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Molecular Evaluation of Genetic Diversity Among Seven Genotypes of Pecan (*Carya Illinoinensis)*

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ABSTRACT

In this study twenty six RAPD primers and six ISSR primers were used to assess the genetic diversity among seven pecan genotypes cultivated in Egypt. Using the RAPD and ISSR analyses, 156 out of 276 and 30 out of 59 alleles, respectively, were detected as polymorphic markers among the seven pecan genotypes. The pecan genotypes were characterized by 59 genotype-specific markers i.e 45 for RAPD and 14 for ISSR that would be considered as useful markers for pecan genotypes. Eleven markers distinguished the Grazona genotype, five markers for the Cheyenne genotype and one marker for each of Desirable genotype, Mahan and Moneymaker.

Keyword: RAPD, ISSR and pecan

INTRODUCTION

Pecan *(Carya illinoinensis)* is a lately decided nut crop and have important economically value for the genus *Carya* Nutt. (Wood, 1994). Pecan genotypes are diploid (2n=2x=32). The trees of pecan are long-lived, large and have a juvenile period of 5-10 years. Nuts and seeds comprise enormous uses, such as snack items or added as side component to appetizing and sweet dishes. Furthermore, they have many applications in vegetarian food, because it can used as important sources of protein, unsaturated fatty acids, tocopherols and other nutrients (Gray, 2005). Pecan has many advantages on human health as it contains anti-hyperglycemic and anti-hyperlipidemic effects (Abdelrahman *et al*., 2008; Villarreal-Lozoya *et al.,* 2007). Also, its fruit contains high nutritional value as it is rich in proteins, carbohydrates, fats, Calcium (Ca), Phosphorus (P), Magnisium (Mg), both of vitamins A and B (Kays, 1991), flavonoid glycosides and aglycones, galloylated glycosides (Nahla et al., 2007, Cuong et al., 1996).

Its homeland location is from north central and eastern United States into southern Mexico (Grauke *et al.* 1995). Pecan is the only nut which needs low chilling requirements; therefore it can be cultivated in different type of Egypt's lands (Hassanen and Gabr, 2013). In the last years of the 20th century pecan trees were successfully grown in scattered areas in Egypt.

Morphological and isozyme markers have been used to characterize the pecan varieties (Grauke, *et al* 1995; Thompson, and Grauke, 1991). However, these marker could be affected by the environment condition and also are limited in number. Hence, different molecular marker have been used including AFLP, RAPD and SSR (Paterson, 1996). Furthermore, study of genetic relationships between different genotypes is necessary to develop favorable strategies for breeding, improvement and employment of genetic resources (Paterson *et al*., 1991). Imbalzamo and Stine (1993) used RAPD marker to identify pecan genotypes in United States. RAPD required low amount of DNA, no primer information and give a lot of markers number. RAPD have been used to characterize the pecan genotype and to assessment their genetic relatedness (Conner and Wood, 2001).

The aim of this study was to assess genetic diversity among seven pecan genotypes. Also, to evaluate the genetic relationships between these genotypes using RAPD and ISSR markers. Furthermore, to identify the specific markers which would be associated with some economic characteristics such as nut yield, earliness in fruit set and kernel oil content in these genotypes.

MATERIALS AND METHODS

Plant materials: The seven pecan genotypes (Wichita, Grazona, Desirable, Burkett, Mahan, Moneymaker, and Cheyenne) examined in this study were kindly provided from Horticultural Research Institute farm, Agricultural Research Center, Giza, Egypt. Table 1 listed the seven pecan genotypes pedigree.

Table 1. Parentage and origin of pecan genotypes

Genotypes	Parentage	Origin	Source date
Burkett	Native	Texas, Callahan Co.	1900
Cheyenne	Clark x Odom	Texas, Brownwood	1942
Desirable	Success x Jewett	Miss., Ocean Springs	Early 1900's
Mahan	Seedling	Miss., Kosciusko	1910
Grazona	seedling	Mesa	1952
Moneymaker	Seedling	La., Mound	≈1885
Wichita	Halbert x Mahan	Texas, Brownwood	1940

DNA extraction: The genomic DNA was isolated using the method of Porebski *et al.* (1997) for the high polyphenol and polysaccharide plants.

