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Evaluation of genetic diversity in durum wheat genotypes (*Triticum turgidum* var. durum) using ISSR markers

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Abstract

This study was investigated to evaluate the genetic diversity among 25 genotypes of durum wheat (23 breeding lines, two local varieties and one improved cultivar) at the biotechnology laboratory of Islamic Azad University, Kermanshah, Iran in 2010-2011. 10 inter-simple sequence repeats (ISSR) markers were used to investigate genetic diversity among tested genotypes. A total of 70 loci were scored by 10 primers. The average polymorphic information content (PIC) was 0.41, and the highest amount PIC was 0.49 related to primer IS12. The highest genetic distance between genotypes based on Dice similarity coefficients was observed between genotypes 23 and 24 and the least genetic distance was between genotypes 13 and 18. Based on Dice similarity coefficients, genetic distance of genotypes varied from 0.38 to 0.90 with mean value equal to 0.64. Cluster analysis based on Ward's method and principal coordinate analysis (PCoA) classified the genotypes into five main groups. The results revealed that ISSR markers could be efficiently used to evaluate genetic variation in the durum wheat genotypeswheat germplasm.

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Introduction

Durum wheat (*Triticum turgidum* var. *durum*) is among the tetraploid (AABB, 2n=4x=28) wheat species with commercial importance that is widely cultivated today (von Buren, 2001; Kilian *et al*., 2009). Genetic diversity of plants determines their potential for improved efficiency and hence their use for breeding, which eventually may result in enhanced food production (Khodadadi *et al*., 2011). 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; Godwin *et al.* 1997; Hollngsworth *et al.* 1998; Blair *et al.* 1999; Amsellem *et al.* 2000).

Microsatellites or simple sequence repeats are tandemly repeated mono-, di-, tri-, tetra- or pentanucleotide units (Sehgal *et al.* 2009). Inter-simple sequence repeats (ISSR) PCR using primers based on di-, tetr- or penta-nucleotide repeats have now become a routine among the researchers (Zietkiewicz *et al*., 1994). For its advantages of simple procedure, low-cost, good stability and high reproducibility, ISSR markers has been successfully used in genetic mapping (Casaoli *et al.* 2001; Cekic *et al.* 2001; Tanyolac, 2003). Najaphy *et al*., (2012) revealed that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. The ISSR markers are increasingly applied in the plant sciences and have detected a sufficient degree of polymorphism in faba bean (Terzopoulos and Bebeli, 2008), safflower (Golkar *et al*., 2011), rice (Blair *et al*., 1999) and barley (Hou *et al*., 2005). In the present work, we analysed a collection of 25 durum wheat genotypes with ISSRs, in order to evaluate their genetic variability.

The main objective of this study was to estimate the genetic diversity among a set of durum wheat genotypes with ISSR markers were used and to quantify the relationship of the genetic materials for future durum breeding programs.

Materials and methods

Materials

In this study 10 ISSR markers were used to investigate the genetic diversity among 25 durum wheat genotypes consisting of 23 breeding lines, two local varieties and one improved cultivar were used at the biotechnology laboratory of Islamic Azad University, Kermanshah, Iran in 2010-2011. (Table 1). Genomic DNA was extracted from young leaves following the cetyl tri methyl ammonium (CTAB) procedure described by Doyle and doyle, (1987). Extracted DNA concentration was quantified using the NanoDrop spectrophotometer and qualified using agarose gel electrophoresis. CTAB buffer was including 100 mM Tris-HCl (pH= 8.0), 4M Nacl (pH=8.0), 0.5 M EDTA (pH=8.0), 0.2 % 2-Merkapto etanol and 2% CTAB.

Table 1. List, pedigree and some characters of 30 wheat genotypes used in this study.

