International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN: 0974-4290
Vol.8, No.10 pp 211-216, 2015

Determination of the Genetic similarity among some Genotypes of Durum Wheat (*Triticum* sp) and Wild species using (ISSR).

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Abstract: This work was carried out at the Molecular Biology Laboratory, Faculty of Agriculture, Damascus University during the season 2013-2014, to determine the genetic similarity among five cultivated durum wheat (Sham3, Sham9, Doma3, Horani, Jurie) and three wild species (*Aegilops ovata*, A. *Triuncialis*, A. *geniculata)* using the ISSR technique. Twenty seven primers were used for this purpose, only 13 proved their effectiveness in showing polymorphism among the examined genotypes. The primers gave 90 alleles whith a polymorphism percentage of 100 %, with PIC value 0.205. The number of bands for each primer varied from 2 bands for the primers (ISSR3, ISSR41) to 10 bands for the primers (ISSR43, ISSR32) with an average of 6.9 bands for each primer. The two genotypes A. *triuncialis* and jurie were the closest to each other. Based on this study, the use of ISSR technique could be a powerful tool to detect genetic diversity among wheat genotypes.

Keywords: Dendrograme, Genotyes, Durum Wheat, Aegilops, ISSR.

Introduction

Wheat is the most important cereal crop, it is a stable diet for more than one third of the world population and contributes more calories and protein to the world diet than any other cereal crop[1]. Genetic diversity of wheat cultivars is very important in reducing genetic vulnerability during plant breeding efforts, in order to estimate the genetic diversity molecular markers provided excellent tools[2]. Inter Simple Sequences Repeats (ISSR) is one of the DNA-based markers that has become widely used in various areas of plant research[3].

ISSR was first described by [4,5]. ISSR-PCR is a technique which involves the use of microsatellite sequences as primers in polymerase chain reaction to generate multi locus markers, it is asimple and quick method that combines most of the advantages of microsatellites (SSRs) and amplified fragment length polymorphism (AFLP) to the universality of random amplified polymorphic DNA (RAPD). ISSR markers are highly polymorphic and they are useful in studies on genetic diversity, gene tagging. Genome mapping and evolutionary biology in awide range of crop plants[6]. Also 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[7]. ISSR are dominant DNA markers widely used by plant breeders for genetic variability analysis, cultivars and genotypes identification and phylogenetics and DNA fingerprinting[8].

Carvalho[9] tasted ISSR markers on fifty-one cultivars of old Portuguese durum wheat (*triticum turgidum* and *triticum durum* sp), the result showed that the ISSR amplified loci ranged from 150 to 3000 bp, and total

mean percentage of ISSR polymorphism was 42.1%. In China, 5 ISSR primers were 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[10]. Malik[11] conducted ISSR tests on 27 varieties of bread wheat in India resulted in 176 bands with 68.42% polymorphism percentage.

Vosough[12] examined genetic diversity within a set of twenty Iranian landraces of wheat using ISSR. Fifteen ISSR primers were used to estimate genetic diversity among genotypes, the primers produced 178 bands, among which 138 bands (about 77%) were polymorphic, with an average of 9.7 bands per primer. Estimate of genetic similarity based on 138 polimorphic bands ranged from 0.35 to 0.83 with an average of 0.61. Sofalian[13] used ISSR markers to study the genetic diversity of 18 type of landraces from Iran and selected 9 cultivars grown in Iran out of 15 ISSR primers. 11 primers were used for assessment. These primers produced 108 DNA fragments, 78 (72.22%) fragments were polymorphic.

Therefore, the objective of this research was to determine the genetic similarity for some genotypes of durum wheat (*Triticum* sp) and wild species *Aegilops* by using inter simple sequence repeats (ISSR).

Materials and methods:

Expremintal site: the research was carried out in laboratory of biotechnology affiliated to the Faculty of Agriculture – Damascus University, Syria. during 2013-2014.

Plant materials: a set of eight genotypes of wheat were used in this study, Three of them are wild species related to Aegilops (*Aegilops ovata*, *A. trinucialis, A. geniculata*), and five are related to durum wheat (*Sham3, Sham9, Doma3,* Horani, Jurie).

DNA extraction

Genomic DNA was extracted from 1g of fresh leaves following the method of Dellaporta[14]. 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

Twenty seven ISSR primers (Table 1) 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 (1): Sequences and temperature annealing for primers.

