, R.W., J.W. Sites, D.G. Buth and C.H. Huafler. 1996. Isozyme Electrophoresis. In: *Molecular Systematics*. (Eds.): Hillis, D.M., C. Moritz and B.K. Mable. (Eds.), pp. 51-119. *Molecular Systematics*. Sinauer Associates, Sunderland, Massachusetts, USA.

Naz, A., Z.A. Swati and I.A. Khan. 2006. Studies on genetic diversity in Pakistani wheat varieties using randomly amplified polymorphic DNA. *Pak. J. Biolo. Sci*., 9(15): 2858-2861.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci*., 70: 3321-3323.

Nevo, E. 1998. Genetic diversity in wild cereals: regional and local studies and their bearing on conservation ex situ and in situ. *Genet. Resour. Crop Evol*., 45: 355-370.

Nevo, E. and H. Cleve. 1978. Genetic differentiation during speciation. *Nature*, 274: 125-126.

Nevo, E., A. Beiles and D. Zohari. 1986. Genetic resources of wild barley in the near east: structure, evaluation and application in breeding. Biol J Linn Soc., 27:355-380.

Nevo, E., A. Beiles and N. Storch. 1983. Microgeographic edaphic differentiation in hordein polymorphism of wild barley. *Theor. Appl. Genet*., 64:123-132.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*., 6:288-295.

Persson, G. 1997. Förädling av vårkorn i Sverige från förr och nu. In Olsson, G. (ed.) Den svenska växtförädlingens historia. Skogs- och lantbrukshistoriska meddelanden nr 20. KSLA, Stockholm.

Pritchard, J.K., M. Stephens and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics*, 155(2): 945-959.

Salamini, F., H. Ozkan, A. Brandolini, R. Schäfer-Pregl and W. Martin. 2002. Genetics and geography of wild cereal domestication in the near east. *Nat. Rev. Genet*., 3: 429-41.

Shakhatreh, Y., N. Haddad, M. Alrababah, S. Grand and S. Ceccarelli. 2010. Phenotypic diversity in wild barley *Hordeum vulgare* L. ssp. *spontaneum* (C. Koch) accessions collected in Jordan. *Genet. Resour. Crop Evol*., 57: 131-146.

Shewry, P.R., R.A. Finch, S. Parmar, J. Franklin and B.J. Miflin. 1983. Chromosomal location of Hor-3, a new locus governing storage proteins in barley. *Heredity*, 50:179-189.

Tang, H.H., Y. Ding and Y.J. Hu. 2002. Genetic polymorphism of hordein in wild relatives of barley from China. *Zuhan Bot. Res*., 4: 251-257.

Wright, S. 1943. Isolation by distance. *Genetics*, 28: 114-138

Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao. 2000. POPGENE, the user-friendly software for population genetic analysis. Molecular Biology and Biotechnology Center, Univ. of Alberta, Edmonton AB.

Yin, Y.Q., D.Q. Ma and Y. Ding. 2003. Analysis of genetic diversity of hordein in wild close relative of barley from Tibet. *Theor. Appl. Genet*., 107: 837-842.

Zhang, Y.B., Z.M. Xiao, S.Y. Qi and W.L. Xin. 1997. Study on a noncontinuous formic acid-page method of wheat gliadin. *China Acad. J*., 13:70-73.

(Received for publication 4 March 2013)