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Characterization of Twenty Wheat Varieties by ISSR Markers

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Abstract: Molecular genetic markers are widely used tools in genotype and species identification. The main objective of this study was to analyze the genetic diversity and relationships among wheat genotypes included hexaploid, tetraploid and diploid varieties. Genomic DNA from 20 wheat genotypes were analyzed using inter simple sequence repeats (ISSR) markers. Eight ISSR primers produced 112 amplified DNA fragments ranging in size from 127–1857 base pairs, 17 fragments were monomorphic (15.2%) and 95 fragments were polymorphic (84.8%) with average of 11.87 polymorphisms per primer. The primer 884A was the best one in distinguishing the wheat varieties, but to identify all varieties we have to use the two primers 884A and 884B together. Similarity values showed clearly substantial differences among the wheat varieties, with genetic similarity ranging from 47 to 94%, with an average of 71%. The 112 markers were used to construct the dendrogram based on UPGMA cluster analysis. The dendrogram indicated that the ISSR markers succeeded in distinguishing most of the 20 varieties in relation to their genetic background and geographical origin, which the tetraploid varieties were put together in one group as well as the diploid varieties. The hexaploid Egyptian varieties were also placed together in one group.

Key words: Wheat · ISSR markers · Genetic similarity · Dendrogram

INTRODUCTION

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 [1]. Inter simple sequence repeats (ISSRs) are one of the DNA-based markers that have become widely used in various areas of plant research [2]. The technique exploits the abundant and random distribution of SSRs in plant genomes by amplifying DNA sequences between closely linked SSRs. This technique has been widely used in studies of cultivar identification, genetic mapping, genetic diversity, evolution and molecular ecology [3]. Najaphy *et al*. [4] revealed that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. Molecular variation evaluated in their study in combination with agronomic and morphological characters of wheat can be useful in traditional and molecular breeding programs. El-Assal and Gaber [5] investigated the discriminating capacity of RAPD, ISSR and SSR markers and their effectiveness in establishing genetic relationship and diversity among eleven wheat cultivars and landraces collected from Egypt and Saudi Arabia. The dendrogram cluster diagram classified the evaluated genotypes in three major clusters corresponding to the cultivation regions. Sofalian *et al.* [6] 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 *et al*. [7] 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). No statistically significant differences were found between genetic diversity parameters of durum and bread wheat. Carvalho *et al*. [8] analyzed fifty-one

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cultivars of Old Portuguese durum wheat belonging to 26 different botanical varieties by using ISSR markers. They found that amplified ISSR loci ranged from 150 to 3000 bp and the total mean percentage of ISSR polymorphism was 42.1%. All primers used allowed the detection of inter-variety and intra-variety ISSR polymorphisms. Carvalho *et al*. [9] 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 *et al*. [1] 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 *et al*. [10] 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 *et al*. [11] 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 [12] 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.

MATERIALS AND METHODS

Materials: A total of 20 genotypes including sixteen hexaploid (*Triticum aestivum* L.), two tetraploid (*Triticum monococcum*) and two diploid (*Triticum urartu*) wheat varieties were used in this study (Table 1).

Table 1: The 20 wheat varieties used in the study and their origins.

No.	Variety	Latin name	Origin
1	Giza 157	*Triticum aestivaum*	Egypt
2	Giza 164	*Triticum aestivaum*	Egypt
3	Gimaza 5	*Triticum aestivaum*	Egypt
4	Sakha 60	*Triticum aestivaum*	Egypt
5	Sakha 93	*Triticum aestivaum*	Egypt
6	Sids 8	*Triticum aestivaum*	Egypt
7	Sahel 1	*Triticum aestivaum*	Egypt
8	Bacora	*Triticum aestivaum*	Egypt
9	Xinjiang Uygur	*Triticum aestivaum*	China
10	Khorasan	*Triticum aestivaum*	Iran
11	Diyala	*Triticum aestivaum*	Iraq
12	Trabulus	*Triticum aestivaum*	Libya
13	Baluchistan	*Triticum aestivaum*	Pakistan
14	Raqqa	*Triticum aestivaum*	Syria
15	Sfaqis [Sfax]	*Triticum aestivaum*	Tunisia
16	Namangan	*Triticum aestivaum*	Uzbakistan
17	East Azarbaijan	*Triticum monococcum*	Iran
18	Kirsehir	*Triticum monococcum*	Turkey
19	At Tafilah	*Triticum urartu*	Jordon
20	Baalbek	*Triticum urartu*	Lebanon

Table 2: List of eight ISSR primers and their nucleotide sequences.

