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Molecular variation of improved durum wheat genotypes based on inter-simple sequence repeats fingerprinting

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Abstract

Durum wheat (*Triticum turgidum* L. var. durum) is an important crop with high protein content and superior cooking quality which used for Pasta production. In present study, the molecular diversity of eighteen durum wheat breeding lines along with one durum wheat check cultivar were evaluated based on inter simple sequence markers. Eight used primers amplified a total of 93 bands, among which 77 bands (about 83%) were polymorphic. A total of 77 polymorphic fragments were scored with average 9.6 polymorphic bands per primer. The polymorphism information content value ranged between 0.27 and 0.36 for ISSR primers. Cluster analysis using UPGMA method and Dice similarity coefficient grouped the 19 genotypes into four separated clusters. The Dice similarity coefficients revealed maximum similarity (0.91) between 20A-URDYT-10 and 20A-URDYT-11 genotypes. PCA based on molecular data distinguished genotypes similar to the cluster analysis results. The results revealed that ISSR technique is a simple, informative and suitable approach for assessment of molecular diversity in durum wheat.

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Introduction

Durum wheat (*triticum turgidum* L. var. durum) is the only tetraploid (AABB, 2n=4x=28) species of wheat with commercial importance that is widely cultivated today (Von Buren, 2001). Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability during plant breeding efforts. Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production. Plant uniformity, which can be resulted by the use of modern plant breeding techniques, can produce plants, which are more efficient by means of different goals including enhanced resistance under stress, however much more research must be performed to indicate the most optimized methods that can be used for the production of efficient plants. This is of very important for the production of food for the world increasing population (Fu and Somers, 2009). There is a range of molecular methods available to study genetic diversity. Amplified fragment length polymorphism (AFLP), isozymes, simple sequence repeats (SSR), random amplified microsatellite polymorphisms (RAMP), random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) have all been used to determine genetic diversity in plant populations (Wang *et al*., 1994; Godwine *et al*., 1997; Hollngsworth *et al*., 1998; Blair *et al*., 1999; Amsellem *et al*., 2000; Mengoni *et al*., 2000). The inter simple sequence repeats (ISSR) are a new kind of molecular markers involving PCR amplification of DNA by a single primer 16-18 bp long composed of a repeated sequence anchored at the 3' or 5' end by 2-4 arbitrary nucleotides. (Zietkiewicz *et al*., 1994). Najaphy *et al.* (2012) revealed that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. Molecular variation evaluated in their study in combination with agronomic and morphological characters of wheat can be useful in traditional and molecular breeding programs. El-Assal and Gaber, (2012) investigated the discriminating capacity of RAPD, ISSR and SSR markers and their effectiveness in establishing genetic relationship and diversity among eleven wheat cultivars and landraces collected from Egypt and Saudi Arabia. The dendrogram cluster diagram classified the evaluated genotypes in three major clusters corresponding to the cultivation regions. Sofalian *et al.* (2009) 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. Carvlho *et al.* (2008) analyzed 51cultivars of Old Portuguese durum wheat belonging to 26 different botanical varieties by using ISSR markers. They found that amplified ISSR loci ranged from 150 to 3000 bp and the total mean percentage of ISSR polymorphism was 42.1%. All primers used allowed the detection of inter-variety and intra-variety ISSR polymorphism. Chowdhury *et al.* (2008) used ISSR markers to fingerprint and estimate genetic diversity in a set of 27 genotypes which comprised Indian bread wheat varieties released for high yield, quality and abiotic stress and trait specific landraces having known pedigrees. They found that the cluster analysis tree placed these genotypes in six groups and is in agreement with their known origin. The genetic relationships estimated by the polymorphism of ISSR markers revealed greater level of genetic variability in Indian bread wheat varieties of wide adaptability and applicability. Pasqualone *et al.* (2000) tested the efficiency of ISSR markers to distinguish a set of 30 Italian durum wheat cultivars and 22 breeding lines. They found that the efficiency was very high and two primers were sufficient to distinguish all the durum wheat cultivars examined. Nagaoka and Ogihara, (1997) reported that the genetic relationships of wheat accessions estimated by the polymorphism of ISSR markers were identical with those inferred by RFLP and RAPD markers, indicating the reliability of ISSR markers for estimation of genotypes. The main objective of this study was to evaluate the genetic diversity among durum wheat breeding lines and measure the relationship of the germplasm for future durum breeding programs.

