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Full Length Research Paper

Genetic diversity in hulless barley based on agromorphological traits and RAPD markers and comparison with storage protein analysis

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Genetic diversity among 63 hulless barley accessions originating from ICARDA (International Centre for Agricultural Research in the Dry Area) was investigated using agromorphological traits and random amplified polymorphic DNA (RAPD) variation. Among 20 agromorphological traits under study, a considerable diversity was observed for grain yield per plot, 1000-grain weight and peduncle length. High estimates of heritability in broad sense were recorded for plant height, number of grains per spike and number of tillers. These traits can be used for indirect improvement of yield. Cluster and Principal component analysis based on agromorphological traits could effectively classify the samples. The RAPD-based genetic similarity ranged from 0.221 to 0.81, with the mean of 0.481. Cluster analysis based on Jaccard Similarity Coefficient divided genotypes into 8 different groups. The average of genetic diversity index for RAPDs and storage proteins were compared and showed that mean of genetic diversity index was lesser for RAPDs than storage proteins. Thus, when resources are a limiting factor and considering the cost of consumables and work time, seed storage proteins must be the technique of choice for a first estimation of genetic variation in hulless barley genetic resource collections. In RAPD analysis, a special band was observed which could be used in identifying hulless barley varieties with low or high β-glucan.

Key words: Genetic diversity, hulless barley, agromorphological traits, RAPDs, storage proteins.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is the 5th most cultivated crop of the world. Hulless or naked barley differs from hulled barley by the loose husk cover of caryopses that is easily separable upon threshing in contrast to hulled barley (Bhatty, 1999). The hulless grain character is controlled by the single recessive gene 'nud' located on the long arm of chromosome 7H (Kikuchi et al., 2003). The domestication of naked barley is believed to have occurred after the hulled type around 6500BC (Zohary and Hopf 2000). Taketa et al. (2004) suggested a monophyletic origin of naked barley as a single mutation event either from wild barley (*Hordeum spontaneum*) or from domesticated hulled barley (*H. vulgare*).

Hulless barley is mainly used as animal feed, but in recent years, its importance is increasing as a human food in non-traditional areas due to its high β-glucan content which acts as an inhibitor of total and LDL cholesterol and triglyceride synthesis (Shimizu et al., 2008). β-glucan also normalizes blood sugar level, heals and rejuvenates the skin (Pins and Kaur, 2006). In addition the crude protein of hulless barley typically exceeds that of comparable hulled types and should be 1.5 - 3% greater (Lasztity, 1996).

Hulless barley is distributed widely in the world, but there is a higher preference for hulless barley in East Asian countries such as China, Korea and Japan and it is especially high in Tibet and the northern parts of Nepal, India and Pakistan. Since the frequency is low in the West, Vavilov (1926) considered Southeastern Asia be a center of origin for hulless barley. It has, however, become clear that this crop was grown in Anatolia (Turkey) and in northern Europe already in ancient times (Hunter, 1952; Helbaek, 1969).

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Hulless barley is very well suited to Iran region fitting well with the cropping systems and potentially providing grain for the poultry industries in addition to current uses. Hulled barleys have not generally been fed due to the high fiber content and hulless barley has not been utilized due to the low yield varieties for the Iran region. (Balouchi et al., 2005).

Assessment of the genetic diversity in a crop species is fundamental to its improvement. Genetic diversity among and within plant species is in danger of being reduced. In wild species genetic diversity may be lost because of severe reduction in population size, whereas in domesticated crops genetic diversity may be lost because of the narrow genetic base in many breeding programs (Cao et al., 1998).

Criteria for the estimation of genetic diversity can be different: pedigree records, morphological traits, biochemical markers and molecular markers.

Diversity in barley breeding program based on morphological traits and pedigree information was measured by Moralejo et al. (1994), Schut et al. (1997), Papa et al. (1998), Abebe et al. (2008) Chand et al. (2008). They showed that Grain yield is an ultimate product of the action and interaction of number of components such as number of tillers, number of grains per spike, 1000-grain weight, plant height, harvest index and etc. In addition the knowledge on nature and magnitude of gene effects controlling inheritance of agromorphological characters related to crop productivity will in turn become helpful in formulating an effective and efficient breeding program.

Biochemical markers also are key tools in the evaluation of genetic variability in both natural populations and germplasm accessions. Storage proteins (Hordein and monomeric prolamins) has a great inter-genotypic variation, and has been used as marker in cultivar identification, genetic diversity studies, and in determining the phylogenic origins in covered and hulless barley (Atanassov et al., 2001; Yin et al., 2003; Fernandez et al., 2006; Listrumaite and Paplauskiene, 2007; Pan et al., 2007; Michelmore et al., 2007; Eshghi and Akhundova, 2009).

In the other hand, molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and population. The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as RAPD, simple sequence repeats (SSR), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP) and inter-simple sequence repeat polymorphic DNA (ISSR), and so on (Saiki et al., 1988; Welsh and McClelland, 1990; Williams et al., 1990; Akkaya et al., 1992; Tragoonrung et al., 1992; Zietkiewicz et al., 1994; Wu et al., 1994; Nagaoka and Ogihara, 1997). These molecular markers had been used in hulless barley for detecting genetic diversity, genotype identification, genetic mapping (Hong et al., 2001; Taketa et al., 2004; Fernandez et al., 2006; Pandey et al., 2006; Feng et al., 2006; Kojima et al., 2007; Pan et al., 2008).

