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Study the Genetic Diversity of Wheat Landraces from Northwest of Iran Based on ISSR Molecular Markers

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ABSTRACT

Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability during plant breeding efforts. In order to estimate the genetic diversity, molecular markers provided excellent tools. In this study Inter-simple sequence repeat (ISSR) markers were used to determine the genetic diversity of 39 bread wheat accessions, including 33 wheat landraces and 6 wheat cultivars from northwest of Iran. Out of 129 amplified scored bands, 106 (82.2%) were polymorphic. Average of amplified and polymorphic bands per primer was calculated as 11.7 and 9.6, respectively. Results indicated high level of polymorphism of wheat landraces based on these markers in contrast to other markers. Cluster analysis suggested that, ISSR markers were efficient tools for estimating intra-specific genetic diversity in wheat and this molecular marker could differentiate the local varieties obtained from different locations. Also the majority of landraces from same geographic region belonged to the same group and this confirmed that ISSR marker could be efficient tool in cultivar identification.

Key Words: Bread wheat; Genetic diversity; ISSR markers; Wheat landraces

INTRODUCTION

Modern semi dwarf wheat cultivars were at the risk of genetic erosion that primarily results in replacement of landraces of crop species with modern breeding cultivars in their native regions. Introducing of modern cultivars is necessary for food production of world populations and un-acceptance of them resulted in reducing of cereal production and was followed by misinterpretation and starvation (Skovmand *et al*., 2002). Selection that was performed during breeding programs increases frequency of finding desirable alleles or their combinations but decreases frequency of other alleles simultaneously. Hence, many of these alleles are deleted from breeding population. Therefore, there are possibilities of genetic diversity diminishing at many breeding programs. The logic remedy of this problem is conservation of landraces and their wild relatives and prevention of disappearance of these native resources (Chen *et al*., 1994). In fact, wide range of genetic diversity is critical to maintaining, enhancing and developing of wheat yield in relation of different condition For instance, it may provide new sources of resistance and tolerance against biotic and abiotic stresses (Skovmand *et al*., 2002). Crop plants were improved after natural and hand-made selection. In some crops, these combined actions resulted in forming of landraces, which under traditional agricultural systems exhibited stable yield. Some of genotypes that existed in population are likely to have satisfactory production under biotic and abiotic stresses (Zeven, 1998).

Plant genome has been characterized with large size and complex organization. Formerly, morphological analysis and sometimes cytogenetic, pedigree or chemical analysis were used in order to study of plant diversity (Volis *et al*., 2001). Applying molecular markers and recognition of polymorphic nucleotide sequences dispersed throughout the genome have provided new possibility for evaluating genetic diversity and determining of inter- and intra-species genetic relationships (Gostimsky *et al*., 2005). Several PCR based molecular markers are available for investigation of genetic diversity. SSR (Tautz, 1989), RAPD (Williams *et al*., 1990), AFLP (Vos *et al*., 1995) and ISSR (Zietkiewicz *et al*., 1994) were the most important of them. The major limitations of these methods were low reproducibility of RAPD, high cost of AFLP and need to know the flanking sequences to design specific primers for SSR markers. ISSR markers overcome most of these limitations (Pradeep *et al*., 2002).

The ISSR molecular markers are semi-arbitrary. Single forward primers with 16-18 nucleotide length comprises repetitive units and anchors 2-4 arbitrary nucleotides at the 3' or 5' end. This method did not required the information about genomic sequences and therefore by means of these primers high level of polymorphism could be realized (Zietkiewicz *et al*., 1994). ISSR markers were successfully used for estimating of genetic diversity in main crops for instance maize (Kantety *et al*., 1995), wheat (Nagoaka & Ogihara, 1997), rice (Blair *et al*., 1999) and barley (Brantestam *et al*., 2004; Hou *et al*., 2005).

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Northwest of Iran is amongst the main centers of origin and diversity of bread wheat (Harlan & Zohary, 1966). The present study was carried out to study genetic biodiversity of wheat landraces in relation to ISSR molecular markers of this region.

