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Investigation of genetic diversity of some durum and bread wheat genotypes using SSR markers

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Abstract

Diversity is very important for breeding objective, since a narrow genetic base of germplasm is very vulnerable to biotic and abiotic stress. Genetic diversity of 40 wheat genotypes was assessed using 30 SSR primers that all of them were generated scorable bands. Totally 71 alleles (ranged between 2 to 4 alleles per each locus) was distinguished. Polymorphic information content (PIC) for all SSR primers was calculated. The highest (0.77) and the lowest (0.13) value of PIC was pertained to Xbarc352 and Xcfd56 Primers, respectively. According to similarity matrix, genetic similarity value ranged from 0.18 to 0.95 with an average of 0.48. The lowest and highest genetic similarity was observed between the Sistan and Arg (Bread wheat, No 27 and 28), Karkheh and Behrang (Durum wheat, No 35 and 38) genotypes respectively. Unweighted pair group method of the arithmetic average (UPGMA), based on Jaccard similarity clustering form a dendrogram with three genotypes group. Clustering somewhat was distinguished durum and bread wheat's. Principle co-ordinate Analysis (PCA), 2D plot was confirmed the results of cluster analysis. Cophenetic correlation showed that molecular data and cluster was corresponded. It was concluded that SSR marker was suitable for evaluated of genetic diversity in wheat genotypes and this genetic diversity can be used in wheat breeding programs.

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Introduction

Wheat is a one important plant in Iran. Improvement of wheat depends on the existence of genetic diversity. The loss of genetic diversity due to modern breeding practice has been reported by several studies (Russell *et al.,* 2000, 2004; Fu *et al.,* 2005). Therefore, it seems necessary to understand the levels and distribution of genetic diversity in existing crop gene pools as a basis for developing strategies of resource management and exploitation. Molecular markers are useful tools to assess genetic diversity and provide the best estimate of genetic diversity since they are independent of the confounding effects of environmental factors. However, some of the molecular marker systems, such as random amplified polymorphic DNA (RAPD) (Williams *et al.,* 1990) and restriction fragment length polymorphisms (RFLP) (Botstein *et al.,* 1980) have been limited use for crop plants due to their low polymorphism rate, particularly in self-pollinating species with a narrow genetic basis (Sharam *et al.,* 1983). On the other hand, simple sequence repeats (SSRs) (Tautz *et al.*, 1989) have been extensively used in wheat due to their high level of polymorphisms, co dominant inheritance and equal distribution in the wheat genome (Roder *et al.,* 1995; Parker *et al.*, 2002).

Several molecular assays have been applied to assess genetic diversity among wheat cultivars (Chen *et al.,* 1994). These molecular markers are different in several ways, such as principle, application, and amount of polymorphism detected and in task and time requirements. Assays based on the polymerase chain reaction (PCR) are considered to meet both the technical and genetic requirements for the characterization of plant and animal genetic resources (Powell *et al.,* 1995). SSR markers have been used to assays genetic diversity of wheat in many studies: Salem *et al.,* (2008) using morphological characters and 48 SSR markers were investigated genetic diversity of the seven wheat varieties, in this study indicated that the number of alleles per locus ranged from 2 to 7 and the allelic polymorphism information content (PIC) value ranged from 0.27 for the Xgwm95 to 0.81 for the Xgwm43 with an average of 0.54. The results revealed that the genotypes differed for morphological characters and SSR markers. The average genetic diversity based on morphological characters (23.49 with a range of 8.51-38.46) was higher than SSR markers (0.53 with a range of 0.42-0.63). Our results suggested that the classification based on morphological characters and genotypic markers of these wheat genotypes will be useful for wheat breeders to plan crosses for positive traits. Genetic diversity of 11 bread wheat cultivars that grown in Turkey were analyzed with 19 microsatellite markers. In this study was found that PIC values were ranged between was between 0.36 and 0.87 with an average value of 0.68. The numbers of observed alleles were between two and nine, with an average value of 5.42 (Akkaya and Buyukunal-Ba, 2003). Prasad *et al.*, (2000) using 20 wheat microsatellite markers studied genetic diversity of 55 elite wheat genotypes. 155 alleles were detected at 21 loci using the above microsatellite primer pairs (only one primer amplified two loci; all other primers amplified 1 locus each). The values of average PIC for these markers were estimated to be 0.71. Cluster analysis was able to distinguish a maximum of 48 of the 55 wheat genotypes. Spanic *et al.,* (2012) assessed the genetic diversity among 30 wheat genotypes using 24 SSR markers and reported that the number of alleles per locus ranged from 1 to 14 with an average number of 8.44 alleles per locus. Cluster analysis based on SSRs data clearly differentiated wheat genotypes.