Primer	Sequence (5'-3')
844A	5' CTC TCT CTC TCT CTC TGC 3'
844B	5' CTC TCT CTC TCT CTA 3'
HB12	5' CAG CAG CAG GC 3'
HBS10	5' GAG AGA GAG AGA CC 3'
HBS11	5' GTG TGT GTG TGT CC 3'
17889A	5' CAC ACA CAC ACA AC 3'
17889B	5' CAC ACA CAC ACA GT 3'
17899A	5' CAC ACA CAC ACA AG 3'

The first eight wheat varieties (1-8) were furnished by the Wheat Research Department, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt. The rest twelve varieties (9-20) were provided by ICARDA, Alepo, Syria. The names and sources of these genotypes are shown in Table 1.

Methods: Ten grains from each of the 20 wheat varieties were germinated on wet filter papers for two weeks and then the genomic DNA was extracted from 0.5 g of fresh leaves following Dellaporta method [13]. ISSR markers were produced by PCR using the genomic DNA and ISSR primers [3]. PCR reactions were conducted using eight primers (Metabion International AG, Martinsried, Germany) as shown in Table 2, to characterize the 20 wheat varieties. PCR technique was performed in 25 µl volume containing: master mix beads, 10 µl buffer (10 X), 1 µl primer (100 pmol), 1 µl DNA template (50 ng) and

13 µlH_2O sterile. The amplification was carried out in a thermocycler programmed as follows: 1 cycle of 94°C/2 min, 35 cycles of (94°C/1 min, 48°C/2 min and 72°C/2 min), 1 cycles of 72°C/7 min. The primer annealing degrees were varied according to the melting point of each primer. Agarose gel was used for separating the PCR products of amplified DNA fragments by electrophoresis. The agaros gel was prepared by dissolving 1.2 g agarose in 100 ml buffer including 40 mM Tris acetate and 2 mM $Na_2EDTA.2H_2O$. The gel was stained with ethidium bromide, photographed under UV light, scanned using a Gel-Documentation system and the data were analyzed using Bio-Rad Model 620 Software Programs, USA. Genetic similarity was estimated using Nei-Li's similarity index [14]. A dendrogram was constructed on the basis of the similarity matrix data by unweighted pair group method with arithmatic average (UPGMA) cluster analysis using the software MEGA program.

RESULTS AND DISCUSSION

Identification of Wheat Varieties: Eight ISSR primers were used to identify and characterize the 20 wheat varieties. Fig. 1 shows the banding patterns produced from each primer for the 20 varieties. The highest number of PCR-amplified fragments by using all primers was present in the hexaploid variety Gimaza 5 (65 fragments), while the diploid variety Baalbek gave the lowest number (44 fragments), as shown in Table 3. The other varieties displayed different numbers of amplified fragments. On the other hand, the primer 884B gave the highest number of amplified fragments (212 fragments) for all studied varieties, while the primer HB12 showed the lowest number of amplified fragments (101 fragments). ISSR specific markers of MW 615 and 402 bp were detected in the electrophoretic patterns of the tetraploid variety East Azarbaijan and the hexaploid variety Bacora respectively using the primer 884B (Fig. 1-A). In addition, two specific ISSR markers of 395 and 277 bp were detected in patterns of the two tetraploid varieties East Azarbaijan and Kirsehir, respectively. These two tetraploid varieties were characterized by seven bands of 1580, 1500, 1484, 395, 277, 211 and 166 bp found only in these varieties. Also, the diploid varieties "At Tafilah and Baalbek" were characterized by three bands of MW 406, 300 and 180 bp (Fig. 1-A).

ISSR specific markers of MW 1250, 800 and 390 bp characterized the three varieties Xinjiang Uygur, East Azarbaijan and Baalbek respectively using the primer 884A (Fig. 1-B). ISSR analysis using primer HBS10 for the 20 wheat varieties (Fig. 1-D) showed one specific fragment of 495 bp, can be used as molecular genetic marker for identifying the diploid variety At Tafilah. ISSR analysis using primer 17889A (Fig. 1-E) illustrated two specific

Table 3: Total bands produced from each primer for the wheat varieties and all amplified fragments in each variety

