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MICROSATELLITE ANALYSIS OF GENETIC DIVERSITY OF WILD BARLEY (*HORDEUM VULGARE* SUBSP. *SPONTANEUM*) USING DIFFERENT SAMPLING METHODS IN IRAN

H. Khodayari, H. Saeidi, A. Akhavan, M. Pourkheirandish and T. Komatsuda

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The genetic diversity patterns of 54 natural populations and 40 individuals of wild barley from different localities in Iran were analyzed using 26 microsatellites markers. At population level, high levels of diversity (0.71), with an average number of 6.92 alleles per locus (ranging up to 17) and high level polymorphism with polymorphism rate averaging 0.67 were observed. Average number of alleles was 38.37 per population (ranged from 36.33 in the Northeast to 38.94 in the West) and 28.42 per individual (ranged from 28.11 in the West to 29 in the North). In dendrograms, accessions collected from the North and Northeast of Iran were grouped away from those collected from the West and Northwest, suggesting the presence of two different wild barley sub genepools in this country. The genetic diversity decreases from the West toward the East. Based on the results of this study the wild Iranian gene pool of barley represents high level of diversity and there is value to search for new useful alleles to incorporate to the breeding programs.

Hamed Khodayari Departmentof of Biology, Faculty of Science, University of Lorestan, Khorramabad, Lorestan, Iran.- Hojatolah Saeidi (correspondence<ho.saeidi@sci.ui.ac.ir>), Azadeh Akhavan, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, Iran.- Mohammad Pourkheirandish, and Tako Komatsuda, plant Genome Research Unit, National Institute of Agrobiological Science (NIAS),2-1-2Kannondai, Tsukuba, Ibaraki, 305-8602, Japan

Key words: wild barley; genetic diversity; gene pool; microsatellites; Iran

تحلیل ریزماهوارکی از تنوع ژنتیکی جو خودرو (*Hordeum vulgare* subsp. *spontaneum*) در ایران با استفاده از روش‌های نمونه‌برداری مختلف

حامد خدایاری، استادیار گروه زیست شناسی دانشکده علوم پایه دانشگاه لرستان
حجت اله سعیدی، دانشیار گروه زیست شناسی دانشکده علوم دانشگاه اصفهان
آزاده اخوان، گروه زیست شناسی دانشکده علوم دانشگاه اصفهان
محمد پورخیراندیش، محقق پسادکتری، موسسه نیاس(NIAS)، تسوکوبا، ژاپن
تاکائو کوماتسودا، استاد موسسه نیاس (NIAS)، تسوکوبا، ژاپن

الگوی تنوع ژنتیکی ٥٤ جمعیت و ٤٠ فرد جو خودرو از رویشگاههای طبیعی مختلف ایران با استفاده از ٢٦ جفت نشانگر ریزماهواره مورد آنالیز قرار گرفت. در سطح جمعیتی، مقادیر بالایی از تنوع (٠.٧١) با متوسط تعداد آلل ٦.٩٢ آلل در هر لوکوس (تا ١٧ آلل در هر لوکوس) ومیزان بالایی از پلی‌مورفیسم به میزان متوسط ٠.٦٧ مشاهده گردید. تعداد متوسط آلل در هر جمعیت ٣٨.٣٧ (از ٣٦.٣٣ آلل در شمال‌شرق تا ٣٨.٩٤ آلل در غرب کشور) و در افراد ٢٨.٤٢ آلل در هر فرد (در دامنه‌ی ٢٨.١١ در غرب کشور تا ٢٩ آلل در شمال کشور) بود. در دندروگرامها، نمونه‌های جمع‌آوری شده از شمال وشمال‌شرق ایران در یک گروه و دور از نمونه‌های جمع‌آوری شده از غرب و شمال‌غرب قرار گرفتند که بیانگر وجود دو زیرخزانه ژنی مخلف در ایران می‌باشد. براساس نتایج این مطالعه، خزانه ژنی جو خودرو ایرانی منابع بالایی از تنوع را به نمایش می گذارد که برای اصلاحگران نباتات جهت یافتن آلل‌های جدید سودمند می‌باشد.

