Biological Forum – An International Journal **7**(1): 214-218(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluation of Molecular Diversity of durum wheat genotypes using ISSR markers

Zahra Zamanianfard*, Alireza Etminan*, Reza Mohammadi** and Lia Shooshtari*
**Department of Plant Breeding, College of Agriculture,*
Kermanshah Branch, Islamic Azad University, Kermanshah, IRAN.
***Dryland Agricultural Research Institute (DARI),*
P.O. Box 67145-1164, Kermanshah, IRAN

(Corresponding author: Alireza Etminan)
(Received 10 December, 2014, Accepted 24 January, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In present study, the molecular diversity of twenty five durum wheat genotypes including 21 breeding lines and 3l and races were evaluated using 11inter simple sequence repeat sprimers. The used primers generated 108 fragments, among which 83 bands (about 77%) were polymorphic. A total of 83 polymorphic bands were scored with average 7.54 polymorphic fragments per primer. The average of polymorphism information content index was 0.31, indicating the efficiency of the markers in discrimination of the populations. Cluster analysis based on UPGMA algorithm and Dice similarity coefficient classified the 25 genotypes into four separated groups. The result of principal coordinate analysis (PCoA) was in congruity with cluster analysis.

Keywords: ISSR, Durum, wheat, landrace

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum) is a major crop in the Mediterranean basin of West Asia, North Africa, and Southern Europe (Elias and Manthey, 2005). This tetraploid crop (AABB, 2n = 4x = 28) is the most suitable wheat for high quality pasta products because of its high protein content (Von Buren, 2001). 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). Genetic diversity among genotypes are useful for genetic development of crop plants. Considering the importance of Genetic diversity in reducing genetic vulnerability, the maintenance of variation in an important goal in breeding programs. Assessment of genetic diversity is the first step for efficient management of genetic resources. There are many different methods to evaluate the genetic variation, among which DNA markers are more efficient and reliable. ISSR marker system is one of the best choices to detect the genetic polymorphism in higher plants is ISSR (Zietkiewicz *et al.*, 1994; Nagaoka and Ogihara, 1997).The sequences amplified by a single primer 16-18 bp long can be used for DNA fingerprinting. Najaphy *et al.*, (2012) revealed that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. El-Assal and Gaber, (2012) investigated the discriminating capacity of ISSR markers in establishing genetic relationship and diversity among wheat genotypes. Sofalian *et al.*, (2009) showed that ISSR markers could be efficiently used to evaluate genetic variation in the wheat germplasm. Chowdhury *et al.*, (2008) used ISSR markers for fingerprinting in a set of 27 genotypes which comprised Indian bread wheat varieties released for high yield, quality and abiotic stress and found that the cluster analysis based on molecular data is in agreement with their known origin. Pasqualone *et al.*, (2000) found a high efficiency of ISSR markers to assess the genetic diversity and distinguish all the durum wheat cultivars examined. The main goals of the present study were to test the efficiency of ISSR primers to measure the relationship between durum wheat germplasm and evaluate the genetic diversity among advanced genotypes for future breeding programs.

MATERIALS AND METHODS

A. DNA extraction

Genomic DNA was extracted from young fresh leaves of 25 durum wheat genotypes (Table 1) following the CTAB procedure described by Saghai-Maroof *et al.*, (1984) with some modifications. The quantity and quality of genomic DNA was tested by the Nano Drop spectrophotometer and agarose gel electrophoresis.

Table 1. The codes/ names of 25durum wheat genotypes.

Code	Name	Code	Name
1	19E-SORA/2*PLATA	13	19E-ALTAR 84/STINT//SILVER_45/3/GUANAY
2	19E-GUAYACAN	14	19E-ALTAR 84/STINT//SILVER_45/3/STOT
3	19E-CBC 501	15	19E-CBC 509 CHILE/SOMAT_3.1
4	19E-CMH82A	16	19E-LYMNO_8/3/RASCON_37
5	19E-SNITAN/3/STOT	17	19E-SRN_1
6	19E-ALTAR 84	18	19E-AINZEN-1//HYDRANASSA30/SILVER_5
7	19E-STOT//ALTAR 84/ALD	19	19E-CBC 503 CHILE
8	19E-AINZEN-1/SORD_3	20	19E-G-1252/Zardak
9	19E-CAMAYO	21	19E-Zardak/3/61-130/414-44//Cak79
10	19E-CBC 509 CHILE/SOMAT_3.1	22	Saji (Check)
11	19E-BCRIS/BICUM	23	Zardak
12	19E-ALTAR 84/STINT	24	Gerdish
		25	Sardari

B. PCR amplification and Electrophoresis

A total of 11 ISSR primers(Table 2), were used for amplification and PCR amplifications were performed in 20µl reaction volume 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 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. 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.

