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Molecular Characterization of Some Syrian Bread Wheat Cultivar

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Abstract: This study was performed to assess genetic variability of nine Syrian bread wheat varieties using ISSR technique. PCR amplifications with 17 ISSR primers gave 101 discernible loci of which 99 (98.01%) polymorphic with PIC value 0.352. The pattern generated by ISSR markers separated the nine wheat varieties into two clusters. The first cluster consists of two subclusters. The first subcluster involved Bohoth4, Douma4, Jolan2 and Douma2, while the second one includes only Bohoth6 which was very far from other varieties. The second cluster also consists of two subclusters. The first subcluster involved Sham4, Sham6,and the second subcluster consists of Sham8 and Sham10 that were also closely related. Based on this study, the use of ISSR technique could be a powerful tool to detect genetic diversity between wheat cultivars.
Keywords: ISSR, genetic diversity, variety, wheat.

Introduction

Wheat is one of the most important cereal crops worldwide in terms of production and utilization. It is a major source of energy, protein, and dietary fiber in human nutrition and animal feeding. It provides approximately one-fifth of the total calorific input of the world's population[1]. Currently about 95% of the wheat grown worldwide is hexaploid bread wheat, with most of the remaining 5% being tetraploid durum wheat[2]. Using new techniques, like Molecular Markers, may help in accelerating the pace of genetic improvement of wheat crop yield, increasing agricultural production and reducing yield gap to achieve food security. Molecular markers provide an excellent tool for obtaining genetic information and their use in the assessment of genetic diversity in wheat (*Triticum aestivum* L.) has increased in the last few years[3]. Inter Simple Sequence Repeats (ISSRs) are a new type of DNA markers which involve the use of microsatellite sequence directly in the polymerase chain reaction (PCR) for DNA amplification[4]. ISSR have been proposed as a new source of genetic markers which overcomes the technical limitation of Restriction Fragment Length Polymorphisms (RFLP) and Random amplified Polymorphic DNA (RAPD)[5]. ISSRs have been used successfully in genome mapping of a variety of crop species including maize, rice, barley and wheat[6 7]. ISSRs are inherited as dominant or rarely as co dominant genetic markers and are random-type markers, so they are suitable for phylogenetic studies, evaluation of genetic diversity and identification of cultivar[8]. El-Assal[9] compared between ISSR markers and RAPD & SSR markers by applying on 11 of Egyptian and Saudi varieties, the number of polymorphic bands detected by each ISSR primer ranged from 5 to 10 with an average of 8.6 bands per primer, whereas the polymorphism percent was 46% with 78 distinct reproducible bands, 36 of the 78 bands were polymorphic. Malik[10] conducted ISSR tests on 27 varieties of breed wheat in India and got 176 bands with 68.42% polymorphism percentage. In China,5 ISSR primers was applied on 8 bread wheat varieties and gave 43 bands, 29 (67,44%) of them was polymorphic, the number of polymorphic bands detected by each ISSR primer ranged from 3 to 8 with an average of 4.8 per primer[11]. Sofalian[12] 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. Carvalho[13] used ISSRs for genetic diversity analyses of an Old Portuguese wheat collection. They found that cultivars were clustered according to their botanical varieties and, in a few cases, with their homonym(s). Carvalho[14] analyzed forty-eight bread wheat cultivars of an Old Portuguese collection by using ISSR markers. They used 18 ISSR primers amplified a total number of 245 ISSR loci, being 233 of them polymorphic. They indicated that most cultivars belonging to the same botanical variety were clustered in the same main group, however an intra-variety ISSR polymorphism was also observed. Sofalian[15] used ISSR markers to determine the genetic diversity of 39 bread wheat accessions, including 33 wheat landraces and 6 wheat cultivars from northwest of Iran. The results indicated high level of polymorphism of wheat landraces based on these markers in contrast to other markers. Cluster analysis suggested 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. Chowdhury[16] used ISSRs 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[17] 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[18] 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. Malik[19] used SSR and ISSR markers to study Genetic diversity for 27 varieties of Indian bread wheat, and they got 191 bands with 65.58% polymorphism percentage for ISSR markers. The PIC value (polymorphic information content) was 0.382 to 0.826.