RAPD and ISSR amplification: For RAPD analysis the PCR was carried out in a Biometra thermal cycler using primers listed in Table 2. The PCR reaction mix includes the following: 10 ng/µL of DNA; 0.5 U of Red Hot *Taq* polymerase (AB-gene House, UK) and 10-X *Taq polymerase* buffer (AB-gene House, UK); 10 mM dNTPs; 50 mM $MgCl_2$; 10 µM each of forward and reverse primers. The PCR profile starts with 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 32 for 1 min, extension at 72°C for 2 min. A final extension 72°C for 7 min was included. The ISSR reaction was done with the same PCR condition using six primers according to Hussein, *et al.,* (2008) and the same PCR program with different annealing temperature listed in Table 3. 2% (w/v) Agarose gel in 1X TAE buffer was used to amplify the product.

Table 2. PCR amplicons obtained from RAPD markers in pecan genotypes

Primer name	Sequence (5' - 3')	Total band	Polymorphic band	Polymorphism%	Band size
OP A-01	CAGGCCCTTC	16	3	18.75	169-1210
OP A-03	AGTCAGCCAC	6	2	33.3	160-962
OP A-04	AATCGGGCTG	15	12	80	310-1272
OP A-05	AGGGGTCTTG	12	3	25	456-1443
OP A-06	GGTCCCTGAC	10	4	40	167-893
OP A-08	GTGACGTAGG	12	9	75	185-2489
OP A-09	GGGTAACGCC	7	4	57.14	187-854
OP A-10	GTGATCGCAG	9	6	66.6	332-1308
OP A-11	CAATCGCCGT	13	6	46.1	313-1377
OP A-12	TCGGCGATAG	9	8	88.8	150-963
OP A-13	CAGCACCCAC	7	2	28.5	113-646
OP A-17	GACCGCTTGT	7	6	85.7	302-1083
OP A-18	AGGTGACCGT	9	6	66.6	226-1098
OP B-02	TGATCCCTGG	9	3	33.3	255-855
OP B-03	CATCCCCCTG	13	6	46.1	186-1641
OP B-04	GGACTGGAGT	11	5	45.4	240-1052
OP B-06	TGCTCTGCCC	12	12	100	185- 963
OP B-13	TTCCCCCGCT	12	6	50	258-1165
OP B-14	TCCGCTCTGG	8	8	100	120-780
OP C-01	TTCGAGCCAG	7	5	71.4	336-1106
OP C-02	GTGAGGCGTC	6	3	50	259-661
OP C-03	GGGGGTCTTT	11	6	54.5	367-1715
OP C-04	CCGCATCTAC	15	12	80	298-1349
OP C-05	GATGACCGCC	15	7	46.6	178-1680
OP K-02	GTCTCCGCAA	13	5	38.4	237-1613
OP M-13	GGTGGTCAAG	12	7	58.3	444-1937
Total		276	156	56.5	

Data analysis: The bands were sized and then binary coded by 1 or 0 for their presence or absence in each genotype. The systat ver. 7 (SSPSS inc.c 1997 spss inc.3/97 standard version) computer programs were used to calculate the pairwise difference matrices (Yang and Quiros, 1993). Cluster analysis was based on similarity matrices obtained with the unweighed pair-group method (UPGMA) using the arithmetic average to estimate the phenogram.

RESULTS AND DISCUSSION

Molecular markers are considered one of the valuable tools for the characterization of genetic materials for plant breeding. RAPDs and ISSRs considered dominant markers. Both techniques require little information about the template DNA, do not required radioactivity, and have high polymorphism levels in many plant species. In order to study the genetic difference among seven pecan genotypes, RAPD and ISSR analyses were used. All the primers produced reproducible PCR products with a clear pattern for each cultivar and showing informative and easily scrabble RAPD and ISSR profiles.

RAPD analysis

Many application were used the RAPD analysis to assess the the relationships between related plants. This technique is quicker and easier to use. In this analysis a single arbitrary primer is used to detect nucleotide sequence polymorphisms in DNA. Oilseed rape genotypes have been identified and described by using RAPD (Ahmad et al. 2007). The different primers revealed different levels of polymorphism among seven pecan genotypes as illustrated in Fig (1). Using the RAPD analysis, a total of 276 alleles were detected among the seven pecan genotypes (Table 2). Only 156 of them were polymorphic markers (56.5 %). The highest number of bands (16 bands) was generated by using the primer OPA-01, while the lowest one was 6 bands and generated with both primers OPA-03 and OPC-02. The highest polymorphism percentages were belonging to the both markers i.e. OPB-06 and OPB-14 (100 %) while the lowest marker was OPA-01 (18.75 %). These results were in agreement with those obtained by Conner and Wood (2001). They study the genetic relatedness between 43 cultivars using 100 RAPD markers.