Genotype code	Name
1	IDYN-88-4
2	IDYN-88-5
3	IDYN-88-6
4	IDYN-88-7
5	IDYN-88-8
6	IDYN-88-11
7	IDYN-88-14
8	IDYN-88-15
9	IDYN-88-17
10	IDYN-88-19
11	IDYN-88-20
12	IDYN-88-23
13	IDYN-88-26
14	IDYN-88-28
15	IDYN-88-31
16	IDYN-88-35
17	IDYN-88-37
18	IDYN-88-43
19	IDYN-88-44
20	IDYN-88-46
21	IDYN-88-47
22	Saji (check)
23	Zardak (durum landrace)
24	Gerdish (durum landrace)
25	Sardari (bread wheat landrace)

ISSR analysis

Ten ISSR primers were used for the PCR. Each 20 µl reaction volume was contained master mix beads, 2 µl buffer (10 X), 1.2 µl primer, 0.4 mM dNTPs, 12.6 mM DDW, 1.5 mM MgCl2, 0.3 U Taq polymerase (Fermentas), and 2 ng of genomic DNA. The amplified products were separated on 2 % agarose gels and stained with ethidium bromide. Images were photographed, captured by Gel Doc 2000TM (Bio-Rad, USA). Amplified products were scored for the presence (1) or absence (0) of bands and binary matrices were assembled for the ISSR markers. The ISSR binary data matrix was used to calculate the Dise similarity coefficient. Cluster analysis was performed via complete linkage method using NTSYS-pc software version 2.02 (Rohlf, 2000).

For each ISSR marker, were recorded total amplified bands, number of polymorphic bands, and percentage of polymorphic bands (PPB). To measure the informativeness of the ISSR markers to differentiate between wheat genotypes were calculated, polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI) and resolving power (RP). PIC was calculated according to the formula of Anderson *et al.*, (1993), as PIC = 1 – Σp_i^2, where p_i is the frequency of the ith allele of the locus in the set of thirty wheat genotypes. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands (Joshi and Nguyen, 1993). MI was determined according to (Powell *et al.*, 1996) as the product of PIC and EMR. RP was calculated using the formula RP=ΣI_b, where I_b is band informativeness and I_b= 1-[2 × (0.5 - p)], where *p* is the proportion of genotypes containing the band (Altintas *et al*, 2008).

Results and discussion

Thirteen ISSR primers were initially screened for their ability to produce polymorphic patterns across the twenty five durum wheat genotypes. Ten primers which were repeatable and produced high resolution bands for all the genotypes were selected for evaluation of genetic diversity in the accessions (Table2).

Table 2. Parameters of genetic variation generated by ISSR markers.

Primer	Sequence (5'-3')	Total amplified bands	No. of polymorphic bands	PPBa	PICb	EMRc	MId	RPe
IS1	5'-(AC)$_8$YA-3'	8	7	%100	0.47	7	3.29	5.48
IS5	5'-(AG)$_8$ C-3'	11	11	%100	0.40	11	4.4	9.36
IS6	5'-(CA)$_8$ G-3'	6	5	%100	0.46	5	2.3	5.72
IS7	5'-(GT)$_8$ -3'	8	7	%100	0.35	7	2.45	6.68
IS9	5'- (CT)$_8$ G-3'	5	4	%100	0.42	4	1.68	6.24
IS10	5'-(GA)$_8$ RC-3'	7	6	%85	0.34	5.1	1.73	8.68
IS12	5'- (TG)$_8$ G -3'	7	6	%100	0.49	6	2.94	8.92
IS13	5'- (AG)$_8$ YT -3'	6	6	%100	0.47	6	2.82	8.2
IS15	5'-(GGAT)$_4$ AT -3'	3	3	%100	0.41	3	1.23	5.48
IS16	5'-DBDA(CA)$_7$ -3'	9	7	%87	0.31	6.09	1.88	5.4
Minimum		3	3	%85	0.31	11	1.23	5.4
Maximum		11	11	%100	0.49	3	4.4	9.36
Mean		7	6.3	%97	0.41	6.01	2.47	7.01

a Percentage of polymorphic bands;

b Polymorphism information content;

c Effective multiplex ratio;

d Marker index;

e Resolving power

10 ISSR primers generated 70 clearly bands across 25 genotypes.. The percentage of polymorphic bands (PPB) ranged between 85 and 100 with an average of 97% (Table 2). Variable efficiencies of different

