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Assessment of Genetic Relationships among Durum wheat Genotypes based on Molecular and Morphological Markers

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ABSTRACT

Background: Molecular variation in combination with morphological characters can be useful in breeding programs**. Objective:** In this study, the genetic diversity of 14 durum wheat breeding lines along with three wheat checks (Zardak, Sardari and Saji) was evaluated based on morphological traits and ISSR data. **Results:** Ten used primers generated 76 polymorphic markers. The PIC values of primers ranged from 0.13 to 0.42 with an average of 0.27. Cluster analysis using the UPGMA algorithm and Jaccard similarity coefficient indicated three major groups. The results of principal coordinate analysis showed a good congruency with cluster analysis. Cluster analysis based on morphological traits separated the genotypes into three distinct groups. **Conclusion:** The results revealed that genetic information obtained from ISSR data can be used in categorizing durum wheat genotypes and also can be used proficiently for selection of good parental material in breeding programs. write the main objective for your paper. write the main and most important results for your paper. write the main conclusion for your paper.

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INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum) is used primarily for the production of pasta, spaghetti and related products because of its high protein quality and gluten strength. Nowadays the use of molecular markers for Evaluation of genetic diversity in crop species, plays an important role in identification of superior genotypes to explore in plant breeding programs [9]. Durum is a cultivated tetraploid wheat with genomes AABB [2n,= 4x = 28], and its endosperm has the hardest texture of all wheat types. The kernels are also larger and more vitreous than those of bread wheat. The durum endosperm contains about twice the concentration of xanthophylls or luteins (not carotene) pigments when compared to that of bread wheat [11, 1]. Durum wheat is mainly produced and consumed in the Mediterranean region; it is used to produce several specific end-products. The wheat grain quality traits are considered to be inherited as quantitative traits as it is known to be controlled by a group of genes and being very affected by environmental variations [4, 2, 5].Genetic diversity of wheat cultivars plays a very important role in reducing genetic vulnerability during plant breeding. molecular markers provided excellent tools to assess the amount of genetic variation among breeding materials [13]. Inter simple sequence repeats (ISSRs) are one of the DNA-based markers that has been widely used in studies of cultivar evolution and molecular ecology [3]. This technique exploits the abundant and random distribution of SSRs in plant genomes by amplifying DNA sequences between closely linked SSRs [14]. Molecular variation evaluated by molecular markers in combination with agronomic and morphological characters of wheat can be useful in traditional and molecular breeding programs. Najaphy *et al*. [6] revealed that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. Sofalian *et al*. [12] showed that ISSR markers could be efficiently used to evaluate genetic variation in the wheat germplasm. Genetic similarity and dissimilarity among genotypes are useful for genetic differentiation of wheat accessions, selection strategies and genetic development of crop plants. Pasqualone *et al*. [7] tested the efficiency of ISSR markers to distinguish a set of 30 Italian durum wheat cultivars and 22 breeding lines. They found a very high efficiency of ISSR primers to distinguish all the durum wheat cultivars examined. In the present study, the genetic diversity in 17 durum wheat genotypes was examined using ISSR markers. The main

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objectives were to quantify the amount and distribution of genetic variation among durum wheat genotypes using genetic diversity measures.

MATERIALS AND METHODS

Plant materials and experimental design:

The seeds of 14 durum advanced breeding lines along with three check cultivars including: a new released durum wheat variety (Saji), a commercial variety of durum wheat(Zardak) and one variety of bread wheat (Sardari), were obtained from Dryland Agricultural Research Sub-Institute (Sararood station), Kermanshah, Iran. The study was conducted at Sararood station during the 2012-2013 cropping season. The experimental layout was a randomized complete block design with three replications.

Measurements and DNA extraction:

Measurements of examined characters were done on five plants which had been randomly chosen in the mid-row of each plot. The studied characters were plant height (PH), The number of grains per spike (NG), harvest index(HI), The number of spikes (NS), spike length(SL), and the thousand-kernel weight (TKW).

Genomic DNA was extracted from young fresh leaves following the CTAB procedure described by Saghai-Maroof et al. [10] with some modifications. The quality and quantity of extracted DNA was tested by comparing the sample with known standards of lambda DNA on 1% agarose gel electrophoresis. The isolated genomic DNA was stored at -20 °C for use.

