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Genetic variation of Iranian barley landraces, commercial varieties and improved breeding lines using SSR markers

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Abstract

In order to estimate the level of genetic diversity of 70 Iranian barley genotypes, commercial varieties and improved breeding lines, 100 microsatellite primers were employed. Genomic DNA was extracted from fresh leaves and amplification reactions were done by PCR. The amplification products were separated on 6% denaturing polyacrylamide gel. Seventy one primers amplified 290 alleles with the range of 2 to 15 and an average of 4.08 alleles per locus. The mean gene diversity, polymorphic information content (PIC) and frequency of major allele were 0.42, 0.38 and 0.68, respectively. Analysis of molecular variance based on separating genotypes into two groups (Iranian barley genotypes vs. commercial varieties and improved lines) indicated an acceptable genetic diversity between these groups. Furthermore, larger proportion of within group variation (88%) of the total molecular variance determined the presence of high genetic variation within the groups. Nei's gene diversity index and Shannon's index were 0.4 and 0.75 for Iranian barley genotypes and 0.39 and 0.7 for commercial and improved lines, respectively, indicating the existence of reliable genetic diversity within both groups. Cluster analysis based on Minimum Evolution algorithm and Jukes-Cantor distance coefficient assigned the genotypes into three groups and within a group. In conclusion based on the molecular data analysis, substantial amount of genetic diversity was observed among the barley genotypes under study which can be utilized in breeding programs for selecting and producing superior barley lines.

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Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crops of Iran (Kohansal and Rahimi, 2013). Also, this crop has worldwide importance due to its compatibility to poor and saline soils. Barley is a founder crop of old world Neolithic food and one of the earliest domesticated crops. Barley is used for animal and human feed and also malt industry. Barley has high yield potential and is an early maturing and short season cereal that is cultivated between extremes of altitudes where other crops are not simply adapted (Von Bothmer *et al.*, 2003). This ability of adaptation is mainly due to wide genetic diversity of barley germplasm that is second largest after wheat (Sun and Gong, 2010). Nowadays, the genetic diversity of barley is decreasing because of the widely use of improved verities and

South west of Iran (eastern part of Fertile Crescent) is the region between primary (Jordan) and secondary (Himalaya and Tibet) habitats of barley where is one of the main diversification centers of this plant. So, Iranian landraces that resulted from introgression between wild and cultivated *Hordeum*species of these habitates, could be a valuable genetic resource of barley germplasm (Badr *et al.*, 2000; Khodayari *et al.*, 2012).

One of the most important aspects about evaluation of barley germplasm is determination of genetic diversity (Sun and Gong, 2010). DNA molecular markers are appropriate instruments for determining genetic diversity, because of their independence from environment and growth stages, abundance and high polymorphism (Garge *et al.*, 2001; Tomka *et al.,* 2013). In *Hordeum* genus several types of polymerase chain reaction (PCR) based markers have being employed for studding genetic diversity, including amplified fragment length polymorphism (AFLPs), sequence tagged sites (STSs) and simple sequence repeats (SSRs) or microsatellites (Liang and Pardee, 1992; Matus and Hayes, 2002; Feng *et al.*, 2006; Kojima *et al.*, 2007; Pan *et al.*, 2008; Ben Naceur *et al.*, 2011; Khodayari *et al.*, 2012). Some of advantages of SSRs such as high polymorphism and reproducibility, locus specificity, co-dominance and genomic prevalence make them as superior marker system for studding genetic diversity (Liu *et al.*, 1996; Powell, 1997; Wang *et al.*, 2009; El-Awady and El-Tarras, 2012).

The purpose of this study was to evaluation the genetic diversity in barley landraces and improved lines from Iran and determination of differences between them and some commercial varieties in aspect of microsatellites polymorphism.