INTRODUCTION

Based on several evidence the cultivated barley (*H. vulgare* L. subsp. *vulgare*) is originated from versatile wild barley (*H. vulgare* subsp. *spontanum* C. Koch.) genepool in the Middle East, where the domestication process was took place (Bothmer et al. 1995; Badr et al. 2000).

The wild barley (*H. vulgare* subsp. *spontaneum;* syn: *H. spontaneum* C. Koch.), occurs naturally in the Mediterranean Region, Greece, Egypt and Southwest Asia and distributed eastward through Iran to Afghanistan, west of Pakistan and the south of Tadjikestan (Bothmer et al. 1995). The centre of diversity of wild barley is considered to be the Fertile Crescent. Populations of this subspecies grow in different habitats with wide range of environmental conditions (Badr et al. 2000; Ivandic et al. 2002). Adaptations to variable environment conditions are reflected in the large morphological and physiological variability in wild barley (Graner et al. 2003).

The populations of spontaneous barley grow widely in mountainous regions of Iran in the North, Northeast, West and Southwest (Bor 1970) with different and stressful environmental conditions. Morrell and Clegg (2007) demonstrated that the wild barley populations from the east of Zagros Mountains (western Iran) are different from those of west in allelic combinations. They argued that the domestication of barley has probably began from the West of Iran in the eastern region of Fertile Crescent and a second domestication event have occurred possibly at the eastern edge of the Iranian Plateau in the Central Asia (Morrel and Clegg 2007). They also have noted that the western landraces of cultivated barley are probably originated from the western genepool (primary center in the Fertile Crescent) and the eastern ones from the eastern genepool (secondary center of diversity) somewhere at the eastern end of the Iran Plateau. Badr et al. (2000) showed that the Iranian germplasm from the southern part of the Fertile Crescent in western Iran (Zagros) are genetically different from the germplasm originated from the west of Fertile Crescent. They believed that the wild populations found in the western Iran have contributed germplasm to the cultivated barley on its way to the Himalayas (Badr et al. 2000).

Regarding the diverse ecological conditions in Iran and the adaptation based variability in wild barley (see for example Nevo et al. 1986a), it can be assumed that the Iranian genepool contains new and different useful alleles which can be utilized in genetic improvement of modern barley cultivars. This opinion is strengthened with the facts that the genetic diversity increased in parallel with the increase in stressed environments (Nevo et al. 1998) and that the wild barley carry many useful genes (Ellis et al. 2000). In the other hand, many reports showed that Iranian genepool of wild barley were genetically most distinct from that of Turkey and other countries (Badr et al. 2000; Ivandic et al. 2002; Pakniyat et al. 1997; Nevo 1992). Evaluating patterns of variation within this wild genepool is fundamental for designing a strategy for germplasm collection and conservation, identifying populations of highest conservation priority and for tracking the origin of domesticated barley.

The value of microsatellite markers for both genetic diversity studies and barley breeding was demonstrated as early as in 1994 (Saghai Maroof et al. 1994; Liu et al. 1996; Struss and Plieske 1998). Later, comprehensive microsatellite genetic maps integrating 369 SSR loci in barley were prepared by Ramsay et al. (2000) and by Li et al. (2003). SSR markers have been successfully used to evaluate genetic diversity of Triticeae species (e.g. Struss and Plieske 1998; Graner et al. 2003; Baek et al. 2003; Fahima et al. 1997; Saeidi et al. 2006; Akhavan et al. 2010; Jenabi et al. 2011; Ahmad 2002).

Considering the advantages of microsatellites as molecular markers and this fact that the morphological characters are not precise indicators of the genetic potential of a germplasm collection (Tanksley and McCouch 1997), this study was conducted to evaluate the genetic structure and diversity across wild barley genepool in different regions of Iran and to examine the geographic influence on the patterns of diversity using microsatellites.

MATERIALS AND METHODS

A total of 54 accessions of wild barley (Fig. 1) were collected from various regions of Iran (19 accessions were provided by the National Plant Gene Bank of Iran) and these were identified morphologically according to Bothmer et al. (1995). Two cultivated barley accessions (two and six rowed) were included as control.

From each accession 15 – 20 seeds were grown in experimental field and bulked genomic DNA was isolated from fresh leaves according to Komatsuda et al. (1998).

