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Anther culture response and genetic relationships between Iranian and European barley (*Hordeum vulgare* L.) cultivars

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Abstract

Barley is a widely adaptable cereal grain, but with a low response from anther cultures to callus induction. In this study, the response to anther culture and the genetic diversity of 16 Iranian and 26 European barley cultivars were evaluated using 20 ISSR (Inter Simple Sequence Repeats) primers. The callus induction phase was investigated based on a completely randomized design with an unequal number of replications. The regeneration phase was studied using a factorial experiment based on a completely randomized design with 3 replications. The factors included the tested cultivars and several growth regulators. All cultivars that responded to anther cultures (8 of 42 cultivars) had European origin. A correlation analysis showed the highest correlation between the callus size and its growth rate. A cluster analysis based on anther culture traits grouped 8 cultivars into 2 classes. The effect of growth regulators was not significant for total regeneration ratio, green and albino plants ratio. The results showed that the efficiency of the anther culture method is largely dependent on the plant genotype. The percentage of polymorphism using ISSR markers ranged from 50 to 78.94. The range of genetic similarity coefficients among barley cultivars was from 0.138 to 0.867. According to the Mantel test, there was no significant correlation between the assumed variation patterns using ISSR molecular markers and anther culture data. The cluster analysis based on the ISSR markers divided 42 barley cultivars into 6 classes, although there was no clear pattern of clustering for differentiation between Iranian and European cultivars. Iranian cultivars showed a higher molecular variation than European ones.

Key words: anther culture, callus induction, genetic diversity, ISSR markers, Mantel test

Introduction

Barley (*Hordeum vulgare* L.) is a widely adaptable crop with a short growing season. It is a member of the grass family, having 14 chromosomes (Maniruzzaman et al., 2014). In terms of the world's crop production, barley is the 4th most important cereal following wheat, rice and corn (Feug et al., 2006). Barley is a component of various health foods, it is also used as animal fodder, and is a source of fermentable material for beer making and certain distilled beverages. Barley cultivars are divided in two-row or six-row cultivars based on the spikelet organization. The head of a two-row barley contains two-rows of kernels along its length, whereas the head of a six-row barley contains six-rows of kernels (Komatsuda et al., 2007).

The production of doubled haploids through androgenesis is essential, because complete homozygous plants can be produced within a year, compared to long-term plant breeding methods. Unfortunately, many important crops are recalcitrant to androgenesis (Islam and Tuteja, 2012). One of the problems in androgenesis via anther or pollen cultures is the occurrence of albinism. This can cause alterations in the developmental pathways of microspores, the survival rates of *in vitro* cultures, the efficiency of androgenic embryo production and the ability of green plants to regenerate (Makowska and Oleszczuk, 2014). A low response of anther cultures to callus induction, low numbers of regenerated plants and production of albino plants affect the efficiency of anther cultures (Turasheva, 2015). On the other hand,

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considering the importance of genetic diversity and its role in population survival, molecular markers proved to be useful tools in the characterization and evaluation of genetic diversity within and between species and populations (Giancarla et al., 2012). One of the PCR based molecular marker techniques is Inter Simple Sequence Repeat (ISSR) (Pradeep Reddy et al., 2002). ISSR analyses have been used for the evaluation of genetic diversity in chickpea (Fazeli and Cheghamirza, 2011), wheat (Abou-Deif et al., 2013), bean (Khaidizar et al., 2012), and barley (Fernandez et al., 2002). ISSR markers can also be used as tools for estimation of the genetic diversity in barley resource collections (Eshghi et al., 2012). The evaluation of some Romanian barley cultivars using ISSR markers has revealed the existence of a high genetic variability among tested cultivars (Giancarla et al., 2012). Therefore, the aims of the present study were to evaluate the response to anther cultures, to study the genetic diversity of Iranian and European barley cultivars and to determine the correlation between variation patterns in studied cultivars based on anther culture data and ISSR molecular markers.

