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Molecular discrimination of the advanced breeding lines of durum wheat (*Triticum turgidum* L.) based on inter simple sequence repeat markers

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Abstract

Durum wheat (*Triticum turgidum* L. var. durum) is the most suitable wheat to produce pasta and spaghetti because of its high protein content. A set of 14 durum breeding lines along with three check cultivars (i.e., Saji, Zardak and Sardari), 10 ISSR primers were used to estimate genetic diversity among genotypes. The primers produced 111 bands, among which 85 bands (about 76.5%) were polymorphic. A total of 85 polymorphic fragments were scored with average 11.1 polymorphic bands per primer. The average of PIC index was 0.33, that showed a good efficiency of primers to separate the genotypes. Cluster analysis using UPGMA method and Dice similarity coefficient categorized the genotypes into five main groups in which the check genotypes were classified in the separated groups.

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Introduction

Durum wheat (*Triticum turgidum* L. var. durum) is an important crop with a yellow endosperm and high protein content that mainly used for the production of pasta and spaghetti. It is the most suitable wheat for high quality pasta products because of its high protein content, excellent amber color, kernel hardness, granular semolina, and superior cooking qualit (Dexter *et al.*, 1990). Durum wheat is a major crop in the Mediterranean basin of West Asia, North Africa, and Southern Europe (Elias and Manthey, 2005).

The level of genetic diversity in durum wheat is being affected by the high selection pressure applied in breeding programs and the gene pool of durum wheat varieties has been narrowed (Maccaferri *et al.*, 2005; Royo *et al.*, 2006). With the number of new similar or closely related wheat cultivars increasing yearly, the identification of cultivars is essential for cultivar registration, certification and breeder's right protection (Zhu *et al.*, 2011). Genetic diversity within populations was considered highly important for possible adaptation to environmental changes for long-term survival of plant species (Bauert *et al.*, 1998).

Genetic diversity might be evaluated by assessing morphological or biochemical traits but evaluation of genetic diversity by DNA markers is more efficient and reliable than others. The genetic diversity in germplasms of Durum wheat has been studied by morphological (Autrique *et al.*, 1996), physiological (Mohammadi *et al.*, 2011), seed storage protein (Carrillo *et al.*, 1990) and DNA- based markers (Autrique *et al.*, 1996; Carvalho *et al.*, 2011). Fingerprinting with DNA-based molecular markers allows precise, objective and rapid cultivar identification compared with field plot growth tests (Zhu *et al.*, 2011). DNA finger printing methods are more plentiful than morphological markers and are not dependent on environmental effects. Several PCR-based markers were developed and applied to assess the genetic variation among populations and genetic resources (Powell *et al.*, 1995).

Inter-simple sequence repeat markers has been successfully used in germplasm identification and genetic diversity analysis (Ash *et al.*, 2003; Wu *et al.*, 2005; Mantel, 1967; Saghai-Maroof *et al.*, 1984). In higher plants, ISSR markers are more and more in demand, because they are known to be abundant, very reproducible, highly polymorphic, highly informative and quick to use (Bornet and Branchard, 2001). In addition, the simple procedure and low cost are the other advantages of ISSR marker system. Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, defined here as a reduction of genetic diversity in time (Manifesto *et al.*, 2001).

With regard to the importance of genetic variation in wheat improvement programs, the objective of this study was to estimate the genetic diversity among a set of of durum wheat genotypes including breeding lines, landraces and modern cultivar using Inter-simple sequence repeat markers.

Materials and methods

Plant materials and DNA extraction

The seeds of 14 durum breeding lines along with three check cultivars (i.e., Saji, Zardak and Sardari), were obtained from Dryland Agricultural Research Sub-Institute (Sararood station), Kermanshah, Iran (Table 1). The seeds were sown under protected conditions in nurseries to produce seedling plants. After two weeks, fresh leaves were collected from each pot, for genomic DNA extraction. The fresh leaves of each sample were ground to a fine powder in liquid nitrogen and total genomic DNA was extracted using a CTAB method (Potter *et al.*, 2002) 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-PCR amplification

A set of 10 ISSR primers (Table2), was used to amplify the genomic DNA of all 17 genotypes. PCR amplifications were performed in 20 μl reaction

volume containing: 2 µl of genomic DNA, 1.2 µl of primer, 0.4 µl dNTPs Mix, 1.5 µl Mgcl2, 2 µl PCR buffer, 0.3 µl Taq DNA polymerase (5 unit/ µl) and 12.6 µl double distilled water.