In a simultaneous study 40 individuals from different regions (west, 20; southwest, 9; northeast, 6; North, 5 individuals) were analyzed separately to compare the results obtained from single plant studies and the results obtained from accessions (bulk seeds). For SSR analysis at individual level, DNA was isolated from single plants.

Twenty seven primer pairs flanking microsatellites ("primers") derived from *Hordeum spontaneum* (Ramsay et al. 2000) were used to evaluate

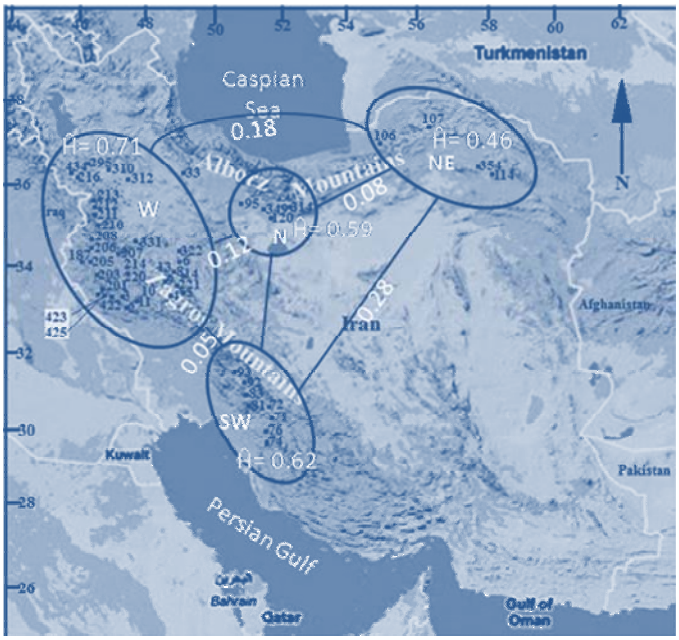

Fig. 1 Distribution of collected accessions of *Hordeum vulgare* subsp. *spontaneum* (W = West, SW = Southwest, N = North, NE = Northeast). The mean genetic diversity values (Ĥ) within each region and the genetic distance (GD) mean values between populations of different regions are shown.

microsatellite variability across accessions. The microsatellite EBmac0555 with no clear DNA fragments in PCRs was not analysed further. Marker names, primer sequences, chromosomal locations and other details regarding microsatellites are listed in table 1.

PCR amplification were carried out in 10 µL, containing approximately 50-100 ng template genomic DNA, 250 nM of each primer pair (see table 1), 0.2 mM of each dNTP, 1.5 mM Mgcl2, 1.2 U EX-*Taq* polymerase (Takara, Tokyo, Japan). PCR amplification was performed as described by Liu et al. (1996) and Ramsay et al. (2000). The amplified DNA segments were separated at 300 mA for 180 min in 1× TBE buffer, and visualized by ethidium bromide (0.5 mg/ml) staining and UV light (Wang et al. 2003). Some samples were denatured and separated on denaturing gel [containing 6% polyacrylamide (19:1 acrylamide: Bis), 8 M urea, TEMED (30 µL), 10% fresh ammonium persulphate (300 µL), 1X TBE] and visualized by silver staining method (Bassam et al. 1991) to confirm the data obtained and to compare the efficiency and resolution of different gels. Gels were scanned into Adobe Photoshop and band sizes entered into a scoring matrix.

Microsatellite data were analysed using PowerMarker software ver 3.25 (Liu and Muse 2005) Polymorphism information content (PIC) which is a measure of allelic variability and evenness at a particular locus was calculated for each locus as described by Anderson et al. (1992). Gene diversity, heterozygosity and allele number per locus were also calculated.

The scores of microsatellite alleles and calculated genetic distances were used to generate dendrograms showing relationships among accessions. Using PowerMarker software and frequency based distances after Nei and Takezaki (1983), UPGMA trees based on number of differences were constructed. Trees based on bootstrap similarity values and neighborjoining methods were also generated.