Materials and methods

Plant materials

The 19 durum wheat genotypes (Table 1) for different breeding objectives were grown under rainfed condition in 2012-2013 cropping season at the Dryland Agricultural Research Sub-Institute (Sararood station), Kermanshah, Iran. Experimental lay out was a randomized complete block design with three replications. Each plot consisted of six rows and six m long with 20 cm row spacing.

DNA extraction

Genomic DNA was extracted from young fresh leaves following the CTAB procedure described by Saghai-Maroof *et al.* (1984) with some modifications. Extracted DNA concentration was quantified by the NanoDrop spectrophotometer and qualified on 1% agarose gel electrophoresis.

PCR amplification

A total of 8 ISSR primers (Table2), were used for amplification and PCR amplifications were performed using Bio-Rad iCycler thermal cycler. The PCR amplification reactions were performed 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 amplified 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.

Band scoring and data analysis

Amplified products were scored in terms of a binary code as present (1) or absent (0). Dice's similarity coefficient was employed to compute pairwise genetic similarities using the DARwin computer software (Perrier et al., 2003).Dice similarity coefficients were used for cluster analysis of genotypes and dendrogram was generated based on UPGMA method. Principal coordinate analysis was performed to generate a two-dimensional representation of genetic relationship among 19 durum wheat genotypes. 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.

Result and discussion

The total number of loci amplified by Eight ISSR primers was 93 bands, of which 77 were polymorphic (Table 2). Data analyses *were* conducted using only the *polymorphic bands*. The number of polymorphic fragments generated by primers, varied from 4 to 14 with an average of 9.6 fragments per primer. (Table 2).

Table 1. The codes/ names of 18 advanced breeding lines of durum wheat genotypes.

Code	Name	Code	Name
1	Saji (Check)	11	20A-URDYT-11
2	20A-G-1252	12	20A-URD-83-12
3	20A-61-130	13	20A-URD-83-13
4	20A-GB-BW-SPII-4	14	20A-ERD-14
5	20A-GB-BW-SPII-5	15	20A-ERD-15
6	20A-GB-BW-SPII-6	16	20A-ERD-16
7	20A-GB-BW-SPII-7	17	20A-ERD-17
8	20A-IDSN-Turkey	18	20A-ERD-18
9	20A-URDYT-9	19	20A-ERDYT-19
10	20A-URDYT-10		

Table 2. The codes and sequences of primers used for ISSR amplification with the number of Total bands(TB), polymorphic bands(PB), percentage of polymorphism(PP), polymorphism information content(PIC) and marker index(MI) for each primer.

Primer sequence*	Code	TB	PB	PP	PIC	MI
5´-ACACACACACACACACYA-3´	IS-1	11	10	%90.91	0.36	3.6
5´-AGAGAGAGAGAGAGAGC-3´	IS-5	13	8	%61.54	0.36	2.88
5´-CACACACACACACACAG-3´	IS-6	12	12	%100	0.31	3.72
5´-CTCTCTCTCTCTCTCTG-3´	IS-9	14	14	%100	0.31	4.34
5´-GAGAGAGAGAGAGAGARC-3´	IS-10	7	7	%100	0.28	1.96
5´-ACACACACACACACACC-3´	IS-11	8	4	%50	0.32	1.28
5´-GACAGACAGACAGACA-3´	IS-14	12	12	%100	0.27	3.24
5´-DBDACACACACACACACA-3´	IS-16	16	10	%62.5	0.31	3.1
Average of values		11.6	9.6	%83.12	0.32	3.02

Genetic similarity matrix generated based on Dice similarity coefficient showed an average similarity between genotypes range from 0.39 (20A-GB-BW-SPII-6 and 20A-ERDYT-19) to 0.91 (20A-URDYT-10 and 20A-URDYT-11) with a mean of 0.69. The cluster analysis using UPGMA method categorized the genotypes into four main groups (Figure 2). According to the dendrogram, the genotype No. 6(20A-GB-BW-SPII-6) was clustered into a group. The genotype No. 19 was also classified individually in a separated group. These two genotypes showed the highest genetic distance. Saji cultivar as the check cultivar was classified with 20A-G-1252 into a separated group. The fourth major cluster was further divided into three sub clusters. The sub clusters comprises 3, 1 and 11 genotypes respectively. Genetic relationship among 19 accessions was also visualized by performing principle coordinate analysis (PCoA) based on ISSR data (Fig. 3). The Principal Component Analysis (PCA) results almost coincided with the results of cluster analysis. Two-dimensional plot generated from PCoA showed a good congruency with cluster analysis and supported the clustering pattern of UPGMA dendrogram (Fig. 3).