Materials and methods

Plant material

Forty-two barley cultivars were planted in the 2013-2014 growing season in the research field of Razi University, Kermanshah (latitude 34°19′N, longitude 47°7′E, and altitude 1322 m.a.s.l) located in the west of Iran, with a moderate-cold and semi-arid climate and mean annual rainfall of 450-480 mm. Sixteen barley cultivars were provided from the Dry Land Agricultural Research Institute (DARI), Iran and 26 from the Genomics Research Center (CRA-GPG), Italy. The characteristics of 42 barley cultivars are presented in Table 1.

Anther culture

Callus induction phase

The callus induction phase was investigated based on a completely randomized design (CRD) with an unequal number of replications. Thirty sterile anthers were placed in petri dishes filled with 20 ml of solid FHG (Hunter, 1988) induction medium supplemented with 2 mg/l 2,4-D and 0.5 mg/l kinetin. The cultured anthers were incubated in a germinator at 25 °C for 4 to 8 weeks in the dark.

Regeneration phase

The regeneration phase was studied in a factorial experiment based on a completely randomized design with 3 replications. The factors included a cultivar and a plant growth regulator. MS medium (Murasnige and Skoog, 1962) containing 3 plant growth regulator concentrations was used for the regeneration phase (Table 2). For plant regeneration, calli were maintained at 25 °C and 16 h of artificial daylight. Green plants were transplanted into laboratory glassware with MS medium without growth regulators. For a gradual adaptation, green plants were transferred to capping small pots. After a gradual adaptation, stage plants were transferred into larger pots.

Evaluated traits

In the induction phase, the following traits were recorded: the percentage of callus induction (the number of calli per 30 anthers), the number of days to callus induction, the size of the callus (mm) and its growth rate. In the regeneration phase, the measured traits were: the total number of regenerated plants and the number of albino and green regenerated plants (the number of plants per five calli).

Inter Simple Sequence Repeat (ISSR) assay

Genomic DNA extraction

Genomic DNA was isolated from 100-200 mg young leaf tissues using the Dellaporta method (Dellaporta et al., 1983). DNA quantity and quality were determined with a spectrophotometer, and DNA samples were diluted to 25 ng/µl with deuterium-depleted water (DDW).

Polymerase chain reactions

PCR was performed using 20 ISSR primers in a 25-µl volume containing 0.8 ng/µl of template DNA, 3.2 mM of $MgCl_2$, 0.05 mM of dNTP, 1 µM primer and 1 U of *Taq* DNA polymerase. ISSR-PCR reactions were performed in a Palm-Cycler in the following conditions: 4 min at 94 °C as an initial step and 40 cycles of 30 s denaturing at 94 °C, for 40 s at the annealing temperature for every primer, 1.5 min at 72 °C for extension, followed by a final extension at 72 °C for 5 min and cooling at 30 °C for 10 min. 10-µl of PCR products were analyzed on 1.2% agarose gel in TAE buffer running at 90 V for 2 h. The gels were stained using ethidium bromide.