Of these techniques, RAPD have become one of the most widely used marker systems in studies related to plant genetic resources. They yield a high number of discrete bands and have been extensively used to document genetic variation in hulless and hulled barley (Chalmers et al., 1993; Tinker et al., 1993; Papa et al., 1998; Hong et al., 2001; Santos et al., 2001; Dakir et al., 2002; Yu et al., 2002; Yong-Cui et al., 2005; Michelmore et al., 2007).

The objective of this study was to estimate the genetic diversity in hulless barley genotypes, using phenological and morphological traits and RAPD markers and comparison them with storage protein analyses.

MATERIALS AND METHODS

Plant materials

The materials used in this study included 63 genotypes of hulless barley, all of which had been provided by ICARDA (Table 1).

Field experiments

Genotypes were evaluated in randomized block design with three replications in Moghan region (Iran), during 2007-2008. Each genotype was grown in 5 rows of 3 m long bed with spacing of 30 cm between the rows. An approximate distance of 15 cm was maintained between plant to plant by hand thinning. Ten competitive random plants from the middle rows of the experimental plots were taken for recording the observations on plant height, number of total tillers per plant, number of fertile tillers per plant, number of internodes, peduncle length, length of flag leaf, width of flag leaf, spike length, number of grains per spike, grain weight per spike, awn length, seed length, seed width, 1000-grain weight, days to maturity, days to heading, biomass, harvest index, grain yield per plant and grain yield per plot. Heritability in broad sense was calculated as ratio of the total genetic variance to the phenotypic variance. To examine interrelationships among the genotypes, a Principal component and Cluster analysis were performed based on these traits.

RAPD analysis

DNA was extracted from leaves of young plants grown in MS culture medium (Murashige & Skoog, 1962) for about three weeks at $20^{\circ}C$ and photoperiod of 13 hours. Total DNA was extracted from 0.4 g of material using the protocol described by Dellaporta et al. (1983). Final Pellets were dissolved in 250 μl TE solutions and kept at $-20^{\circ}C$. The DNA concentration of all samples was determined in agarose gels comparing the DNA from the samples with λ DNA solutions of different concentration. Reaction were performed in a 25 μl volume containing the reaction buffer (10 mM Tris –HCl pH 8.0 ,50 mM KCl, 1.5 mM $MgCl_2$) plus 2 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μ 10-base primer, approximately 35 ng of template DNA, and 2 units of Taq DNA polymerase. The thermal cycle was used $94^{\circ}C$ for 3min; then 45 cycles of $94^{\circ}C$ for 1min, $35^{\circ}C$ for 2min; and finally $72^{\circ}C$ for 5min. The 16 RAPD10-mers used (Table 3) were selected from among 75 RAPD primers in a preliminary test for oligos that amplified numerous discrete fragments.

PCR products were separated by electrophoresis on 1.4% agarose gels, run in 1 × TBE buffer (0.089 M Tris–borate and 0.2

Table 1. Name or pedigree and number of row of hulless barley genotypes used for diversity analysis

No.	Name or pedigree		No.	Name or pedigree	
1	BF 891 M-591	6 row	33	INON/3/CHAMICO/TOCTE//CONGONA..	2 row
2	BF 891 M-597	6 row	34	Atahualpha/CV.Tuwaitha	2 row
3	HIGO / LINO	6 row	35	BF891-614	6 row
4	SB91488	6 row	36	ICNBF8-617 (SEL.5AP)	6 row
5	BF 891M-609 (SEL.1AP)	6 row	37	CI 10590/CEDRO//OLMO/3/CHINA/LINO	6 row
6	SB 91925	2 row	38	MOLA/ALELI/MORA/3/CONDOR-BAR…	2 row
7	AMAPA/3/ROBUR-BAR/EGYPT20 …	2 row	39	ICNBF8-653	6 row
8	Rabano /5/CM67-B/Centeno//Cam…	2 row	40	ZARA/BEREMJO/4/DS4931//GLORIA- …	6 row
9	ICNBF 8-611 (SEL.2AP)	6 row	41	Atahualpa	2 row
10	PETUNIA 1	6 row	42	RABANO/4/DS4931//GLORIA-BAR/ …	6 row
11	ICNBF 93-369	2 row	43	PENCO/CHEVRON-BAR/3/ATACO/ …	2 row
12	ICNBF-582	6 row	44	CHENG Du 891/PENCO/CHEVRON…	2 row
13	Alpha/Durra//Himalaya-26	2 row	45	PENCO/CHEVRON-BAR//CANTUA/3/…	2 row
14	ICB-102607	2 row	46	BF891M-654	6 row
15	Pamir-167/Himalaya-26	2 row	47	ORA/NB1054/3/MOLA/SHYRI//ARUP*/…	2 row
16	Himalaya-13/Rhn-o3	6 row	48	Alanda-01	6 row
17	BF 891 M-622 /3/ Arar // 2762 / …	6 row	49	LINO//ALISO/C13909.2/4/CEDRO// …	6 row
18	Mala/SHAYRI//RUPO*2/JET/3/ …	6 row	50	Chamico/tecte//Congona	6 row
19	PETUNIA2	2 row	51	REGENT-BAR/CONOR-BAR/3/MOLA/…	2 row
20	ICNBF 93-328	6 row	52	PENCO/CHEVRON-BAR//CHENG DU …	2 row
21	MJA/BRB2/QUINA/3/CABUYA/4/ …	2 row	53	ALELI/VIRNGA	6 row
22	CERRAJA/3/ATACO/ACHIRA// HIGO	6 row	54	RHODS//TB-B/CHZO/3/GLORIA-BAR/...	6 row
23	ASL-2/5/Cr.115/Pro//BC/3/Api/CM…	6 row	55	PINON/3/QUINN/ALOE/CARDO/4/CIRU	2 row
24	Harmal	2 row	56	DC-B/SEN/3/AGAVE/YANALA/TUMBO…	2 row
25	Rihane-03	6 row	57	BBSC Congana	6 row
26	ICNBF8-613	6 row	58	WI 2291	2 row
27	BF891M-614	6 row	59	Moroc 9-75	2 row
28	BF891M-584	6 row	60	ICNBF8-654	6 row
29	BF891M-592	6 row	61	LINO/HIGO/4/CEDRO//MATNAN/EH…	2 row
30	ANCA/2469//TOJI/3/SHYRI/4/…	2 row	62	TOCTE/PINON/PALTON	2 row
31	Atahualpha/Iraqi Black	2 row	63	TOCTE/TOCTE//BERROS/3/PETUNIA...	2 row
32	Atahualpha/IPA 7	2 row			