Materials and methods:

Materials

Grains of nine closely related wheat cultivars were provided by General Commission for Agricultural Research, Damascus, Syria. (Table 1)

Table 1: Wheat cultivars used and its pedigree

pedigree	Cultivar Name	NO.
S-201	Bohoth 4	1
CROE,S	Bohoth 6	2
SHUHA-17/GHURAB-1	Jolan 2	3
VEE,S/BOW,S//ALD,S,-PVN,S	Douma 2	4
ACSAD529/4/C182.24/C168.3/3/cno*2/7c//Cc/TOB	Douma 4	5
FLK,S,-HORK	Sham 4	6
(W3918/JUP) Niser	Sham 6	7
JAY//URES-81 JUPATECO-73/BIUE	Sham 8	8
KAUZ/KAUUZ/STAR	Sham 10	9

DNA extraction

Genomic DNA was extracted from young leaves using the modified CTAB method[20]. DNA was dissolved and diluted to a final concentration of 40 ng/μL with 1×TE buffer (10 mM/L Tris-HC1, pH 8.0; 1 mM/L EDTA) and stored at -20 ºC for further use.

ISSR analysis

Fifteen ISSR primers (Table 2) were used in the study. ISSR polymerase chain reaction (PCR) amplification was conducted in a 25 μL volume containing 50 ng of genomic DNA, 1 U Taq DNA polymerase, 1.5 mM Mg2+, 0.25 mM dNTPs, and 0.2 μM primer. The PCR protocol consisted of an initial denaturation at

94ºC for 4 min, followed by 40 cycles of 94ºC for 45 s, annealing according to the temperature of each primer, as shown in the table (2) for 45 s, 72ºC for 1 min, and a final extension step of 72ºC for 10 min. All PCR reactions were carried out in a thermal cycler C1000 (Bio-Rad, USA). PCR products were separated on 1.5% agarose gels, stained with GelRedTM (Biotium, USA) and photographed under UV light using Image LabTM software Version 2.0.1 (Bio-Rad, USA).

Table 2. Sequences of ISSR primers used in the study

C	Primer Sequence	Primer name
50	$(AG)_8T$	ISSR-1
52	$(GA)_8C$	ISSR-2
50	$(CA)_8A$	ISSR-3
52	$(CA)_8G$	ISSR-4
50	$(AC)_8T$	ISSR-5
56	$(GA)_8CG$	ISSR-6
56	$(AC)_8GG$	ISSR-9
56	$(CA)_{11}$	ISSR-20
52	$(AG)_8C$	ISSR-23
52	$(GA)_8T$	ISSR-33
52	$(CA)_6ACAG$	ISSR-35
52	(TG)8G	ISSR-37
52	(AC)8TT	ISSR-40
56	(AC)8GG	ISSR-41
54	(TG)8GA	ISSR-42

Analysis of data:

Only reproducible and well-defined bands in the replications were considered as potential polymorphic markers, for each primer, the bands were scored as 1 (present) or 0 (absent), and genetic similarity was estimated using Nei-Li's similarity index[21]. A dendrogram was constructed on the basis of the similarity matrix data by unweighted pair method with arithmatic average (UPGMA) cluster analysis using the software POPGENE program. PIC (polymorphic information content) values was calculated according to the equation:

$$\mathbf{PIC = [\Sigma 2(PI(1- PI)^2]}$$

Whereas : PI bands frequency resulting from using the primers in all samples[22].