Table 1. Pedigree and some characteristics of the analyzed barley cultivars

No	Cultivar	Pedigree	Geographic location	Growth habit	Grain type	Row type
1	Fajr 30	Lignee131/ Gerbet//Alger- Ceres/ jonoob	Iran	F	hulled	6
2	Gorgan 4	Herta	Iran	F	hulled	2
3	Aras	Arumir	Iran	S	hulled	2
4	Makooei	Star	Iran	F	hulled	6
5	Zarjo	1-28-9963	Iran	F	hulled	6
6	AfzaL	Chahafzal	Iran	F	hulled	6
7	Jonoob	Gloria " s"/ Copal "s"	Iran	F	hulled	6
8	Karoon	Strain- 205	Iran	F	hulled	6
9	Jo Danmark	Denmark55	Iran	W	hulled	2
10	Sahra	L. B. LRAN/ Una8271// Giorias "s" Com	Iran	F	hulled	6
11	Mahoor	Wi2291/Wi2269//Er/Amp	Iran	W	hulled	2
12	Yoosef	Lignee527/chn-01//Gustoe/4/Rhn-08/3/DeirAlla 106//DI71/strain 205	Iran	S	hulled	6
13	Nimrooz	Trompillo, CMB74A-432-25B-1Y-IB-IY-OB	Iran	F	hulled	2
14	Reyhan	Rihane-03/4Alanda/lLignee5 27/Arar/3/Centinela/2*	Iran	S	hulled	6
15	Sararood	Chicm/An57//Albert	Iran	W	hulled	2
16	Nosrat	Karoon/Kavir	Iran	F	hulled	6
17	Astartis	(IABO × Arda3) × Amillis	Europe	W	naked	2
18	Cometa	PO202.169 × FO 3358	Europe	W	hulled	2
19	Explora	[(Onice\Arma\\Onice\Mirco\\\Jaidor) × (Plaisant\Jaidor\Express)] × Gotic	Europe	W	hulled	6
20	Rodorz	Baraka × Gotic	Europe	W	hulled	2
21	Martino	FIOR 3007 × Federal	Europe	W	hulled	6
22	Aquirone	FIOR 5186 × Naturel	Europe	W	hulled	2
23	Ponente	(Vetulio × Arma) × Express	Europe	W	hulled	6
24	Alce	(Tipper × Igri3) × [(Tipper × Alpha) × (Sonja × Wb117/18)]	Europe	W	hulled	2
25	Sirio	FIOR 2136 × Arco	Europe	W	hulled	2
26	Panaka	Amillis × Diadem	Europe	W	hulled	2
27	Pariglia	Airone × Arco	Europe	W	hulled	2
28	Sfera	(Katy × HJ54/30) × Igri × Arda) × (Tipper × Sonja)) × Amillis	Europe	W	hulled	2
29	Alimini	FIOR 2551 × Federal	Europe	W	hulled	6
30	Aldebaran	Rebelle × Jaidor	Europe	W	hulled	6
31	Vega	Rebelle × FIOR 1341	Europe	W	hulled	6
32	Aliseo	(Plaisant × Gerbel) × Express	Europe	W	hulled	6
33	Nure	(FIOR 40 × Alpha2) × Baraka	Europe	W	hulled	2
34	Airone	Gitane × FIOR 763	Europe	W	hulled	2
35	Aiace	FO 1078 × FO 1638	Europe	W	hulled	2
36	Scirocco	FIOR 1000 × Express	Europe	W	hulled	6
37	Trebbia	selection from FiorSynt 3	Europe	W	hulled	6
38	Alfeo	Tipper × Igri	Europe	W	hulled	2
39	Zacinto	IABO 329 × Arda	Europe	W	naked	2
40	Arda	Igri × HJ 51-15-3	Europe	W	hulled	2
41	Doria	(Nure × Zita) × (Nure × PO 202.169)	Europe	S	hulled	2
42	Tidone	(Okos × 273 cat.) × Igri	Europe	S	hulled	2

F – facultative; W – winter; S – spring

Table 2. Plant growth regulators and their concentrations used in *in vitro* regeneration phase of growth

Level	Plant growth regulator
1	BAP: 0.5 mg/l + NAA: 0.5 mg/l
2	BAP: 1.0 mg/l + NAA: 0.5 mg/l
3	BAP: 1.0 mg/l

Statistical analysis

The analyses of the data collected from anther cultures were performed using MSTAT-C, SAS and SPSS software. The distribution of the data was tested before the analysis, and the arcsinx data transformation was performed in cases where the distribution was not normal. After ISSR marker scoring based on the presence (1) or absence (0) of amplified fragments for all 42 cultivars, the ISSR binary data matrix was formed and the dice similarity coef cient was calculated. The cluster analysis was conducted based on the complete linkage method and principle coordinate analysis (PCoA) with NTSYS software version 2.02. XLSTAT software was used for the Mantel test to assess the correlation between the similarity coefficient matrixes of ISSR markers and the tissue culture data. The analysis of Molecular Variance (AMOVA) based on ISSR marker data was conducted using Arlequin ver 3.5 and Gen Alex ver 6.502 software. For the analysis of the population genetic parameters, Pop Gen software version 3.2 was used. For the evaluation of genetic diversity and assessment of the efficiency of 6 polymorphic primers, the following factors were calculated: the total ampli ed loci (TAL), the number of polymorphic loci (NPL), the percentage of polymorphism, the polymorphism information content (PIC), the effective multiplex ratio (EMR) that is of the fraction of polymorphic loci and the number of polymorphic loci for an individual assay (Kumar et al., 2009), resolving power (Rp) according to Prevost and Wilkinson (1999) and marker index (MI) based on Powell et al. (1996).