Our objective was to investigate genetic diversity among some durum and bread wheat cultivars and varieties grown in Iran, using molecular data obtained from SSR profiles.

Materials and methods

Plant Materials, DNA Isolation and Markers Analysis

In this study, 24 SSR markers were applied. Primer sequences were obtained from Grain genes database (http://grain genes.org). Frothy wheat genotypes (including 32 bread and 8 durum) were used that

obtained from the seed and Plant Improvement Institute, Karaj, Iran (Table 1). DNA extraction was performed according to the modified CTAB-method (http://www.diversityarrays.com). The quantity of DNA was measured under 0.8% agarose gel electrophoresis. DNA concentration was estimated using Picodrop (Pico 200). The final DNA concentration of each template stock was adjusted to 50 ng/µl. PCR for SSRs marker was done according to Roder *et al*., (1998). The amplification products were electrophoresed on 3.5% agarose gels and for staining, 3 µL Gel Red and dye (the 1.5:1.5 ratio) was added to each sample. The gel was scanned with Bio-Rad Gel Doc.

Data Collection and statistical analysis

Each SSR band was scored as present (1) and absent (0) for the different genotypes. Genetic similarity between two genotypes was calculated using the Jaccard similarity coefficient (Jaccard, 1908), and dendrogram obtained by clustering according to the un-weighted pair group method with arithmetic average UPGMA algorism using the NTSYS-pc software version 2.02 (Rohlf, 1992). The polymorphism information content (PIC) for each primer was calculated according to the formula: PIC = 1- ΣPij^2, where P_{ij} is the frequency of j^{th} allele of i^{th} locus, summed across all the alleles for the locus over all genotypes (Anderson *et al*., 1993).

Table 1. Name or Identity of wheat genotypes.

No	Name/Identity	Growth type	No	Name/Identity	Growth type
1	Bayat	S	21	Sabalan	W
2	Falat	S	22	Maroon	S
3	Heirmand	S	23	Kavir	S
4	Darab-2	S	24	Hamoon	S
5	Atrak	S	25	Bam	S
6	Chamran	S	26	Akbari	S
7	Star	S	27	Sistan	S
8	Dez	S	28	Arg	S
9	Aflak	S	29	Yavarous	S
10	Baaz	S	30	Kohdasht	S
11	Shahpasand	W	31	Ohadi	W
12	Omid	W	32	Dehdasht	S
13	Roshan	F/S	33	Rijav	F
14	Tabassi	F	34	Rasad	W
15	Sholleh	S	35	Karkheh	S
16	Sorkhtokhm	S	36	Aria	S
17	Adl	F	37	Dena	S
18	Sardari	W	38	Behrang	S
19	Azar-2	W	39	Seimareh	S
20	Zagross	S	40	Saji	F

S, W and F: Spring, winter and facultative growth type

Number 29, 32, 35, 36, 37, 38, 39, 40 Durum and other are bread wheat.

Results and discussion

Thirty SSR markers were used to characterize and evaluate the genetic diversity of fourteen wheat genotypes. 71 alleles were detected. The number of alleles per locus ranged from 2 to 4 with an average number of 2.36 alleles per locus (Fig 1 and Table 2). These results are comparable with the results of other authors (Salem *et al*., 2008; Drikvand *et al*., 2013). This level of polymorphism is lower than the average of 5.7, 8.44 and 10 alleles per locus reported by Zhang *et al*., (2006), Spanic *et al*., (2012) and Fahima *et al*., (1998). Some of these studies have been conducted on wild wheat, this higher genetic variation in wild wheat's could be attributed to the considerable amount of natural out crossing that occurs in these genotypes (Tsegaye *et al*., 1996). In addition, the landraces that are selected from local germplasm have a wide range of diversity. However, genotypes under study have been cultivated extensively and that are product of repeated inbreeding would have lower genetic diversity comparisons of wild genotypes or landraces. The lower level of polymorphism may be

attributed to a narrow genetic diversity of these wheat genotypes. The PIC values ranged from 0.13 for the Xwmc52 locus to 0.77 for Xbarc352 and Xbarc86 with the average value of 0.45 (Table 2).