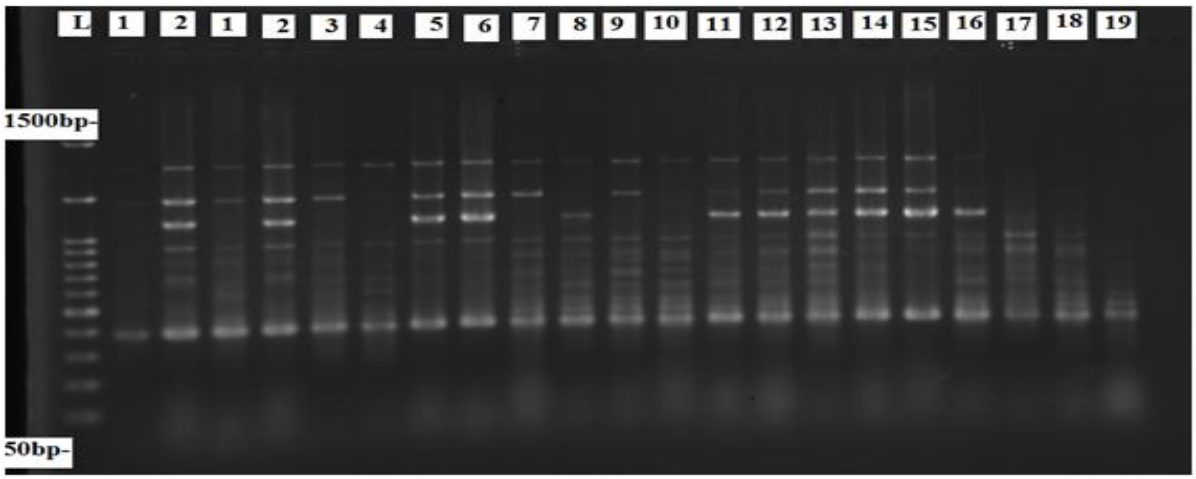


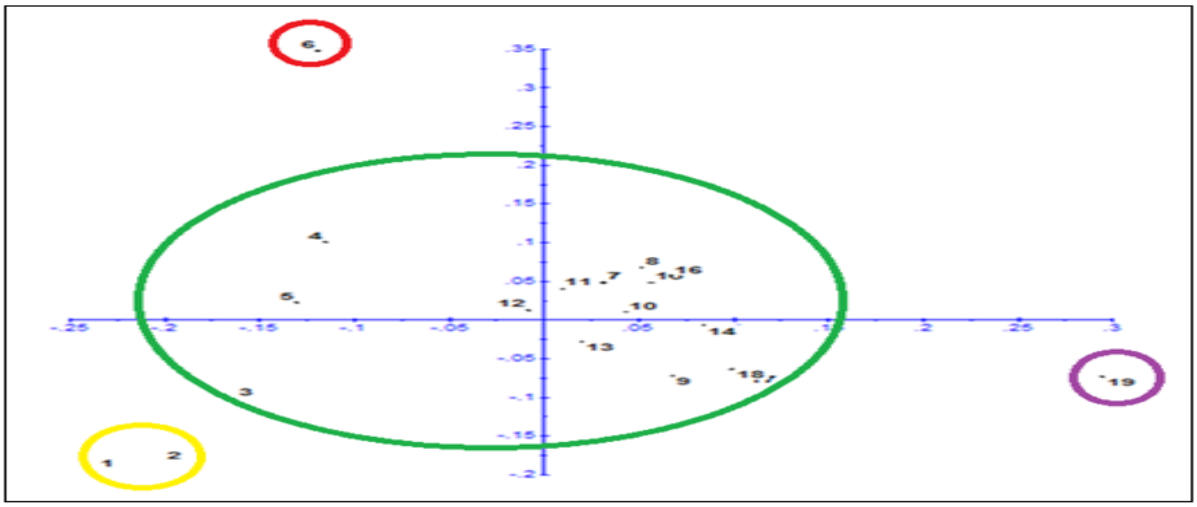
Fig. 1. ISSR marker profile of the primer 1 in 19 durum wheat genotypes.