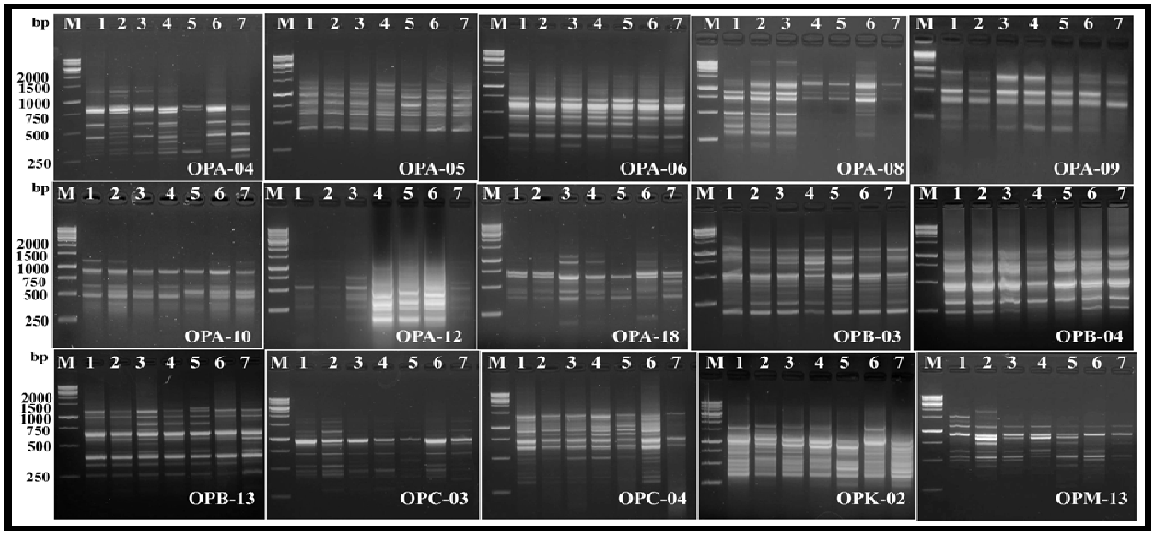

Fig. 1. RAPD profile demonstrating polymorphism among the seven pecan. M refers to DNA marker of 1Kb ladder. Lanes 1-7 represent (Wichita, Grazona, Desirable, Burkett, Mahan, Moneymaker, and Cheyenne respectively)

ISSR analysis

ISSR markers use microsatellite sequences that are disseminated across the plant genome and extremely variable. Compared with RAPDs, ISSR achieving higher reproducibility and required time and money less than AFLPs. For this advantages ISSR are used in many studies, especially on genetic diversity (Shafiei-Astani *et al*. 2015), DNA fingerprinting (Shen *et al*. 2006). For ISSR analysis, a total of 59 alleles were detected among the seven pecan genotypes (Table 3 and Fig.2). Only 30 of them were polymorphic markers (50.8 %). The highest number of bands (16 bands) was generated by using the primer 844A, while the lowest one was 6 bands generated by 17898A primer. The highest polymorphism percentage belonged to HB-8 markers (90.9 %) while the lowest belonged to 17899B marker (22.2 %).

Table 3. PCR amplicons obtained from ISSR markers in pecan genotypes

	Primers name	Sequences	Annealing temp. °C	Total band	Polymorphic band	Polymorphism%	Bands size
1	17898B	(CA)6GT	49	8	4	50	457-1000
2	17899B	(CA)6GG	51	9	2	22.2	380- 1309
3	17898A	(CA)6AC	49	6	2	33.3	534-1668
4	17899A	(CA)6AG	49	8	4	50	82- 508
5	844A	(CT)8AC	62	16	10	62.5	96-1184
6	HB-8	(GA)6GG	48	12	8	90.9	250-1400
	Total			59	30	50.8	