C. Band scoring and data analysis

Banding pattern of the ISSR markers in the individuals were scored as presence (1) and absence (0) of the band. The calculation genetic similarities between all 25 genotypes was achieved using Dice? s similarity coefficient. The similarity matrix was subjected to cluster analysis using un weighted pair group method with arithmetic means (UPGMA) clustering procedure and a dendrogram was generated using the DARwin computer software (Perrier *et al.*, 2003).

Principal coordinate analysis was performed to generate a two-dimensional representation of genetic relationship among 19durum wheat genotypes.

To evaluate the efficiency of selected primers for investigation of genetic diversity, the polymorphism information content (PIC) was calculated according below:

$$PIC = 1- \sum P^2i$$

where p represent band frequency and ∑ 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 11 ISSR primers amplified 108 clear and scorable bands across 25 genotypes, of which 83 were polymorphic (Table 2). The size of ISSR fragments varied from 300 bp to 2100 bp. The number of polymorphic fragments generated by primers, varied from 3 to 11with an average of 7.54 fragments per primer. Minimum, maximum and average values of polymorphism information content index (PIC) were found to be 0.19, 0.39 and 0.31, respectively (Table 2). Since the maximum value of PIC for dominant markers such as ISSRs is 0.5 the average value of PIC (0.31) showed a good efficiency of the used primers in discrimination of the individuals. Although the low PIC value obtained by some ISSR markers maybe only due to low number of ISSR loci studied. Similar results have been reported by other workers (Ebrahimi *et al.*, 2010; Pirseyedi *et al.*, 2010; Soriano *et al.*, 2011). Also the amounts of the marker index(MI) of primers calculated based on the PIC and polymorphic bands is showed in Table 2. As shown by Table 2 the highest MI (3.42) was observed with primer UBC-844 that generated 9 polymorphic fragments across 25 genotypes. The polymorphism percentage ranged from 55.6 ~ 100% showing abundant genetic diversity at the population level (Ma *et al.*, 2000; Liu and Jia, 2003; Sun *et al.*, 2004). The average of genetic similarity between genotypes was found 0.74. The genetic similarities range from 0.4 to 0.92 showing a relative high level of polymorphism among genotypes. The cluster analysis using UPGMA method classified the genotypes into four main groups (Fig. 2). According to the dendrogram, the genotype No. 25 (Sardari) was classified individually in a separated group.

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
ACACACACACACACACYA	Is- 1	10	7	70	0.36	2.52
AGAGAGAGAGAGAGAGC	Is- 5	15	9	60	0.35	3.11
CACACACACACACACAG	Is- 6	9	5	55.6	0.28	1.41
CTCTCTCTCTCTCTCTG	Is- 9	10	8	80	0.32	2.53
GAGAGAGAGAGAGAGARC	Is- 10	11	11	100	0.26	2.90
GACAGACAGACAGACA	Is- 14	9	9	100	0.33	2.93
DBDACACACACACACACA	Is-16	9	9	100	0.19	1.72
CTCTCTCTCTCTCTCTRC	UBC- 844	10	9	90	0.38	3.42
CACACACACACACACARG	UBC -848	12	7	58.3	0.39	2.73
TCTCTCTCTCTCTCTCRT	UBC -853	8	6	75	0.25	1.48
VDVCTCTCTCTCTCTCT	UBC- 886	5	3	60	0.30	0.90
Average of values		9.81	7.54	%77.17	0.31	2.33