µM EDTA) and visualized with ethidium bromide. Gels were photographed under UV light with Polaroid 667 films. Reproducibility of the RAPD analytical procedure was investigated with repeated analysis of samples. Only those bands which showed consistent amplification were chosen for use in this study. After identification of the polymorphic bands, different patterns were identified among genotypes, and then, using Nei (1973) method and each patterns frequency, the genetic diversity index was calculated for each primer.

$$H = 1 - \sum Pi^2$$

Where; H is genetic diversity index and Pi is each pattern's frequency. 0 and 1 coefficients were calculated for all the genotypes, depending on the presence (1) or absence (0) of the bands. It was also used in obtaining other results as well as similarity coefficient matrix of Jaccard (Jaccard, 1908). Furthermore, in order to classify the accessions, Cluster analysis was done using UPGMA (Unweighted Pair Group Method with Arithmetic Means) calculating similarity coefficients matrix and dendrogram was done by NTSYS-PC (Numerical Taxonomy and Meltivariate Analysis system) program (Rohlf, 1992).

We have compared genetic diversity of these accessions with storage proteins polymorphism (monomeric prolamins and hordeins) (Eshghi and Akhundova, 2009).

Finally, the similarity between matrices based on different marker system (agromorphological data, storage proteins and RAPD) were calculated using the standardized Mantel coefficient (Mantel 1967).

RESULTS AND DISCUSSION

Agromorphological traits

Variance analysis of the traits showed that there existed a significant difference among the genotypes under study with respect to all traits (results not shown).

Principal component analysis was carried out for all traits under study. The first six components could justify