marker systems have been reported for detecting DNA polymorphism in wheat. Joshi and Nguyen (1993), observed 1.8 polymorphic bands per RAPD primer among 15 wheat cultivars, while SSRs with 6.2 alleles/ bands were more polymorphic (Plaschke *et al.*, 1995). Nagaoka and Ogihara (1997), detected 3.7 polymorphisms per ISSR primer, while Carvalho *et al.*, (2009) reported 12.9 polymorphic bands per primer using 18 ISSR primers in 48 wheat accessions. We detected a high level of polymorphism among the durum wheat genotypes using ISSR markers, indicating high efficiency of the ISSR marker technique to reveal genetic diversity in the case of durum wheat. These results are in agreement with those obtained by Najaphy *et al.* (2012), who found that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. Carvalho *et al.*(2008) found that the total mean percentage of ISSR polymorphism was 42.1% among 51 cultivars of old Portuguese durum wheat. The results of Sofalian *et al.*, (2008) indicated high level of polymorphism of wheat landraces based on ISSR markers in contrast to other markers. The lowest polymorphism value (57.1%) was obtained with the IS10 primer [$(GA)_8$ RC] (Table 2). Primers based on more infrequent tetranucleotide SSRs amplified few bands in rice Blair *et al.* (1999), while they detected more polymorphism in Dent and Popcorn Kantety *et al.* (1995). In our case, the ISSR primers with dinucleotide motifs (GA)n, (CT)n and (AG)n produced a high level of polymorphism (Table 2). These results are in agreement with those of Carvalho *et al.*, (2009), who reported that dinucleotide primers were more suitable for amplifying ISSRs in bread and durum wheat. SSRs seems to be randomly distributed in the genome, and (GA)n dinucleotide repeats are most abundant in plant species (Steinkellner *et al.*, 1997; Wang *et al.*, 1994).

Polymorphism information content (PIC)

The PIC values varied among the ten primers from 0.31 to 0.49 with an average of 0.41. The lowest and highest PIC indices were recorded for primer IS16 and IS12, respectively. The moderate values of PIC for the ISSR primers could be attributed to the diverse nature of the wheat accessions and or highly informative ISSR markers used in this study. The PIC index has been used extensively in many genetic diversity studies (Talebi *et al.*, 2010; Tatikonda *et al.*, 2009; Thudi *et al.*, 2010).

Marker index (MI) and effective multiplex ratio (EMR)

MI is a feature of a marker and was calculated for all the primers. The MI values ranged between 1.23 and 4.4. The maximum MI (4.4) was observed for the primer IS5 and the minimum MI (1.23) was obtained with ISSR primers IS15. The primers that showed higher polymorphism had higher EMR values. This feature varied from 3 to 11 with a mean value of 6.01. EMR is the product of the fraction of polymorphic bands and the number of polymorphic bands and MI is the product of PIC and EMR, therefore the higher polymorphism provides higher EMR.

Resolving power (RP)

The estimates of RP ranged from 5.4 to 9.36 with an average of 7.01 per primer. The highest RP was recorded for the primer IS5(9.36) and the lowest value was scored with the primers IS16 (5.4) and IS15 (5.48). Prevost and Wilkinson (1999), introduced the RP index that provides a moderately accurate estimate of the number of genotypes identified by a primer. Three of the ISSR primers (IS5, IS12 and IS10) possessed high RP values (8.68, 8.92 and 9.36, respectively) and therefore seem to be the most informative primers for distinguishing the genotypes. RP was not significantly associated with the other molecular indices e.g. PPB, PIC, EMR and MI in this study. The resolving power provides no information on the ability of a primer to reflect the genetic or taxonomic relationships of a group of genotypes under study (Prevost and Wilkinson, 1999).

Genetic relationships among durum wheat genotypes

The dendrogram obtained from the method Ward, in comparison with the Ward method had higher

cophenetic correlation and no chaining. Genetic similarity (Fig. 1). ranged between 0.38 and 0.90 (data not shown). The dendrogram classified the thirty wheat genotypes into five clusters (Fig. 2). First cluster included genotypes 14, 2, 25 and 13. Genotypes 11, 9, 10 and 12 were grouped in the second cluster.