The amplifications were performed in a Biorad thermocycler using the following profile with an initial 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. Amplified products were separated by electrophoresis on 1.5% agarose gel containing 0.5 mg/ml ethidium bromide in TBE buffer and photographed on UV light by a Vilber gel documentation.

Band scoring and data analysis
Since ISSR markers are dominant, amplified fragments were constructed by scoring 0 and 1 for absence and presence of bands, respectively. The generated data matrixes were subjected to statistical analysis using the DARwin computer software (Perrier *et al.*,2003). Genetic similarity estimates were calculated and relationships of the genotypes were estimated from the markers data using the UPGMA clustering method based on Nei's (1978) unbiased genetic distance. The polymorphism information content (PIC) was used to characterize the efficiency of each primer to reveal polymorphic loci. The PIC index was calculated as: PIC=1- $\sum Pi^2$, where p represent band frequency and q represent no-band frequency.

Results and discussion
Of the all 15 tested primers, 10 ISSR primers generated 111 clearly bands across 17 genotypes. The size of ISSR fragments varied from 200 bp to 2800 bp. Analysis of banding patterns revealed 85 polymorphic bands with an average of 11.1 fragments per primer. The highest and the lowest number of polymorphic bands per assay were 14 and 4 bands, respectively (Table 2).

Table 1. The codes/ names of tested genotypes.

Entry	Genotype	Entry	Genotype	Entry	Genotype	Entry	Genotype
1	Saji (check)	6	18E-M84859	11	18-EM142005	16	18E-M142069
2	Zardak	7	18E-M141979	12	18E-M142017	17	18E-M142070
3	Sardari	8	18E-M141982	13	18E-M142025		
4	18E-TOPDY	9	18-EM141994	14	18E-M142038		
5	18E-RASCON	10	18E-M141995	15	18E-M142045		

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

Primer code	Primer sequences	No. of bands scored	No. of polymorphic bands	PIC	MI
IS10	5'-GAGAGAGAGAGAGAGAGARC-3'	14	14	36	5.1
IS 5	5'-AGAGAGAGAGAGAGAGAGC-3'	13	11	32	3.0
IS 7	5'-GTGTGTGTGTGTGTGTGTC-3'	11	10	25	2.3
IS14	5'-GACAGACAGACAGACA-3'	8	4	38	0.8
IS16	5'-DBDACACACACACACACA-3'	14	7	25	0.9
IS 1	5'-ACACACACACACACACYA-3'	13	11	26	2.5
IS 9	5'-CTCTCTCTCTCTCTCTG-3'	12	9	32	2.1
IS 6	5'-CACACACACACACACAG-3'	8	6	45	2.0
IS13	5'-AGAGAGAGAGAGAGAGYT-3'	8	8	28	2.2
IS11	5'-ACACACACACACACACACC-3'	10	5	40	1.0

The average of PIC index was 0.33, that showed a good efficiency of primers to separate individuals, but Some primers were more efficient in differentiating between individuals than others. For example the maximum PIC (0.45) was observed for the primer IS-6 and Primers IS-7 and IS-16 showed the lowest amount of PIC (0.28) that represented their not good separation power. The Marker index (MI) was calculated for all primers. A summary of the MI calculated based on the PIC and polymorphic bands for each primer, is reported in Table 2. The MI values

for ISSR primers, ranged from 0.8 to 5.1 with an average of 2.19.

