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Evaluation of Genetic Diversity in Durum Wheat Advanced Lines

Soheila Mehdiabadi*, Reza Mohammadi**, Ali Reza Etminan* and Lia Shooshtari*

**Department of Plant Breeding, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, IRAN*

***Assistant Professor of Dryland Agriculture Research Sub-Institute, Kermanshah, IRAN*

(Corresponding author: Alireza Etminan)

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ABSTRACT: The present study was conducted to evaluate the genetic diversity within a set of 16 advanced lines of durum wheat (*Triticum turgidum*) using 9 ISSR primers. A total of 73 polymorphic fragments were scored with average 8.1 polymorphic bands per primer. Cluster analysis using UPGMA method and Dice similarity coefficient categorized the genotypes into 4 separated groups. Estimates of genetic similarity ranged from 0.34 to 0.86 with an average of 0.68. The frequency distribution of genetic distance between pairs of accessions based on marker data indicates a normal distribution. The results showed a considerable polymorphism among durum advanced lines, reflecting the efficiency of ISSRs in the assessment of the genetic diversity.

Keywords: diversity, wheat, ISSR, advance line.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum) is a tetraploid (AABB, 2n = 4x = 28) wheat with high protein quality and gluten strength when compared to that of bread wheat (Quaglia *et al.,* 1998; Von Buren, 2001). The endosperm of durum wheat has the hardest texture of all wheat types that make it suitable to produce the pasta, spaghetti and related products (Dexter et al., 1990). More than 80% of the spring durum cultivars released in the developing world, covering more than 50% of the area planted to this crop, are semi dwarf types, either from The International Maize and Wheat Improvement Center (CIMMYT) crosses or from crosses involving at least one CIMMYT parent (Abdlulaziz Al-Doss *et al.,* 2011). The study of genetic diversity is the first step of a breeding program and genetic resources are very valuable for breeders (Etminan *et al.,* 2010). The successfulness of a breeding program depends on many thing such as knowledge of key traits, Type of gene action, genetic systems controlling their inheritance, genetic variation and environmental factors that influence the expression of gens (Rashidi, 2011). The understanding of genetic variation and genetic similarities present within individuals or populations are useful for the efficient use of genetic resources in breeding programs (Safavi *et al.,* 2012). Genetic variation might be evaluated by assessing morphological or biochemical traits but molecular markers, provide the best assessment of genetic variation because they are plentiful and are not dependent on environ-mental effects. This made this evaluation more efficient and reliable (Dongre *et al.,* 2007). Molecular markers, in particular, DNA based markers provide reliable genetic information because of the in-dependence of the confounding effects of environmental factors (Powell *et al.,* 1995). The use of molecular markers have provided the new tools for more accurate determination of the genetic diversity within and between of species (Sofalian *et al.,* 2008). The inter simple sequence repeat markers are one of the DNA based molecular markers involving PCR amplification of DNA by a single primer and provided sufficient polymorphism for evaluating genetic diversity of wheat genotypes (Zietkiewicz *et al.,* 1994; Najaphy *et al.,* 2012). ISSR markers are easy to handle, highly informative, and repeatable (Mahdy Abouzied, 2011). These markers have been used for fingerprinting of bread wheat varieties released for high yield and quality successfully (Chowdhury *et al.,* 2008).

With regard to the necessity of determinism of genetic diversity in wheat improvement programs, the main goals of this study were to estimate the genetic diversity among advanced genotypes for future breeding programs using Inter-simple sequence repeat markers and evaluate the efficiency of ISSRs for assessment of the genetic diversity in durum wheat germplasm.

MATERIALS AND METHODS

A. DNA extraction

To evaluate the molecular diversity of 16 cultivars of durum wheat (Table 1), the seeds of genotypes were sown under protected conditions in nurseries to produce seedling plants.