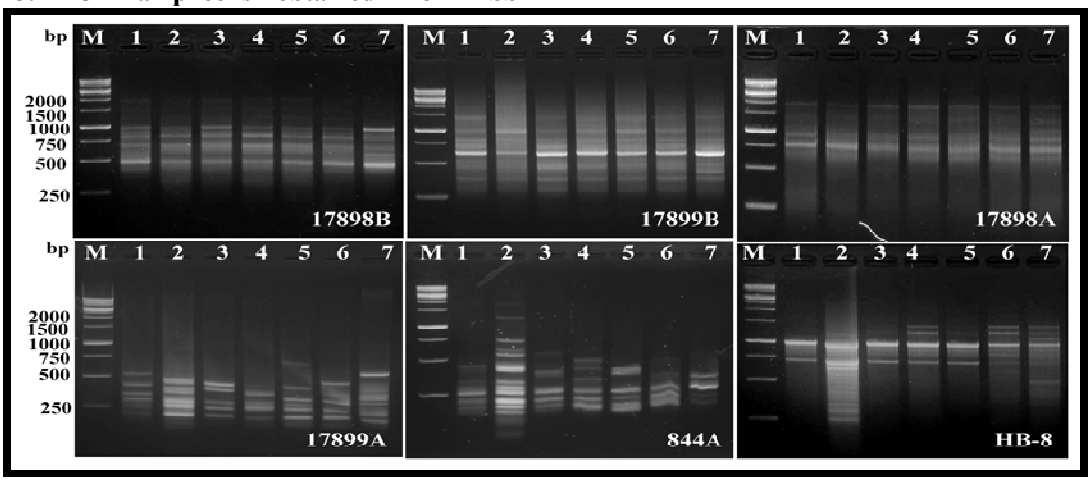

Fig. 2. ISSR profile demonstrating polymorphism among the seven pecan. M refers to DNA marker of 1Kb ladder. Lanes 1-7 represent (Wichita, Grazona, Desirable, Burkett, Mahan, Moneymaker, and Cheyenne, respectively)

Genotype identification

Many application for RAPD have been reported including effective use for genetic resources collection, genotype purity determination and mislabeled accessions identification (Ahmad, 1999).

45 out of the 156 polymorphic RAPD markers were genotype-specific (28.8 %). The seven pecan genotypes were characterized by 45 (19 positive and 26 negative) unique RAPD markers (Fig. 3A). A total of 19 random primers out of 26 revealed the presence of 45 unique markers, which represent an average of 2.36 markers per primer. The Mahan, Moneymaker, Cheyenne, Derirable, Burkett, Grazona and Wichita genotypes were characterized by 10, 10, 9, 2, 7, 5, and 2 unique RAPD markers (positive and /or negative markers), respectively. Therefore, these RAPD markers would be used as associated markers for the pecan genotype.

Moreover, all ISSR primers produced unique markers ranged from one for 17898A, 17899A and 844A to six for HB-8 as presented in Fig. 3B. 14 unique ISSR markers were generated by 6 primers with an average of 2.33 markers per primer. Grazona was characterized by 6 specific unique markers followed by Cheyenne by 5 markers. One unique marker was only obtained and characterized Desirable, Mahan and Moeymaker as using 17899B, 17899A and HB-8 primers, respectively. In ISSR, a single primer composed of the repeated motif is utilized in the amplification of DNA. The primer HB-8 that was based on the repetitive motif GA produced more amplicons (6 amplicons) than the primers based on any other repetitive motifs used in this study. This result was in agreement with those obtained by Safari *et al*. (2013). From all of these observations, it would be indicated that the microsatellites content of repetitive motif $(GA)_n$ are more frequent than the repetitive motif targeted by the other ISSR primers successfully in generating reliable amplicons in pecan genotype.