These finding in our study almost were in accordance with previous studies (Landjeval *et al.*, 2006; Bryan *et al.*, 2007 and Akkaya and Buyukunal-Bal, 2003). Botstein *et al.* (1980) reported that PIC value 0.5 > PIC > 0.25 is an informative marker. A high mean PIC value can be attributed to the use of more informative markers. The similarity coefficients between all genotypes ranged from 0.18 to 0.95 and averaged 0.48. The similarity coefficient showed that the two most closely related in bread wheat genotypes were Sabalan and Ohadi (No 21 and 31) with the

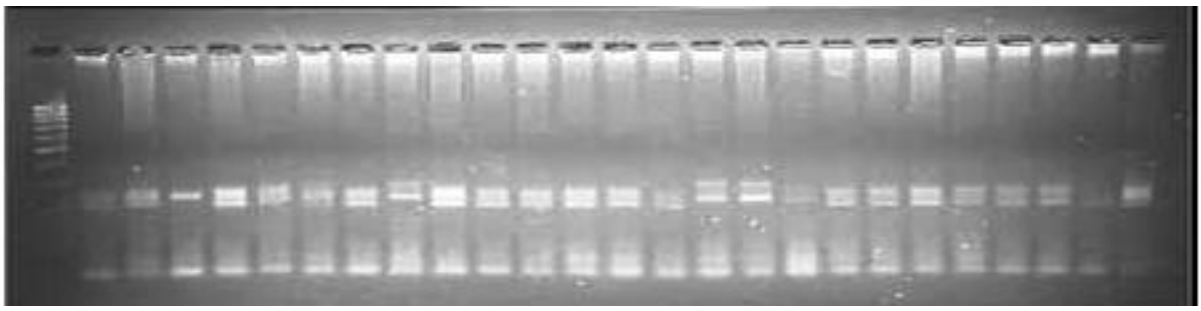

Fig. 1. Banding pattern of some wheat genotypes using Xcfa2153 primer in agaros gel.

Table 2. Name, annealing temperature and polymorphic information content of primers.

No	Primer	Annealing temperature	Number of amplified bands	Number of polymorphic bands	Polymorphic %	PIC
1	Xbarc54	60	2	1	50	0.50
2	Xbarc61	52	2	2	100	0.44
3	Xbarc86	52	2	1	50	0.73
4	Xbarc148	52	2	2	100	0.37
5	Xbarc149	60	2	2	100	0.45
6	Xbarc164	50	4	2	50	0.61
7	Xbarc200	52	2	1	50	0.25
8	Xbarc320	55	2	1	50	0.30
9	Xbarc352	55	2	2	100	0.77
10	Xbarc1060	55	2	1	50	0.22
11	Xcfa2153	59	4	2	50	0.55
12	Xcfa2164	60	4	2	50	0.45
13	Xcfd5	60	2	2	100	0.31
14	Xcfd13	60	2	1	50	0.47
15	Xcfd18	60	2	1	50	0.44
16	Xcfd42	66	2	2	100	0.62
17	Xcfd56	60	4	2	50	0.14
18	Xgwm5	50	2	2	100	0.46
19	Xgwm257	60	2	2	100	0.48
20	Xgwm261	55	3	3	100	0.58
21	Xgwm285	60	2	2	100	0.51
22	Xgwm577	55	2	2	100	0.22
23	Xwmc52	61	2	1	50	0.13
24	Xwmc215	61	2	2	100	0.35
25	Xwmc453	61	2	2	100	0.54
26	Xwmc596	61	2	2	100	0.50
27	Xwmc662	61	4	2	50	0.39
28	Xwmc722	61	2	2	100	0.56
29	Wms304	55	2	2	100	0.40
30	Wms513	60	2	2	100	0.53

highest similarity index (0.85), and in durum wheat Karkheh and Behrang (No 35 and 38) had the highest similarity index (0.95). On the other hand, the two most distantly genotypes in bread wheat's were Arg and Sistan (0.18) and in durum wheat were Yavarous and Karkheh, with low similarity index (0.27). In the winter growth type wheat's, the highest and lowest similarity coefficient was observed between Sabalan and Ohadi (0.85), Rasad and Sabalan (0.20), respectively.

The estimates of a similarity coefficient between pairs of genotypes ranged from 0.18 to 0.95. The average value of similarity coefficient was 0.48. This average value was suggesting that the 40 genotypes used in the present study were diverse. This similarity coefficient value reported in earlier studies. Yildirim *et al.*, (2011) in evaluation of genetic diversity among Turkish durum wheat landraces by SSR markers reported that the coefficient of similarity among all genotypes ranged from 0.35 to 0.74. Prasad *et al.*, (2000) in estimating genetic diversity among wheat genotypes using 20 wheat microsatellite markers reported that the genetic similarity coefficient between pairs of genotypes ranged from 0.05 to 0.88 with an average of 0.23. Maccaferri *et al.*, (2003) using SSR markers studied genetic diversity of durum wheat and showed that the average genetic similarity is 0.44. The variation in genetic similarity coefficient values may be attributed either to the differences in number of genotypes or microsatellites primers that used to detect DNA polymorphism.

