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Genetic Diversity in Spring Wheat Landraces from Northwest of Iran Assessed by ISSR Markers

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Abstract

Inter Simple Sequence Repeat (ISSR) markers were used to study the genetic diversity of 18 spring growth type landraces from Iran and selected 9 cultivars grown in Iran out of 15 ISSR primers .11 primers were found to have enough polymorphism and they were used for assessment. These primers are composed of di, tetra and penta-nucleotide sequences. From a total of 108 DNA fragments produced, 78 (72.22%) fragments were polymorphic. The UPGMA clustering algorithm classified the varieties into three major groups .Majority of landraces located in each group were originated from common locations. The results revealed that ISSR markers could be efficiently used to evaluate genetic variation in the wheat germplasm. Genetic similarity and dissimilarities among genotypes will be useful for genetic differentiation of wheat accessions, selection strategies and genetic development of crop plants.

Keywords: genetic diversity, ISSR markers, wheat landraces and grown cultivars

Introduction

Old wheat landraces (*Triticum aestivum*) are an important genetic resource that can be used to improve modern varieties by means of introducing new alleles or combination of genes (Ciaffi *et al.*, 1992). In the Near East, primary habitats of wheat progenitors are situated in the northern and eastern parts of the Fertile Crescent (Harlan and Zohari, 1996). The study of the genetic diversity in this species (including studies involving wheat evolution) may provide significant information regarding their potential for breeding purposes. Furthermore heterogeneity of the wheat landraces is more complicated and could not be analyzed systematically (Nevo and Payne, 1987).

Molecular markers discriminate high levels of polymorphism between cultivars and could be used efficiently to estimate the genetic similarities, which would enable researchers to study the possible relationships between markers and traits. Consequently an accurate selection can be applied in any Marker Assisted Selection programs. Bread wheat, however, exhibits an extremely low level of polymorphism by using RFLP (Restriction Fragment Length Polymorphism - Roder *et al.*, 1998).

Inter simple sequence repeats (ISSR) was first described by Zietkiewicz *et al.* (1994) and Kantety *et al.* (1995). ISSR-PCR is a technique which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multilocus markers. It is a simple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD). ISSR markers are highly polymorphic and they are useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology in wide range of crop plants (Blair *et al.*, 1995). The used ISSR primers can be di-, tri-, tetra- or penta-nucleotide SSR motifs found at microsatellite loci, giving a wide array of possible amplification products (Blair *et al.*, 1995). Simmons *et al.* (2007) in a study of efficiency of dominant markers in phylogenic study concluded that although using dominant markers create some problems , the problems are insufficient to reject the use of these markers in phylogenetic studies among closely related species.

Genetic diversity of the nuclear genome in the wheat has been well evaluated using ISSR markers by the following criteria: construction of genetic map (Li *et al.*, 2007), variety detection (Nagaoka *et al.*, 1997) and marker development for rust resistance gene (Gold *et al.*, 1999). In order to appraise the level of polymorphism in rice genome (Akagi *et al.*, 1997; Bervele *et al.*, 1997; Parsons *et al.*, 1997), and to study the genetic diversity in the maize inbred lines (Kantety *et al.*, 1995), millet (Slamath *et al.*, 1995), and sorghum (Yang *et al.*, 1996) and many other plant species this markers were used (Gupta *et al.*, 1994; Mcgregor *et al.*, 2000).

Little information is available regarding genetic variation in wild wheat relatives from Iran. It is supposed that the wild populations of Triticum species in this region may contain high levels of genetic diversity. The objectives of the present study was to investigate the molecular diver-

sity among the wheat landraces used in this work and to compare the molecular diversity between selected local landraces and improved varieties using ISSR markers.

Materials and methods

Plant material

18 spring landraces and 9 cultivars cultivated in Iran (Tab. 1) were used. The cultivars have mainly spring growth habit and in order to better finding differences, two winter cultivars were used as outgroup. The landraces were obtained from the National Plant Gene Bank of Iran (NPGBI). Plants were grown in greenhouse in 2007. Young leaves were harvested from all the genotypes when the seedlings reached 3-4 leaf stage, scored between 4 and 7, in Zadoks scale (Zadoks *et al.*, 1974). The harvested leaves were used for DNA extraction. DNA extraction and amplification

DNA was extracted from wheat young leaves (Saghai-Maroof *et al.*, 1984). The extracted DNA was diluted to obtain a final concentration of 25 ng/µL in order to use it in the PCR amplification.