The polymorphism information content values for ISSR primers in the present assessment ranged from 0.27 to 0.36. The average of PIC index was 0.32, reflected a relative high allelic diversity among the genotypes. A summary of the MI calculated based on the PIC and polymorphic bands for each primer, is reported in Table 2. The highest and the lowest values of MI were observed with primer IS-9(4.34) and Primers IS-11 (1.28) respectively. Karaca and Izbirak (2008), in analysis of genetic diversity in Turkish durum wheat cultivars using ISSR markers reported 57.9% for average of polymorphism. (Karaca and Izbirak , 2008).

Fig. 2. UPGMA Dendrogram based on Dice similarity coefficient.

These results showed that ISSR markers can indicate considerable polymorphism in durum wheat germplasm which has lead to the suggestion that they can be used for characterization and fingerprinting purposes. The result also showed this genotypes have high genetic diversity which can be used in rainfed durum wheat breeding.

In The present study, the percentage of polymorphic bands ranged from 50 ~ 100%. It is generally acknowledged that the genetic diversity of the plant is abundant when the percentage of polymorphic bands reach about 50% at the population level (Ma et al., 2000; Liu and Jia, 2003; Sun et al., 2004). This study revealed that ISSR technique is a simple, informative and suitable approach for assessment of molecular diversity in durum wheat. Genetic diversity reflect the ability of species to adapt to the environment and the potential to be used and transformed (Wang et al., 2011).



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.

References

Amsellem L, Noyer JL, Lebourgeois T, Hossaertmckey M. 2000. Comparison of genetic diversity of the invasive weed *Rubus alceifolius* Poir. (Rosaceae) in its native range and in areas of introduction, using amplified fragment length polymorphism (AFLP) markers. Molecular Ecology **9(4),** 443-455. http://dx.doi.org/10.1046/j.1365294x.2000.00876.x

Bauer I, Drinic SM, Drinic G, Micic DI. 2007. Assessing temporal changes in genetic diversity of maize hybrids using RAPD markers. Cereal

Research Communications **35(4),** 1563-1571. http://dx.doi.org/10.1556/CRC.35.2007.4.3

Blair MW, Panaud O, Mccouch SR. 1999. Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (*Oryza sativa* L.). Theoretical and Applied Genetics **98(5),** 780-792.

Carvalho A, Brito JL, Maces B, Pinto HG. 2008. Molecular characterization of a Portuguese collection of durum wheat. Options, Mediterraneennes. Serie A. Seminaires Mediterraneens **81,** 59-61. 3 ref.

Chowdhury RMVK, Kundu SJS, Jain RK. 2008. Applicability of ISSR markers for genetic diversity evaluation in Indian bread wheat genotypes of known origin. Environmental Ecology **26,** 126-131.

El-Assal SED, Gaber A. 2012. Discrimination capacity of RAPD, ISSR AND SSR markers and their effectiveness in establishing genetic relationship and diversity among Egyptian and Saudi wheat cultivars. American Journal of Applied Sciences **9(5),** 724-735. http://dx.doi.org/10.3844/ajassp.2012.724.735

Fu YB. 2006. Impact of plant breeding on genetic diversity of agricultural crops: Searching for molecular evidence. Plant Genetic Resources **4(1),** 71-78. http://dx.doi.org/10.1079/PGR2006116

Fu Y, Somers D. 2009. Genome-Wide Redution of Genetic Diversity in Wheat Breeding. Crop Science **49(1),** 161-168. http://dx.doi.org/10.2135/cropsci2008.03.0125

Godwin ID, Aitken EAB, Smith LW. 1997. Application of inters simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis **18(9),** 1524-1528. http://dx.doi.org/10.1002/elps.1150180906

Hollngsworth PM, Tebbitt M, Watson KS, Gornall RJ. 1998. Conservation genetics of an artic species, *Saxifgra rivularis* L. Botanical journal of the Linnean Society **128(1),** 1-14. http://dx.doi.org/10.1111/j.10958339.1998.tb02104.x

Karaca M, Izbirak A. 2008. Comparative analysis of genetic diversity in Turkish durum wheat cultivars using RAPD and ISSR markers. Journal of food, Agriculture & Environment **6(3-4),** 219-225.