ISSR amplification:

A set of 10 ISSR primers (Table2), was used to amplify the genomic DNA of all 17 genotypes. The PCR reactions were carried out in 20 µl volumes containing 2 µl PCR buffer (10x), 1.5 µl $MgCl_2$ (50 mM), 0.4 µl dNTPs (10mM), 1.2 µl primer (10pmol/µl), 0.3 µl Taq DNA polymerase (5unit/µl), 12.6 µl DDW and 2 µl of genomic DNA. The PCR reactions were performed in a Bio-Rad iCycler thermal cycler programmed for an initial denaturing step of 94°C for 4 min(to activate *Taq*DNA polymerase), followed by 35 cycles of denaturing at 94°C for 30 s, annealing (considering Tm of primers) for 45s and extension at 72°C for 2 min. This was followed by a final extension stage for 7 min at 72°C. The PCR products were separated on 1.5% agarose gel in TBE buffer. The DNA bands were visualized by staining the gels with ethidium bromide and photographed under UV light using gel documentation system.

Data analysis:

Morphological traits data were analyzed using MSTAT-C for analysis of variance and mean comparison of traits. Cluster analysis was used to classify the genotypes based on morphologic and agronomic characters. All statistical analyses were carried out using SPSS software version 16.0 (SPSS, 2007).

To analysis of molecular data, amplified fragments were constructed by scoring 0 and 1 for absence and presence of bands, respectively. Jaccard's similarity coefficient was used to calculate similarity between pairs of genotypes. The similarity matrix was subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated using the DARwin computer software Perrier et al.[8]

The polymorphism information content (PIC) was calculated as:

$PIC=1- \sum P^2i$

where p represent band frequency and q represent no-band frequency to characterize the efficiency of each primer to reveal polymorphic loci. The Marker Index (MI) was also calculated for each primer as:

$MI = PIC \times PB$

where PB is the number of polymorphic bands generated by the primers.

Table 1: The codes and names of three cultivars (1,2 and3) and 14 lines of Durum wheat.

Name	NO.
Saji (check)	1
Zardak	2
Sardari	3
19E- TOPDY	4
19E- RASCON	5
19E- M84859	6
19E- M141979	7
19E- M141982	8
19E- M141994	9
19E- M141995	10
19E- M142005	11
19E- M142017	12
19E- M142025	13

19E- M142038	14
19E- M142045	15
19E- M142069	16
19E- M142070	17

RESULTS AND DISCUSSIONS

The 10 primers produce 81 bands across 17 genotypes, of which 76 were polymorphic. The number of polymorphic fragments generated by primers, varied from 4 to 12 with an average of 7.6 fragments per primer. (Table2). The average of polymorphism information content index was 0.27. The lowest and the highest PIC value were recorded for primer IS-16 and UBC-848, respectively. A summary of the Marker Index(MI) calculated based on the PIC and polymorphic bands for each primer, is reported in Table 2. The maximum MI (3.12) was observed for the primer IS-14 and the minimum MI (1) was obtained with UBC-844.

Genetic similarity matrix generated based on Jaccard's similarity coefficient. Jaccard's similarity coefficient ranged from 0.33 to 0.91. A dendrogram based on UPGMA analysis with ISSR data is shown in Figure 1. Cluster analysis based on Jaccard similarity coefficient categorized the genotypes into three main groups (Fig. 1). The bread wheat genotype (Sardari) was classified in a single group. The genotype No. 14(19E-M142038), was also classified individually in a separated group.

The third group included most of the genotypes (13 durum breeding lines along with 2 durum wheat check cultivars) that was further divided into sub-clusters. The principal coordinate analysis (PCo) was performed with ISSR data in order to establish the relationship among samples and comparison to cluster analysis (Figure 3). The results of PCO analysis showed a good congruency with cluster analysis. In considering the distribution of genotypes, the use of more primers was recommended to gather more genomic information. Sofalian *et al*. [12] revealed that ISSR markers could be efficiently used to evaluate genetic variation in the wheat germplasm. Pasqualon et al. [7] found that the efficiency of ISSR markers was very high to distinguish all the examined durum wheat cultivars.

Table 2: Primers used for ISSR amplification with the number of bands, PIC and MI per primer.

MI	PIC	No. of polymorphic bands	No. of bands scored	Primer sequences*	Primer code
2.34	0.26	9	9	ACACACACACACACACYA	P_1=IS1
1.56	0.39	4	5	AGAGAGAGAGAGAGAGC	P_5=IS5
1.32	0.22	6	7	CTCTCTCTCTCTCTCTG	P_9=IS9
3	0.30	10	10	GAGAGAGAGAGAGAGARC	P_{10}=IS10
3.12	0.26	12	12	GACAGACAGACAGACA	P_{14}=IS14
1.3	0.13	10	10	DBDACACACACACACACA	P_{16}=IS16
1	0.25	4	4	CTCTCTCTCTCTCTCTRC	P_{844}=UBC844
2.52	0.42	6	6	CACACACACACACACARG	P_{848}=UBC848
2.48	0.31	8	8	TCTCTCTCTCTCTCTCRT	P_{853}=UBC853
1.4	0.20	7	10	VDVCTCTCTCTCTCTCT	P_{886}=UBC886