After two weeks, the genomic DNA was extracted from young fresh leaves following the CTAB procedure described by Saghai-Maroof *et al.,* (1984) with some modifications. The DNA samples were qualified and quantified by spectrophotometer analysis and agarose gel electrophoresis. Then the isolated genomic DNA was stored at -20 °C for use.

Table 1: The codes and name of 16 genotype of durum wheat.

Number	Genotype	Number	Genotype
1	Saji (check) 21//RASCON_39/TILO_1	9	19E-CF4-JS
2	G-1252	10	19E-PNE/2*RASCON_37
3	61-130	11	19E-SORA/2*PLATA_12
4	19E-BCRIS/BICUM	12	19E-1A.1D 5+10-6/2*WB881
5	19E-D94528/3/2*STOT//ALTAR 84/ALD	13	19E-INTER_16/SNITAN
6	19E-CBC509HILE/SOMAT_3.1	14	19E-CF4JS40
7	19E-MINIMUS/COMBDUCK_2	15	19E-P91.272.3.1/3*MEXI75
8	19E-MINIMUS/COMBDUCK_2	16	19E-SORA/2*PLATA_12

B. PCR amplification and Electrophoresis

A set of 9 ISSR primers (Table 2), was used to amplify the genomic DNA of all 16 genotypes. PCR reactions were carried out in 20 µl volumes containing: 2 µl PCR buffer (10x), 1.5 µl $MgCl_2$ (50 mM), 0.4 µldNTPs (10mM), 1.2 µl primer(10pmol/µl), 0.3 µlTaq 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 with an initial step of 94°C for 4 min(to activate TaqDNA polymerase), followed by 35 cycles of denaturing at 94°C for 30s, 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 and amplified fragments were visualized by staining the gels with ethidium bromide and photographed under UV light using gel documentation system.

C. Scoring and analysis of molecular data

To analysis of molecular data, banding pattern of the ISSR markers were scored by 0 and 1 for absence and presence of bands, respectively.

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) and polymorphism information content(PIC) for each primer.

Primer sequence*	Code	TB	PB	PP	PIC
5'-ACACACACACACACAC.YA-3'	IS1	11	11	100	0.398
5'-AGAGAGAGAGAGAGAG.C-3'	IS5	8	8	100	0.273
5'-GACAGACAGACAGACA-3'	IS6	10	10	100	0.255
5'-GAGAGAGAGAGAGAGA.RC-3'	IS7	5	5	100	0.190
5'-CACACACACACACACA.RC-3'	IS9	10	5	50	0.153
5'-GTGTGTGTGTGTGTGT.C-3'	IS10	6	6	100	0.273
5'-CTCTCTCTCTCTCTCT.G-3'	IS11	11	11	100	0.305
5'-ACACACACACACACAC.C-3'	IS14	10	10	100	0.340
5'-DBDACACACACACACAC.A-3'	IS16	7	7	100	0.387

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

Dice similarity coefficient was used for calculation of genetic similarities between pairs of genotypes. The similarity matrix was subjected to cluster analysis based on UPGMA method and a dendrogram was generated using the DARwin computer software (Perrier *et al.,* 2003). To evaluate the efficiency of selected primers for investigation of genetic diversity, the polymorphism information content (PIC) was calculated according below:

$$PIC=1-\sum P2i$$

where p represent band frequency and q represent no-band frequency to characterize the efficiency of each primer to reveal polymorphic loci.