MATERIAL AND METHODS

Plant materials consist of 33 wheat landraces from northwest of Iran and 6 wheat cultivars provided by Seed and Plant Improvement Institute, Karaj, Iran. Fresh leaves were used for DNA extraction according to Saghai-Maroof's method (Saghai-Maroof *et al*., 1994) with slightly modifications.

ISSR markers. In this study, 15 ISSR primers were used for estimating of genetic diversity of plant genotypes. But only the polymorphic ones were used for analyzing. ISSR primers synthesized by Biotechnology Laboratory of British Columbia University (Vancouver, British Columbia, Canada) were used for PCR. The polymorphic ISSR markers are given in Table I. An amplification reaction was performed according to CIMMYT protocol (Hoisington *et al*., 1994) using Bio-Rad Thermal Cycler. PCR products were then run on 1.6% agarose gels in Tris-acetic acid-EDTA (TAE) buffer for 2 h. Ethidium bromide staining was used for detection of amplified bands (Fig. 1). Since ISSR primers are dominant markers, amplified bands were scored for presence (1) or absence (0) of bands. Statistical analysis of data was performed by means of NTSYS-pc software (Rohlf, 2000). Cluster analysis demonstrating genetic relationships of accessions were generated using un-weighted pair-group method using arithmetic averages (UPGMA) and simple matching coeficient.

RESULTS AND DISCUSSION

Total number of amplified products was 129 bands ranging from 450 to 2500 bp and 106 bands (82.2%) of them were polymorphic. Maximum and minimum bands generated by UBC 881 (19 bands & 100% polymorphic) and UBC 812 (one band & monomorphic), respectively. The average of amplified and polymorphic bands per each primer was 11.7 and 9.6, respectively. Amplified bands information related to each primer is giving in Table II.

The studied primer sequences were composed of di-, tri-, tetra- and panta-nucleotide repeat sequences. The highest polymorphism observed in the case of panta- and tetra-nucleotide repeat primers (UBC 881 & UBC 876, respectively) was in agreement of this result. Song *et al*. (2002) in the study of microsatellite primers in wheat proposed that the polymorphism rate would higher when the motifs comprise three or four nucleotides. Also, Nagoaka and Ogihara (1997), while studying hexaploid wheats observed maximum polymorphism in relation to tetra nucleotide primer repeats.

Diversity detected by di-nucleotide repeat primers were found at lower levels. In the group of di-nucleotide,

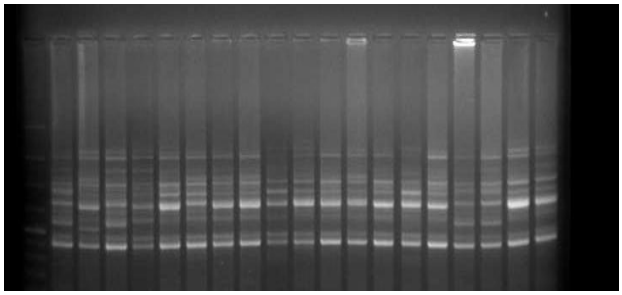
Table I. ISSR marker list that was polymorphic and using for determining genetic diversity of wheat landraces

Primer name	Sequences (3´ – 5´)	Annealing temperature (°C)
UBC 814	$(CT)_8A$	52°C
UBC 815	$(CT)_8G$	52°C
UBC 822	$(TC)_8A$	54°C
UBC 834	$(AG)_8TT$	52°C
UBC 840	$(GA)_8TT$	47°C
UBC 845	$(CT)_8TT$	45°C
UBC 852	$(TC)_8AA$	49°C
UBC 876	$(GATA)_2(GACA)_2$	51°C
UBC 881	$(GGGGT)_3G$	53°C

Table II. Primer names and polymorphism degree (polymorphic/ amplified bands ratio)