In order to determine the geographic pattern of the genetic diversity, 4 geographic regions were assigned based on topology and our knowledge of ecological conditions and then the samples were divided into four groups according to their geographic origin (Fig. 1): West (W) including western regions of Zagros Mountains (Provinces Lorestan, Ilam, Kermanshah, Kordestan and Azerbaijan-e-Gharbi; 35 accessions), Southwest (SW) including the southwest of Zagros Mountains (Fars, Kohgiluieh-va-Boyerahmad, Chaharmahal-va-Bakhtyari Provinces and south of Isfahan province; 8 accessions), North (N) including the southern slopes of Alborz Mountains (Tehran, Qazvin and Zanjan province; 7 accession), North-East (NE) including the eastern and southern slopes of

Table 1. SSR markers, chromosomal location, Tm, allele size range (bp), number of alleles, gene diversity, PIC and heterozygosity for the 26 SSR loci used for genetic diversity analysis in bulked DNA study. Chrom. (Chromosome)

SSR marker	Chrom. number	Tm (°C)	Allele size	Allele number	Gene diversity	PIC	Hetero-zygosity
HvHVA1	1H	55	132-136	2	0.375	0.304	0
WMC1E8	1H	55	197-222	4	0.484	0.443	0.25
Bmac0211	1H	55	172-192	7	0.808	0.78	0.437
Bmac0032	1H	61	214-292	17	0.918	0.913	0.562
Bmac0213	1H	55	144-168	6	0.757	0.721	0.533
Bmac0093	2H	58	150-165	4	0.369	0.347	0
EBmac0607	2H	55	148-172	5	0.701	0.646	0.375
EBmac0415	2H	55	242-265	5	0.760	0.717	0.312
Bmag0125	2H	61	130-138	4	0.750	0.703	0
Bmac0067	3H	58	136-175	7	0.646	0.615	0.416
HvLTPPB	3H	55	200-225	5	0.677	0.628	0.812
Bmag0006	3H	58	174-210	5	0.650	0.609	0.375
HVM70	3H	57,60	180-230	6	0.741	0.706	0.404
HVM03	4H	55	163-217	14	0.906	0.899	0.375
HVMLOH1A	4H	55	155-180	3	0.541	0.464	0.5
EBmac0679	4H	58	140-153	4	0.710	0.658	0.375
EBmac0906	4H	60,57	150-200	11	0.826	0.805	0.0208
EBmatc0040	5H	55	178-180	2	0.468	0.359	0.75
Bmac0096	5H	55	150-200	11	0.886	0.877	0.976
Bmag0613	6H	55	160-180	7	0.711	0.677	0.25
EBmac0602	6H	58	200-248	10	0.822	0.802	0.562
Bmag0135	7H	58	124-187	13	0.900	0.892	0.25
EBmag0794	7H	55	152-195	10	0.853	0.837	0.562
Bmac0031	7H	60	175-200	4	0.650	0.595	0.687
Bmag0341	7H	62	214-228	6	0.816	0.791	0
HVM04	7H	55	190-255	11	0.758	0.723	0.979
Mean				6.92	0.711	0.674	0.414

Alborz Mountains (Golestan, Khorasan-e-Shomali and Khorasan-e-Razavi provinces; 4 accessions). Polymorphism Information Content (PIC) and gene diversity were calculated for all SSR markers in each region and for each SSR in all regions. The average of Nei's genetic distance was calculated among accessions within each geographic region and among accessions from all regions.

In the study of single plants, 40 individuals were divided into 9 groups (Table 2) according to their geographic origin. The analysis of molecular variance (AMOVA; implemented in ARLEQUIN software ver 3.1) was carried out to partition the variation to the: among geographic regions, among subregions and within populations (among individuals). Population pairwise FSTs and frequency of alleles within each region were also calculated using ARLEQUIN software ver 3.1 (Excoffier et al. 2005).

RESULTS

Analysis of data

The 26 SSR loci revealed 180 alleles in wild barley accessions as shown in table 1. All of the SSRs were polymorphic when the results of all accessions were considered. The number of alleles ranged from 2 (EBmatc40 and HvHVA1) to 17 (Bmac032) with an average of 6.92 alleles per locus. The primer HvLTPPB detected two loci with two and three alleles. Five SSR markers showed more than 10 alleles in all accessions (table 1). Many of the alleles were exclusive, meaning that they were unique to a single accession. A relatively direct correlation between the total number of alleles, polymorphic alleles of each locus and PIC was observed.