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

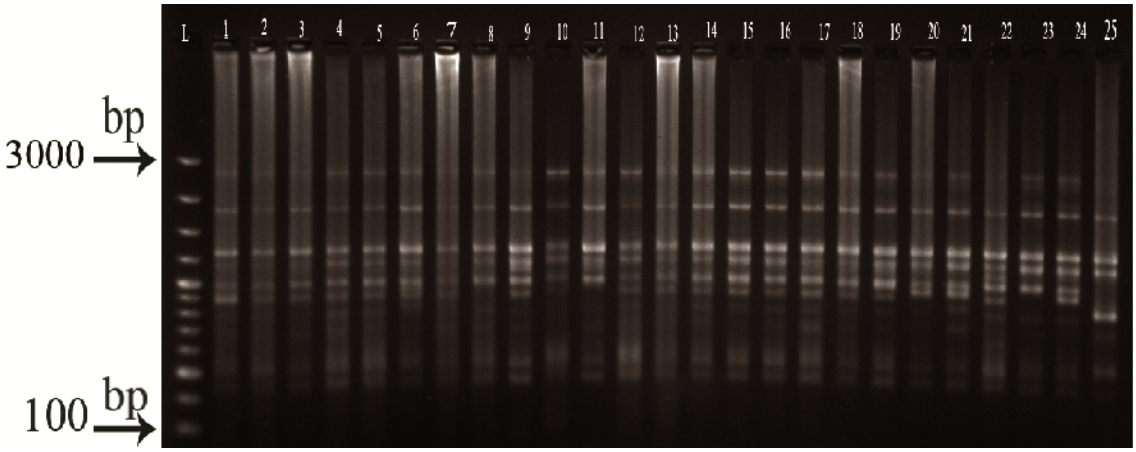

Fig. 1. ISSR marker profile of the primer IS-9 in25durum wheat genotypes.

It is considerable that this genotype was really different from other genotypes in genetic structure. Sardari is a bread wheat variety whereas, the other 24 genotypes are tetraploid (AABB, 2n = 4x = 28) wheat. The principal coordinate analysis (PCo) was performed with ISSR data in order to establish the relationship among genotypes and comparison to cluster analysis. The Principal Component Analysis (PCA) results almost coincided with the results of cluster analysis and supported the clustering pattern of UPGMA dendrogram (Fig. 3). 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). Genetic diversity reflect the ability of species to adapt to the environment and the potential to be used and transformed (Wang *et al.*, 2011). These results showed that ISSR markers are informative and suitable for fingerprinting purposes and could be efficiently used to evaluate genetic variation in the durum wheat germplasm. The result also revealed that there is a high genetic diversity among tested genotypes which can be used in rain fed durum wheat breeding.

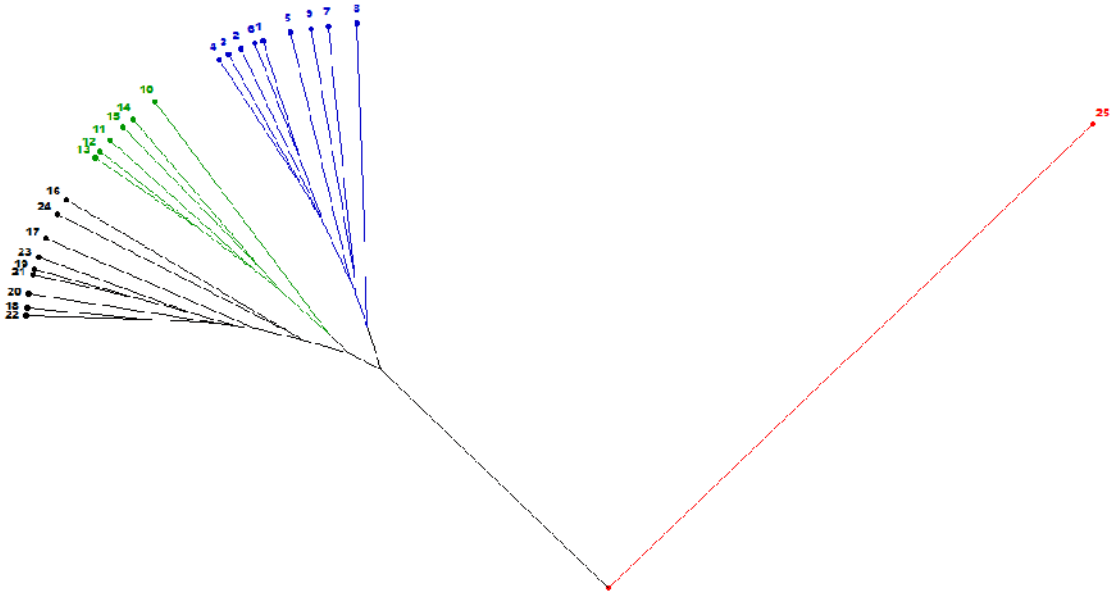

Fig. 2. UPGMADendrogram based on Dice similarity coefficient.