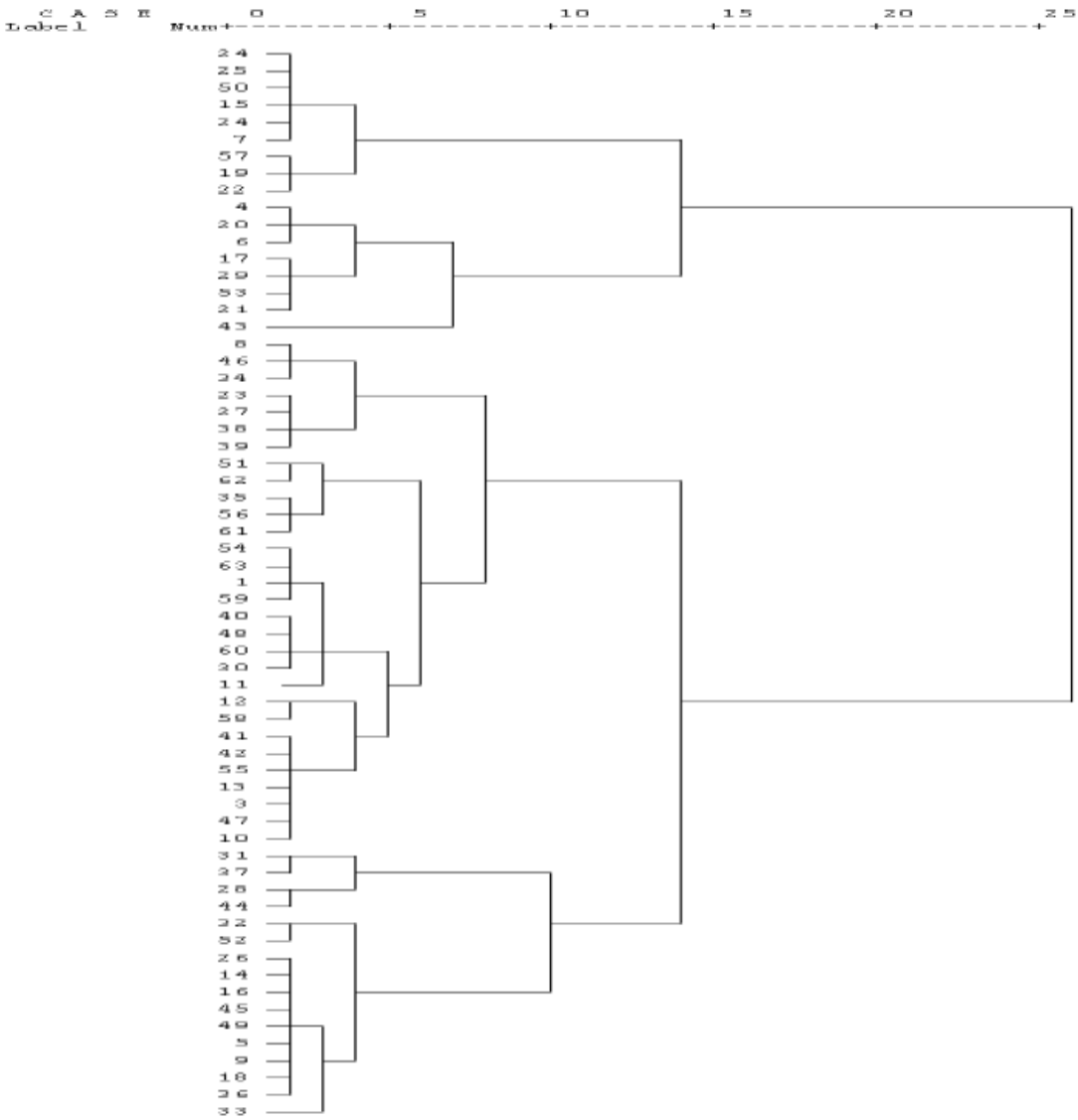

Figure 1. Dendrogram showing the relationship among 63 genotypes hulless barley based on agromorphological traits.

more than 73% of the whole variance in the genotypes. The first component could justify the most amount of variance among genotypes (28.66%). Traits that had correlation with this component included: plant height (r = -0.365**), total number of tillers (r = 0.661**), number of fertile tillers (r = 0.735**), peduncle length (r= -0.404**), spike length (r = 0.517**), number of grains per spike (r = 0.686**), grain weight per spike (r = 0.629**), biomass (r = 0.595**), harvest index (r = 0.671**), grain yield per plant (r = 0.77**), and grain yield per plot (r= 1.00**). Regarding high correlation between first component and yield and other traits associated with that, this component can be called yield component. Genotypes that were selected by this component included genotypes 4, 20, 6, 17, 29, 53, and 21. Cluster analysis was carried out for 63 genotypes with Ward method (Richard and Johnson, 1996) (Figure 1). If the cutting is done on the distance 5, genotypes are divided into 8 groups. As it is shown, those significant genotypes which were selected in Principal component analysis are now in the same discrete group in Cluster analysis. Genotype 4 with yield in plot of 3.92 ton per hectare (t/h) and yield in plant of 10.4 g, genotype 20 with yield in plot of 3.77 t/h and yield in plant of 10.2 g, genotype 29 with yield in plot of 3.77 t/h and yield in plant of 9.6 g scored the highest yield among genotypes under study. After, genotypes 17, 21, 6 and 53 were introduced as having the highest yields with yields in plot of 3.76, 3.76, 3.51, 3.35, and 3.45 t/h, respectively.

The values of genetic variance, broad sense heritability, genetic coefficient of variation, minimum and maximum values of the traits under study have been a trait shows how much of the variety available among the

Table 2. Estimates of Maximum, minimum, mean, genetic variance, genetic C.V., environment C.V. and broad sense heritability (h^2_{bs}) for 20 agromorphological traits.

Traits		Max	Min	Mean	Genetic variance	Genetic CV%	Environmental CV%	h^2_{bs}
Plant height	(cm)	113.6	68.2	91.87	80.09	9.74	8.7	**0.79**
Number of total tillers		5.2	2.1	3.387	0.221	13.87	16.5	0.68
Number of fertile tillers		5	1.8	3.065	0.244	16.12	17.4	**0.72**
Number of internodes		5.6	3.7	4.522	0.1069	7.23	8.8	0.67
Peduncle length	(cm)	13.7	4.9	9.967	3.460	**18.66**	23.2	0.66
Length of flag leaf	(cm)	16	9.4	11.859	2.029	12.01	13.3	0.71
Width of flag leaf	(cm)	1.7	0.73	1.192	0.004	5.2	10.7	0.42
Spike length	(cm)	8.8	4.8	6.732	0.1186	5.1	6.8	0.63
Awn length	(cm)	14.7	4.1	10.448	2.689	15.69	12.5	0.82
Number of grains per spike		58.43	26.2	42.497	53.162	17.16	16.7	**0.76**
Grain weight per spike	(g)	2.7	0.93	1.758	0.080	16.13	24.3	0.57
Seed length	(cm)	0.98	0.61	0.768	0.007	10.87	8.2	0.84
Seed width	(cm)	0.39	0.29	0.340	0.0007	7.78	5.2	0.87
1000-grain weight	(g)	47.6	24.2	37.437	59.61	**20.62**	17.3	0.81
Days to maturity		211.2	177.1	192.85	32.041	2.93	2.7	0.78
Days to heading		154.3	125.2	138.63	32.17	4.09	4.2	0.74
Bimass	(g)	39.1	14.2	29.248	25.989	17.43	**30.8**	0.49
Harvest index	(%)	51.2	32.7	41.075	15.23	9.50	15.5	0.53
Grain yield per plant	(g)	10.4	3.4	6.841	1.314	16.75	23.7	0.60
Grain yield per plot	(kg/h)	3919.8	1372.4	2657.365	**334533.8**	**21.76**	**34.1**	0.55

C.V.: Coefficient of variability

phenotypes of the samples can be attributed to genetic affects and influential the environment is. Obviously, if the heritability of a trait is high, we can apply selection to improve that trait. The amount of heritability for yield was 0.55. Most probably, high genetic variance for this trait has led to calculating its heritability more than its actual value. In the experiments carried out in the same year and the same location, genotype × environment interaction effect variance is not separable from genetic variance, and this might lead to calculating the genetic variance more than what it actual is. None the less, genetic variation coefficient for this trait was within an acceptable range. Yield is a polygenetic trait and many genes are involved in it, and since environment has a considerable share and heritability of this trait is usually low (Muhammad and Konak, 2005; Baghizadeh et al., 2003). Therefore, in order to improve this trait, breeders have to study traits related to that with high heritability and those in which environmental effects and genotype × environment interaction effects are less obvious.