The maximum MI (5.1) was observed for the primer IS10 and the minimum MI (0.8) was obtained with IS14. This feature has been used to evaluate the discriminatory power of molecular marker systems in some plant species e.g. apricot (ISSR, MI = 3.74) (Kumar *et al.*, 2009), Jatropha (AFLP, MI = 25.13) (Tatikonda *et al.*, 2009).
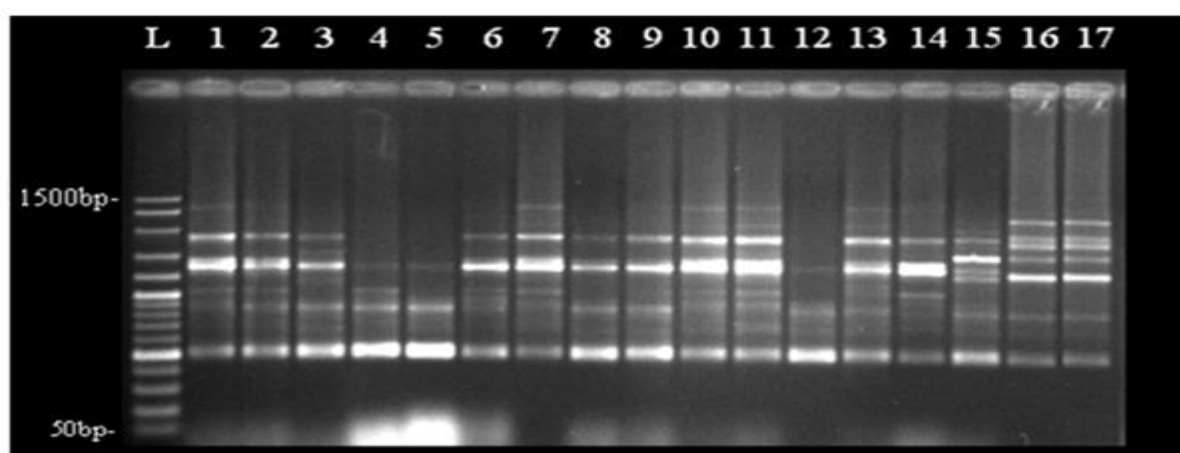

Fig. 1. Banding patterns among the 17 durum wheat genotypes using simple sequence repeat (ISSR) using primer IS 5.
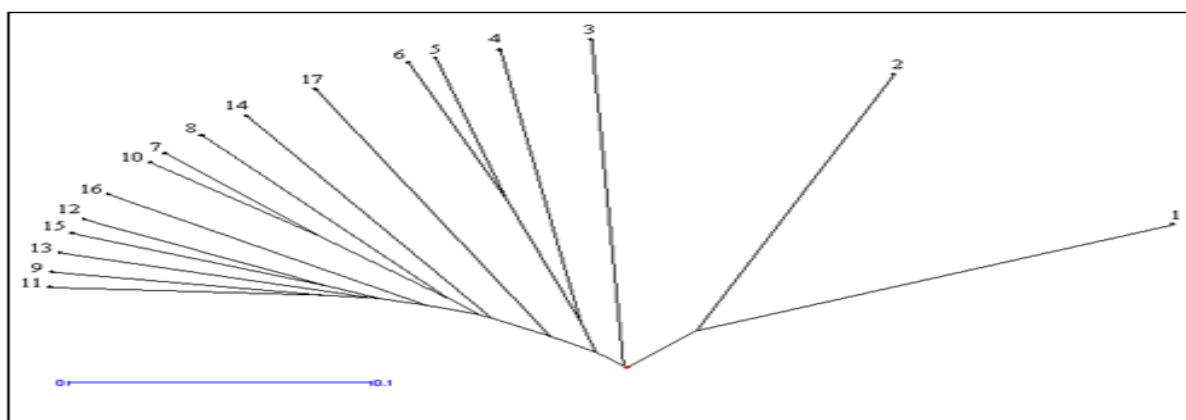

Fig. 2. Dendrograms generated with hierarchical UPGMA cluster analysis based on ISSR data for 15 tested genotypes.