*Single letter abbreviations for mixed-base positions: Y = (C,T), R = (A,G), B = (C,G,T), D = (A,G,T) , V=(G,A,C)

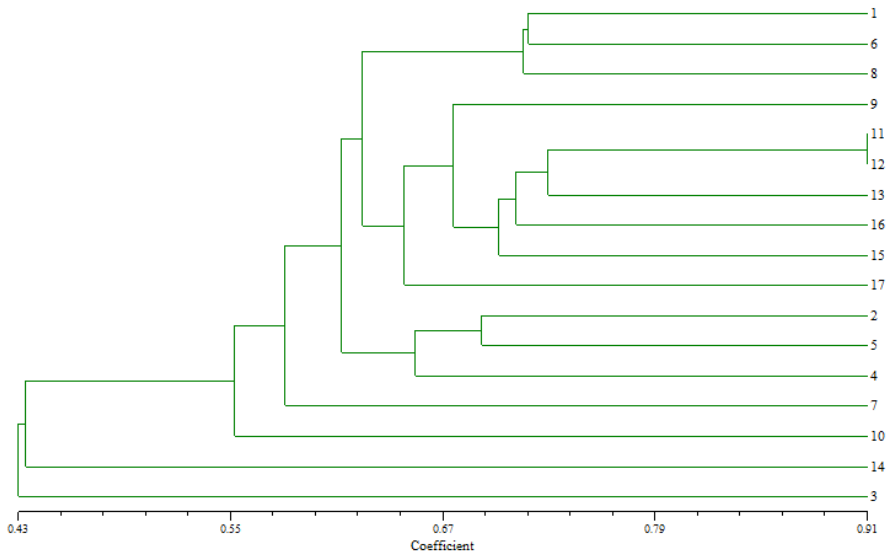

Fig. 1: Dendrogram of ISSR analysis on 17 durum wheat genotypes based on Jaccard's similarity coefficient.

The results of analysis of variance for plant height (PH), The number of grains per spike (NG), harvest index(HI), The number of spikes (NS) and spike length(SL) indicated that genotypic differences were highly significant (P<0.01). Significantly variation (P<0.05) among genotypes was observed for the thousand-kernel weight (TKW). These results indicated the genetic diversity among the genotypes tested. The cluster analysis based on agro-physiologic traits resulted in dendrogam shown in the figure 3. According to the dendrogram, there is no clear clustering corresponding to the classification of genotypes based on molecular data, but, the bread wheat check genotype(Sardari) was classified in a separated group like classification based on ISSR data.

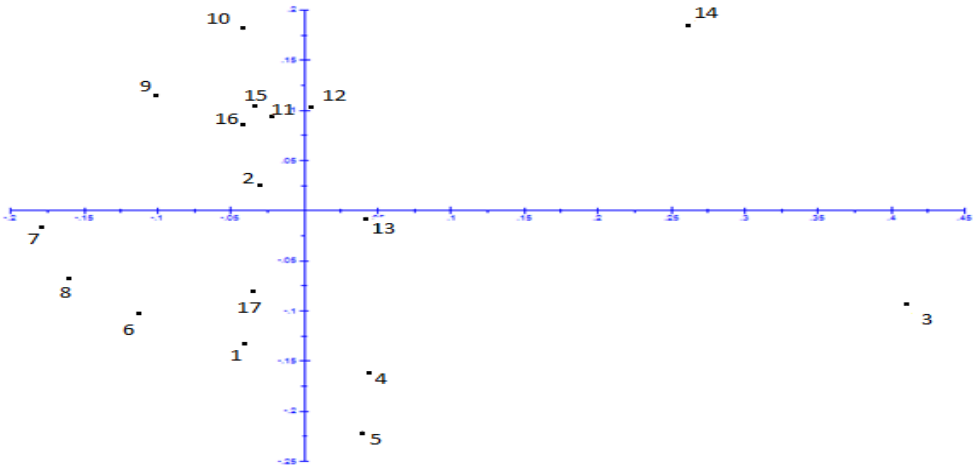

Fig. 2: Plot of the first and second coordinate in durum wheat genotypes according to ISSR markers

Table 3: Mean squares for morphologic traits of durum wheat genotypes.