When all populations were pooled, the PIC values ranged from 0.304 for SSR marker HvHVA1 to 0.913 for Bmac032, averaged 0.711. Seven SSRs were extraordinarily polymorphic with PIC value ≥0.80

Table 2. Four geographic regions and nine sub regions of Iran, from which individual plants of wild barley were selected for single plant studies

Region*	Subregion	Locality	No. of individuals
W	1	Darehshahr, Ilam, Eivan, Sarableh	5
	2	Mamoolan, Veisian, Khoramabad, Zagheh, Dorood	6
	3	Kermanshah, Islamabad, Mahidasht, Kamiaran	4
	4	Sanandaj, Sarvabad, Marivan, Bookan, Mahabad	5
SW	5	Shiraz, Takht-e-Jamshid, Marvdasht, Eghlid	4
	6	Noorabad-e-Mamasani, Babameidan, Yasooj, Lordegan	5
NE	7	National Park of Golestan.	2
	8	Bojnoord	4
N	9	Tehran, Firoozkooh	5

*W = west, SW = southwest, N = north, NE = northeast (See regions in Fig. 1).

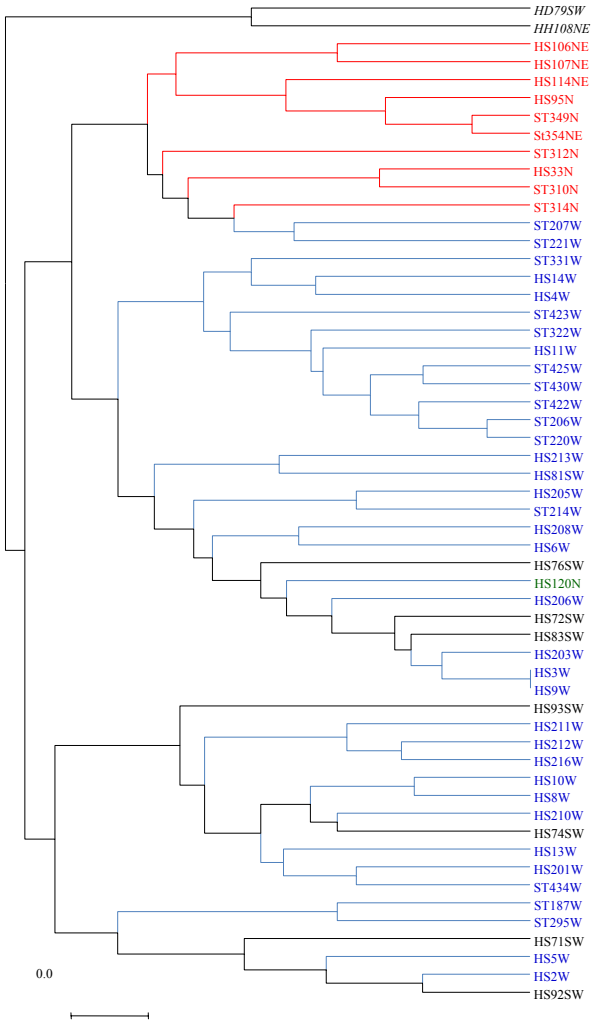

Fig. 2. The Nei`s genetic distance (Nei 1983) based dendrograms generated using microsatellite data and UPGMA clustering method, showing relationships among (a) 40 individual plants and (b) 54 Iranian populations of wild barley (*Hordeum vulgare* subsp. *spontaneum*). Accession numbers are shown with geographic origin (N, NE, W and SW) and taxon (S: *Hordeum vulgare* subsp. *spontaneum*). HD79SW and HH108NE are *H.vulgare* subsp. *vulgare* var. *distichon* and *H. vulgare* subsp. *vulgare* var. *hexastichon* respectively which are used as control.

Table 3. Comparison of allele number and number of unique alleles in populations and individuals from different regions. Abreviations: No. (Number), pop (population), indiv (Individual), Unq (Unique), W (west), SW (southwest), N (north) and NE (northeast).