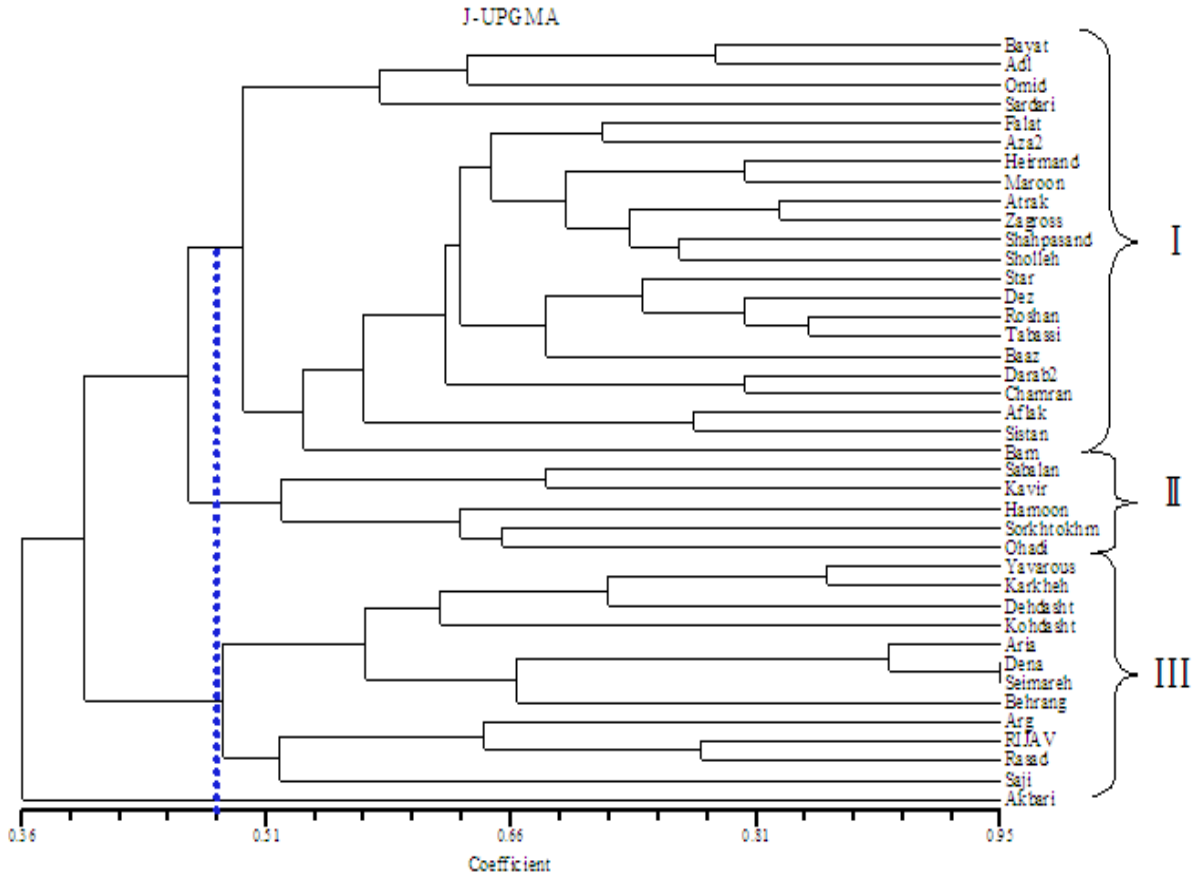

Fig. 2. Dendrogram of wheat genotypes using UPGMA method based on Jacquard's coefficient.

Based on Jaccard similarity and Unweighted pair group method of the arithmetic average (UPGMA), cluster analysis of genomic SSR similarity matrix, dendrogram was constructed as shown in Fig. 2. Three major groups can be distinguished by truncating the dendrogram at mean similarity

coefficient value of 0.48 (Jamshidi, 2011), the major group (group I) consists of 22 bread wheat genotypes, the majority of winter growth type wheat's are in this group. Five bread wheat's formed group II. Eight-drum wheat's were grouped in cluster III but this cluster includes five bread wheat genotypes, that is probably due to the common genome (A and B) that there are between bread and durum wheat. Genotypes were grouped in the same cluster they have similar genetic base. The cluster analysis almost enabled the grouping of all the genotypes used in the present study. Spanic *et al.*, (2012) stated that awareness of genetic diversity is a best tool for the selection of genotypes in wheat breeding programs.