Although the highest broad sense heritability in this experiment was that of seed width (0.87), seed length (0.84), awn length (0.82), and 1000-grain weight (0.81) broad sense heritability of plant height, number of grains per spike, and number of fertile tillers was relatively high (Table 2). Thus simple selection procedure in early segregating generations will be effective for these traits. Since these traits were the principal components of yield in this study, they can be used in indirect improvement of the genotypes under study.

Budak (2000) and Muhammad and Konak (2005) also revealed that additive and partial dominance genetic effects were important for plant height and number of tillers. They reported high heritability for these traits. Although Muhammad and Konak (2005) found additive type of inheritance for number of grains per spike in hulless barley accessions, Rohman et al. (2006) and Ordas et al. (2008) reported non-additive gene effects for this trait. In another research, Chand et al. (2008) also, reported high broad sense heritability for 1000-grain weight and number of grains per spike.

The highest genetic variation coefficient and, as a result, the highest diversity among the genotypes under study were found for grain yield per plot (21.76%), 1000-grain weight (20.62%), and peduncle length (18.66%). Chand et al. (2008) reported high diversity for number of grains per spike and grain yield per plant. At the same time, Abebe et al. (2008) studied the diversity of the Ethiopian barley germplasm through morphological traits and found a considerable diversity for days to heading, days to maturity, biomass, plant height and 1000-grain weight. In another research, Okeno (2001) reported sig-

Table 3. Primer sequences, total number of bands amplified and analyzed, polymorphic bands, genetic diversity index, size range of amplified products, the total and mean primer.

Primer	Sequence (5'-3')	Total bands	Bands analyzed	Polymorphic bands	Genetic diversity index	Fragment size
S29	TGATCCCTGG	17	17	12	0.891	470-1200 bp
S18	CCACAGCAGT	**10**	**10**	8	0.772	230-860 bp
S32	TCGGCGATAG	**31**	**29**	**21**	0.783	350-2500 bp
S39	CAAACGTCGG	18	18	13	0.903	150-900 bp
S134	TGCTGCAGGT	25	24	**19**	0.891	500-2400 bp
A-01	CAGGCCCTTC	13	12	12	0.784	400-1300 bp
F-03	CCTGATCACC	17	17	12	**0.725**	280-2100 bp
OPC-06	GAACGGACTC	20	20	**18**	**0.936**	180-2200 bp
OPC-07	GTCCCGACGA	14	14	14	0.844	500-2500 bp
OPC-08	TGGACCGGTG	18	18	16	**0.923**	380-1500 bp
F-09	CCAAGCTTCC	21	19	17	**0.920**	200-1900 bp
OPC-14	TGCGTGCTTG	20	20	12	0.761	580-2500 bp
OPC-15	GACGGATCAG	16	16	16	0.913	430-2100 bp
OPC-16	CACACTCCAG	13	13	**7**	0.812	180-1700 bp
OPD-02	GGACCCAACC	17	17	11	0.882	450-2000 bp
OPD-03	GTCGCCGTCA	26	26	14	0.796	160-2200 bp
Total	-	296	290	222	-	150-2500 bp
Mean/primer	-	18.5	18.12	13.87	0.848	-

nificant genotypic variation, for length and width of flag leaf, plant height and yield per plant, indicated possibility of selection response in these traits in spring barley.

RAPD markers

Among 75 primers being used, 16 primers that included a considerable polymorphism were selected for the analyses. Through these primers, 296 bands were produced from among which 290 strong bands were analyzed. Among the genotypes under study, 222 fragments (76.55%) were polymorph. This is a high level of polymorphism expressed by arbitrary primers compared to reports of other RAPD studies in autogamous species.

Cao and Oard (1997) detected only 14.6% of polymorphic fragments in rice cultivars recommended for commercial production in Louisiana. Ko et al. (1994) found 67% for other rice cultivars originated from different places of the world, and for pea, Samec and Nasinec (1995) reported 18.8% of polymorphic bands. In barley, Noli et al. (1997) detected 26.5% of polymorphic fragments in European cultivars, and Russel et al. (1997) found 67% polymorphic bands in 12 winter barleys and 12 spring barleys.

The most and the least replicated DNA fragments were related to primers S32 and S18 respectively. The highest numbers of polymorphic bands were those of primers S32, S134, and OPC-06, and the lowest number of polymorphic bands was that of primer OPC-16. The average number of polymorphic bands for each primer was 13.87. Among the primers under study, the primer OPC-06 with genetic diversity index of 0.936 had the most diversity (Figure 2), and next came the primers OPC-08 with diversity index of 0.923 and the primer F-09 with diversity index of 0.92. The least genetic diversity was that of F-03 with the genetic diversity index of 0.725. The genetic diversity average among all the genotypes for all primers was calculated as 0.848.

Among the bands created by the primer S39, a replicated DNA fragment (150 bp) was observed which was found only in genotypes with low β-glucan content (Figure 3). The t-test statistically confirmed this relation. As a result, this primer can be used a SCAR marker to help quickly identify genotypes with low or high β-glucan content. In a similar study, Yu et al. (2002) reported the correlation between this primer and β-glucan content in hulless barley genotypes.