PCR amplification

The ISSR amplification was carried out in a 25µL volume, according to CIMMYT laboratory protocols (Hoisington *et al.*, 1994). The amplifications were performed in a BioRad thermocycler. The PCR products were detected by 1.6% agarose gel electrophoresis that was stained with ethidium bromide. Then, the PCR products were visualized in a ultraviolet light using transluminator.In order to better distinguish the bands we used the molecular ladder contained known fragments.

Data analysis

The PCR product bands were scored as [1] for the presence and [0] for absence. The obtained data were used for analyses of genetic associations in the examined wheat material. A similarity matrix was constructed using the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis for personal computers) software, version 2.1 (Rohlf, 2000). For all pairs, wise comparisons were done, according to Jaccard's similarity coefficient. A dendrogram was constructed from the similarity matrix using the UPGMA method (Unweighted Pair-Group Method with Arithmetical Averages) and the SAHN subprogram (Sequential, Agglomerative, and Hierarchical and Nested clustering).

Results and discussions

ISSR marker analysis

In order to distinguish the potential polymorphism l of the studdied landraces, eleven primers were selected from 15 using primers (Tab. 2). Nine of the selected studdied primer sequences contained di-nucleotide sequences composed of $(GA)_8T$, $(GA)_8A$, $(CT)_8A$, $(CT)_8G$, $(TC)_8A$, $(AG)_8TT$, $(GA)_8TT$, $(CT)_8GG$ and $(TC)_8AA$ that were anchored with the T, A or G nucleotides at the 3′ends in turn. Together with the above mentioned sequences another primer sequences was composed of a tetra-nucleotide $(GATA)_2(GACA)_2$, and other one had a panta-nucleotide sequence $(GGGTG)_2$ (Tab. 2). A total of 108 DNA fragments were scored with an average of 9.8 fragments per primer and 78 of them (72.2%) were polymorphic. The potential of ISSR markers to generate genetic information through polymorphic fragments depends on the microsatellite frequency and their distribution in the genome wide scale of the species (Morgante *et al.*, 1993). The results showed that polymorphism index varied from 82% to 100% for different primers with a mean of 72.2% (Tab. 2). The highest polymorphism observed in the case of panta and tetra nucleotide repeat primers (UBC 881 and UBC 876, respectively). In agreement of this result, in the study of microsatellite primers in wheat it was assumed that the polymorphism rate would be higher when the motifs are composed of three or four

Tab. 1. List of cultivars and landraces used for assessment of genetic diversity by ISSR markers

Growth type	Wheat Variety
Cultivar	
Capple Desprez	Winter
Sardari	Facultative
Azar	Spring
Morgan	Winter
MV17	Winter
Zagros	Spring
Shahriar	Spring
Zarrin	Spring
Ataii	Spring
Landraces Variety*	
11487	Spring
11488	Spring
12185	Spring
11492	Spring
11199	Spring
11479	Spring
10527	Spring
10528	Spring
12190	Spring
12191	Spring
12078	Spring
12072	Spring
12070	Spring
12071	Spring
12194	Spring
12199	Spring
12073	Spring
12186	Spring

*The numbers refer as code number of each landrace in national seed bank of Iran

Tab. 2. ISSR Primers using in the study of wheat landraces and improved varieties, total number of fragments, number of polymorphic fragments, percent of polymorphism produced, and annealing temperature

Primer Sequences (3'-5'))	Total Number of fragments	Number of polymorphic fragments	Polymorphic Fragments (%)	Fragment Size (bp)
$(GA)_8T$	8	8	100	700
$(GA)_8A$	1	0	0	600
$(CT)_8A$	12	12	100	650
$(CT)_8G$	11	11	100	700
$(TC)_8A$	9	9	100	300
$(AG)_8TT$	12	12	100	1100
$(GA)_8TT$	9	8	89	750
$(CT)_8GG$	11	9	82	300
$(TC)_8AA$	12	12	100	600
$(GATA)_2(GACA)_2$	14	14	100	1000
$(GGGTG)_2$	15	15	100	950
Total	108	78	72.22	