Results and discussion

Anther cultures

Variance and correlation analyses

Only 8 out of 42 studied barley cultivars produced calli from anthers. The obtained calli were transferred to and subcultured in regeneration media. The analysis of variance (data not shown) indicated highly significant differences in the number of days to callus induction between tested cultivars. The European cultivar "Airone" showed the highest percentage of callus induction (18.33%), and the lowest value was observed for the cultivar "Alfeo" (3.33%). In the regeneration phase, the effect of growth regulators was not significant for total regeneration ratio, or green and albino plants ratio. To understand the relationship between anther culture traits in barley cultivars, Pearson correlation coefficients were calculated (Table 3). The results showed that the callus size had a positive and highly significant correlation with the callus growth rate. Moreover, a positive and highly significant correlation between total regeneration ratio and the albino plant ratio was observed. Similar results were reported by Chaghamirza and Arzani (1999) and Kahrizi and coworkers (2011), where the authors reported that the efficiency of the anther culture method is largely dependent on plant genotype and culture medium.

Cluster analysis

A cluster analysis based on the square Euclidean distance matrix using the average linkage (between group) method classified 8 cultivars into 2 clusters (Fig. 1). One cluster consisted of "Astartis", "Airone", "Zacinto" and "Pariglia" cultivars,which all are two-row barleys of European origin. The other cluster included "Explora", "Ponente", "Alfeo" and "Alce" cultivars, of which "Explora" and "Ponente" were European six-row barley cultivars and the others were European two-row barley cultivars. In the compared clusters, the cultivars in the first cluster had a higher green plant ratio (0.05), callus growth rate (0.36), callus size (6.09 mm) and number of days to callus induction (57). The cultivars in the second cluster showed a higher total regeneration ratio (0.26), albino plant ratio (0.26) and callus induction percentage (19.73). The mean values of traits for each cluster are listed in Table 4. A discriminant analysis confirmed this clustering with 100% accuracy via the average linkage method (Table 5).

Inter Simple Sequence Repeat (ISSR) assay

In the present study, 20 ISSR primers were tested and only 6 primers had amplified clear and polymorphic bands and were, therefore (subsequently), applied for

Table 3. Correlation coefficients among the anther culture traits in 8 barley cultivars

Anther culture traits	Number of days to callus induction	Callus size [mm]	Callus induction [%]	Callus growth rate	Albino plant ratio	Green plant ratio	Total regeneration ratio
Number of days to callus induction	1.00						
Callus size [mm]	0.08	1.00					
Callus induction [%]	−0.47	−0.54	1.00				
Callus growth rate	0.15	0.98**	−0.62	1.00			
Albino plant ratio	0.55	−0.08	−0.35	0.03	1.00		
Green plant ratio	−0.48	−0.38	0.21	−0.49	−0.35	1.00	
Total regeneration ratio	0.36	−0.27	−0.27	−0.18	0.89**	0.12	1.00

** – significant at $P < 0.01$

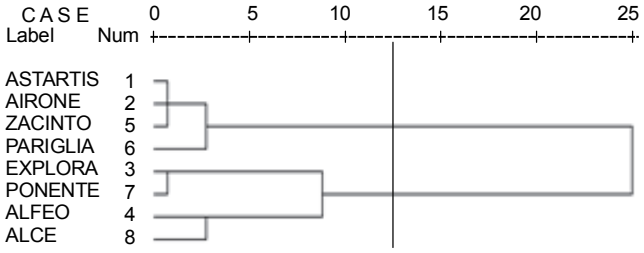

Fig. 1. A cluster dendrogram of eight barley cultivars based on anther culture traits via average linkage method