Results and Discussion:

Identification of Wheat Varieties

Of the seventeen primers tested in wheat material, fifteen generated numerous very clear variable bands with 98.01% reproducibility. Over the 15 primers, 99 of the 101 DNA fragments observed on the gels were polymorphic, and that was not corresponded with Malik's results[10] (Table 3). The number of variable-sized fragments per primer ranged from 2 for ISSR-33 to 13 for ISSR-1. Primers ISSR-1, ISSR-40, ISSR-2, and ISSR-3 have given a clear polymorphism in comparison to the others. (Table 3) (Figure 1). PIC (polymorphic information content) values ranged from 0.296 (ISSR-35) to 0.375 (ISSR-2) with an average of 0.352 for selected primers. And this result was not corresponded with Malik[19] (0.382 to 0.826) (Table 3).

Table 3. Number of bands, percentage of polymorphism and PIC values.

Primer name	Number of band total	Number of polymorphic bands	Polymorphism %	PIC
ISSR-1	13	13	100	0.353
ISSR-2	10	10	100	0.375
ISSR-3	8	8	100	0.353
ISSR-4	6	5	83.33	0.365
ISSR-5	6	6	100	0.355
ISSR-6	5	5	100	0.370
ISSR-9	6	5	83.33	0.368
ISSR-20	3	3	100	0.374
ISSR-32	6	6	100	0.365
ISSR-33	2	2	100	0.328
ISSR-35	5	5	100	0.296
ISSR-37	5	5	100	0.370
ISSR-40	11	11	100	0.305
ISSR-41	7	7	100	0.360
ISSR-42	8	8	100	0.341
Total	101	99	98.01	
Mean	6.73	6.6		0.352