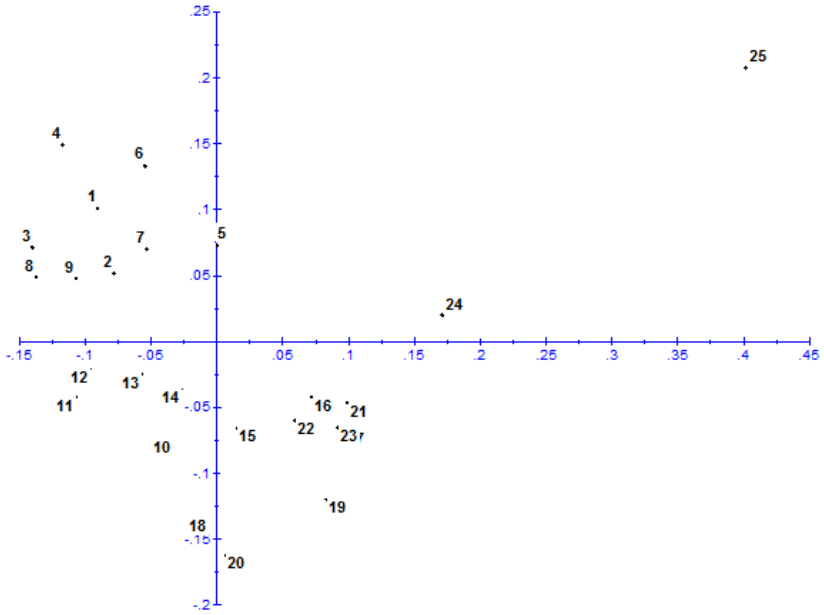

Fig. 3. Principle coordinate analysis according to ISSRs on 25 durum wheat genotypes.

REFERENCES

Chowdhury, R.M.V.K., Kundu, S.J.S. & Jain, R.K. (2008). Applicability of ISSR markers for genetic diversity evaluation in Indian bread wheat genotypes of known origin. *Environmental Ecology*, **26**, 126-131.

Ebrahimi, S., Sayed-Tabatabaei, B.E. & Sharifnabi, B. (2010). Microsatellite isolation and haracterization in pomegranate (*Punica granatum L.*). *Iranian J Biotechnol*, **8**(3): 159-163.

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

Elias, E.M. & Manthey, F.A. (2005). Durum wheat breeding at North Dakota State University. Pages 939-962. C. Royo, M.M. Nachit, N. DiFonzo, J.L. Araus, W.H. Pfeiffer, and G.A. Slafer eds. *Food Products Press*. New York.

Hasnaoui, N., Buonamici, A., Sebastiani, F., Mars, M., Trifi, M. & Vendramin, G.G. (2010). Development and characterization of SSR markers for pomegranate (*Punica granatum* L.) using an enriched library. *Conservation Genet Resour*, **2**(1): 283-285.

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.

Liu, G.H. & Jia, B.L. (2003). The study of genetic diversity of Ulmuspumila. *J. Ar var. sabulosa id Land Resour. Environ.* **17**: 123-128.

Maccaferri, M., Sanguineti , M.C., Noli, E. & Tuberosa, R. (2005). Population structure and long-range linkage disequilibrium in a durum wheat elite collection. *Molecular Breeding*, **15**: 271-289.

Ma, X.J., Wang, X.Q. & Xu, Z.X. (2000). RAPD vriation within and among populations of ginseng cultivars. *Acta Botanica Sinica*, **42**(6), 587-59.

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.

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

Pirseyedi, S.M., Valizadehghan, S., Mardi, M., Ghaffari, M.H., Mahmoodi, P., Zahravi, M., Zeinalabedini, M. & Khayam Nekoui, S.M . (2010). Isolation and Characterization of Novel Microsatellite Markers in Pomegranate (*Punica granatum* L.). *Int. J. Mol. Sci.*, **11**: 2010-2016.

Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. &Allard, R.W. (1984). Ribosomal Spacer Length Polymorphism in Barley: Mendelian Inheritance, Chromosomal Location and Population Dynamics. *Proc. Natl. Acad. Sci. U.S.A.*, **83**: 1757-1761.

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.

Soriano, J.M., Zuriaga, E., Rubio, P., Llacer, G., Infante, R. & Badenes, M.L. (2011). Development and characterization of microsatellite markers in pomegranate (*Punica granatum* L.). *Mol Breeding*, **27**:119-128.

Sun, K., Chen, W., Ma, R.J. & 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, D.N., Mu, C.C., Gao, Z. & Feng, F.J. (2011). Inter-simple sequencerepeat (ISSR) analysis of genetic diversity of JuglansmandshuricaMaxim populations. *Nonwood Forest Res*. **29**: 22-29.

Zietkiewicz, E., Rafalski, A. & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics.* **20**: 176-183.