Analysis of the RAPD markers was carried out with UPGMA method and with the help of Jaccard similarity coefficients. Different authors have used different coefficients to make these estimations, such as Simple Matching Coefficient, Jaccard Similarity Coefficient, Nei and Li coefficient (Nei and Li, 1979), among others (Santos et al., 2001; Dakir et al., 2002; Yu et al., 2002; Yong-Cui et al., 2005; Michelmore et al., 2007). Maybe the most important criterion for the choice of an adequate coefficient to apply on RAPD analyses is that the method does not consider the absence of bands as a similarity. This premise is important in RAPD data because the

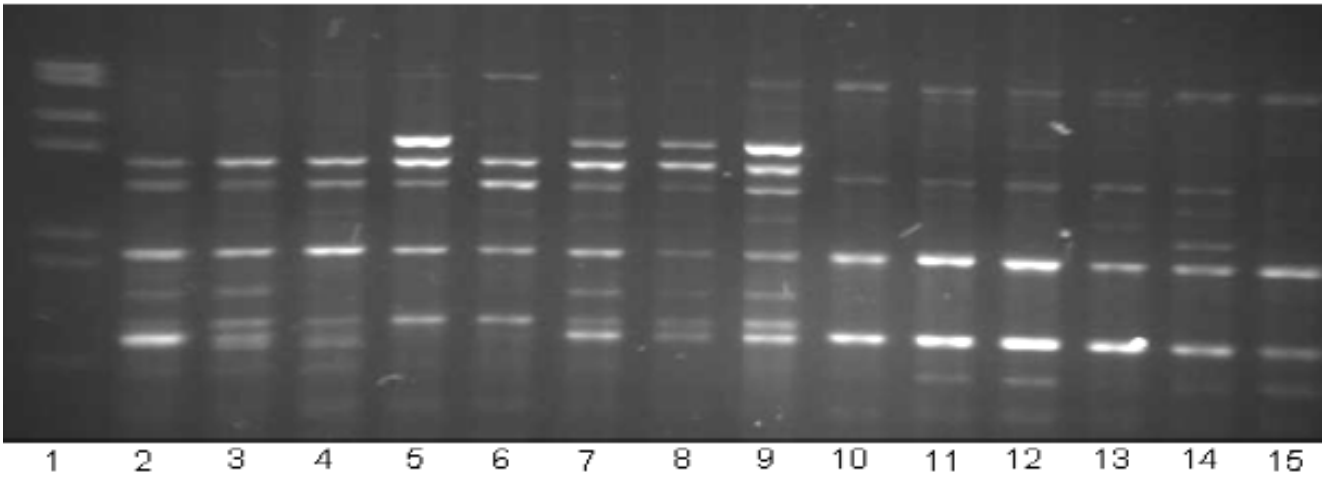

Figure 2. RAPD analysis of 14 hulless barley genotypes with primer OPC-06. Lane 1: DNA marker; 2: SB 91925 3: BF891M-614; 4: ICNBF 93-369; 5: WI 2291; 6: BF 891 M-591; 7: Moroc 9-75; 8: ICNBF-582; 9: ICB-102607; 10: Atahualpha/IPA 7; 11: HIGO / LINO; 12: PETUNIA2; 13: ICNBF8-613; 14: BBSC Congana; 15: Harmal.

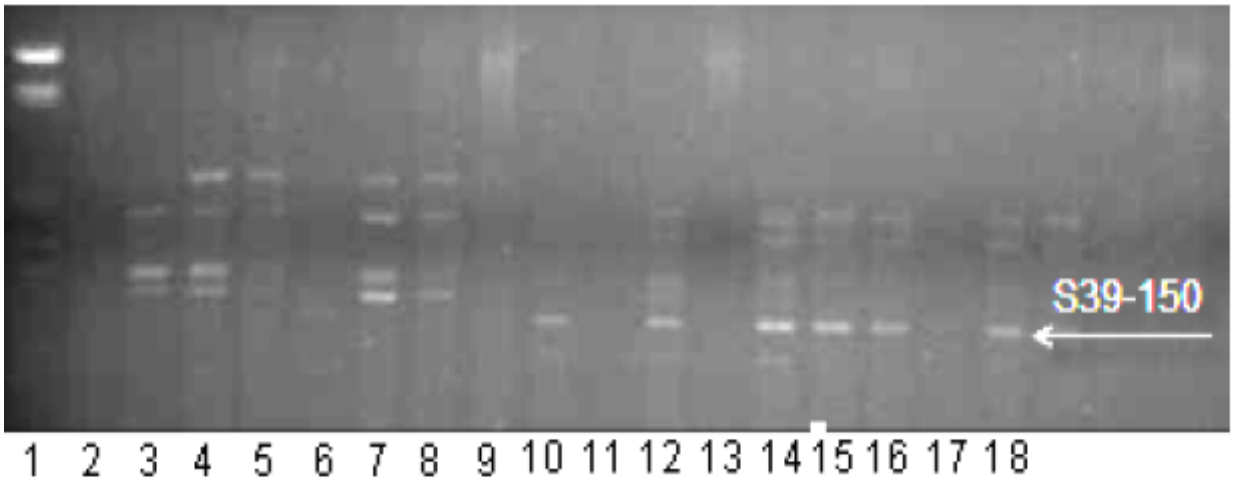

Figure 3. RAPD analysis of 17 hulless barley genotypes with primer S39. Lane 1: DNA marker; 2: BF 891M-609 (SEL.1AP); 3: CNBF 8-611 (SEL.2AP); 4: ICNBF8-617 (SEL.5AP); 5: Rabano /5/CM67-B/Centeno//Cam…; 6: ALELI/VIRNGA; 7: PINON/3/QUINN/ALOE/CARDO/4/CIRU; 8: AMAPA/3/ROBUR-BAR/EGYPT20…; 9: Alpha/Durra//Himalaya-26; 10: BF 891 M-622 /3/ Arar // 2762 / …; 11: CERRAJA/3/ATACO/ACHIRA// HIGO; 12: Atahualpha/IPA 7; 13: Rihane-03; 14: Atahualpha/CV.Tuwaitha; 15: MOLA/ALELI/MORA/3/CONDOR-BAR…; 16: Alanda-01; 17: Moroc 9-75; 18: REGENT-BAR/CONOR-BAR/3/MOLA/… .