Materials and methods

Plant materials and DNA extraction

The plant materials used in this study (Table 1) were 70 barley (*Hordeum vulgare* L.) genotypes, including 47 Iranian and two Chinese landraces, 12 commercial varieties and nine improved breeding lines which all had been provided by dry land agricultural research institute of Iran (DARI).

Table 1. List of studied barley genotypes.

No.	Name	Code	No	Name	Code
1	Iran/Kerman1	72562	37	Iran/ Torbatejam	72482
2	Iran/Gorgan1	72565	38	Iran/ Azarbayejan	72553
3	Iran/Gorgan2	72566	39	Iran/ Miandoab2	72588
4	Iran/Gorgan3	72566	40	Iran26	72646
5	Iran/Gorgan4	72566	41	Iran27	72646
6	Iran/Kerman2	72568	42	Iran28	72680
7	Iran/Kerman3	72581	43	Iran29	72680
8	Iran1	72584	44	Iran30	72685
9	Iran/Miandoab1	72587	45	Iran31	72686

No.	Name	Code	No	Name	Code
10	Iran2	72602	46	Iran32	72704
11	Iran3	72611	47	Iran33	72715
12	Iran4	72646	48	Iran34	72715
13	Iran5	72647	49	Iran35	72744
14	Iran6	72647	50	Iran36	72747
15	Iran7	72649	51	Unknown1	Advanced line
16	Iran8	72650	52	Unknown2	Advanced line
17	Iran9	72653	53	Unknown3	Advanced line
18	Iran10	72655	54	Unknown4	Advanced line
19	Iran11	72664	55	Unknown5	Advanced line
20	Iran12	72665	56	Unknown6	Advanced line
21	Iran13	72666	57	Unknown7	Advanced line
22	Iran14	72668	58	ICARDA1	Rihane-03
23	Iran15	72672	59	Iran37	Makoee
24	Iran16	72673	60	Iran38	Sahand
25	Iran17	72674	61	Iran39	Abidar
26	Iran18	72675	62	ICARDA2	Dayton / Ranney
27	Iran19	72684	63	ICARDA3	Yea/168
28	Iran20	72689	64	ICARDA4	Denmark
29	Iran21	72703	65	Turkey1	Obruk-86
30	Iran22	72712	66	Iran40	Gara-arpa
31	Iran23	72726	67	Unknown8	EC-79
32	China1	72372	68	Turkey2	Bulbul
33	China2	72382	69	Russia1	Dicktoo
34	Iran24	72472	70	Russia2	Radical
35	Iran25	72472	71	Russia3	Dobrynya

Genomic DNA was extracted from fresh leaves tissue of young plants using CTAB method (Saghai-Maro of *et al.*, 1994). Extracted DNA samples were then quantified and qualified by using both spectrophotometer and %0.8 agarosegel electrophoresis methods. DNA samples were diluted to 25 ng/µl for PCR reactions.

SSR analysis

Screening of genotypes was accomplished by means of 71 pair of SSR primers (Table 2). PCR reactions were carried out in 10 µl volume containing5 µl *Taq* DNA polymerase master mix, 0.5 µl of each primer, 1 µl genomic DNA and 3 µl deionized water. Sinagen company master mix was used in this study that included *Taq* DNA polymerase, DNTPs, $MgCl_2$ and PCR buffer. Thermal cycles for SSR amplification included 95°C for 4 min for initial denaturation step, followed by 35 cycles of three steps including denaturation for 1 min at 95°C, annealing for 55 second at 53-62°C and extension for 1 minute at 72°C with a final extension for 7 minutes at 72°C. The amplified fragments were separated on %6 denaturing polyacrylamide sequencing gels (Fig. 1) in 1X TBE buffer (Kadri *et al.*, 2009). DNA fragments were visualized via silver nitrate staining method (Bassam *et al.*, 1991).

Table 2. Chromosomal location and annealing temperature of SSR primers.