RESULTS AND DISCUSSION

In the evaluation of genetic diversity among 16 durum wheat genotypes using 9 ISSR primers, 73 clear polymorphic bands were amplified. The number of polymorphic fragments generated by primers, varied from 5 to 11with an average of 8.1 bands per primer. (Table 2). In the previous studies the genetic diversity among fifteen varieties of wheat was studied by 20 RAPD primers and the percentage of polymorphism was found (64.84%) (Iqbal *et al.,* 2012). The genetic similarities ranged from 0.34 to 0.86 that showed a moderate to high level of polymorphism. In the previous studies 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 and 106 clear polymorphic bands (about 82.2%) were amplified. The frequency distribution of genetic distance between pairs of accessions based on marker data indicates a relative normal distribution (Fig. 1). The same results have been reported in *B. carinata* genotypes (Genet *et al.,* 2005). Based on the frequency distribution diagram, the most frequent genetic distance were found within the range of 0.33 to 0.39. The cluster analysis using UPGMA method categorized the genotypes into four main groups (Fig. 3). According to the dendrogram, three genotypes including 1(Check), 9 and 16 were clustered individually and the other remaining genotypes were grouped into a separated group. Genetic relationship among 16 genotypes was also visualized by performing (Fig. 3). The results of principle coordinate analysis (PCoA) based on ISSR data showed a good congruency with cluster analysis and supported the clustering pattern of UPGMA dendrogram (Fig. 4).

The polymorphism information content values for ISSR primers in the present assessment ranged from 0.15 to 0.39. The average of PIC index was 0.28, reflected a moderate to high allelic diversity among the genotypes. These results revealed that ISSR markers can be used as an informative and suitable tools for determination of polymorphism in durum wheat germplasm. These results also showed a considerable level of genetic diversity among genotypes which can be used in durum wheat breeding programs.

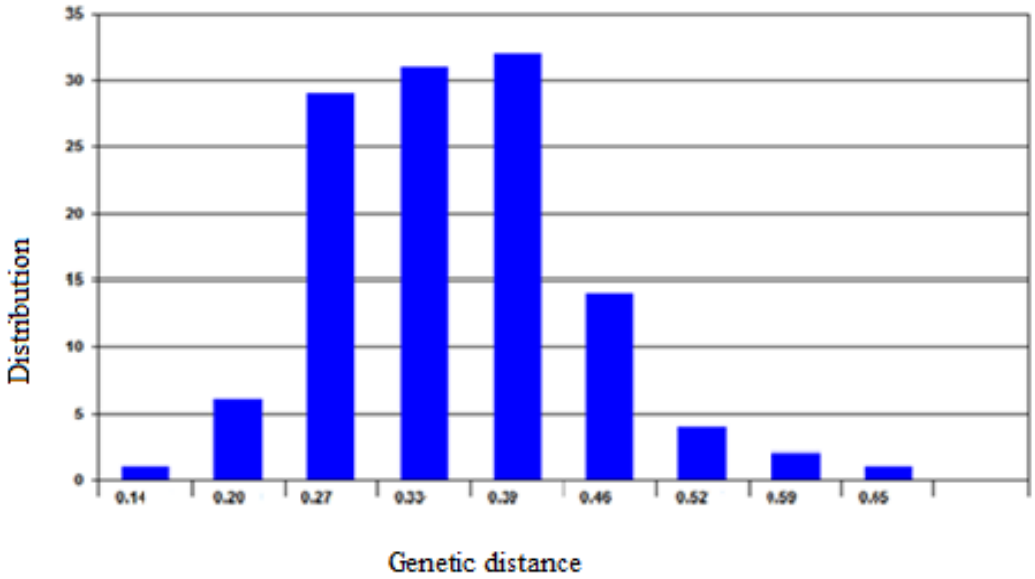

Fig. 1. Distribution of the genetic distance.