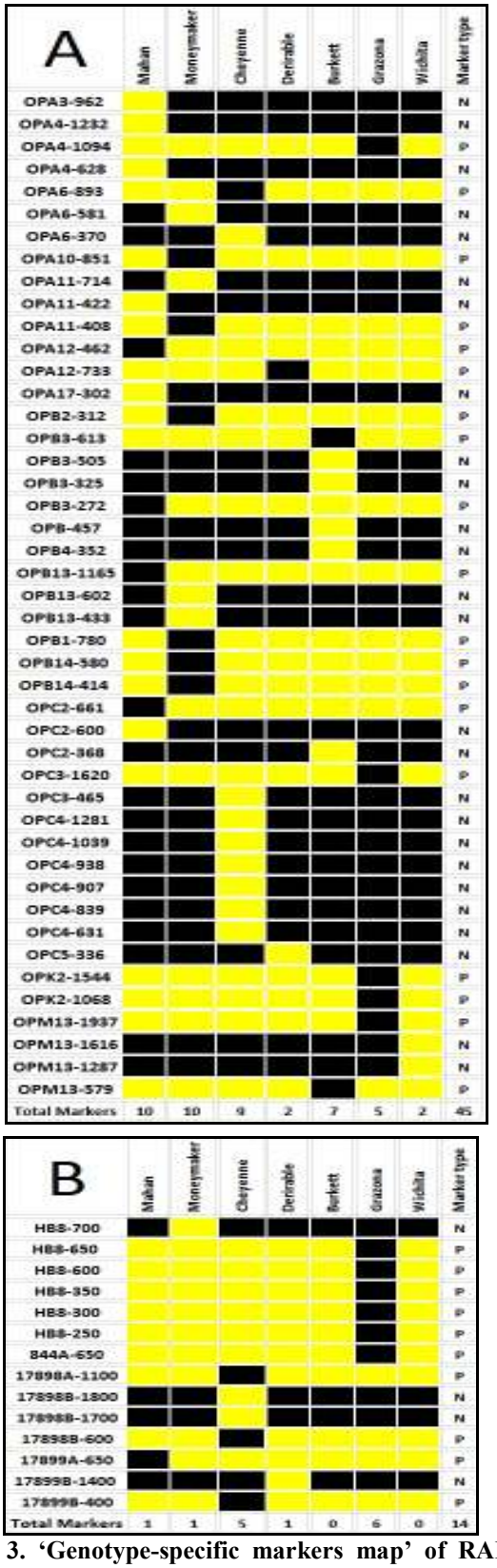

Fig. 3. 'Genotype-specific markers map' of RAPD and ISSR in seven pecan cultivars; A: RAPD genotype-specific markers, B: ISSR genotype-specific markers, N: negative marker, P: positive marker. Band names are at the left of each row, designated according to primer name and fragment size. Shaded blocks (Black color) represent the presence of DNA bands.

The genotype identification results indicated that all techniques applied in this study i.e RAPD and ISSR succeeded in showing different genotype-specific molecular markers which can be relied upon in distinguishing between studied pecan genotypes. Moreover, these genotype-specific markers can be used as molecular markers associated with economic characteristics. All techniques would be considered suitable tools for sufferable fingerprinting diagnostic markers for all studied pecan genotypes.

Genetic similarity among pecan genotypes by DNA markers

One of the important point for clustering and analyzing diversity is to determine the genetic variation between individuals (Kosman and Leonard, 2005). Assessment of genetic relationships could have a great impact in plant breeding programs. This may be by using the selection of parents and reducing the number of accessions needed to ensure sampling a broad range of genetic variability (Mohammadi and Prasanna, 2005).

The generated RAPDs were used to determine the genetic similarity among the pecan genotypes. The genetic similarity ranged from 90% between Moneymaker and Cheyenne to 75% between Desirable and Cheyenne (Table 4). The scoring data resulting from ISSR were analyzed as presented in Table (5).

Table 4. Pecan genotypes similarity matrix based on RAPD analysis.

	Wichita	Grazona	Desirable	Burkett	Mahan	Moneymaker
Grazona	83					
Desirable	79	80				
Burkett	81	81	86			
Mahan	82	85	80	85		
Moneymaker	83	84	77	83	88	
Cheyenne	81	84	75	79	88	90

Table 5. Pecan genotypes similarity matrix based on ISSR analysis.

	Wichita	Grazona	Desirable	Burkett	Mahan	Moneymaker
Grazona	73					
Desirable	83	69				
Burkett	86	67	89			
Mahan	90	70	90	88		
Moneymaker	82	62	86	94	85	
Cheyenne	76	63	76	85	75	87

The estimated genetic similarity among the seven pecan genotypes ranged from 62% to 94%. The highest genetic similarity was among Burkett and Moneymaker, while the lowest genetic similarity was between Grazona and Moneymaker. To obtain more balanced values for genetic similarity among genotypes and an equilibrated dendrogram representation of the relationships among

these genotypes, data of RAPD and ISSR profiles were combined and summarized in Table 6.

Table 6. Pecan genotypes similarity matrix based on the combine date between RAPD and ISSR analysis.

	Wichita	Grazona	Desirable	Burkett	Mahan	Moneymaker
Grazona	81					
Desirable	80	78				
Burkett	82	79	86			
Mahan	84	82	82	85		
Moneymaker	83	80	78	84	87	
Cheyenne	80	80	75	80	86	89

Combining data showed that the highest similarity was 89% between Moneymaker and Cheyenne while the lowest similarity was 75% between Desirable and Cheyenne. These results are consistent with the results obtained by Conner and Wood (2001). They mentioned that the Genetic distances among some pecan genotypes varied from 0.91 to 0.46 with an average value of 0.66 among all genotypes. This was according to the Nei and Li similarity coefficient.