Primer name	Number of amplified bands	Number of polymorphic bands	Polymorphic/ amplified bands (%)
UBC 810	3	0	0
UBC 812	1	0	0
UBC 814	10	7	70
UBC 815	15	12	80
UBC 822	11	8	72.7
UBC 834	10	7	70
UBC 840	14	11	78.5
UBC 845	19	18	94.7
UBC 852	11	8	72.7
UBC 876	16	16	100
UBC 881	19	19	100

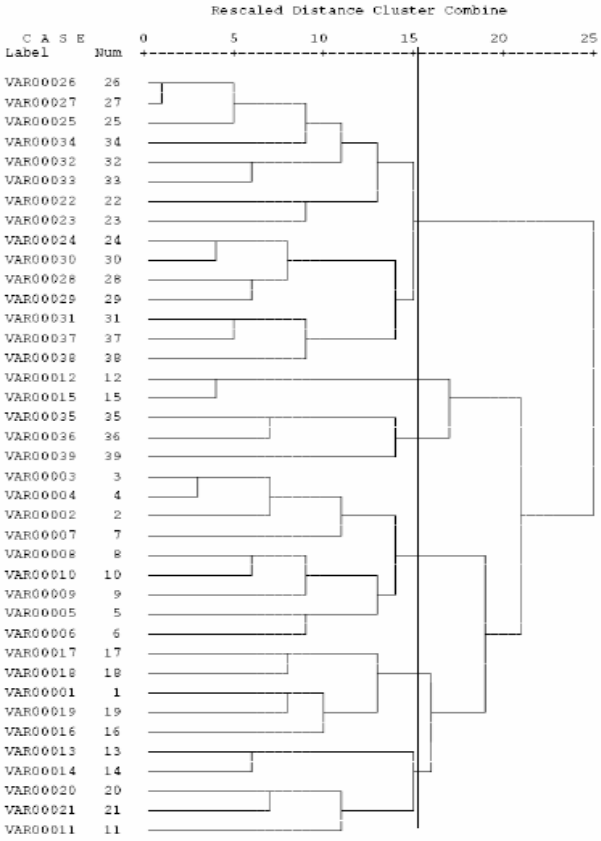
Fig. 1. The results of amplification were detected by means of agarose gel electrophoresis



GA repeats primers indicated lower level of polymorphism in contrast to other primers, as previously mentioned by Akkaya *et al*. (1992) with respect to microsatellite primer in soybean. We speculated that the primer sequences compose of GA repeats present the low level of polymorphism in plants but not in animal or human genomes. UBC 812 and UBC 810 belonged to this group. UBC 814, UBC 815 and UBC845 belonged to (CT) repeats primers group. They demonstrated different levels of polymorphism (70, 80 & 94.7%, respectively). Primers (UBC 822 & UBC 852) with (TC) repeats had same level of polymorphism (72.7%). In the set of primers, there was one (AG) repeats primer (UBC 834) with 70% polymorphic bands.

Based on data achieved by ISSR-PCR, cluster analysis performed to generate dendrogram (Fig. 2). Plant genotypes were divided to 6 main groups. Whole of developed wheat cultivars except for sample 10001 lied in one group. In other

Fig. 2. Dendrogram of studding landraces based on ISSR markers



groups landraces with near genetic code belonged to same geographical region that were expected to be more similar to each other, genetically- classified in the same groups. Groups 2, 3, 4 and 5 included this landraces, 10521 10532 (group 2), 12076, 12079, 12200 (group 3), 10333, 10422, 10426, 10428 (group 4) and 11197, 11198, 11202, 11204 (group 5). Members of each group occupied in a specific geographical zone but landraces from two different zone located in groups 1 and 6. It seemed that because north-west of Iran was one of the centers of origin of wheat (Harlan & Zohary, 1966), some landraces from different location had some similarities.

CONCLUSION

ISSR markers are highly polymorphic and repeatable even for intra-specific purposes in wheat varieties and could reflect real genetic relationships among wheat accessions. They are potentially useful markers to identify wheat landraces and might be used for cultivar identification. In the crop plants such as bread wheat, the polymorphism rate according to other molecular markers was low. The existence of polymorphic markers was an excellent choice in order to use in different breeding aims.

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