Primer	sequence ('5 - '3)	Temperature annealing
ISSR-2	GAGAGAGAGAGAGAGAC	52
ISSR-3	CACACACACACACACAA	50
ISSR-4	CACACACACACACACAG	52
ISSR-5	ACACACACACACACACAT	50
ISSR-6	GAGAGAGAGAGAGAGACG	56
ISSR-7	TCTCTCTCTCTCTCTCGA	54
ISSR-8	TCTCTCTCTCTCTCTCAG	54
ISSR-9	ACACACACACACACACGG	56
ISSR-13	GTCCCATATTCAACTGTGTTAAAGT	68
ISSR-14	CCAGGTGTGTGTGTGTGT	56
ISSR-15	GTGTGTGTGAGAGAGAGA	54
ISSR-17	KKVRVRV(TG)6	50

ISSR-18	CCTCTCTCTGTGTGTGTG	56
ISSR-20	CACACACACACACACACACA	56
ISSR-22	GAGAGAGAGAGAGAGAGAGA	54
ISSR-32	AGAGAGAGAGAGAGAGT	52
ISSR-33	GAGAGAGAGAGAGAGAT	52
ISSR-34	CTCTCTCTCTCTCTCTT	52
ISSR-35	CACACACACACAACAG	52
ISSR-36	TCTCTCTCTCTCTCTCC	52
ISSR-37	TGTGTGTGTGTGTGTGG	52
ISSR-40	ACACACACACACACACTT	52
ISSR-41	ACACACACACACACACGG	56
ISSR-43	TGTGTGTGTGTGTGTGAA	52
ISSR- 230/4	CACACACACACACACACACACACA	51
ISSR- 230/5	GAGAGAGAGAGAGAGAGA	50
ISSR- 230/6	CACACACACACACACACACAC	56

R: G/ A K: G/ T V: G/ C /A

Analysis of data:

Data were analyzed using pop gen model 1.31 program. Genetic similarity was estimated using Nei and Li[15]. A dendrogram was constructed on the basis of the similarity matrix data by unweighted pair group method with arithmetic average (UPGMA). Polymorphism information content values (PIC) of ISSR method was calculated according to Botstein [16]. **PIC = {Σ 2Pi (1-Pi)^2}.**

Whereas: Pi: is frequency of allels which were obtained from using primer in all studied samples.

Results and Discussion:

Polymorphism:

Twenty seven ISSR primers were used, 13 of them produced 90 bands. These primers were polymorphic (100%), the number of bands ranged from two bands with (ISSR37, ISSR3), to 10 bands with (ISSR32, ISSR43), with an average of (6.9) bands per primer. The polymorphism information content (PIC) was calculated for all primers, it ranged from (0.131) to (0.351) with an average of (0.205). And this result was not in agreement with Malik[11].fig (1), table (2).

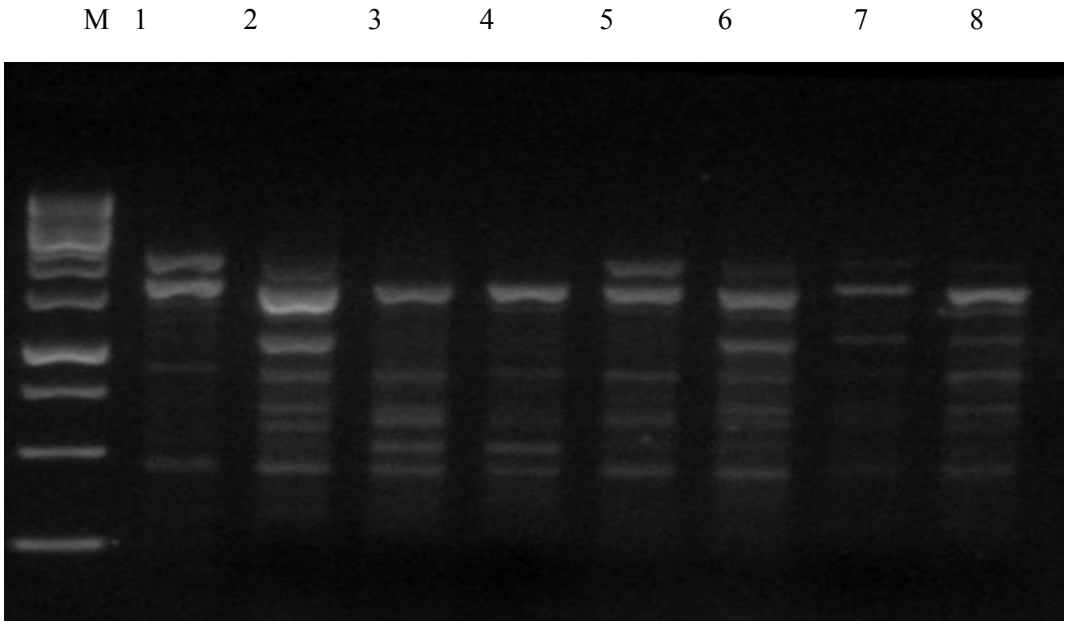

Fig.1: ISSR patterns obtained from the 8 genotypes of wheat using primer ISSR43.