	Populations				**Individuals**			
Region	No. of pops	Mean alleles/ pop.	No. of Unq alleles	Mean Unq alleles/ pop.	No. of indiv	Mean alleles/ indiv.	No. of Unq alleles	Mean Unq allele/ indiv.
W	35	38.94	8	0.23	20	28.2	5	0.25
SW	8	37.62	3	0.38	9	28.9	1	0.11
N	7	37.28	2	0.29	5	28	1	0.2
NE	4	37.25	3	0.75	6	28.5	1	0.166
Total	54	37.78	16	0.41	40	28.4	8	0.181

(table 1). When all 26 microsatellites were taken in account, at population level, average number of alleles was 37.78 (= 1.47 allele per locus) per population (ranged from 37.25 in the Northeast to 38.94 in the West). 16 unique alleles in 14 populations (~25% of populations) were found (table 3).

The accessions from each geographic region were grouped and all the gene diversity indices (Nei and Takezaki 1983) and genetic distance within groups (geographic regions) were calculated (Fig. 1). There were no significant differences in inter-populational genetic distances from each geographic region and between accessions from different geographic regions.
Average diversity statistics were calculated for each SSR in all accessions and for all SSRs in accessions from each region of origin. Based on the data of all 26 SSRs, there were no significant differences in genetic diversity calculated within regions. The within region averaged genetic diversity was ranged from 0.46 (Northeast) to 0.71 (West). There was a correlation between gene diversity and the number of accessions collected from each region.

The frequency of alleles within each geographic region was calculated. The frequency of alleles confined to a single group was higher in the accessions from the W (0.635; $\chi 2$ test of independence: $P<0.001$) than in the accessions from the SW (0.551), NE (0.593) and N (0.25).

Maximum number of polymorphic alleles were observed in Kordestan province in the West of Iran (51 alleles) and minimum one in the National Park of Golestan (1 allele) in the Northeast of country. The number of polymorphic alleles in the Lorestan province accessions (39 polymorphic alleles) from the West was also high.

No unique allele was confined to populations of a single geographic region. Seven out of 35 western accessions (20%) showed 1-2 unique alleles, whereas 3 unique alleles were found in 2 of 4 (50%) northeastern accessions (table 3).

The highest similarity (0.88) was found between populations HS203 (collected from the Ilam) and HS212 (collected from the Sarvabad) both collected from the West but distant localities. The lowest similarity (0.07) was observed between populations HS93 and HS95, collected from Lordegn (SW) and Boomehen (N) respectively. The average genetic similarity between wild barley and cultivated barley was 0.695.

In genetic distance based dendrogram generated based on SSR data, there were no significant groupings related to their geographic origin, however, more accessions collected from the North and Northeast were loosely clustered. The markers were able to group geographically closely related collections (fig. 2). The two cultivated barley accessions were grouped well away from the wild accessions indicating that the genome of cultivated barley is well differentiated from that of wild progenitor.

Results obtained from analysis of single plants:

In microsatellite analysis at individual level, gene diversity (average = 0.651) and PIC (average = 0.624) were similar to that calculated at population level (bulked DNA), but heterozygocity (average = 0.128) was significantly lower than that of populations (average = 0.414). The population pairwise FSTs were calculated to measure the genetic differentiations between individuals. When average FST values were calculated using the allelic information of all SSRs, genetic differentiation was found to be highest between individuals originated from Lordegan (SW) and Golestan (NE) and lowest between individuals sampled from Lorestan and Sanandej (both from the West). Among all four geographical groups, genetic

Table 4. Attribution of variation portions among 4 geographic regions and 9 subregions (see Table 2.) and among individuals within subregions calculated based on analysis of molecular variance (AMOVA).

Source of variation	d.f.	Sum of squares	Variation components	Percentage of variation
Among Regions	3	95.245	1.27207 Va	9.61
Among Subregions	10	198.714	4.59271 Vb	34.69
Among Individuals	25	184.400	7.37600 Vc	55.71
Total	38	478.359	13.24079	

differentiation was substantial with the mean FST value amounting to 9.6 (P<0.001). Based on the genetic similarities and generated dendrogram (data not shown) 9 subregional groups were detected (Fig. 2). In cases that more than one individual of an accession were analysed they were mainly grouped in dendrogram. When all 26 microsatellites were taken in account, an average of 28.42 alleles (1.1 allele per locus per individual) per individual (ranged from 28 in the North to 28.9 in the Southwest) were observed that were significantly lower than that of accessions (bulked DNA analysis). The unique alleles observed in individuals and also genetic similarities calculated between individuals were not similar to their relevant populations.