	Primers								
Varieties	884B	884A	HBS10	HB S11	17889A	17889B	17899A	HB-12	*
Giza 157	10	7	6	8	5	6	10	7	59
Giza 164	11	6	5	7	6	6	11	7	59
Gimaza 5	10	7	9	8	6	6	11	7	65
Sakha 60	12	9	7	8	5	6	11	6	64
Sakha 93	9	5	4	7	6	6	9	6	52
Sids 8	10	9	8	8	6	6	11	6	64
Sahel 1	11	10	5	7	6	6	9	6	60
Bacora	12	7	4	8	6	6	9	7	59
Xinjiang Uygur	10	12	4	9	5	6	8	7	61
Khorasan	10	10	8	9	5	8	5	6	61
Diyala	10	7	6	8	2	0	8	4	45
Trabulus	9	7	5	8	6	6	7	5	53
Baluchistan	9	9	5	8	5	6	8	4	54
Raqqa	10	9	5	8	7	6	10	6	61
Sfaqis[Sfax]	12	4	5	9	6	6	10	7	56
Namangan	13	7	6	9	7	6	9	2	59
East Azarbaijan	14	7	5	10	7	5	7	3	58
Kirsehir	14	5	4	9	6	6	5	3	52
At Tafilah	8	8	6	8	5	6	5	2	48
Baalbek	8	6	4	8	6	8	4	0	44
Total bands	212	152	111	164	113	117	167	101	1137

* Refer to presence of all amplified fragments in each variety.

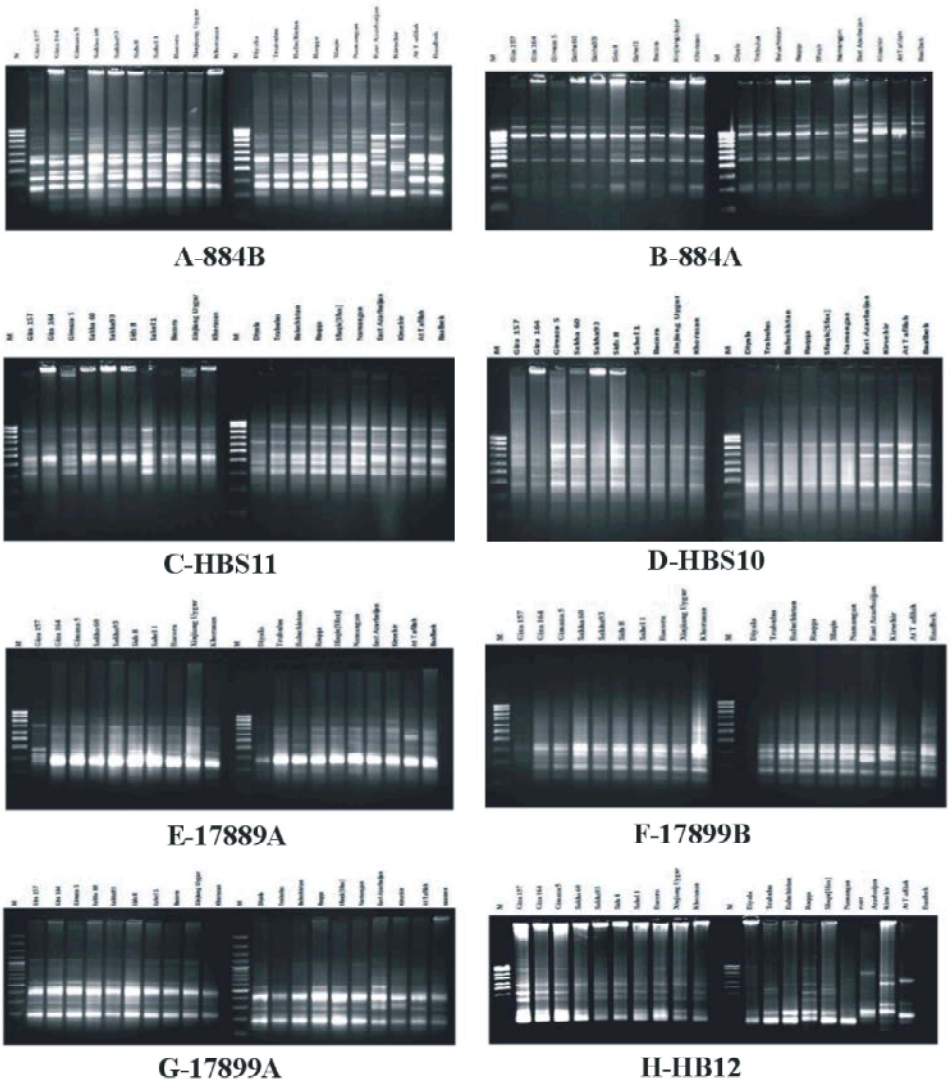

Fig. 1: Electrophoretic profiles of PCR products by using 844B (A), 844A (B), HBS11 (C), HBS10 (D), 17889A (E), 17899B (F), 17889A (G), HB12 (H) ISSR primers for 20 wheat varieties. M: DNA marker. The arrangement of varieties is the same in Table 1.