M: 1000 pb molecular weight marker,1:A. *ovata*, 2:A. *triuncialis*, 3: A.*geniculata*, 4:jurie, 5:sham9, 6:doma3, 7:sham3, 8:horani

Table (2): primers and polymerphism percentage and pic:

Primers	Total bands	Polymorphic bands	Polymorphism percentage %	Polymorphism information content PIC
ISSR-3	2	2	%100	0.274
ISSR-5	7	7	%100	0.291
ISSR-6	8	8	%100	0.331
ISSR-9	6	6	%100	0.351
ISSR-14	8	8	%100	0.181
ISSR-15	7	7	%100	0.221
ISSR-18	9	9	%100	0.181
ISSR-22	7	7	%100	0.190
ISSR-32	10	10	%100	0.231
ISSR-35	5	5	%100	0.326
ISSR-37	2	2	%100	0.233
ISSR-41	9	9	%100	0.131
ISSR-43	10	10	%100	0.132
Total	90	90	%100	2.671
Mean	6.9	6.9	%100	0.205

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 and Li[15] . 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.

The genetic similarity was examined among 8 genotypes of wheat by applying percent disagreement values (PDV), so the high values refer to great genetic variation among genotypes. The lowest value of (PDV) was 0.11 between two genotypes (*A. triucialis and Jurie)*. This reflects a high degree of genetic similarity, followed by *(Jurie and Sham3)* (PDV =0.13). then *(A. Ovata and A. Triuncialis)* (0.46), the highest PDV value (0.64) was between (Sham9 and *A*. geniculata) indicating of great genetic variation. Table (3).

Table (3): Percent Disagreement values (PDV) produced by 27 ISSR primers.

Genotypes	A. ovate	A.triuncialis	A.geniculata	Jurie	Sham9	Doma3	Sham3	Horani
A. ovate	****							
A.triuncialis	0.46	****						
A.geniculata	0.44	0.31	****					
Jurie	0.29	0.11	0.37	****				
Sham9	0.31	0.31	0.64	0.33	****			
Doma3	0.29	0.25	0.29	0.23	0.29	****		
Sham3	0.29	0.14	0.33	0.13	0.29	0.19	****	
Horani	0.33	0.25	0.41	0.19	0.33	0.23	0.27	****

Cluster analysis:

Based on data achieved by ISSR –PCR, cluster analysis performzed to generate a dendrogram, the UPGMA clustering grouped the eight wheat genotypes into two major clusters, the first cluster contained only *A. geniculata*, which is the farthest (19.83) (wild species collected from the country side of Damascus). The second cluster contained the other genotypes divided into two sub-clusters(15.38), the first sub-cluster contained *Sham9*, the second sub-cluster contained *A. ovata* which is separated from the others in a sub sub cluster (13.5), while the second sub sub-cluster contained (*A. triuncialis*; Jurie; Sham3; Doma3; Horani). This

suggested that *A. triuncialis* is one of the wild ancestors of cultivated wheat. The two genotypes *(A. triuncialis and* Jurie*)* were the closest (5.42). then Sham3 (6.76), followed by Doma3 (11.22), then Horani (11.77).