The dendrogram generated with hierarchical unweighted pair group method with arithmetic mean (UPGMA) cluster analysis based on data obtained by ISSR primers and Dice similarity coefficient categorized the genotypes into five main groups. According to the analysis, the first cluster contained two check cultivars of durum wheat (Saji and Zardak)

and Sardari as the bread wheat check cultivar was classified individually in a separated group. One of the landraces (genotype number 17 was also classified separately). Genotypes number 4, 5 and 6 were clustered as three individual groups and the other landraces were classified in a separated group (Figure 2). The cophenetic correlations between the similarity

matrix and corresponding dendrogram were found to be 0.87 (Mantel, 1967). 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. 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 and Olivieri, 1993).
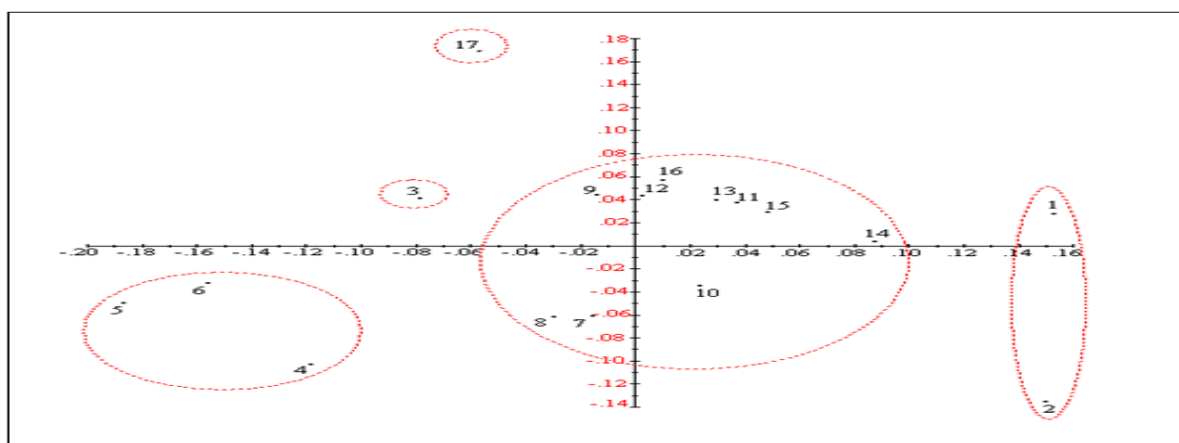

Fig. 3. Plot of the first and second coordinate in 17 durum wheat genotypes according to ISSR markers.

References

Autrique E, Nachit M, Monneveux P, Tanksley SD, Sorrells ME. 1996 . Genetic Diversity in Durum Wheat Based on RFLPs, Morpho-physiological Traits, and Coefficient of Parentage **36(3),** 735-742.
http://dx.doi.org/10.2135/cropsci1996.0011183X003600030036x

Ash GJ, Raman R, Crump NS. 2003. An investigation of genetic variation in *Carthamus lanatus* in New South Wales, Australia, using inter-simple sequence repeats(ISSR) analysis. Weed Res (Oxford). **43(3),** 208–213.
http://dx.doi.org/10.1046/j.1365-3180.2003.00335.x

Bauert MR, Kalin M, Baltisberger M, Edwards PJ. 1998 . No genetic variation within isolated relict populations of Saxifraga cernua in the Alps using RAPD markers. Mol. Ecol.**7(11),** 1519-1527.
http://dx.doi.org/10.1046/j.1365-294x.1998.00470.x

Bornet B, Branchard M. 2001. Nonanchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol. Biol. **19(3),** 209_ 215.
http://dx.doi.org/10.1007/BF02772892

Carrillo JM, Vazquez JF, Orkellana J. 1990. Relationship Between Gluten Strength and Glutenin Proteins in Durum Wheat Cultivars. Plant Breeding **104(4),** 325–333.
http://dx.doi.org/10.1111/j.14390523.1990.tb00443.x

Carvalho A, Guedes-Pinto H, Lima-Brito JE. 2011. Genetic Diversity in Old Portuguese Durum Wheat Cultivars Assessed by Retrotransposon-Based Markers. Plant Molecular Biology Reporter **30(3),** 578-589.
http://dx.doi.org/10.1007/s11105-011-0367-5

Dexter JE, Matsuo RR, Kruger JE. 1990. The spaghetti making quality of commercial durum wheat samples with variable alpha-amylase activity. Cereal Chemistry **67(5),** 405-412.