No	Primer	Chromosome	Ta (°C)	No	Primer	Chromosome	Ta (°C)
1	GBM1422	4H	52	37	scssr09398	6H	55
2	HVM77	4H	51	38	GBM1270	6H	60
3	Bmac0273	5H	53	39	GBM1215	6H	55
4	GBM1252	4H	60	40	GBM1355	6H	60
5	Bmac0067	3H	55	41	GBM1212	6H	60
6	GBM1425	3H	57	42	Bmac0018	6H	60
7	Bmac0209	3H	59	43	GBM5012	6H	62
8	GBM1495	3H	60	44	Bmag0003	6H	58
9	scssr25691	3H	54	45	Bmgttttt0001	6H	62
10	GBM1405	3H	62	46	scssr02093	6H	62
11	Bmag0306	3H	55	47	Bmag0613	6H	61
12	HVM70	3H	52	48	EBmac0602	6H	61
13	scssr02306	5H	59	49	scssr05599	6H	57
14	GBM1176	5H	53	50	GBM1140	6H	56
15	scssr07106	5H	60	51	scssr00103	6H	61
16	GBM5028	5H	53	52	GBM1274	6H	55
17	Bmac0047	7H	56	53	GBM1275	6H	60
18	GBM1325	5H	59	54	GBM1087	6H	62
19	GBM1222	5H	55	55	GBM1404	6H	58
20	Bmag0323	5H	59	56	GBM1126	7H	56
21	HVLEU	5H	59	57	HvWaxy4	6H	56
22	Bmag0005	5H	58	58	GBM5060	7H	61
23	GBM1399	5H	58	59	GBM1464	7H	58
24	GBM1426	5H	57	60	GBM1432	7H	56
25	scssr16991	5H	62	61	Bmac0273	7H	56
26	scssr15334	5H	62	62	GBM1237	7H	58
27	GBM1483	5H	61	63	GBM1492	7H	60
28	scssr05939	5H	59	64	EBmac0827	7H	60
29	GBM1231	5H	57	65	GBM1516	7H	58
30	GBM1141	5H	56	66	Bmac0167	7H	55
31	GBM5008	5H	57	67	Bmag0571	6H	56
32	GBM1436	5H	59	68	Bmag0746	7H	53
33	GBM1463	5H	60	69	GBM1303	7H	56
34	GBM1490	5H	55	70	GBM1472	7H	62
35	scssr03907	5H	60	71	Bmac0064	7H	56
36	BAC84c21	6H	55				

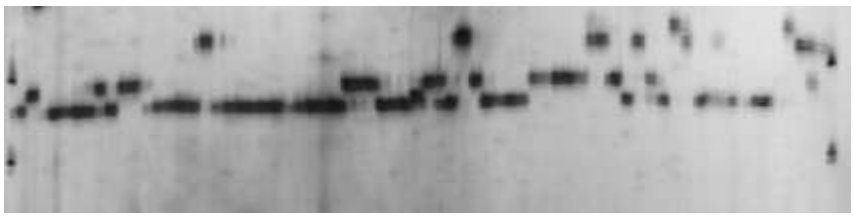

Fig. 1. SSR bands of various cultivars of Barley on %6 denaturing polyacrylamide gel.

Data analysis

Banding patterns of polymorphic SSR markers were scored as co-dominant method (A, B, C ...) and then recoded to binary 1 and 0 mode by considering alleles of each microsatellite locus as dummy variables.

To determine the efficiency and differentiation power of markers, the polymorphism information content (PIC, Smith *et al.*, 1997) and the gene diversity index (H_e, Nei, 1973) for each locus were calculated using the following formulas:

$PIC=1-\sum p_i^2-2\sum p_i^2 p_j^2$

$H_e=1-\sum p_i^2$

Where p_i and p_j are the frequencies of i^{th} and j^{th} alleles, respectively. These parameters were estimated by Power Marker software version 3.25 (Liu and Muse, 2005).