Kraic F, Mocák J, Roháčik T, Sokolovičová J. 2009. Chemometric characterization and classification of new wheat genotypes. Nova Biotechnologica **9(1),** 101-106.

Liu GH, Jia BL. 2003. The study of genetic diversity of Ulmus pumila. J. Ar var. sabulosa id Land Resour. Environ **17,** 123-128.

Ma XJ, Wang XQ, Xu ZX. 2000. RAPD vriation within and among populations of ginseng cultivars. Acta Botanica Sinica **42(6),** 587-59.

Mengoni A, Gor A, Bazzicalupo M. 2000. Use of RAPD and microsatellite (SSR) variation to assess genetic relationships among populations of tetraploid alfalfa, *Medicago sativa*. Plant Breeding **119(4),** 311-317. http://dx.doi.org/10.1046/j.1439-0523.2000.00501.x

Nagaoka T, Ogihara Y. 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theoretical and Applied Genetics **94(5),** 597-602. http://dx.doi.org/10.1007/s001220050456

Najaphy A, Parchin RA, Farshadfar E. 2012. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Australian Journal of Crop Science **6(2),** 326-332.

Pasqualone A, Lotti C, Bruno A, Vita P, Fonzo N, Blanco A. 2000. Use of ISSR marker for cultivar identification in durum wheat. Options

Méditerranéennes: Serie A. Seminaires Méditerranéennes **40,** 157-161. 7 ref.

Reif JC, Zhang P, Dreisigacker S, Warburton ML, van Ginkel M, Hoisington D, Bohn M, Melchinger AE. 2005. Wheat genetic diversity trends during domestication and breeding. Theor Appl Genet **110(5),** 859-864. http://dx.doi.org/10.1007/s00122-004-1881-8

Sofalian O, Chaparzadeh N, Dolati M. 2009. Genetic diversity in spring wheat landraces from northwest of Iran assessed by ISSR markers. Notulae Botanicae Horti Agrobotanici Cluj-Napoca **37(2)**, 252-256.

Sofalian O, Chaparzadeh N, Javanmard A, Hejazi MS. 2008. Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. International. Journal of Agriculture & Biology **10(4),** 466-468.

Sun K, Chen W, Ma RJ, Chen X. 2004. A study on the genetic diversity of subpopulations of Hippophae rhamnoides ssp. Sinensis at Ziwuling, Gansu. Journal of Lanzhou University (Natural University) **40(3),** 72-75.

Von Buren M. 2001. Polymorphism in two homeologous gamma-gliadin genes and the evolution of cultivated wheat. Genetic Research and Crop Evolution **48(2),** 205-220. http://dx.doi.org/10.1023/A:1011213228222

Wang DN, Mu CC, Gao Z, Feng FJ. 2011. Inter-simple sequence repeat (ISSR) analysis of genetic diversity of Juglans mandshurica Maxim populations. Nonwood Forest Res. **29,** 22-29.

Wang Z, Weber JL, Zhang G, Tanksley SD. 1994. Survey of plant short tandem DNA repeats. Theoretical and Applied Genetics **88(1),** 1-6. http://dx.doi.org/10.1007/BF00222386

White J, Law JR, MacKay I, Chalmers KJ, Smith JSC, Kilian A, Powell W. 2008. The genetic diversity of UK, US, Australian cultivars of Triticum aestivum measured by DArT markers and considered by genome. Theoretical and Applied Genetics **116(3),** 439-453. http://dx.doi.org/10.1007/s00122-007-0681-3

Zietkiewicz E, Raflaski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeats (SSR)-anchored polymerase chain reaction amplification. Genomics **20(2),** 176-183. http://dx.doi.org/10.1006/geno.1994.1151