DNA amplification in all 42 barley cultivars. Amplification details of the primers used are presented in Table 6. Fifty-one of the 77 amplified bands were polymorphic. The average number of amplified loci per primer was 12.83. Sizes of amplified loci ranged from 147 to 2853 bp. The range in the numbers of polymorphic fragments was from 2 to 15 with an average of 8.50 fragments per primer. ISSR markers are dominant, where the maximum value of PIC is 0.5 (Ghasemi Ghehsareh et al., 2015). In this study, the average value of PIC was 0.34 and this showed the good level of efficiency of the polymorphic primers used. The average values of PIC for the ISSR primers could be related to the diverse nature of barley cultivars and/or highly informative ISSR markers used (Najaphy et al., 2011). The UBC811 primer had a maximum amount of MI and EMR and a maximum percentage of polymorphism. The highest Rp and PIC were observed for UBC826 and UBC840 primers, respectively. The polymorphism percentage varied from 50 to 78.94%.

Cluster analysis and principal coordinate analysis (PCoA)

A cluster analysis based on ISSR markers classified 42 barley cultivars into 6 clusters. The dendrogram obtained using the dice similarity coefficient is shown in Figure 2. This classi cation was confirmed by the AMOVA analysis with a PhiPT (an analog of F_{ST} or pairwise F_{ST}) equal to 0.03. In this study, no clear pattern of clustering for differentiation between Iranian and European cultivars was observed. The results obtained from the principal coordinate analysis showed that the first 3 most informative coordinates explained 70.14% of the total variations. Based on a two-dimensional graph in the principle coordinate analysis, 42 barley cultivars were categorized into 3 groups (Fig. 3).

Assessment of genetic similarity coefficients between barley cultivars

The Dice Genetic Similarity (GS) coefficient was evaluated based on data for ISSR markers and ranged between 0.14 and 0.87. Olgun and coworkers (2015) reported that in the evaluation of genetic variation in barley genotypes using ISSR markers, similarity matrix values varied from 0.16 to 0.87. The means, maxima, minima and coefficients of variation (CV) of genetic similarity coefficients are presented in Table 7. The highest genetic similarity coefficient was observed between "Aras" and "Reyhan" cultivars that had Iranian origin and a hulled spike. The highest genetic similarity coefficient in European cultivars was observed between "Vega" and "Arda" cultivars. Maximum genetic similarity coefficients among two-row and six-row barley cultivars were observed be-

Table 4. Average values of anther culture traits in each cluster in 8 barley cultivars

Total regeneration ratio	Green plant ratio	Albino plant ratio	Callus growth rate [mm/day]	Callus induction [%]	Callus size [mm]	Number of days to callus induction	Cluster
10.00	0.05	0.05	0.36	12.90	6.09	57.00	1
0.26	0.00	0.26	0.26	19.73	4.25	36.25	2

Table 5. A discriminant analysis of grouping based on anther culture traits of 8 barley cultivars

Groups of cluster analysis	Predicted group membership		
	1	2	
1	4	0	4
2	0	4	4
1	100.0	0	100
2	0	100.0	100

Table 6. Sequences and characteristics of ISSR primers used for DNA amplification in 42 barley cultivars

No	Primer names	Sequence (3′-5′)	Annealing temperature [°C]	TAL	NPL	Polymorphism [%]	PIC	EMR	Rp	MI
1	UBC811	(GA)8C	56	19	15	78.94	0.37	11.84	8.24	4.45
2	UBC822	(TC)8A	56	10	5	50.00	0.26	1.50	1.81	0.66
3	UBC824	(TC)8G	54	9	5	55.55	0.26	2.77	1.70	0.73
4	UBC826	(AC)8C	56	18	12	66.66	0.40	8.00	8.35	3.27
5	UBC840	(GA)8TT	55	8	5	62.50	0.46	3.12	4.00	1.44
6	UBC856	(AC)8Y*A	58	13	9	69.23	0.28	6.23	4.18	1.78
Total				77	51	–	–	–	–	–
Mean				12.83	8.5	63.81	0.34	5.74	4.72	2.07

