Genetic Diversity of Cultivated Barley Landraces in Iran Measured Using Microsatellites

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***Abstract*—The cultivated barley (*Hordeum vulgare* subsp. *vulgare*) is one of the major crops in the world. In this study the genetic diversity of 32 individuals of two-rowed and six-rowed Iranian landraces barley evaluated using 17 microsatellite markers. A high level of polymorphism information content (PIC; average = 0.651) and an average of 8.117 allele per locus were observed. In dendrograms constructed based on the SSR data, the two group of cultivars (var. *distichon* and var. *hexastichon*) were separated. Based on the results of this study, it can be concluded that there is a high level of genetic diversity between the barely landraces in Iran and that the barely Iranian gene pool is valuable source to search for new useful alleles for crop improvement.**

***Index Terms*— Genetic diversity, barley, Iran, microsatellite.**

I. Introduction

Cultivated barley (*Hordeum vulgare subsp. vulgare*) is one of the most important crop cereals in the tribe Triticeae (Poaceae) that cultivated over the temperate regions [1]. Based on several reports it has been originated from Fertile Crescent in Near East or from Tibetan in the west China [2], [3]. Many studies have demonstrated that Tibetan wild barley populations were clearly different from the Fertile Crescent wild barley in respect to their distribution, ecology; morphology, archaeology, cytogenetics and isozyme complement [4], [5]. Iran is placed in the southeastern edge of Fertile Crescent from where based on several evidences; the cultivation processes of barley have taken placed [3]. Morphological characters are insufficient for the discrimination of barley varieties, and recognition of barley cultivars on the basis of kernel morphology is very hard [6].

In *Hordeum* species molecular markers, such as RFLP, AFLP, STS and microsatellites have made potential the description of different cultivars, the understanding of phylogenetic relations, and genetic mapping [7]. SSRs have been increasingly used as molecular markers. Molecular markers, such as RFLP, AFLP, STS and microsatellites, provide the mainly powerful methods to assay genetic diversity, to construct genetic mapping and to categorize different varieties [7]. In *Hordeum* species molecular markers, such as RFLP, AFLP, STS and microsatellites have made potential the description of different cultivars, the understanding of phylogenetic relations, and genetic mapping [7]. SSRs have been increasingly used as molecular markers.

Regarding the high variable geographical and ecological conditions in Iran, the Iranian landraces can be considered as valuable gene sources for modern cultivar improvement. The study of genetic diversity in a germplasm such as barley landraces is fundamental to perform a strategy for landrace conservation and to find germplasem with higher priority. The aims of the present study were: (1) to determine variability a set of microsatellite markers, and (2) to use them for genome analysis, distinguishing between varieties, genotypes, and the estimation of genetic diversity in Iranian barley landraces germplasm collection.

II. Methodology

A total of 32 individuals of barley landraces were collected from various regions of Iran (from the altitude of 43m to 2051m) by two of the authors (Khodayari and Saeidi) and these were identified morphologically according to Bothmer *et al.* [1] (Table I). The accession numbers and geographic origins of samples are shown in Table I. In order to assessing genetic diversity, 17 SSR markers derived from wild barley were used [8]. Seeds from each individual plant were grown in experimental field and DNA was isolated from fresh leaves according to Komatsuda *et al.* [9]. For SSR analysis at individual level, the DNA was isolated from individual plants.

PCR amplifications were carried out in 10 μL, containing approximately 50-100 ng template genomic DNA, 250 nM of each primer, 0.2 mM of each dNTP, 1.5 mM Mgcl2, 1.2 U EX-Taq Polymerase. PCR amplifications procedure of SSR markers was performed by an initial denaturation step of 5 min at 94 ℃ followed by 30 cycles of three steps: denaturation for 30 s at 94 ℃, annealing for 30 s at 55–60 ℃, extension for 30 s at 72 ℃ with a final extension for 7 min at 72 ℃ [8]. Along with size marker tracks (100bp DNA ladder, Promega), PCR products were mixed with loading buffer (2:1) and loaded on 12% non-denaturing polyacrylamide gels (Fig. 1).

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TABLE I: ACCESSIONS CODES, ROW TYPE, ALTITUDE (METER) AND LOCALITY OF THE BARLEY LANDRACES FROM IRAN USED IN THIS STUDY. H: SIX-ROWED BARLEY, D: TWO-ROWED BARLEY.