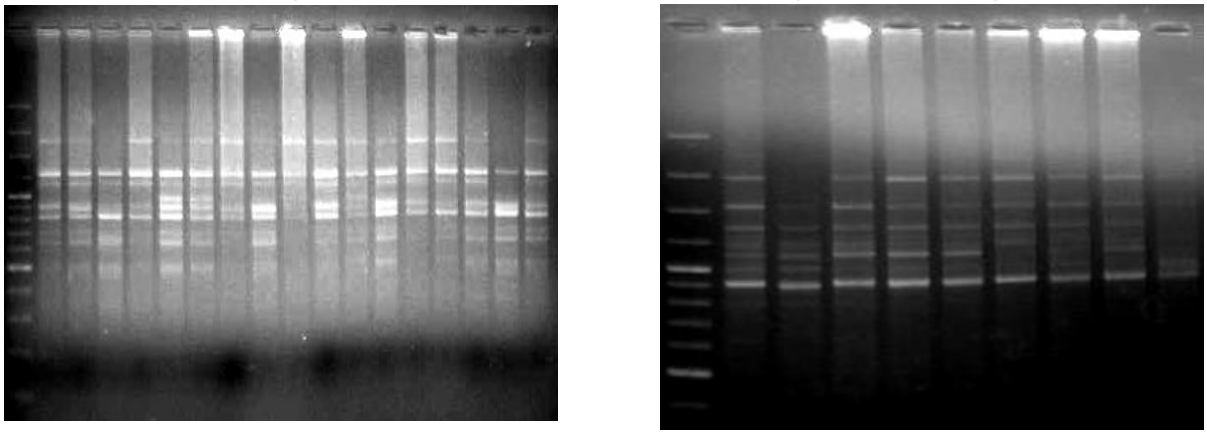
nucleotides (Song *et al.*, 2002). In the study of hexaploid wheats from Kihara Genetic Center;it was also observed a maximum polymorphism in relation to tetra nucleotide primer repeats (Nagaoka *et al.*, 1995).

ISSR marker analysis

In order to distinguish of the potential polymorphism of the studied landraces ,eleven primers were selected from 15 primers (Tab. 2). Nine of the selected primers sequences contained di-nucleotide sequences composed of $(GA)_8T$, $(GA)_8A$, $(CT)_8A$, $(CT)_8G$, $(TC)_8A$, $(AG)_8TT$, $(GA)_8TT$, $(CT)_8GG$ and $(TC)_8AA$ anchored with the T, A or G nucleotides at the 3′ends in turn. Together with the above mentioned sequences another primer sequences was composed of a tetra-nucleotide $(GATA)_2(GACA)_2$, and other one had a penta-nucleotide sequence $(GGGTG)_2$ (Tab. 2). A total of 108 DNA fragments were scored with an average of 9.8 fragments per primer. According to Morgante and Olivieri (2002), the potential of ISSR markers to generate genetic information through polymorphic fragments depends on the microsatellite frequency and their distribution in the genome wide scale of the species. The results showed that there isa high polymorphism between studdied landraces and cultivars (Tab. 2). The highest polymorphism was observed in the case of penta and tetra nucleotide repeat primers (UBC 881 and UBC 876, respectively), Also in the study of microsatellite primers in wheat it was supposed that the polymorphism rate would become higher when the motifs are composed of three or four nucleotides (Song *et al.*, 2002).