M 1 2 3 4 5 6 7 8 9

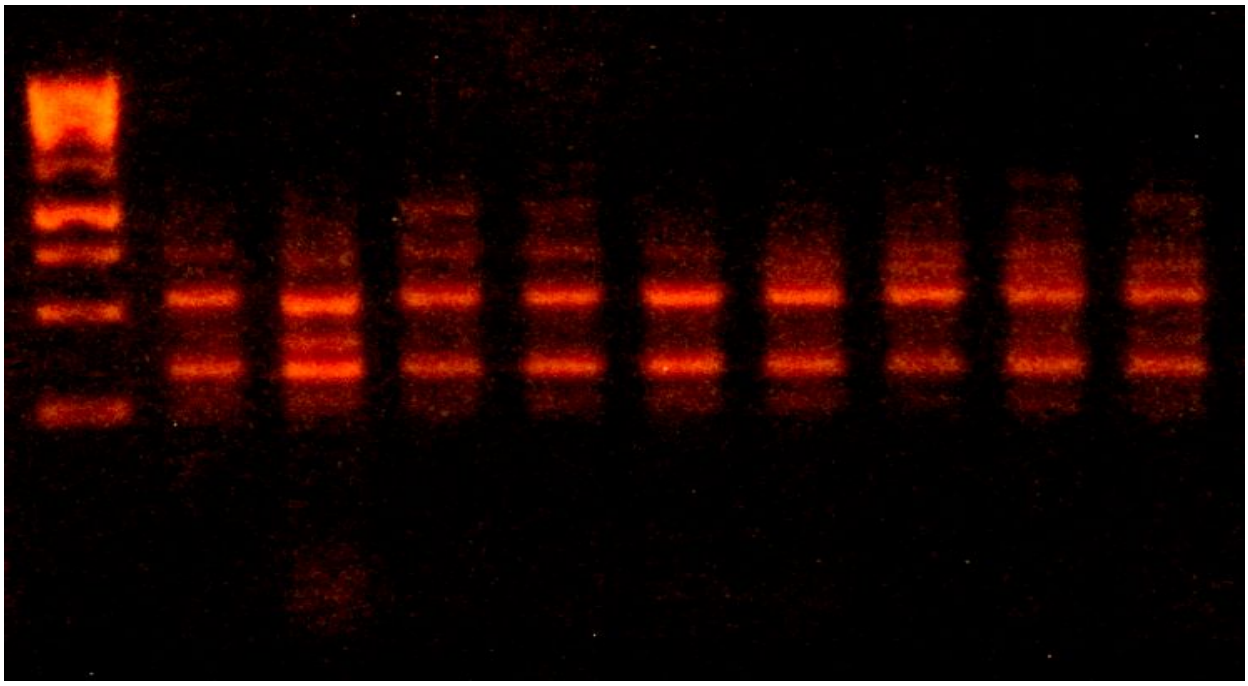

Figure 1. ISSR patterns obtained from the 9 varieties of wheat by using primer ISSR40.
M: 1000 pb molecular weight marker, 1: Bohoth4, 2: Bohoth6, 3: Jolan2, 4: Douma2, 5: Douma4, 6: Sham4, 7: Sham6, 8: Sham8, 9: Sham10.

Genetic Similarity

The ISSR marker data were collected and used to analyze genetic diversity through cluster analysis. A UPGMA tree was prepared using genetic similarity and was estimated using Nei-Li's similarity index[14]. A

dendrogram was constructed on the basis of the similarity matrix data by un weighted pair method with arithmatic average (UPGMA) cluster analysis using the software POPGENE program.

The PDV (Percent Disagreement Values) generated were used to determine the genetic distances among the wheat varieties. Where high values of this matrix shows the genetic variation, and when it increases the genetic variation between studied cultivars. For ISSR markers, PDV values ranged from 0.1032 between bohoth4 and Douma4 which show a high degree of genetic similarity, to 0.636 between Jolan 2 and Sham 6 which show a high degree of genetic variation. (Table 4).

Table 4: Percent Disagreement values (PDV) produced by 15 ISSR primers.

	Bohoth4	Bohoth6	Jolan2	Douma2	Douma4	Sham4	Sham6	Sham8	Sham10
Bohoth4	****								
Bohoth6	0.4353	****							
Jolan2	0.3345	0.4506	****						
Douma2	0.2942	0.4978	0.2555	****					
Douma4	0.1032	0.3765	0.3623	0.2942	****				
Sham4	0.4353	0.4978	0.5819	0.4353	0.3765	****			
Sham6	0.3909	0.6176	0.6360	0.4818	0.3623	0.2812	****		
Sham8	0.4055	0.5645	0.5819	0.4055	0.3765	0.3483	0.2812	****	
Sham10	0.4661	0.5996	0.5474	0.3765	0.4353	0.4353	0.3345	0.2683	****

The wheat cultivars were grouped in two clusters (1.93). Cluster 1 divided into two sup- cluster, sup-cluster 1 included Bohoth4 & Douma4 and They were the closest to each other genetically (5.16) and Jolan 2 & Douma2 (12.78), while the sup- cluster 2 included Bohoth6 alone (22.00). Cluster 2 divided into two sup-cluster, sup- cluster 1 included Sham4 &Sham6 (14.06) and sup- cluster 2 included Sham8 & Sham10 (13.41), these results could be related to the ancestors. (Fig. 2).