Cluster analysis

The scoring data resulting from each marker type assay was used to determine the genetic relationships among the pecan genotypes. The data were used to compute the similarity matrices and then the UPGMA analysis were used in cluster analysis to generate dendrogram. The relationships among genotypes have been represented as a dendrogram. In RAPD analysis, the dendrogram divided the pecan genotypes into two main clusters (Fig 4), the first one included five genotypes, and branched into three sub-clusters; first sub-cluster included Wichita and Grazona with same linkage distance and Desirable alone, while Moneymaker represent the second sub-cluster alone. The third sub-cluster included Cheyenne genotype. The second cluster included the Burkett and Mahan genotypes with same linkage distance. Based on ISSR data, the cluster analysis divided the pecan genotypes into two main clusters (Fig 5).

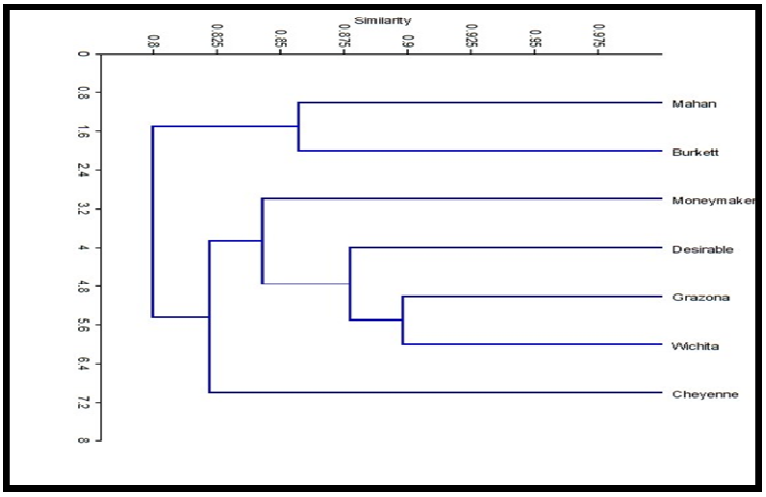

Fig. 4. Cluster analysis of the seven pecan genotypes as revealed by RAPD data.

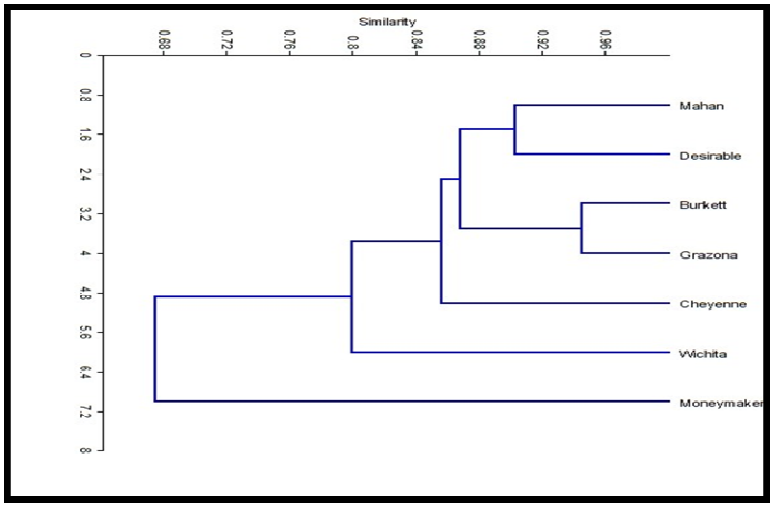

Fig. 5. Cluster analysis of the seven pecan genotypes as revealed by ISSR data.

The first cluster included six genotype i.e Wichita, Grazona, Desirable, Cheyenne, Burkett and Mahan. The Moneymaker genotype was in the second cluster. A dendrogram was constructed based on the combined data from different types of markers, RAPD and ISSR, as shown in Fig. (6). The dendrogram for combined data showed that Wichita, Grazona, Desirable, Cheyenne and Moneymaker genotypes were present in the main cluster, while Burkett and Mahan genotypes were located in the other main cluster. Moreover, the dendrogram of combined data was very similar to RAPD dendrogram than ISSR dendrogram.