SOV	df	Mean squares						
		PH	NG	NS	HI	TKW	SL	
Replication	2	21.45**	4.07^{ns}	2103.1*	12.4^{ns}	55.5^{ns}	1.1^{ns}	
Genotype	16	103.85**	61.83**	1775.3**	230.4**	58.1*	2**	
Error	32	3.078	6.53	662.3	18.9	30.5	0.6	
CV%		0.45	8.68	28.09	13.7	21.7	12.1	

*, ** significant at 5% and 1% level of probability, respectively.
Ns: non-significant

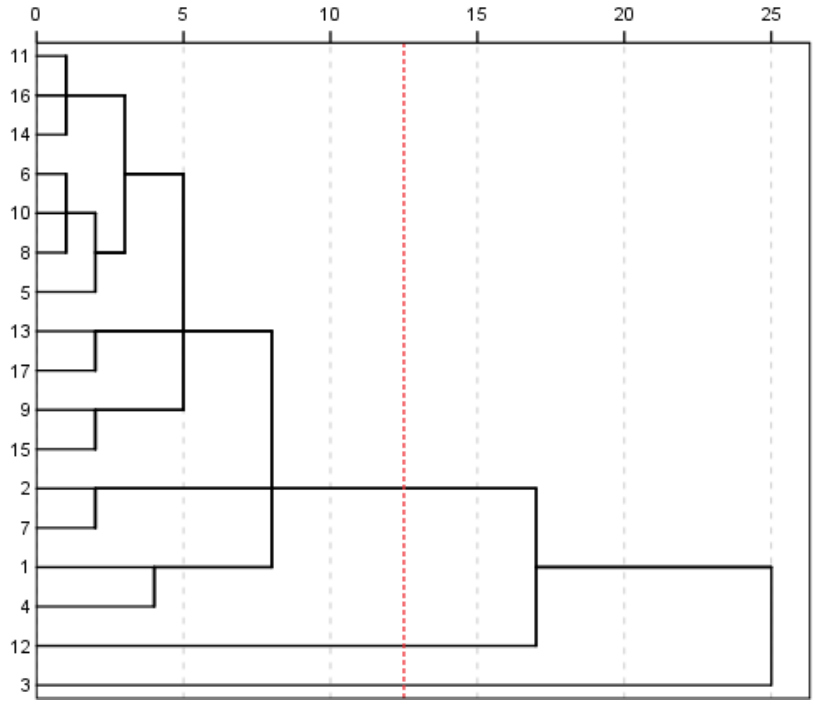

Fig. 3: classification of 17 durum wheat genotypes based on Morphological traits.

As the results showed, the measurements of genetic diversity based on morphological traits are not completely similar to classification of genotypes based on molecular data.

A possible explanation for the difference between the two classification method may be that the loci of the genes responsible for the morphological traits were not associated with ISSR loci. In other words, we don't have enough information about the linkage between ISSR loci and the genes that control morphological traits. For example, a trait like plant height may be controlled by many genes distributed throughout the genome.

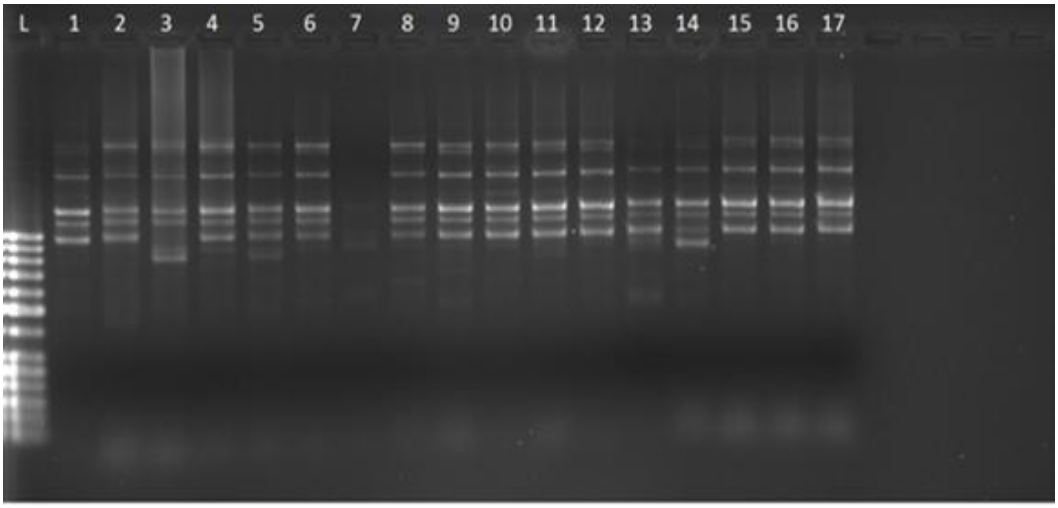

Fig. 4: Banding patterns among the 17 durum wheat genotypes amplified by primer IS 9.

The result of present study showed a relative high diversity in tested materials and revealed that ISSR markers are able to distinguish the polymorphism among durum wheat genotypes with moderate efficiency .Large amount of genetic variation which exists between breeding lines can be used efficiently for development of cultivated genotypes, planning further germplasm collection and the selection of parents in future breeding programmes.

The combination of the morphological variability that was determined in field studies and molecular diversity data, provides useful information for management of germplasm resources, and assessment the role of genetic background in yield production.

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