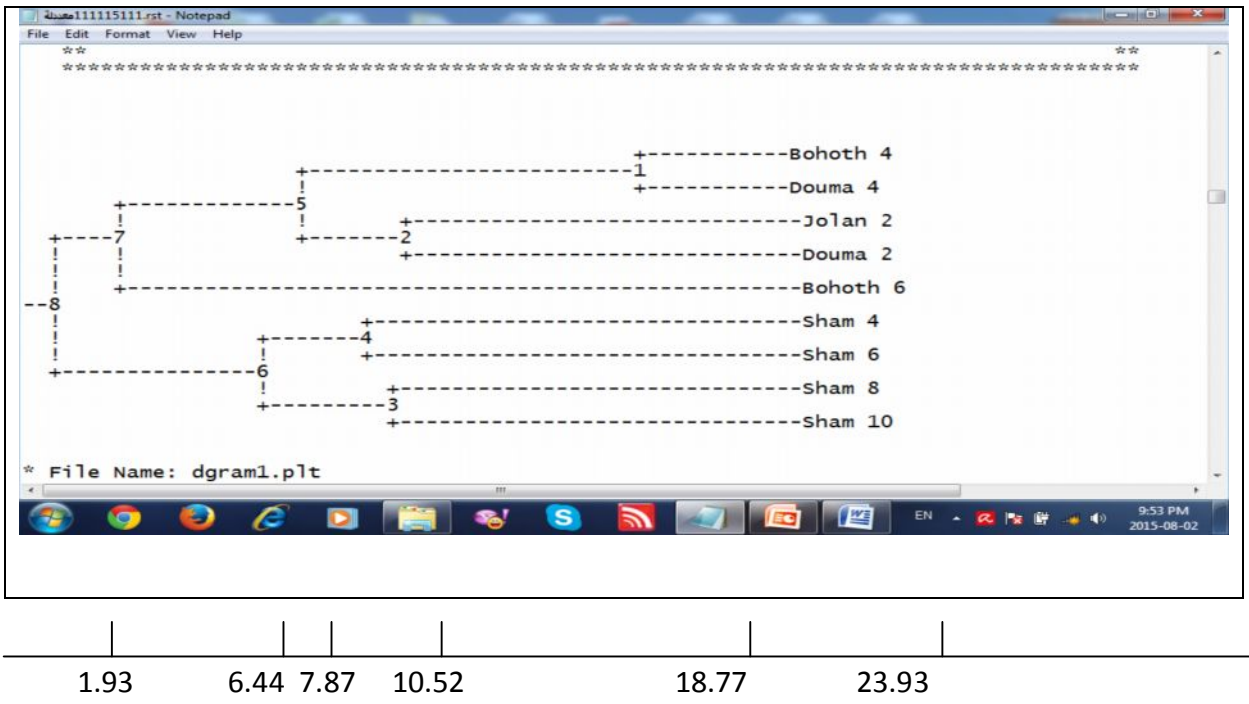

Fig. 2: UPGMA based cluster tree of 9 wheat genotypes with 15 ISSR markers.

Conclusions:

1. This study facilitate chosen the parents used in breeding programs (hybridization) depending on their genetic distance.
2. it is suggested that ISSR marker system were found to be efficient in discriminating each cultivars at the molecular level and can be used for genetic diversity analysis for germplasm conservation.
3. The high rate of polymorphism between wheat varieties indicated that the ISSR method is efficient to analyse the genetic diversity in wheat varieties.

References:

1. Food and Agriculture Organization (FAO). FAOSTAT database. Available online: http://www.faostat.fao.org.
2. Slađana Žilić , Miroljub Barać, Mirjana Pešić , Dejan Dodig and Dragana Ignjatović-Micić (2011). Characterization of Proteins from Grain of Different Bread and Durum Wheat Genotypes. *Int. J. Mol. Sci. 2011, 12, 5878-5894; doi:10.3390/ijms12095878.*
3. Roy, J.K., Lakshmikumaran, M.S., Balyan, H.S. and Gupta, P.K. 2004. AFLP-based genetic diversity and its comparison with diversity based on SSR, SAMPL, and phenotypic traits in bread wheat. Bio. Gen. 42:43-59.
4. Sanchez de la Hoz, M.P., Davila, J.A., Loarce, Y. and Ferrer, E. 1996. Simple sequence repeat primers used in polymerase chain reaction amplification to study genetic diversity in barley. Genome 39: 112-117.
5. F. Ben El Maati , M. Jlibéne and M. Moumni 2004. Study of the polymorphism of common wheat using ISSR markers. *Journal of Food,* Agriculture & Environment Vol.2 (3&4) : 121-125. 2004.
6. Roder, M.S., Plaschke, J., Konig, S.U., Borner, A., Sorrells, M.E., Tanksley, S.D. and Ganal, M. W. 1995. Characterisation of PCR-amplified microsatellite loci in wheat. Mol. Gen. Genet. 246: 327-333.
7. Saghai-Maroof, M. A., Biyashev, R. B., Yang, G. P., Zhang, Q. and Allard, R. W. 1994.Extraordinarily polymorphic microsatellite DNA in barly: species diversity, chromosomal locations population dynamics. Proc. Natl Acad. Sci. USA 91: 5466-5470.
8. Rakoczy-Trojanowska M. and Bolibok H. 2004. Characteristics and a comparison of three classes of microsatellite-based markers and their application in plants. Cell.Mol. Biol. Lett. 9:221-238.
9. El-Assal Salah El-din and Gaber Ahmed 2012. Discrimination Capacity of RAPD, ISSR and SSR markers and of their effectiveness establishing genetic relationship and diversity among Egyptian and Saudi wheat cultivars. *American Journal of Applied Sciences 9(5): 724-735, 2012.*
10. Malik R, Kundu S, Sareen S, Kumar R, Shoran J and B Mishra(2008). KSSR and ISSR markers for assessing DNA polymorphism and genetic diversity among bread wheat varieties of India. *Directorate of Wheat Research, Post Box No. 158, Karnal-132001, India.*
11. Yanfang Zhu, Jin Hu, Rui Han, Yang Wang, Shuijin Zhu 2011. Fingerprinting and identification of closely related wheat (*Triticum aestivum* L.) cultivars using ISSR and fluorescence- labeled TP-M13-SSR markers. Australian Journal of Crop Science, 5(7): 846-850 (2011).
12. Sofalian, O., N. Chaparzadeh and M. Dolati, 2009. Genetic diversity in spring wheat landraces from northwest of Iran assessed by ISSR markers. Notul. Bot. Hort. Agric., Cluj-Napoca, 37: 252-256.
13. Carvalho, A., J.L. Brito, B. Macas and H.G. Pinto, 2009. Genetic diversity and variation among botanical varieties of Old Portuguese wheat cultivars revealed by ISSR assays. Biochem. Genet., 47: 276-294.
14. Carvalho, A., J.L. Brito, B. Macas, H.G. Pinto, 2008. Genetic variability analysis of a collection of Old Portuguese bread wheat using ISSRs. Options Mediterraneennes. Ser. A, Sem. Medit., 81:35-38. 4 ref.
15. Sofalian, O., N. Chaparzadeh, A. Javanmard and M.S. Hejazi, 2008. Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. J. Agric. Biol., 10: 466-468.
16. Chowdhury, R.M.V.K., S.J.S. Kundu and R.K. Jain, 2008. Applicability of ISSR markers for genetic diversity evaluation in Indian bread wheat genotypes of known origin. Environ. Ecol., 26: 126-131.
17. Pasqualone, A., C. Lotti, A. Bruno, P. Vita, N. Fonzo and A. Blanco, 2000. Use of ISSR markers for cultivar identification in durum wheat. Options, Mediterraneennes. Ser. A, Sem. Medit., 40: 157-161. 7 ref.

18. Nagaoka, T. and Y. Ogihara, 1997. Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet., 94: 597-602.
19. Malik Rekha, Sareen Sindhu, Kundu Sushila, Shoran Jag (2012). The Use of SSR and ISSR Markers for Assessing DNA Polymorphism and Genetic Diversity among Indian Bread Wheat Cultivars. *Progressive Agriculture* Online ISSN : 0976-4615.
20. Murray, M.G. and Thompson, W. F. (1980). Rapid isolation of high molecular weight DNA. Nucleic Acids Res 8: 4321- 4325.
21. Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variations in terms of restriction endonucleases. Proc. Nat. Acad. Sci., 76: 5269-5273.
22. Mohammadi. S.A.; Prasanna. B.M. 2003. Analysis of genetic diversity in crop plants: salient statistical tools and considerations cropscie . 43: 1235-1248.

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