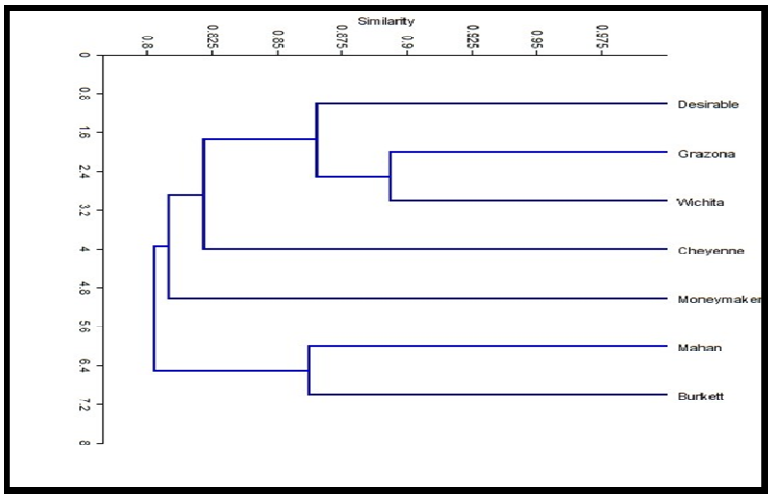

Fig. 6. Cluster analysis of the seven pecan genotypes as revealed by combined data.

Finally, this investigation results indicated that DNA markers showed effective tools for assessing the genetic variability and the genetic relationships between different pecan genotypes. The generated markers were sufficient to differentiate between the seven pecan genotypes. The genotype-specific markers were determined and these markers would be considered as useful markers for high nut yield, earliness in fruit set and high kernel oil content production in pecan breeding programs. This help to developing a molecular genetics map that would lead to the application of marker-assisted selection tools in genetic improvement of pecan.

Conflict of interest:

Authors have no conflict of interest.

REFERENCES

Abdelrahman EH, Salama MM, Abdallah HM, Sleem AA (2008) Pharmacognostical study of *Carya illinoinensis* (wangenh) k. koch (juglandaceae). Al Azhar J Bio Med Sci 28:222–243

Ahmad F (1999) Random amplified polymorphic DNA (RAPD) analysis reveals genetic relationships among annual *Cicer* species. Theor Appl Genet 98:657-663

Ahmad N, Munir I, Khan I, Ali W, Muhammad W, Habib R, Khan R, Swati Z (2007) PCR-based genetic diversity of rapeseed germplasm using RAPD markers. Biotechnology 6(3):334– 338

Conner PJ, Wood BW (2001) Identification of pecan cultivars and their genetic relatedness as determined by randomly amplified polymorphic DNA analysis. J Amer Soc Hort Sci 126(4):474-480

Cuong NM, Sung TV, Kamperdick C, Adam G (1996) Flavonoids from *Carya tonkinensis*. Die Pharmazie 51:128.

Hassanen SA, Gabr MF (2013) In vitro Rooting of Pecan (*Carya illionensis* Wang - C. Koch). World Applied Sciences Journal 21 (3): 315-319

Hussein TS, Tawfik AA, Khalifa MA (2008) Molecular identification and genetic relationships of six strawberry varieties using ISSR markers. International Journal of Agriculture and Biology 10:677-80

Imbalzamo M, Stine M (1993) Variation of random amplified polymorphic DNA(RAPD) markers in pecan. M.Sc Thesis, Louisiana State University 116 p

Kays SJ (1991) Post harvest quality. In Wood, B.W. and Payne. G.A. (eds.) Pecan husbandry: challenges and opportunities, First National Pecan Workshop Proceedings, USDA ARS-96, p 194-228.

Kosman E, Leonard K J (2005) Similarity coefficient for molecular markers in studies of genetic relationships between individuals for haploid, diploid and polyploid species. Molecular Ecology 14:415-424

Grauke LJ, Thompson TE, Marquard RD (1995) Evaluation of Pecan [*Carya illionensis* (Wangenh) K. Koch] germplasm collections, and designation of core subset. HortScience 30 (5):950-954

Gray J (2005) Nuts and seeds. Encyclopedia of Human Nutr 381-388

Mohammadi SA, Prasanna BM (2005) Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Sci 43:1235-1248

Nahla AA, Haidy AG, Mohammed MA (2007) Phenolic constituents with promising antioxidant and hepatoprotective activities from the leaves extract of *Carya illinoinensis*. Nat Prod Indian J 3:151-158

Paterson AH, Tanksley SD, Sorrells ME (1991) DNA markers in plant improvement. Adv Agron 46:39-90