The genotypes were separated into two groups (Iranian landraces vs. other genotypes) and Analysis of molecular variance (AMOVA) was performed by Gen ALEx version 6.4 software (Peakall and Smouse, 2010) based on two mentioned groups. Nei's gene diversity index (Nei, 1973) and Shannon information index (Shannon, 1948) were calculated as within group variation indices by Pop Gen version 1.32 software (Yeh *et al.*, 1997).

For studding genetic relationships between genotypes phylogenetic tree based on minimum Evolution method was performed using the MEGA version 5.05 software (Tamura *et al.*, 2011). Associations among the genotypes from different groups were assessed by Principle Coordinate Analysis (PCoA) using the Gen ALEx version 6.41 software (Peakall and Smouse, 2010).

Results and discussion

Power statistics of SSR markers

In this study, 71 pairs out of 100 pairs of employed primers produced polymorphic bands suitable for SSR analysis. A total of 290 alleles were observed in the 71 SSR loci with an average of 4.08 alleles per locus. The number of alleles by each SSR primer pair varied from 2 (HVM77, GBM1425, HVM70, GBM1176, HVLEU, GBM5028, GBM1325, GBM1426, GBM1141, GBM1436, GBM1463, GBM1215, GBM1140, GBM1274, GBM1275, GBM1404 and GBM1432) to 15 (scssr03907). Pandey *et al.* (2006) detected a total of 165 alleles with average of 6.1 alleles per locus through 107 barley genotypes. The difference in the number of alleles indifferent studies is usually affected by differences in the number of genotypes; their genetic base and employed micros at ellites position in genome.

Diversity index of SSR loci ranged from 0.05 (HVM77, GBM1325, GBM1274, GBM1404) to 0.86 (EBmac0827) with an average of 0.42 for each primer pair. Also, the highest and lowest PIC values which is a criterion of allelic variability were 0.84 (EBmac0827) and 0.05 (HVM77, GBM1325, GBM1274 and GBM1404), respectively, with the average of 0.38 for each locus. In comparison whit the results, diversity index in barley was reported 0.03 to 0.89 by Stam *et al.* (2007). In other study on barley, similar average value of PIC (0.38) was reported by Pillen *et al.* (2000) for SSRs. These results indicated that whatever number of microsatellite alleles in a locus increases, the amount of PIC and diversity index will be more so that there is a positive correlation between these three parameters. High values of these three parameters demonstrate the more power of marker for estimating genetic variation and determining population structure (Matus and Hayes, 2002).

Analysis of molecular variance (AMOVA)

AMOVA was performed based on two defined separate groups of genotypes (Iranian barley landraces vs. commercial varieties and improved lines). The results indicated that the main portion of genetic diversity (%88 of whole genetic diversity) was attributed to within groups, while %12 was between groups (Table 3). Therefore obvious larger proportion of within group variation of the total molecular variance determined the presence of high genetic

variation within Iranian landraces as well as commercial varieties and improved lines. Nei's gene diversity index and Shannon information index were separately calculated for each population which these components were 0.4 and 0.75 for Iranian landraces and 0.39 and 0.7 for commercial and improved lines, respectively. Due to the modality of these indicators, which expresses the genetic richness, a good level of genetic diversity was found for both populations. Confirming the results, Abderrazek *et al.* (2008) reported %45.6 of between and %54.4 of within population genetic variation in a study on worldwide collection of barley landraces based on DNA markers indicating more variation within populations comparing between them. This result can explain persistence of a high and enough genetic variation within local populations for breeding studies.

Table 3. Analysis of molecular variance (AMOVA) of barley genotypes.

Source of variation	Degree of freedom	Sum of squares	Variance	Percentage variation (%)
Among groups	1	147.63	4.03	12
Within groups	68	1966.78	28.92	88

Principal Coordinates Analysis (PCoA)

In Principal Coordinates Analysis, the first three vector sexplained %76.57 of total molecular variance. PCoA distinctly separated Iranian landraces from commercial and improved lines via second vector. The results of PCoA and AMOVA were closely correlated, so that according to Fig. 1, the high within group variation of genotypes and low variation between them observed in PCoA bi plot considering the position of genotypes According to our results, Leisova and Kucera (2010) reported a clear distinction between the barley landraces of different countries and commercial barley in Principal Coordinates Analysis based on SSRs.