Accession code	Row-type	Altitude	Locality
H2	six-rowed	690	Ilam, Darrehshahr
H12	six-rowed	852	Lorestan,Poledokhtar, Chamemehr
H94	six-rowed	2028	Isfahan, Mobarakeh
H100	six-rowed	43	Mazandaran, Neka
H108	six-rowed	915	Khorasan-e-shomali, Badranloo
H115	six-rowed	1200	Semnan, Damghan
D10	two-rowed	907	Lorestan, Mamoolan, Domrud
D79	two-rowed	2051	Fars, kazerun, Dashte arjan
D88	two-rowed	1588	Yasooj, Toot
D110b	two-rowed	1288	Khorasan-e-Shomali, Sisab, Nodeh
D110w	two-rowed	1288	Khorasan-e-Shomali, Sisab, Nodeh
D201	two-rowed	855	Ilam, Mishkhas, Chenaran
D215	two-rowed	1587	Kordestan, Marivan, Sarshio

The amplified DNA segments were separated at 300 milliamp for 180 min in 1× TBE buffer, and visualized by Ethidium bromide (0.5 mg/ml) staining and UV light [10]. Amplification and scoring of microsatellite loci were repeated until all loci had been reliably scored in all accessions (Fig. 1). Gels were scanned into Adobe Photoshop and band sizes entered into a scoring matrix.

The microsatellite data were analyzed by PowerMarker software ver 3.25 [11], and diversity parameter such as

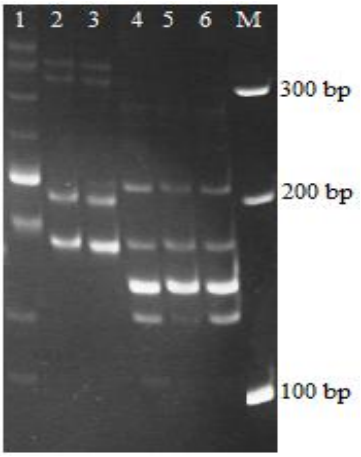

Fig. 1. Example of microsatellite variation in *Bmac0213* locus. Each band indicates an allele at the locus. Lanes 1-3 = *H. vulgare* subsp. *vulgare* var. *distichon* individuals; lanes 4-6 = *H. vulgare* subsp. *vulgare* var. *hexastichon* individuals and *M* = Molecular marker.

(Polymorphism Information Content), Heterozygosity, and inbreeding coefficient (*f*) were calculated. *PIC* which is a measure of allelic variability and evenness at a particular locus was calculated for each locus using formula:

$$PIC = 1-\sum (P_i)^2$$

where *Pi* is the proportion of samples carrying the *ith* allele of a particular locus [11].

A genetic distance based UPGMA tree showing relationships among accessions was generated. Trees based on bootstrap similarity values and neighbor joining methods were also constructed (data not showed). Analysis of molecular variance (AMOVA) implemented in ARLEQUIN software ver. 3.0 was performed to partition the diversity to among accessions and among different geographic region [12].

III. RESULTS AND DISCUSSION

TABLE II: SSR MARKERS, ALLELE NUMBER (AL. NO.), GENE DIVERSITY, HETEROZIGOSITY (HT.) PIC AND INBREEDING COEFFICIENT (F) FOR THE 17 SSR LOCI USED FOR GENETIC DIVERSITY ANALYSIS OF CULTIVATED BARLEY (BOTH TWO-ROWED AND SIX-ROWED) INDIVIDUALS.

SSR Marker	No. of alleles	*Ht.*	*PIC*	*f*
HvLTPPB	16	0.53	0.881	0.417
Bmac0213	16	0.16	0.921	0.836
EBmac0602	10	0.00	0.853	1
Bmac0032	16	0.00	0.905	1
Bmag0135	14	0.03	0.892	0.966
Bmag0613	10	0.00	0.817	1
HVM0003	13	0.00	0.878	1
Bmac0211	7	0.00	0.784	1
EBmatc0040	2	0.00	0.375	1
HvHVA1	4	0.06	0.576	0.905
WMC1E8	2	0.00	0.358	1
Bmag0006	7	0.00	0.697	1
HvMLOH1A	3	0.00	0.115	1
EBmac0607	2	0.00	0.058	1
Bmac0031	4	0.59	0.521	0/025
EBmag0794	5	0.00	0.676	1
EBmac0415	7	0.00	0.799	1
Mean	8.117	0.08	0.653	0.885