Cluster analysis

Based on data achieved by ISSR-PCR, cluster analysis performed to generate a dendrogram. The UPGMA clus-



A B

Fig.1. ISSR banding pattern generated by primer $(CT)_8G$ of studding studied wheat landraces and crop cultivars. (in part A) from left to right CAPPLE DESPREZ, Sardari, Zagros, Morgan, Ataii, Azar, MV17 and Shahriyar)

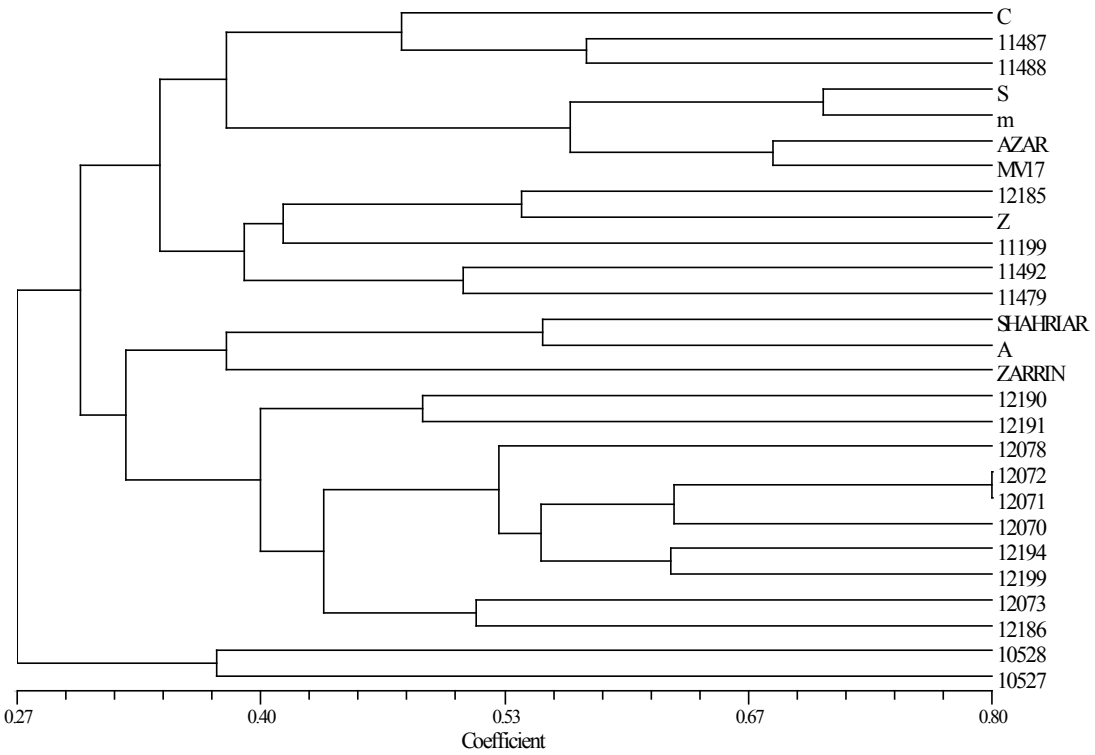


Fig. 2. Cluster analysis based on ISSR markers for the study of genetic relationships among wheat landraces and cultivars (C, Capple despres, S, Sardari, M, Moran, Z, Zagros and A, Ataii)

tering algorithm grouped the 27 wheat varieties into three clusters designated as 1, 2 and 3 (Fig. 2). According to our results twelve varieties were grouped in cluster 1, thirteen varieties were grouped in cluster 2 and in cluster 3 only two entries (10527-10528) were located. These two lines had high bread making qualities and they were different from the others (Valizadeh *et al.*, 2008). It was confirmed that the ISSR molecular markers prepare an excellent molecular tool in genetic variation identification.

Our results confirmed that the ISSR markers had the potential to detect genetic variability in both modern and local varieties (Fig. 1) .The modern checked cultivar belonging to the winter type was located in the first group. In this group all the landraces originated from common region exceptingthe 12185 code number landrace. (Fig. 2). In addition, second group also comprised landraces originated from common region. as well as the third group. According to these results, it was confirmed that the ISSR molecular markers could properly differentiate wheat varieties and could be used in variety identification purposes.

Conclusions

ISSR markers were highly polymorphic even for intra specific purposes in wheat varieties and could reflect the genetic relationships among wheat accessions. They can be considered as a potentially useful tool for studies of genetic diversity and they can be also used for the cultivar identification. It can be inferred that these markers could be used in wheat breeding for different purposes.

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