Y* – pyrimidine base; TAL – total ampli ed loci; NPL – number of polymorphic loci; PIC – polymorphism information content; EMR – effective multiplex ratio; Rp – resolving power; MI – marker index

tween "Cometa" and "Sirio" cultivars (0.84) and between "Fajr 30" and "Martino" cultivars (0.81). The range of variation for genetic similarity coefficients among European cultivars was greater than that among the Iranian ones, while the coefficient of variation for Iranian cultivars was 18.4 and for European cultivars 17.4. On the other hand, Iranian cultivars showed a higher molecular variation than European ones.

Analysis of molecular variance (AMOVA)

The analysis of molecular variance was performed to divide the total genetic variation between and within 2 populations of Iranian and European cultivars. The result of the AMOVA analysis based on ISSR markers is shown in Table 8. In the current study, the AMOVA analysis showed that genetic variation between the populations represented 0.49% of the total variation and within the populations this was 99.51%. This means that there were higher variations among the tested cultivars within the populations. The population differentiation (F_{ST}) value calculated between populations was 0.004. Wright (1978) suggested that a value of F_{ST} from 0 to 0.05 may be considered as indicating little genetic differentiation. In other words, no noticeable genetic differentiation was found between Iranian and European cultivars.

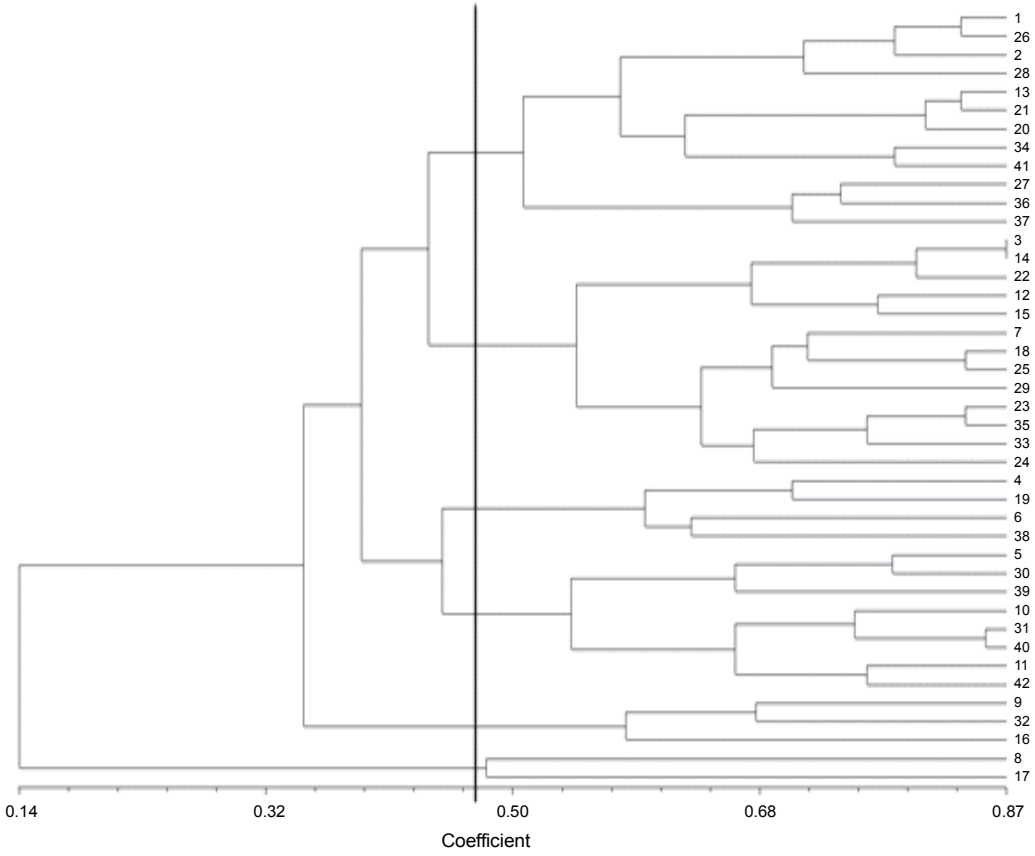

Fig. 2. A dendrogram of 42 barley cultivars based on ISSR markers using the complete linkage method