The 17 primer pairs were assayed on all 32 individuals; there were 161 alleles (Table II). As shown in Table II, the microsatellites were highly polymorphic. The overall average number of alleles per loci was 8.11 ranged from 2 (*EBmatc0040*, *EBmac0607* and *WMC1E*8) to 16 (*HvLTPPB*, *Bmac0032* and *Bmac0213*) (Table II). Seven loci (*HvLTPPB*, *Bmac0213*, *EBmac0602*, *Bmac0032*, *Bmag0135*, *Bmag0613* and *HVM0003*) of the 17 loci showed more than 10 alleles per locus. The highest number of alleles was detected by microsatellite markers *HvLTPPB*, *Bmac0213* and *Bmac0032* with 16 alleles, whereas the *EBmatc0040*, *WMC1E8* and *EBmac0607* with only 2 alleles detected, had the lowest allele number. There are some investigations that have been reported an average of 18 alleles per locus based on four SSRs estimate in the 207 genotypes [13] and there are other reports that have assay average numbers of alleles in barley, varying from 2.1 to 12.2 [14]. The Locus *HVM003* revealed 13 alleles (Table II) whereas other researchers found 2 alleles for this locus in Tibetan barley [5]. This

supports the hypothesis of separate evolutionary systems leading to Iranian barley from Tibetan barley. Number of polymorphic alleles in 16 individuals of tow-rowed and in the same number of individual's six-rowed barley landraces were detected 58 and 43, respectively. It is indicated that two-rowed barley landraces have more genetic diversity than six-rowed; probably because of the most of breeding programs in Iran are achieved on six-rowed genotypes. The highest number of polymorphic allele belongs to the population D215 from mountainous region in Marivan (Kordestan province at the West of Iran) with 17 polymorphic allele and accession H12 was without polymorphic allele.

Considering the total germplasm collection, PIC values ranged from 0.058 (*EBmac0607*) to 0.921 (*Bmac0213*) with the average of 0.653 for the 17 SSRs surveyed in both the var. *distichon* and var. *hexastichon* accessions (Table II). The average PIC values the average within the var. *hexastichon* group was 0.548 (Table III). Within the var. *distichon* group, PIC values ranged from 0.00 (*EBmatc040* and *WMC1E8*) to 0.880 (*Bmac003*2), while the PIC values within var. *hexastichon* ranged from 0.00 (*EBmatc040, HvHVA*1, and *EBmac607*) to 0.852 (*Bmac0032*) (Table III). In the before studies on barley, Similar PIC values for SSRs are reported [7], [15].

Presence of many unique alleles can be taken as another indication of high genetic diversity in Iranian germplasm of cultivated barley landraces; however, it can also be resulted by the high rate of mutation at SSR loci.

TABLE III: NUMBER OF ALLELES AND PIC FOR EACH SSR LOCUS IN IRANIAN LANDRACES OF *HORDEUM VULGARE SUBSP. VULGARE VAR. DISTICHON* (TWO-ROWED) AND *VAR. HEXASTICHON* (SIX-ROWED).

	No. of alleles		PIC	
Locus	Two-rowed	Six-rowed	Two-rowed	Six-rowed
HvLTPPB	9	11	0.797	0.793
Bmac0213	10	9	0.852	0.842
EBmac602	7	5	0.778	0.700
Bmac0032	11	9	0.880	0.852
Bmag135	10	5	0.864	0.716
Bmag613	8	5	0.815	0.700
HVM03	6	7	0.743	0.754
Bmac0211	3	5	0.555	0.617
EBmatc40	1	1	0.000	0.000
HvHVA1	3	1	0.455	0.000
WMC1E8	1	2	0.000	0.304
Bmag06	4	5	0.530	0.668
HvMLOH1A	3	1	0.214	0.000
EBmac607	2	1	0.110	0.000
Bmac31	3	3	0.446	0.555
EBmag794	3	5	0.427	0.711
EBmac415	5	7	0.746	0.769
Mean	5.235	4.823	0.542	0.528

We have examined the level of Heterozygosity and inbreeding factor (f) within and between 17 microsatellite loci from a sample of Iranian barley landraces (Table II). The Heterozygosity mean and Inbreeding coefficient (f) in the barley accessions was calculated 0.080 and 0.885 respectively (Table II). This estimation confirms that *Hordeum vulgare* is an inbreeding species. Barley landraces had a rate of self-fertilization of 88.50%, which results in a very low level of heterozygosity (8.00%). Morrell *et al.* [16] estimated inbreeding rate of 98% for 25 individuals of wild barley accessions.