In analyses of molecular variances (AMOVA, table 4), a main portion of total diversity (55.71%) was attributed to the diversity among individuals within subregions. Other main portion of diversity (34.69%) was attributed to the differentiations among subregions. 90.4% of total variation was attributed to the within regions diversity. Among all four geographic groups, genetic differentiation was substantial with the mean FST value amounting to 9.6 (P<0.001).

DISCUSSION

The genetic variations present within wild progenitors of cultivated cereals, especially those growing in the center of diversity are of vitally importance and globally interest for plant breeders, who are looking for new alleles to broaden the genetic base of modern cultivars. Iran is located in the southeastern part of Fertile Crescent (where is considered as the center of diversity of wild barley and possibly center of origin of cultivated barley) with a wide range of ecological conditions. Therefore, evaluating genetic diversity in Iranian wild barley germplasm would be of high value.

In this study, the accessions were collected from nearly all geographic regions with different ecological conditions to sample all the possible variations of wild barley in Iran. The populations of spontaneous barley can be found mainly in the mountainous regions along the Alborz and Zagros Mountains.

In morphological analysis (data not shown) high level of diversity was observed but these could not be classified into different varieties within subsp. *spontaneum*. Accordingly, the microsatellites analysed here showed a wide range of alleles and high level of polymorphisms. Regarding the topology of dendrogram and calculated genetic distances, the genetic diversity present within Iranian wild barley germplasm is not clearly correlated with the geographic regions, indicating that the genetic diversity present within this germplasm in Iran is distributed all over the different geographic regions, perhaps due to the: 1) occurring gene flow between populations over long distances, or 2) the SSRs are differentiated among populations free of natural selection pressures, or 3) the selection of markers used, by chance, did not sample the geographic-related variation or, 4) the presence of mosaic pattern of ecological diversity in the geographic range of the wild barley in Iran resulted in a mosaic pattern of its diversity. Regarding to very diverse geographic structures and high degree of climatic heterogeneity in Iran, it can be suggested that the genetic diversity in wild barley, at least partly, has adaptation bases in this region. A large adaptation based morphological and physiological variability in wild barley was previously reported by Graner et al. (2003) which is supported by our data. Also Nevo et al. (1986a,b) and Hübner et al. (2009) demonstrated a strong correlation between genetic diversity and genetic based physiological traits such as tolerance to stresses (e.g. to salt, drought and temperature).

The presence of many unique alleles can be taken as another indication of high and adaptation based genetic diversity in Iranian germplasm of spontaneous barley. The presence of high number of unique SSR alleles can be accompanied with a high number of novel functional alleles, which can be utilized in crop improvement.

There were no significant correlation between groupings in dendrogram and geographical latitude, locality altitude and rainfall, indicating that diversity in wild barley can be influenced by other or a

combination of ecological factors.

Most accessions collected from the North and Northeast were loosely grouped in dendrogram, away from accessions collected from Zagros Mountains. This situation can be interpreted as establishing two different sub genepools of wild barley along Alborz (North and Northeast of Iran) and Zagros Mountains (West and Southwest of Iran). These tow genepools can be correlated with the eastern and western wild barley genotypes proposed by Morrell and Clegg (2007). Morrell and Clegg (2007) demonstrated that the accessions originated from the west and east of Zagros Mountains represent different allelic combinations. They argued that the western landraces of cultivated barley are probably originated from the western genepool (primary center) of wild barley and the eastern ones from the eastern genepool (secondary center of diversity) somewhere at the eastern end of the Iran Plateau. In this study, accessions collected from the North and Northeast of Iran showed different genetic relationships from the accessions collected from the Zagros Mountains and clearly supports the Morrell and Clegg (2007) idea of tow centers of diversity of wild barley. The populations of northern and northeastern Iran are probably parts of the secondary center of diversity spanned from Caspian regions to the Central Asia. Accessions collected from the Southwest and West of Iran were divided into 5 groups regardless their geographic origin indicating high genotypic heterogeneity in wild barley genepool along Zagros Mountains.