Paterson AH (1996) Genome Mapping in Plants. ed. Academic Press. R.G. Landes Company, Georgetown, Texas, USA 330 p

Porebski S, Bailey LG, Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rpt 15:8-15

Safari S, Mehrabi A, Safari Z (2013) Efficiency of RAPD and ISSR markers in assessment of genetic diversity in *Brassica napus* genotypes. Int J Agri Crop Sci 5:273-279

Shafiei-Astani B, Ong AHK, Valdiani A, Tan SG, Yong CSY, Ahmady F, Alitheen NB, Ng WL, Kaur T (2015) Molecular genetic variation and structure of Southeast Asian crocodile (*Tomistoma schlegelii*): comparative potentials of SSRs versus ISSRs. Gene 571:107-116

Shen J, Ding X, Liu D, Deng G, He J, Li X, Tang F, Chu B (2006) Intersimple sequence repeats (ISSR) molecular fingerprinting markers for authenticating populations of Dendrobium officinale Kimura et Migo. Biol Pharm Bull 29(3):420-422

Thompson TE, Grauke LJ (1991) Pecans, and hickories (*Carya*). In: Moore JN, Ballington JR (eds). Genetic esources of temperate fruit and nut crops. Intl Soc Hort Sci Wageningen, The Netherlands p 839-904

Villarreal-Lozoya JE, Lombardini L, Cisneros-Zevallos L (2007) Phytochemical constituents and antioxidant capacity of different pecan [*Carya illinoinensis* (Wangenh.) K. Koch] cultivars. Food Chem 102:1241-1249

Wood BW (1994) Edible tree nuts: Pecan and other hickories, In: Arntzen CJ, Ritter EM (eds). Encyclopedia of agricultural science, vol. 2. Academic Press, New York. p 1-8

Yang X, Quiros C (1993) Identification and classification of celery cultivars with RAPD markers. Theor Appl Genet 86:205-212

التقييم الجزيئى للتنوع الوراثى فى سبع تراكيب وراثية لجوز البقان

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قسم الوراثة – كلية الزراعة – القاهر الجيزة – مصر

معهد بحوث البساتين - مركز البحوث الزراعية – مصر

جوز البقان أشجار كبيرة ومُعمرة ، تستخدم ثمارها كغذاء فهى مصدرا هاما للبروتين، والأحماض الدهنية غير المشبعة ، التوكوفيرول والكربوهيدرات والدهون والكالسيوم والفوسفور وفيتامينات A و B وغيرها، كما أن لها فوائد عديدة على صحة الإنسان حيث تحتوي على مضادات لسكر الدم ولدهون الدم. في هذه الدراسة تم إستخدام ٢٦ بادئ لتكنيك الـ RAPD و ٦ بادئات لتكنيك الـ ISSR لدراسة التنوع الوراثي بين ٧ تراكيب وراثية مختلفة للبقان المزروع تحت الظروف المصرية. وبإستخدام الـ RAPD و ISSR تم الحصول على عدد ١٥٦ من ٢٧٦ حزمة للـ RAPD و ٣٠ من ٥٩ حزمة للـ ISSR أظهرت إختلافات وراثية بين التراكيب الوراثية المختلفة للسبعة أصناف بقان. وقد تم الحصول على ٥٩ واسم (مُعلم) مُميز للتراكيب الوراثية المختلفة للبقان حيث أظهر الـ RAPD ٤٥ واسم و الـ ISSR ١٤ واسم والتي يمكن إستخدامها كواسمات (مُعلمات) جزيئية لتراكيب البقان الوراثية المختلفة. وكانت هذه الواسمات ١١ واسم جزيئي محدد للصنف Grazona و ٥ للصنف Cheyenne وواحد للصنف Desirable و Mahan و Moneymaker. وبإستخدام النتائج المتحصل عليها من الـ RAPD و ISSR تم الحصول على شجرة القرابة الوراثية Cluster analysis للتراكيب الوراثية المستخدمة ، حيث أظهرت أن السبع تراكيب تقع فى مجموعتين ، مجموعة تضم الـ Mahan و Burkett بينما تضم المجموعة الثانية باقى التراكيب الوراثية. وبذلك يتضح أن التكنيكات المستخدمة فى هذه الدراسة فعالة فى تحديد معلمات وراثية مُميزة للتراكيب الوراثية محل الدراسة وبذلك يمكن الأعتماد عليها فى برامج التربية والتحسين وتحديد درجات القرابة بينها.