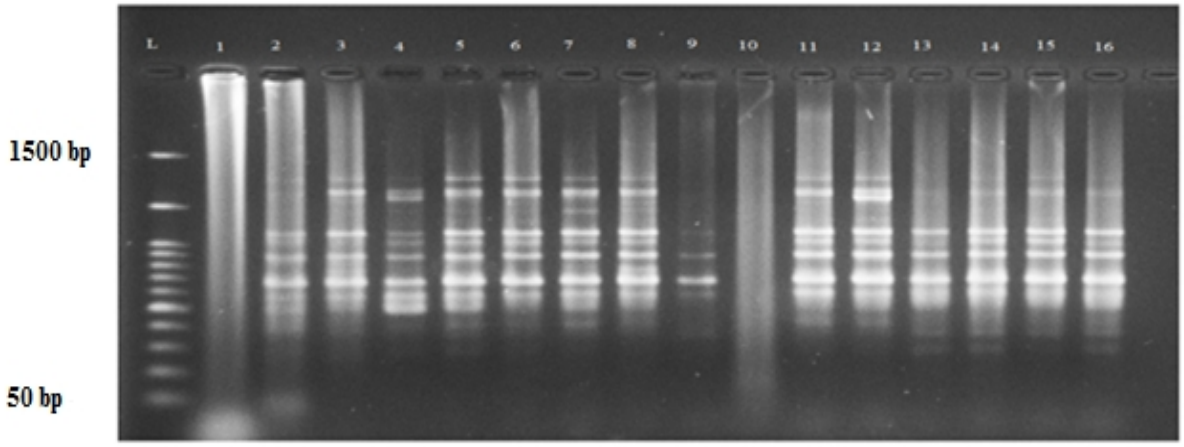

Fig. 2. The banding patterns of 16 durum wheat genotypes using primers IS6.

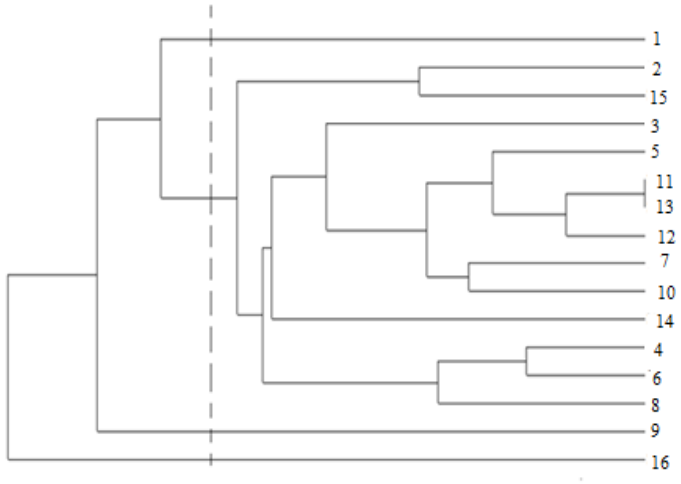

Fig. 3. Molecular analysis dendrogram of cluster analysis (UPGMA).

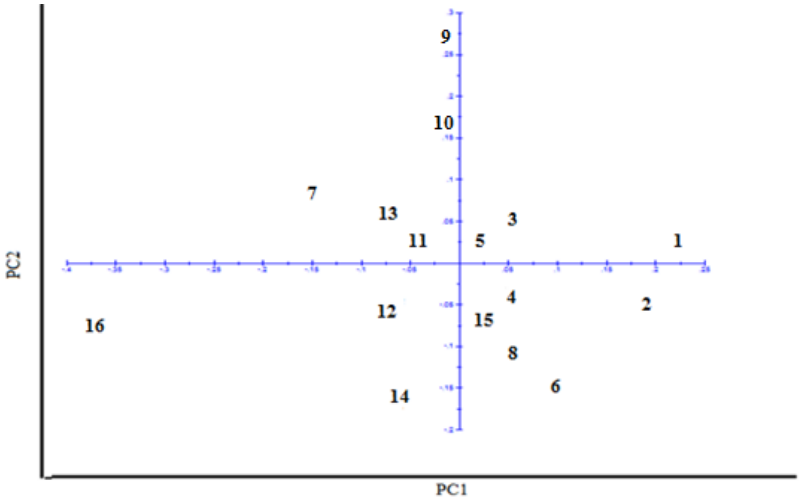

Fig. 4. Principle coordinate analysis according to ISSRs on 16 durum wheat genotypes.

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