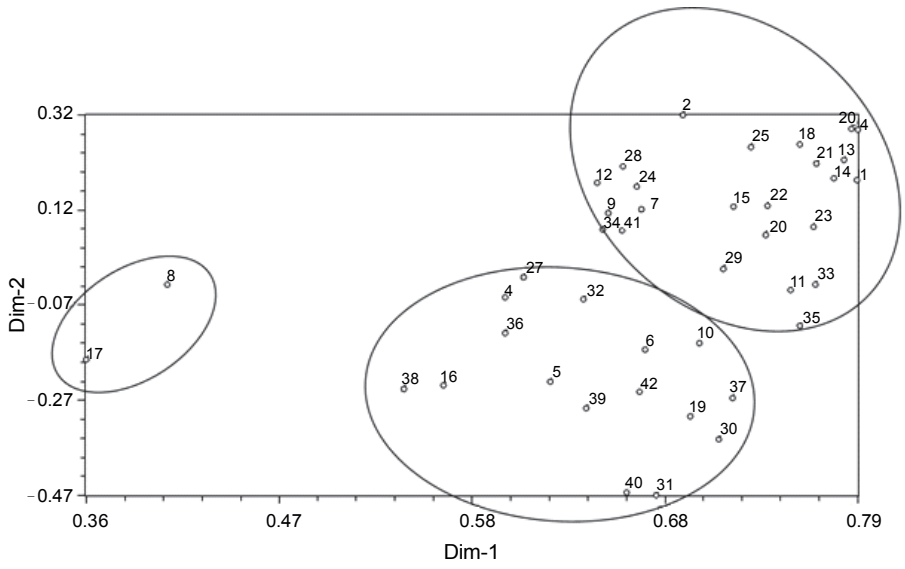

Fig. 3. Distribution of 42 barley cultivars for the first 2 coordinates using a principal coordinate analysis based on ISSR markers

Population genetic parameters

Population genetics is important for conservation and ecology studies, especially with rare and endangered plants species. The results of population genetic analyses based on ISSR markers in various groups of barley cultivars studied are presented in Table 9. In this study, Iranian cultivars showed a higher allele frequency, gene diversity, Shannon index and number and percentage

Table 7. The genetic similarity coefficients between individuals in various groups of 42 barley cultivars using ISSR markers

Cultivars	Maximum	Minimum	Mean ± standard deviation	Coefficient of variation
All cultivars	0.87	0.14	0.61 ± 0.108	17.6
Iranian cultivars	0.87	0.35	0.61 ± 0.112	18.4
European cultivars	0.85	0.14	0.61 ± 0.107	17.4
Two-row cultivars	0.84	0.17	0.62 ± 0.116	18.6
Six-row cultivars	0.81	0.30	0.61 ± 0.103	17.0
Iranian two-row cultivars	0.81	0.58	0.70 ± 0.081	11.6
Iranian six-row cultivars	0.78	0.35	0.57 ± 0.111	19.6
European two-row cultivars	0.84	0.24	0.60 ± 0.114	19.1
European six-row cultivars	0.79	0.50	0.66 ± 0.068	10.3
Winter cultivars	0.85	0.17	0.62 ± 0.096	17.1
Spring cultivars	0.71	0.50	0.62 ± 0.000	9.0
Hulled cultivars	0.85	0.14	0.62 ± 0.107	15.6
Naked cultivars	0.43	0.43	0.43 ± 0.000	0.0

Table 8. An analysis of molecular variance based on ISSR markers for 2 populations of Iranian and European cultivars