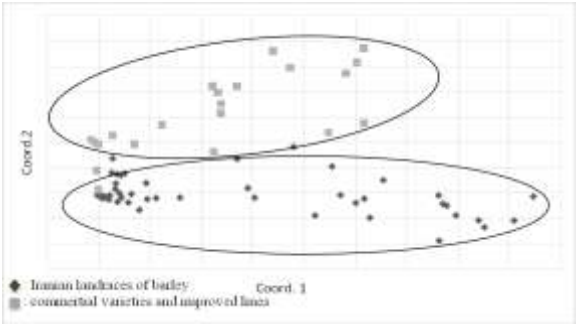

Fig. 2. Principal coordinate analysis for barely genotypes.

Cluster analysis

Cluster analysis based on Minimum Evolution algorithm and Jukes-Cantor distance coefficient assigned the genotypes into four groups (Fig. 3). This analyze is beneficial tool for studying genetic relations among barley genotypes and revealed that most of genotypes grouped according to their origins. First and second groups containing 23 and 22 genotypes, respectively, which in this groups, Iranian barley genotypes, commercial varieties and improved breeding lines was distinct from each other so that first group was included 21 commercial varieties and improved breeding lines and second group was included 19 Iranian barley genotypes. The third and fourth categories were included 5 and 20 Iranian barley genotypes, respectively. The presence of Iranian barley genotypes in all three groups showed high genetic diversity in these genotypes. Naeem *et al.* (2011) have differentiated local barley of Pakistan and other genotypes from different country in their created dendrogram.

Finally, according to the results about the number of alleles and amount of PIC for SSR loci, could be stated that there is a high level of genetic diversity among Iranian barley landraces. Also, Iranian barley landraces showed different evolutionary nature from other commercial cultivars in term of SSRs. So, they could be useful for the development of breeding programs. The results confirmed that the traditional landraces like Iranian barley landraces are important gem resource for breeding programs.

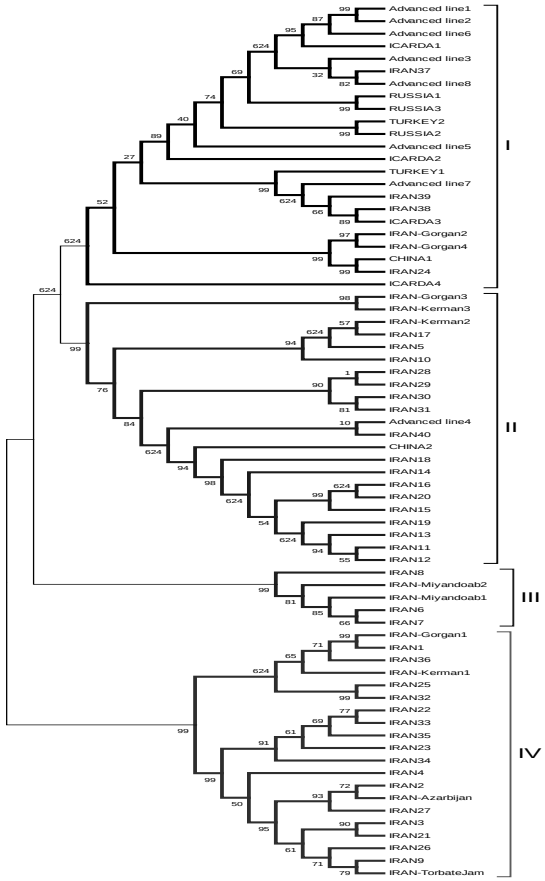

Fig. 3. Dendrogram showing the relationship among 71 barley genotypes based on SSR.

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