absence of one band can be due to different mutations which alter the priming site (Selbach and Cavalli-Molina, 2000). Therefore, Jaccard Similarity Coefficient, which does not consider absence of bands as a similarity, was used in the present analysis. As shown in Figure 4, if the cutting is done from a distance of 0.516, genotypes will be divided into 8 different groups. Though genotype 16 lied in a discrete group alone – which indicated the genetic distance of this genotype from other genotypes – the last group accommodated 58.7% of the genotypes in itself. The most genetic similarity was observed between genotypes 5 and 13 with the similarity coefficient of 0.89 and then between genotypes 51 and 35 with the similarity coefficient of 0.86. The least genetic similarity was observed between genotypes 43 and 21 with the similarity coefficient of 0.215 and then between genotypes 1 and 63 with the similarity coefficient of 0.311. The whole matrix average similarity for all the genotypes was calculated as 0.481.

No phenomenon in plant breeding has yet been as influential on increasing agricultural products as hybrid varieties. With regard to this fact, crossing two individuals with less genetic affinity can lead to genotypes with more capabilities through heterosis. One of the main applica-

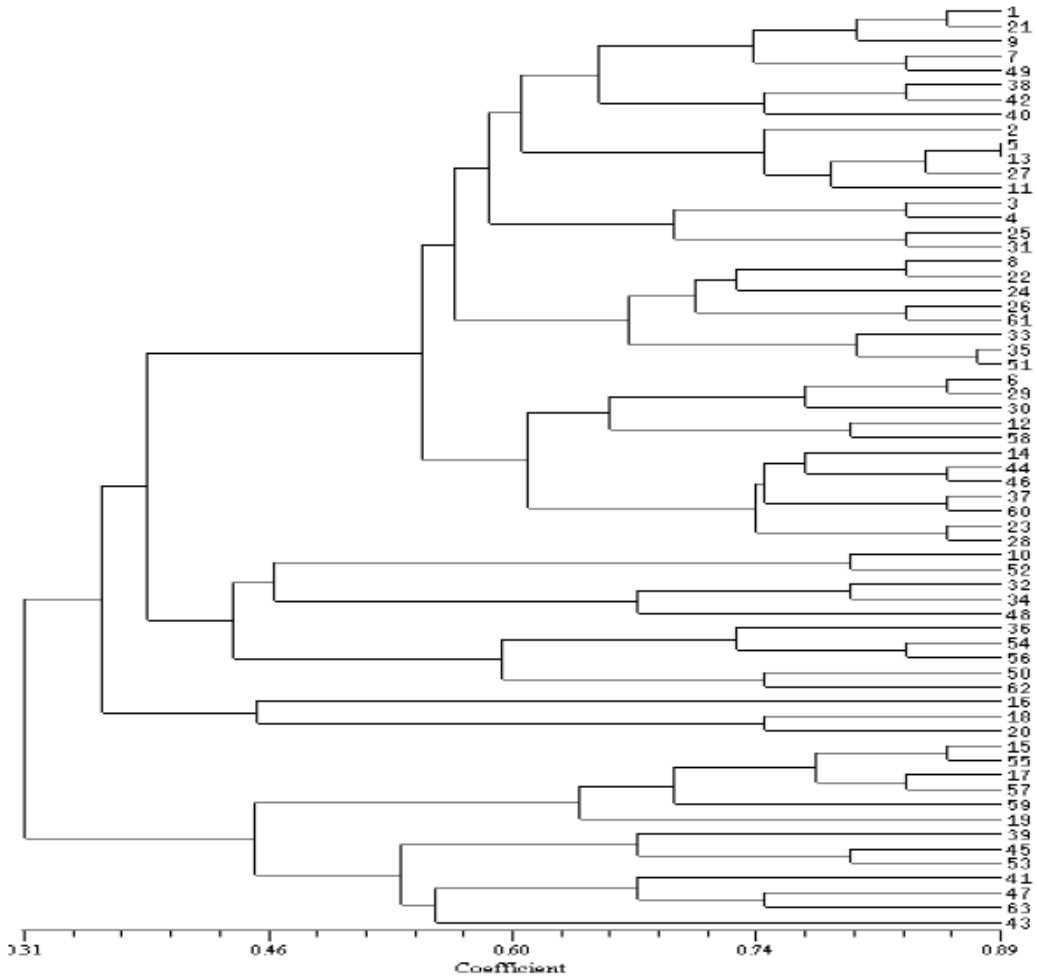

Figure 4. Dendrogram showing the relationship among 63 genotypes hulless barley based on RAPD markers.

tions of these clusters is the estimation of the genetic distance between genotypes. Therefore, these results can be used in identifying appropriate parents for crossings and creating superior hybrids in hybridization.