ISSR markers of MW 477 and 390 bp presented in the varieties Namangan and Baalbek, respectively. ISSR analysis using primer 17889B (Fig. 1-F) gave one specific fragment of MW 421 bp in the variety Khorasan. ISSR marker patterns using primer 17899A (Fig. 1-G) manifested one specific fragment of 590 bp was detected in the pattern of East Azarbaijan variety. ISSR analysis using primer HB-12 (Fig. 1-H) showed that the diploid variety "At Tafilah" pattern has two specific fragments of MW 643 and 255 bp. One specific fragment of 800 bp was found in East Azarbaijan variety, while two specific fragments of MW 725 bp and 624 bp were showed in Kirsehir variety. A specific fragment of MW 300 bp was found in the two tetraploid varieties "East Azarbaijan" and "Kirsehir".

No ISSR specific markers were detected by using primer HBS11 (Fig. 1-C). Therefore, seven primers out of the eight used in this study produced specific amplified fragments which can be used to identify the nine varieties East Azarbaijan, Bacora, Kirsehir, Xinjiang Uygur, Baalbek, At Tafilah, Namangan, Baalbek and Khorasan. ISSR analysis by using primer HBS11 showed five common fragments detected in all varieties at MW of 971, 884, 660, 480 and 400 bp, while three common fragments were presented in all varieties at MW 443, 395 and 205 bp by using primer 17899A. Two common fragments were

presented in all varieties of MW 649 and 380 bp by using primer HBS10. Three common fragments of MW 883, 900 and 300 bp was presented in all studied varieties by using the primers 884B, 884A and 17889A, respectively. One common fragment of 416 bp was observed in all hexaploid and tetraploid wheat varieties. Five common fragments detected in the tetraploid varieties at MW of 1580, 1500, 1484, 277 and 211 bp, while two common fragments were detected in the diploid varieties at MW of 406 and 300 bp by using primer 884B. The primer HB12 revealed one common fragment of MW 300 bp in the patterns of diploid varieties.

The ISSR markers of the primer 884B succeeded in characterizing the studied wheat varieties into 17 different patterns, which each variety appeared in a unique pattern except Giza 157 and Xinjiang Uygur, Giza 164 and Sahel 1. At Tafilah and Baalbek (diploid) were classified in three patterns (each two in one pattern). Also, ISSR markers of the primer 884A identified the 20 varieties into 17 patterns which the two hexaploid varieties Diyala and Trabulos were presented in the same pattern and also the two hexaploid varieties Baluchistan and Raqqa showed the same pattern (Fig. 1-B). Therefore, these results revealed that the studied varieties have different genetic background. The polymorphism revealed by the eight ISSR primers used for identification of wheat varieties is shown in Table 4. Primer HB12 gave the highest number of polymorphic loci in all varieties (14 fragments) with 100% polymorphism followed by the primer 884B showed 96.4 % polymorphism, while primer 17889B gave the lowest number of polymorphic loci (4 fragments) with 44.4 % polymorphism. One ISSR specific marker was detected for each of primers HBS10 and 17899A with MW 495 and 590 bp, respectively. Two specific markers were detected for each of the primers 17889A (477 and 390 bp), 17889B (421 and 400 bp) and 884B (615 and 402 bp). Three specific markers (1250, 800 and 390 bp) out of 17 amplified fragments were detected for the primer 884A and five specific markers (800, 725, 643, 624 and 255 bp) out of 14 amplified fragments were detected for the primer HB12. No ISSR specific markers were detected for the primer HBS11.