Comparing the results of this study with those of Forster et al. (1997), Badr et al. (2000) and Buyukunal and Akkaya (2002) which showed obviously higher diversity among wild barley genotypes from Israel compared to Turkey and Iran, and those of Feng et al. (2006) which showed a relatively low genetic diversity (average; 0.4928) in accessions collected from the west China, clearly show a gradual declining in genetic diversity of wild barley from the Fertile Crescent (center of diversity) to the eastern margins of geographic range of this taxon. As the genetic diversity decreases from the west to the east in Iranian genepool (this study) it can be concluded that the parental genepools founded in the west and then distributed eastward.

At individual level, SSR markers were able to group the geographically related collections [e.g. accessions collected from Lorestan and Ilam provinces (west), from Kordestan and Kermanshah (west), from around Tehran and Firuzkooh (north), and those collected from Kohgiloye-va-Buyerahmad (southwest)], indicating the presence of a higher level genetic diversity (local) partitioning in Iranian wild barley germplasms. Presence of local pattern of diversity that sometimes display sharp geographic differentiation over short distances, was reported by Nevo et al. (1986b) based on the results obtained from alozyme analyses, a conclusion that supported by the results of this study. Some other previous studies on the genetic diversity in *Hordeum spontaneum* (now *H. vulgare* subsp. *spontaneum*) indicated that the variation in this taxon is often correlated with ecogeographical differentiation (Saghai Maroof et al. 1994; Nevo et al. 1986b; Dawson et al. 1993). The above mentioned groupings of geographically related collections in this study can be correlated with the ecogeographical differentiations.

In analyses of molecular variances (AMOVA), a main portion of total diversity (55.71%) was attributed to the diversity among individuals within sub-regions. Other main portion of diversity (34.69%) was attributed to differentiations among subregions indicating that diversity present within wild barley germplasm is divided in local groups that can be taken as another evidence for ecogeographical based genetic diversity. 90.4% of total variation was attributed to the within regions diversity, suggesting the presence of high variability of wild barley over short distances.

When the results of individual plants analyses was take in account, a high level of homozygosity throughout the 26 loci with an overall heterozygosity of <3% and mean Inbreeding Coefficient (0.88) were observed. Comparing the allele number in individuals (1.1 allele per locus per individual) and accessions (1.47 alleles per locus per accessions) indicated that the heterozygosity in populations (pooled DNA analysis) is significantly higher than that observed at individual level, probably due to the presence of different genotypes (inbred lines) within each population. Nevo et al. (1986b) based on allozyme diversity analysis, indicated that the Iranian wild barley is extremely rich genetically, but the variation is carried primarily by different homozygotes in the population due to the predominant self-pollination.

Regarding to high levels of diversity detected among individuals, presence of many unique alleles in individuals and this fact that some alleles observed in individuals were not evident in their relevant populations (bulked DNA analyses), it can be suggested that the future genetic analyses must be performed at individual level. A simultaneous genetic analysis of Iranian cultivated barley land races and the wild germplasm would also be suggested to probe the possible introgressions and to tracking the origin of these cultivated land races. .

CONCLUSION

The results obtained from this study could be useful for

improving the understanding of diversity in and managing of wild barley germplasm collection and conservation. The high level of genetic variation among Iranian populations indicates the usefulness of each population, particularly those that have high number of polymorphic alleles (e.g. Slopes of Zagros Mountains: Kordestan, Kermanshah, Ilam and Lorestan provinces), as a valuable genetic source for selection of superior genotypes for cultivated barley improvement. The populations growing in stressful environments have wider genetic background, and therefore they are of higher value for breeding proposes. We would expect that a greater sampling would be valuable if there was a requirement to search for characters (map-based analysis) but not a broad diversity analysis such as that carried out here. Geographic related diversity analysis such as this study probably would not result in new and different findings, but analysis of local germplasms in more depth would be interesting to clarify the population genetic structures. Ecological adaptability related genetic analysis would also be valuable to search for new useful alleles to improve the modern cultivars of cultivated barley.

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