Storage proteins

In the analysis of the hordeins, we observed no polymorphism in the area D hordein (Eshghi and Akhundova, 2009). However, in that research 10 patterns in the area C hordein, and 13 patterns in the area B hordein were observed and totally 32 bands and 32 patterns were observed. The average of genetic diversity index for these proteins was calculated as H = 0.856. In the analysis of the monomeric prolamins, which was performed with the Acid-PAGE method, 15, 9, 24, and 20 patterns were observed for the ω, γ, β, and α areas, respectively. The average of the genetic diversity index for these proteins was H = 0.889, and, totally 33 bands as well as 57 patterns were observed (Eshghi and Akhundova, 2009).

Comparison between agromorphological traits, RAPD polymorphisms and storage proteins

Comparing the results obtained from agromorphological traits, storage proteins, and RAPD markers showed that the correlation between distance matrix of the genotypes based on agromorphological traits and the matrix of the monomeric prolamins and hordeins polymorphism were calculated as 0.074 and -0.145, respectively, and that of the matrix of agromorphological and RAPD markers was calculated as 0.098. None of these was significant. Also, the correlation between the matrix of monomeric prolamins and hordeins with RAPD markers were calculated as 0.083 and 0.163 respectively, which were not significant either. Therefore, genetic diversity pattern apparently differed based on different methods and clas-

sifying the germplasm yielded different results when different methods were applied.

Throughout three years in their research, Atanassov et al. (2001) studies the diversity of the agromorphological traits along with the results obtained from hordeins polymorphism among hulless barley accessions originating from three different breeding centers. They did not observe any relation between morphological traits and storage proteins. They showed that hordein polymorphism and variation of agromorphological traits considered together would indisputably help the breeder to diversify the sources of germplasm and optimize the choice of parents to be used in crossing programs in hulless barley.

In a research conducted by Papa et al. (1998), twelve local populations (20 strains per population) were evaluated for 13 RAPD markers, six isozyme loci and five morphophenological traits. They showed that although each method used to determine genetic diversity among landrace populations under study revealed different aspects of diversity, no relation was reported between these methods. Meanwhile, Hamza et al. (2004) showed that the correlation between SSR markers and morphological traits was highly significant, and the correspondence between the clustering based on DNA markers and morphological data was relatively good.

Semagn (2002) suggested two reasons for low correlation between DNA markers and morphological as well as protein data: (1) DNA markers cover a larger proportion of the genome, including coding and noncoding regions, than the morphological markers. (2) DNA markers are less subjected to artificial selection compared with morphological markers. Martnez et al. (2005) and Salem et al. (2008) believed that the correspondence between different methods might be improved by analyzing more morphological characters, storage proteins and DNA markers.

It seems that since each of these methods demonstrates different aspects of diversity in different populations, simultaneous application of these methods can present researchers a brighter view of diversity. But the assessment of genetic variation in large samples of plant genetic resources requires a high costing time and amount of consumables. Thus, reliable, affordable and economical techniques should be preferred, at least in the first screening of genetic variability. Obviously, assessment of agromorphological traits takes several months and requires considerable costs; even in some cases, die to influential role of environment and mutual effects of genotype × environment in the emergence of these traits, it is necessary to repeat the experiments throughout several years and in different places.

In this research genetic diversity index of storage proteins was greater than that of RAPD markers.

Genetic variation existing in a set of barley landrace samples collected in Morocco was estimated by Dakir et al. (2002). Two kinds of genetic markers, seed storage protein (hordeins) and random amplified polymorphic DNA, were used. Although no relation was identified between storage proteins and RAPD markers, on average the diversity of the storage proteins was more than the diversity of the RAPD markers. In their research Hordeins analysis showed more alternatives per band on gels and a higher percentage of polymorphic bands.

The occurrence of intravarietal variability in Brazilian hulless barley varieties has been detected by Santos et al. (2001), with isozymes data and RAPD markers, and by Fernandez et al. (2006), in a study with hordein analysis and RAPDs. The variability detected by RAPD markers was higher than that detected by isozymes, but it was lower than that obtained by the analysis of the hordein polypeptide patterns, as may be expected for each kind of molecular regarding their metabolic role and evolutionary dynamics.

In another research fourteen populations from ICARDA's hulless barley collection were evaluated by 20 RAPD markers, hordein polymorphism and ten morphological traits (Michelmore et al., 2007). The diversity level of the population studied was often different for morphological traits, hordeins and RAPDs. However, the genetic diversity index observed in storage protein analysis was slightly larger than found in morphological or RAPD analyses.

Electrophoresis of seed storage proteins is a technique capable of giving an estimate of the genetic variation with and between accessions due to the relatively high number of genetic markers which can be scored. Furthermore, since endosperm half seeds are used, the technique is not destructive (Dakir et al., 2002). The RAPD technique is certainly highly useful for this purpose since it affords us with a great number of polymorphic markers which allows us to distinguish between uniform cultivars or even individuals in heterogeneous accessions (Selbach and Cavalli- Molina 2000; Kraic et al. 1998). Although RAPD is currently an easy and standard technique and the laboratory equipment is economical, storage protein analysis is technically easier and cheaper and needs less sophisticated equipment. Considering the cost of consumables in Iran for us, the analysis of the 1200 seeds for hordeins had a similar cost to that of the 100 seedlings or RAPDs. Therefore if resources are the main limiting factor, we find that seed storage proteins must be the technique of choice for the first estimation of genetic variation in plant genetic resource collections.

Modern breeding process has dramatically narrowed the variation of important traits, especially among common hulless barley cultivars which are widely used in breeding programs. Whereas that all of investigated genotypes were from ICARDA, high level of diversity was observed in the samples through agromorphological traits, storage proteins and RAPD markers. These results show that we can use from this diversity in future breeding programs, widely, as suggested by Atanassov et al. (2001), Michelmore et al. (2007) and Jilal et al. (2008).

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