The amplification results by PCR for the studied wheat varieties with eight ISSR primers indicated distinct differences for identification of these varieties. A total of 112 amplified DNA fragments ranging in size from 127– 1857 bp were presented, whereas 95 fragments were polymorphic and 17 fragments were monomorphic. Therefore, out of 112 loci, 15.2 % were monomorphic and 84.8 % were polymorphic with average of 11.87 polymorphisms per primer. The number of DNA fragments for each primer varied from 9 (17889B) to 28 (884B) with an average of 14 fragments per primer. Three DNA primers (884A, 884B and HBS10) out of eight used in ISSR-PCR analysis succeeded in distinguishing most of the studied wheat varieties in a unique banding pattern when each primer was used alone. The primer 884A identified 16 varieties, 884B (14 varieties) and HBS10 (14 varieties). Therefore, the primer 884A was the best one in distinguishing the wheat varieties, but to identify all varieties we have to use the two primers 884A and 884B together. The high rate of polymorphism between wheat varieties was revealed by the previous three ISSR markers indicated that the method is efficient to analyze the genetic diversity in wheat varieties. These results are in agreement with those obtained by Najaphy *et al*. [4] who found that ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity of wheat genotypes. Sofalian *et al*. [6] revealed that ISSR markers could be efficiently used to evaluate genetic variation in the wheat germplasm. Carvalho *et al*. [8] found that the total mean percentage of ISSR polymorphism was 42.1% among fifty-one cultivars of Old Portuguese durum wheat. The results of Sofalian *et al*. [1] indicated high level of polymorphism of wheat landraces based on ISSR markers in contrast to other markers. Pasqualone *et al*. [11] found that

Table 4: The polymorphic loci amplified by the eight primers.

Primer	loci	Polymorphic	Monomorphic	Polymorphism%	Specific bands
884B	28	27	1	96.4	2
884A	17	16	1	94	3
HBS11	11	6	5	54.5	0
HBS10	11	9	2	81	1
17889A	10	9	1	90	2
17889B	9	4	5	44.4	2
17899A	12	10	2	83.3	1
HB12	14	14	0	100	5
Total loci	112	95	17	84.8	16

Table 5: Genetic similarity percentages of the 20 wheat varieties based on ISSR banding patterns.

Varieties	Giza 157	Giza 164	Gimaza 5	Sakha 60	Sakha 93	Sids 8	Sahel 1	Bacora	Xinjiang Uygur	Khorasan	Diyala	Trabulus	Baluchistan	Raqqa	Sfaqis	Namangan	Azarbaijan	East Kirsehir	afilah	At T Baalbek
Giza 157	1																			
Giza 164	92	1																		
Gimaza 5	91	94	1																	
Sakha 60	92	91	91	1																
Sakha 93	87	90	86	87	1															
Sids 8	88	89	89	90	89	1														
Sahel 1	89	89	88	93	87	88	1													
Bacora	93	90	91	89	85	85	93	1												
Xinjiang Uygur	89	82	84	86	82	85	86	88	1											
Khorasan	84	78	82	86	82	85	85	83	85	1										
Diyala	71	64	67	72	65	72	73	71	74	71	1									
Trabulus	78	75	77	80	78	81	86	82	79	84	80	1								
Baluchistan	81	75	77	80	80	83	86	82	82	84	82	90	1							
Raqqa	88	82	82	86	84	87	88	88	87	85	80	88	91	1						
Sfaqis[Sfax]	81	81	82	82	82	82	85	85	77	78	72	88	84	87	1					
Namangan	75	69	71	75	73	76	77	75	75	75	76	84	86	86	83	1				
East Azarbaijan	57	54	55	56	59	58	55	57	59	57	50	58	60	61	61	66	1			
Kirsehir	52	53	54	55	58	56	56	54	57	56	48	61	61	58	66	64	83	1		
At Tafilah	55	54	57	56	56	62	59	57	60	63	53	67	67	64	62	67	57	66	1	
Baalbek	53	52	55	51	56	57	55	55	56	59	47	63	63	60	62	64	54	63	84	1

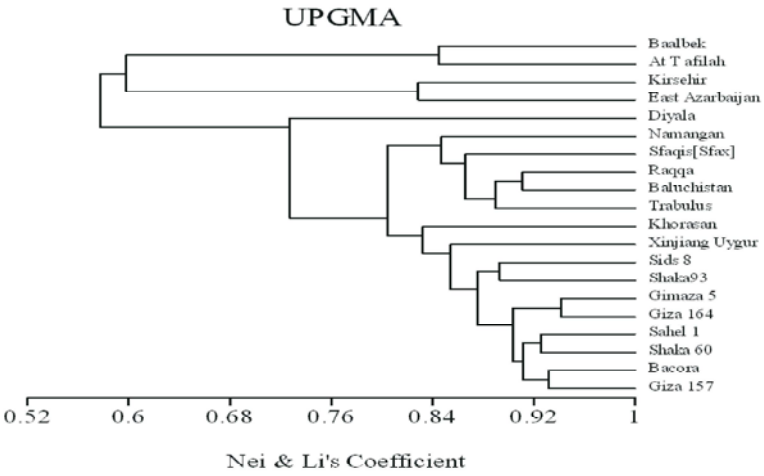

Fig. 2: Dendrogram representing the genetic relationship among the 20 wheat varieties using UPGMA cluster analysis of Nei-Li's similarity coefficient generated from ISSR markers.