Elias EM, Manthey FA. 2005. End products. In: Royo C, Nachit MN, Di fonzo N, Araus JL, Pfeiffer WH, Slafer GA, eds Durum wheat breeding. Current approaches and future strategies. New York: Food Academic Press, The Haworth Press 63–86.

Kumar M, Mishra GP, Singh R, Kumar J, Naik PK, Singh SB. 2009. Correspondence of ISSR and

RAPD markers for comparative analysis of genetic diversity among different apricot genotypes from cold arid deserts of trans-Himalayas. PhysiolMolBiol Plants **15(3),** 225-236.
http://dx.doi.org/10.1007/s12298-009-0026-6

Maccaferri M, Sanguineti MC, Noli E, Tuberosa R. 2005. Population structure and long-range linkage disequilibrium in a durum wheat elite collection. Molecular Breeding **15(3),** 271-290.
http://dx.doi.org/10.1007/s11032-004-7012-z

Manifesto MM, Schlatter AR, Hopp HE, Suarez EY, Dubcovsky J. 2001. Quantitave evaluation of genetic diversity in wheat germplasm using molecular markers. Crop Sci **41(3),** 682-690.
http://dx.doi.org/10.2135/cropsci2001.413682x

Mantel M. 1967. The Detection of Disease Clustering and a Generalized Regression Approach. Cancer Res **27(2),** 209-220.

Mohammadi R, Sadeghzadeh D, Armion M, Amria M. 2011. Evaluation of durum wheat experimental lines under different climate and water regime conditions of Iran. Crop and Pasture Science **62 (2),** 137-151.
http://dx.doi.org/10.1071/CP10284

Morgante M, Olivieri AM. 1993. PCR-amplified microsatellite as markers in plant genetics. Plant J. **3(1),** 175-182.
http://dx.doi.org/10.1046/j.1365-313X.1993.t01-9-00999.x

Perrier X, Flori A, Bonnot F. 2003. Methods for data analysis. In: Hamon P, Seguin M, Perrier X, Glazmann JC (eds) Genetic diversity of cultivated tropical plants. Science Publishers, Inc and Cirad, Montpellier, 31–63.

Potter D, Gao FY, Aliello G, Leslie C, McGranahan G. 2002. Intersimple sequence repeat markers for fingerprinting and determining genetic relationships of walnut (*Juglans regia*) cultivars. Am Soc Horticult Sci **127(1),** 75–81.

Powell W, Orozco-Castillo C, Chalmers KJ, Provan J, Waugh R. 1995. Polymerase chain reaction-based assays for the characterisation of plant genetic resources. Electrophoresis **16(1),** 1726-30.
http://dx.doi.org/10.1002/elps.11501601285

Royo C, Nachit MM, Di Fonzo N, Araus JL, Pfeiffer WH, Slafer GA. 2006. Durum Wheat Breeding. Current Approaches and Future Strategies. Biologi Plantarum **50(4),** 794-798.
http://dx.doi.org/10.1007/s10535-006-0137-7

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal Spacer Length Polymorphism in Barley: Mendelian Inheritance, Chromosomal Location and Population Dynamics. Proc. Natl. Acad. Sci. U. S. A. **81(24),** 8014-8018.

Tatikonda L, Wani SP, Kannan S, Beerelli N, Sreedevi TK, Hoisington DA, Devi P, Varshney RA. 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatrophacurcas* L., a biofuel plant. Plant Sci **176(4),** 505-513.
http://dx.doi.org/10.1016/j.plantsci.2009.01.006

Wu W, Zheng YL, Chen L, Wei YM, Yang RW, Yan ZH. 2005. Evaluation of genetic relationships in the genus Houttuynia Thunb. in China based on RAPD and ISSR markers. Biochem Syst Ecol **33(11),** 1141–1157.
http://dx.doi.org/10.1016/j.bse.2005.02.010

Zhu Y, Hu J, Han R, Wang Y, Shuijin Z. 2011. Fingerprinting and identification of closely related wheat (*Triticumaestivum*L.) cultivars using ISSR and fluorescence-labeled TP-M13-SSR markers. Aust J of Crop Science **5(7),** 846-850.