In analysis of molecular variances (AMOVA), a main portion of total diversity (60.7%) was attributed to differentiations among populations within groups (Table IV). Other main portions of diversity (21.1%) were explained by the diversity within populations and 18.2% percentage of variances was clarified by the two-rowed/ Six rowed barley differentiation. The high FST value (78.9%) indicated that genetic differentiation between *var. hexastichon* and *var.distichon* is a considerably high (Table IV). In some previous studies on the Nordic and Baltic materials such as Kolodinska Brantestam *et al.* [17], the AMOVA rowed barley differentiation. The high FST value (78.9%) indicated that genetic differentiation between *var. hexastichon* and *var.distichon* is a considerably high (Table IV). In some previous studies on the Nordic and Baltic materials such as Kolodinska Brantestam *et al.* [17], the AMOVA(population) of *H. vulgare* subsp. *vulgare* var. *distichon* both from the central Zagros Mountains (D10 from Lorestan and D88 from Yasooj), that were not correlated to their origins. As it have shown in the dendrogram, two-rowed barley accessions include two subgroups (I and II) and six-rowed barley accessions also divided to two subgroups (I and II). Subgroup I comprising H115 indivduales that it is a new elite accession of six-rowed barley and subgroup II include another six-rowed accessions. Subgroup III comprising D10 and D88 both from Central Zagros and subgroup IV includes another two-rowed accessions from the Western Zagros and the Northeast of Iran. There is no correlation between geographic origins and topology of accessions on dendrograms. The dendrograms (Fig. 2) confirms the high level of genetic diversity within the accessions and level of diversity present within the var. *distichon* accessions is more than that of the var. *hexastichon* germplasm. Mean genetic similarity between var. *ditichon* and var. *hexastichon* was 0.76.

In general the clustering patterns of individuals were not correlated to geographical origin at the population's level. Other researchers have reported that classifications of cultivated barley accessions based on SSRs reflect geographic origin [5], [17] and [18].

TABLE IV: ANALYSIS OF MOLECULAR VARIANCE (AMOVA) OF TWO-ROWED AND SIX-ROWED IRANIAN BARLEY LANDRACES. THE STATISTICS INCLUDE VARIANCE COMPONENT ESTIMATES (COMP V), THE PERCENTAGE OF THE TOTAL VARIANCE (COMP %) CONTRIBUTED BY EACH COMPONENT. THE PROBABILITY (P) OF OBTAINING A MORE EXTREME COMPONENT ESTIMATE WAS OBTAINED WITH 1,023 PERMUTATIONS. *P < 0.001

Source of variation	d.f.	Sum of squares	Comp V	Comp%
Between two-rowed and six-rowed accessions	1	68.062	2.605 *	18.20
Among accessions within the same row types	11	264.583	8.688 *	60.69
Within accessions	19	57.417	3.020 *	21.11
Total	31	390.062	14.316	

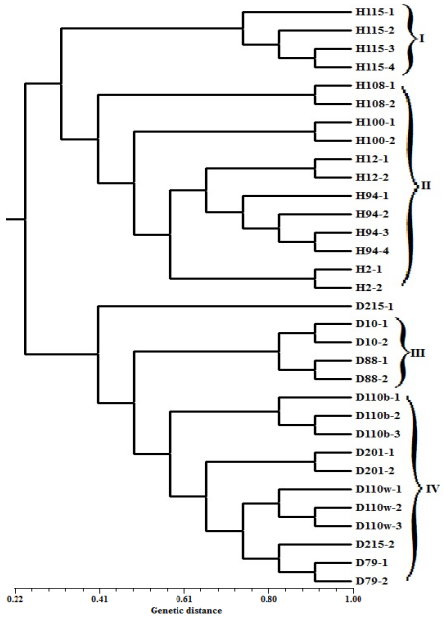

Fig. 2. The Nei`s genetic distance (Nei 1983) based dendrograms generated using microsatellite data and UPGMA clustering method, showing relationships among 32 individual plants of Iranian landraces barley *H.vulgare* subsp. *vulgare* var. *distichon* (D) and *H. vulgare* subsp. *vulgare* var. *hexastichon* (H) supported by adequate data and critical details.

IV. CONCLUSION

The traditional landraces and wild relatives of cultivated cereals are important gene sources to broaden the genetic bases of modern cultivars, which have narrowed gene pool due to intensive breedings. The gemplasms presented in the center of diversity and those grow in the regions with high degree of geographical and ecological diversity would be of highest importance. Based on the results of this study, high level of genetic diversity was observed in Iranian barley germplasm (especially in tow-rowed in the West of Iran). The genetic diversity was distributed all over the different regions and there were no groupings related to the origin of accessions. The two-rowed and six-rowed varieties were separated indicated that these two varieties are genetically distant and have probably different origins.

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