The cophenetic correlation coefficient between the dendrogram and the original distance matrix for SSRs was significant and relatively high (0.83). These results are correspondence with the results Naghavi *et al.*, (2004) and Ismaeli *et al.*, (2010). High cophenetic correlation coefficient between the dendrogram and the original distance matrix for SSRs was a good representation of the relationships among the genotypes, as reported by Rohlf, (1998), earlier where a correlation of 0.82 was considered to be significant.

Principal co-ordinate analysis (PCoA) was carried out on the mean pairwise genetic distances to display the genetic relationship of genotypes in the PCoA 2D plot (Fig. 3). PCoA clearly demonstrated that durum wheat's and few of bread genotypes were scattered in left side, and bread wheat genotypes were scattered in right side of plot. Two bread wheats (Sistan and Ohadi) were located in the middle. PCoA almost showed similar distinct group to all genotypes and confirmed results of cluster analysis.

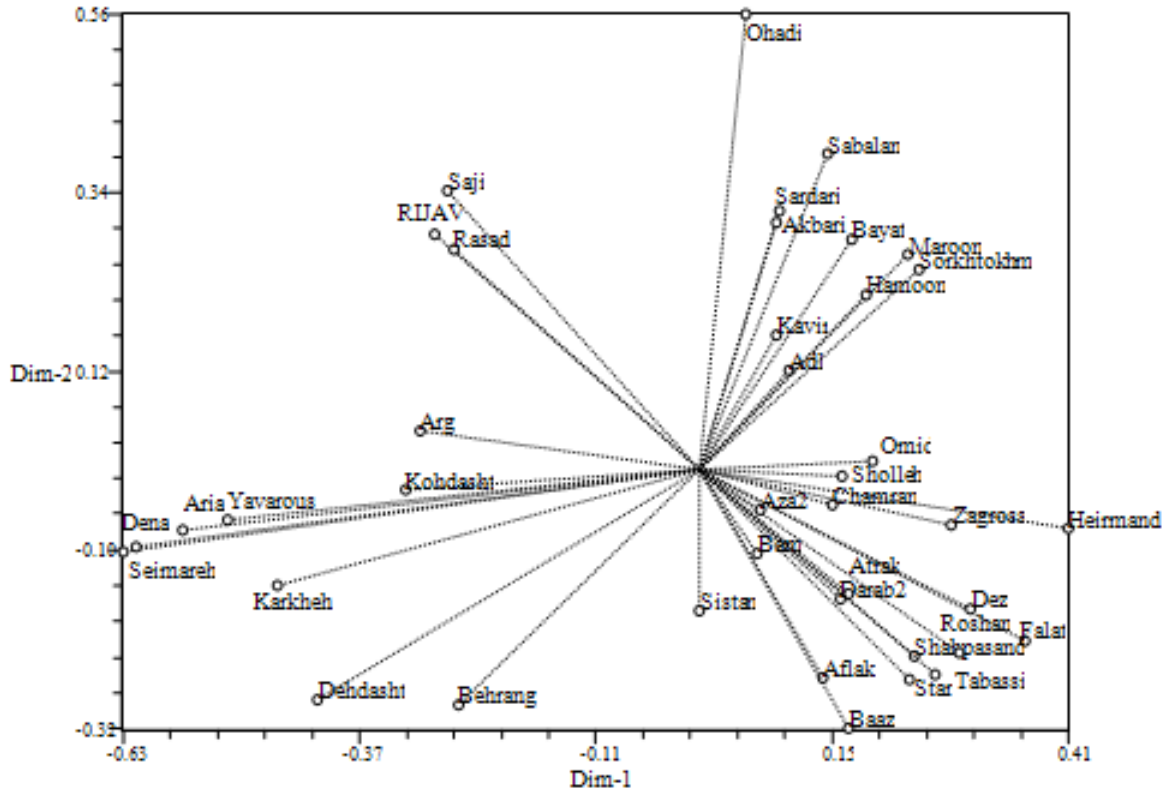

Fig. 3. Principle co-ordinate Analysis of wheat genotypes using SSR data (2D).

Conclusion

We investigate the genetic relationships among some Iranian bread and durum wheat genotypes. SSR primers indicated high level of polymorphism. The genotypes almost showed diverse and distinct SSR patterns. Most of the genotypes were spring and bread types, which presented closest genetic similarities. Cluster and PCoA analysis somewhat

could be distinct durum and bread wheat genotypes. The results have shown that it is possible select genotypes for the highest genetic diversity using SSRs, and using of them in crossing breeding programs (with respect their ploidy level). To achieve better results in crossing programs, we recommended also these genotypes classified using morphological and agronomic traits.

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