Source of variation	Degree of freedom	Sum of squares	Variance components	Percentage of variation	F_{ST} value
Between populations	1	5.1616	0.03	0.49	0.004
Within populations	40	204.788	5.11	99.51	
Total	41	210.405	5.14		

Table 9. Population genetic parameters based on ISSR molecular markers in the various groups of 42 barley cultivars

Population	Number of polymorphic loci	Percentage of polymorphic loci	Gene diversity	Shannon index	Mean number of observed alleles
All cultivars	51	100.00	0.354 ± 0.134	0.528 ± 0.164	2.00 ± 0.00
Iranian cultivars	50	98.04	0.347 ± 0.146	0.516 ± 0.184	1.98 ± 0.14
European cultivars	49	96.08	0.339 ± 0.147	0.506 ± 0.189	1.96 ± 0.20
Two-row cultivars	50	98.04	0.359 ± 0.149	0.523 ± 0.191	1.98 ± 0.140
Six-row cultivars	51	100.00	0.339 ± 0.130	0.511 ± 0.158	2.00 ± 0.00
Iranian two-row cultivars	34	66.67	0.268 ± 0.213	0.390 ± 0.299	1.66 ± 0.48
Iranian six-row cultivars	49	96.08	0.336 ± 0.146	0.503 ± 0.186	1.96 ± 0.20
European two-row cultivars	48	94.12	0.343 ± 0.152	0.509 ± 0.200	1.94 ± 0.28
European six-row cultivars	42	82.35	0.283 ± 0.181	0.425 ± 0.248	1.82 ± 0.39
Winter cultivars	51	100.00	0.348 ± 0.138	0.520 ± 0.172	2.00 ± 0.00
Spring cultivars	37	72.55	0.267 ± 0.195	0.397 ± 0.272	1.76 ± 0.45
Hulled cultivars	38	100.00	0.350 ± 0.136	0.524 ± 0.166	1.60 ± 0.30
Naked cultivars	17	33.33	0.138 ± 0.197	0.201 ± 0.287	1.33 ± 0.48

of polymorphic loci than European ones. The mean of hulled cultivars was higher than the mean of naked cultivars for all tested parameters. In the comparing experiments involving two-row with six-row barley cultivars within Iranian and European groups, the obtained results showed that in Iranian cultivars, six-row cultivars and in European cultivars two-row cultivars had the highest values for the all parameters tested. The variation in genetic parameters values in various studies could be related to the differences in the number of genotypes, their genetic basis and the number of markers used (Shuorvazdi et al., 2014).

Mantel test

Using Mantel testing, the clustering of 8 barley cultivars (the respondent cultivars to anther cultures) based on ISSR markers was compared to the anther culture traits of the relevant grouping. There was no significant correlation $r = -0.05$ and $P = 0.05$) that could be related to the inappropriate distribution of ISSR markers. Mantel correlations indicated that the genetic diversity among 8 barley cultivars was not structured in response to anther culture. In other words, the variation pattern obtained from anther culture data was not matched by variation pattern based on ISSR data.

Conclusions

An understanding of genetic similarities and differences between cultivars can be useful for complementation and can add to phenotypic knowledge useful in the development of barley cultivars and the efficient use of these cultivars in breeding programs. The results indicated significant differences between cultivars for anther culture traits; moreover, all respondents to anther cultures cultivars were of European origin. A cluster analysis based on ISSR markers gave no clear pattern of clustering for a differentiation between Iranian and European cultivars. In the molecular assessment, the average PIC value was 0.34, which indicated a good level of efficiency for the ISSR primers used. The range of variation for genetic similarity coefficients among European cultivars was higher than among Iranian cultivars; nevertheless, the coefficient of variation for Iranian cultivars was greater. Thus, Iranian cultivars showed a higher molecular variation than the European ones. The analysis of molecular variance (a weak value of F_{ST}) did not indicate any noticeable genetic differentiation between Iranian and European cultivars. This means that there were more variations among the individuals or cultivars within both groups: the Iranian and the European. The values of genetic parameters were different in various groups of 42 barley cultivars, which could be related to the differences in the number and genetic background of the cultivars in each group.

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