the efficiency of ISSR markers was very high and two primers were sufficient to distinguish all the examined durum wheat cultivars. Nagaoka and Ogihara [12] 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.

Genetic Similarity: The genetic similarity index and dendrogram tree of the 20 wheat varieties under study were performed using Nei-Li's similarity index [14] on the basis of ISSR amplified fragments as presented in Table 5 and Fig. 2. The similarity values showed clearly substantial differences among the wheat varieties. The genetic similarity ranged from 47 to 94%, with an average of 71%. Some distinctive varieties showed high genetic similarity with others, such as Giza 64 and Gimaza 5 (94%), Sahel 1 and Sakha 60 (93%), Sahel 1 and Bacora (93%). On the contrary, some varieties displayed low genetic similarity, such as Diyala and Baalbek (47%), Diyala and Kirsehir (48%), Diyala and East Azarbaijan (50%), Sakha 60 and Baalbek (51%). It is clear that the hexaploid varieties such as Diyala and Sakha 60 have different

genetic background comparing with the tetraploid varieties East Azarbaijan and KIrsehir and the diploid variety Baalbek. The dendrogram resulting from UPGMA cluster analysis showed that the twenty wheat varieties could be divided into two main clusters. The first cluster divided into two sub-clusters, the first sub-cluster contained the two diploid varieties Baalbek and At Tafilah with similarity 84%, while the second sub-cluster contained the two tetraploid varieties East Azarbaijan and Kirsehir with similarity 83%. The second cluster was divided into two sub-clusters; the first sub-cluster contained only one hexaploid variety "Diyala" from Iraq. The second sub-cluster contained two main groups; the first group included five hexaploid varieties; Namangam, Sfaqis, Raqqa, Baluchistan and Trabulus. The second group contained the eight Egyptian hexaploid varieties grouped with the two hexaploid varieties Xinjiang Uygur from China and Khorasan from Iran. These results indicated that the ISSR markers succeeded in distinguishing most of the 20 varieties in relation to their genetic background and geographical origin, which the two tetraploid varieties were put together in one group as well as the two diploid varieties. The eight hexaploid Egyptian varieties were also placed together in one group. These results are in agreement with those reported by Carvalho *et al*. [9] who analyzed forty-eight bread wheat cultivars of an Old Portuguese collection by using 18 ISSR markers. They found that most cultivars belonging to the same botanical variety were clustered in the same main group. The results also are in agreement with the findings of Malik *et al*. [15] who showed that the cluster analysis tree placed 27 genotypes in six groups in agreement with their known origin. Sofalian *et al*. [1] 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.

REFERENCES

1. 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.
2. 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.
3. Yang, W., A.C. Olivera, I. Godwin, K. Schertz and J.L. Bennetzen, 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. Crop Sci., 36: 1669-1676.
4. Najaphy, A., R.A. Parchin and E. Farshadfar, 2012. Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Aust. J. Crop Sci., 6: 326-332.
5. El-Assal, S.E.D. and A. Gaber, 2012. Discrimination capacity of RAPD, ISSR and SSR markers and their effectiveness in establishing genetic relationship and diversity among Egyptian and Saudi wheat cultivars. Am. J. Appl. Sci.,. 9: 724-735.
6. 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.
7. 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.
8. Carvalho, A., J.L. Brito, B. Macas and H.G. Pinto, 2008. Molecular characterization of a Portuguese collection of durum wheat. Options, Mediterraneennes. Ser. A, Sem. Medit. 81: 59-61. 3 ref.
9. 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.
10. 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.
11. 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.
12. 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.

13. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation. Plant Mol. Biol., 1: 19-21.
14. 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.
15. Malik, R., S. Sareen, S. Kundu and J. Shoran, 2010. The use of SSR and ISSR markers for assessing DNA polymorphism and genetic diversity among Indian bread wheat cultivars. Prog. Agric., 12: 82-89.