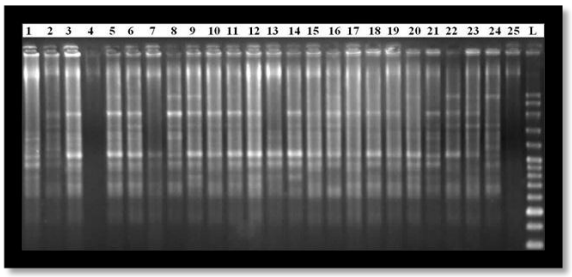

Fig. 1. ISSR products amplified with primer IS13, visualised on an agarose gel 2% stained with ethidium bromide. Each lane contains a different durum wheat cultivar (identified by its code number). M . Molecular weight marker Gene Ladder 100 bp Plus (Fermentas).

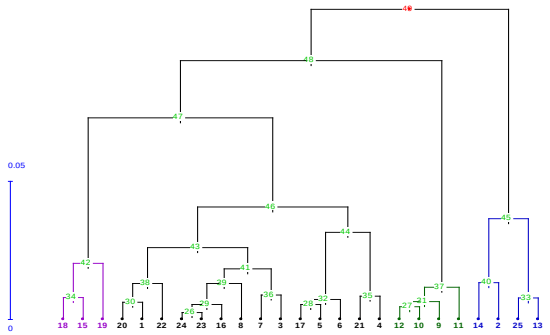

Fig. 2. dendrogram of 25 durum wheat genotypes based on ISSR marker data.

Third cluster contained five genotypes (4, 21, 6, 5 and 17). Fourth cluster contained a total of 9 genotypes (3, 7, 8, 16, 23, 24, 22, 1 and 20). Genotypes 19, 15 and 18 were grouped in fifth cluster. These results are in agreement with those reported by Carvalho *et al.*, (2008), who analyzed forty-eight bread wheat cultivars of an Old Portuguese collection using 18 ISSR markers. They found that most cultivars belonging to the same botanical variety were clustered in the same main group. Sofalian *et al.*, (2008) also reported that ISSR markers are efficient tools for estimating intra-specific genetic diversity in wheat and these molecular markers could differentiate the local varieties obtained from different locations. These results were in agreement with the findings of Arzani and Rezaei (2011).

The principle coordinate analysis results are illustrated in (Fig. 3) The 25 genotypes were grouped into five groups based on two-dimensional graph. First group consisted of genotypes 13, 2 and 25. Second group included 5 genotypes 11, 9, 10, 8 and 12. The third group contained genotypes 4, 5, 14, 16, 21 and 14. Forth group contained a total of 8 genotypes (1, 3, 7, 6, 23, 24, 22 and 20). Fifth group included genotypes 15, 18 and 19. The results of the two methods (Cluster analysis and principle coordinate analysis) were comparable. Both of them classified the 25 durum wheat genotypes in 5 groups and verified similar grouping of the genotypes with some minor disagreements.

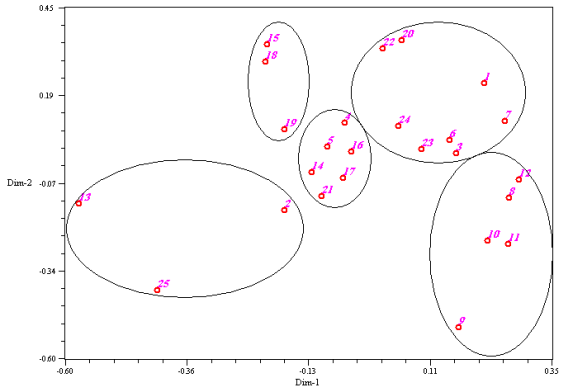

Fig. 3. Scatter plot of durum wheat genotypes using principle coordinate analysis based on ISSR data.

Conclusions

The ISSR technique provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of durum wheat genotypes. The parameters MI and RP can be recommended for selecting informative primers. the genetic variation assessed by ISSR in combination with agro-physiological traits of durum wheat can be useful in traditional and molecular breeding programs.

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