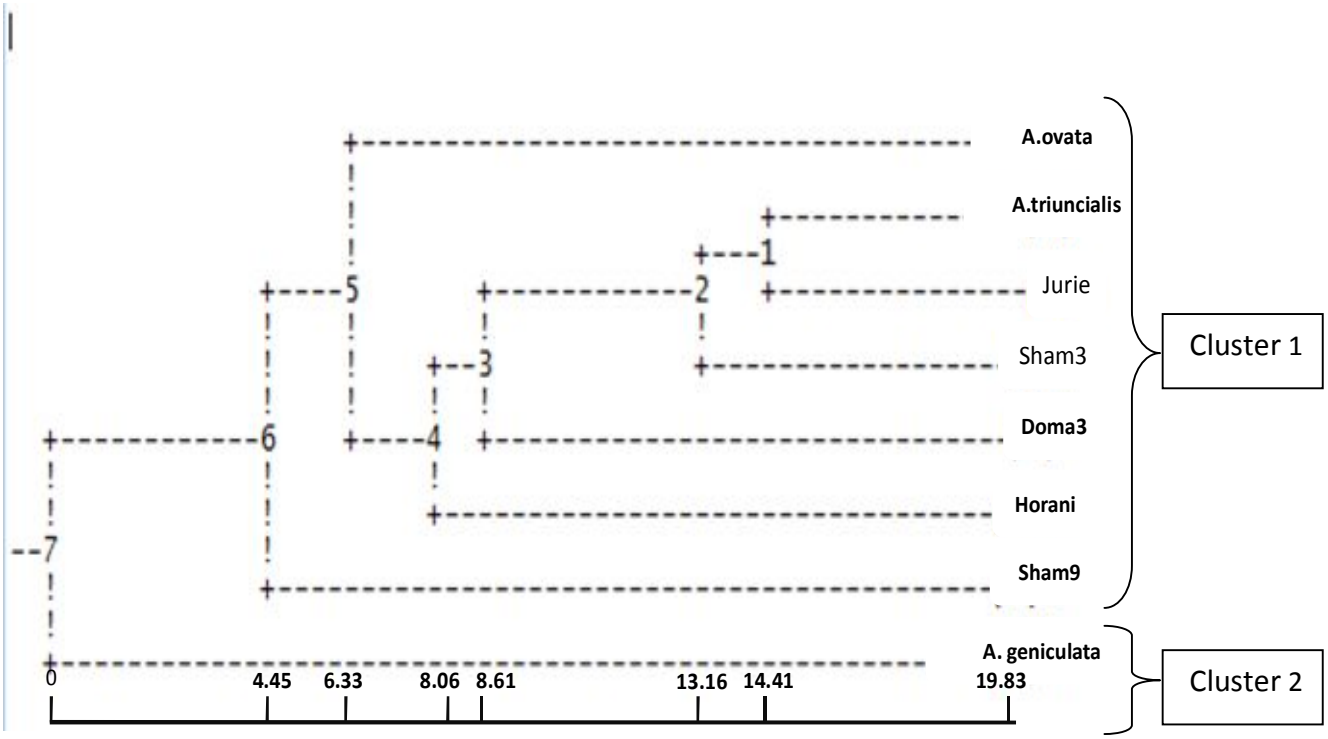

Fig (2): cluster analysis for studied genotypes based on the genetic distance derived from ISSR using UPGMA.

Conclusions

1. ISSR technique showed a high efficient to identify polymorphism and determine the genetic similarity among wheat genotypes. The polymorphism percentage was 100%, and genetic similarity was 72%.
2. The use of the wild species A. *geniculata*, which is the farthest from the other studied samples in breeding programs, (hybridization) depending on its genetic distance.

References:

1. Abd-EL-Haleem, S.H.M., Reham, M.A., Mohamed, M.S. 2009. Genetic analysis and RAPD polymorphism in some durum wheat genotypes. Glopal J Biotech Biochem 4(1):1-9.
2. 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.
3. Karaca, M. and A. Izbirak, 2008. Comparative analysis of genetic diversity in Turkish durum wheat cultivars using RAPD and ISSR markers. J. Food Agric. Environ., 6:219-225.
4. Zietkiewicz E, Rafalski A, Labuda A. (1994). Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics 20:178–183.
5. Kantety, R. V., X. Zeng, j. Bennnetzen and B. E. Zehr,1995. Assessment of genetic diversity in dent and popcorn Zea mays (L.) inbred lines using (ISSR). Amplification. Molecular Breed. 1:365-373.
6. Blair, M.W., Panaud, O. and McCouch, S.R. (1999). Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (Oryza sativa L.). Theor. Appl. Genet. 98 780-792.
7. Bornet, B. and M. Branchard, 2001. Non-anchored inter simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting . Plant Mol Bio Res., 19:209-215.

8. Abou-Deif, M. H., Rashed, M.A., Sallam, M.A.A., Mostafa, E.A.H., and Ramadan, W.A. (2013). Characterization of Twenty Wheat varieties by ISSR Markers. Middle- East Journal of Scientific Research. 15(2):168-175.
9. Carvalho, A., Lima-Brito, J., Macas and H. Guedes-Pinto, 2011. Molecular chracterisation of Portuguese collection of durum wheat. Options Mediterraneennes, Series A. 81:59-61.
10. Yangfang Zhu, Jin Hu, Rui Han, Yang Wang, Shuijuin Zhu. 2011. Fingerprinting and identification of related wheat (triticum aestivum L) cultivars using ISSR and fluorescence labeled TP-M13- SSR markers. Australian Journal of Crop Science, 5(7): 846-850.
11. Malik R., Sareen S., Kundu S., Shoran J. 2013. The use of SSR and ISSR markers for assessing DNA polymorphism and genetic diversity among Indian Bread Wheat cultivars. Progressive Agriculture :82-89.
12. Vosough A., Farshadfar E., Etminan A. 2013. Assessment of Genetic Diversity in some Iranian Landraces of Bread wheat(triticum aestivum) using Inter Simple Sequence Reoeat Markers. Advances in Environment Biology.7(14):4719-4723.
13. 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.
14. Dellaporta, SL., Wood J., and Hicks J.B. 1983. Aplant DNA minipreparation version Π. Plant Molecular Biology Reporter.1:19-12.
15. Nei, M. and W.H. Li, 1979. Mathematical model for polymorphism and genetic diversity among Indian studying genetic variations in terms of restriction bread wheat cultivars. Prog. Agric., 12: 82-89.
16. Botstein, D. R. L., White, M. Skolinck, and R.W. Davis. 1980. Constraction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

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