Genetic Relationships Among some Pears Genotypes Using RAPD and AFLP Molecular Markers

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ABSTRACT

Genetic variability and relationships among seven pear genotypes (3 wild type genotypes of *pyrus syriaca* Boiss and three local Syrian pears cultivars, in addition to Egyptian Licontei cultivar) were achieved using 32 RAPD primers and 10 AFLP primer combinations. The level of polymorphism for all genotypes as revealed by RAPD and AFLP was 81.47%, and 92.5%, respectively. RAPD and AFLP revealed different genetic similarity (according to Jaccard coefficient) among the seven pear genotypes. The highest similarity was between WT1 and WT3 (62%), while 54% between Abu-Satel and WT2 by RAPD and AFLP, respectively. The dendrograms derived from the two techniques discriminate between Licontei and Syrian pear genotypes, RAPD cluster revealed genetic similarity between Romi and Licontei, while AFLP clustered Licontei genotype in a separate cluster. RAPD and AFLP techniques characterized the seven pear genotypes by a large number of unique markers throughout 67 and 227 unique markers, respectively. Licontei genotype was characterized by the highest number of unique markers (70), followed by Romi which was characterized by 50 unique markers, while, Abu-Satel was characterized by the lowest number of unique markers (31). RAPD technique revealed high Hav (p) (0.189), while AFLP technique revealed high marker index (207.04). The correlation coefficient between RAPD and AFLP marker types was high (0.945). Consequently, this investigation showed that RAPD and AFLP techniques were useful to identify the different closely related pear genotypes.

Key Words: AFLP, Genetic relationship, *Pyrus syriaca*, RAPD**.**

INTRODUCTION

The genus pyrus belongs to the subfamily Pomoedeae of the Rosaceae family. The basic chromosome number of pyrus X = 17 (Cheveareu *et al*., 1989). The genus Pyrus, native to the Northern Hemisphere of the Old World, consists of about 20 species, half of which are found in Europe, North Africa, and Asia minor; while the other half is in Asia, The precise origin of the European pear is still unknown but in Asia, the culture of pear goes back 2500–3000 years (Janick, 2000).

The genetic variability in Pyrus germplasm has been accumulated

through hybridization and naturally, through seed based propagation. Moreover, systematic characterization and evaluation in pear is still confined for the selection of desirable types (Ahmed *et al*., 2010). Though, morphological and phenological characterization provides basic data about the ecotypes, it is still not sufficient to assess genetic diversity in pear genotypes. In other wards, such assessment is not possible using morphological traits only (Dimpy *et al*., 2011).

The species *Pyrus Syriaca* Boiss is one of the main pear species that is widely distributed in Syria, Lebanon, Palestine, Turkey, Iraq, and Jordan (Mouterde, 1966). Many genotypes of *Pyrus syriaca* Boiss. and related cultivars are distributed in different regions of Syria from semiarid to humid areas within different altitudes from 200 to 1800m above the sea (Muzher, 1998).

Biochemical markers such as isozymes were used for cultivar identification in pear. Esterase and peroxidase isozymes were used to discriminate between six Syrian pear genotypes (Elshihy *et al*., 2004). More recently, DNA fingerprinting have also applied on pear species to overcome the drawbacks of morphological and biochemical markers.

Random amplified polymorphic DNA (RAPD) established by Williams *et al*. (1990) have been used for the cultivar characterization and identification in several woody species (Zhou and Li, 2000) because of simplicity, versatility and ability to generate high rates of polymorphism (Teng *et al*., 2002a). Many reports indicated that RAPD analysis can discriminate pear genotypes and suggest this technique as a reliable, inexpensive method and an important tool on the study of genetic diversity and genetic resources management of pears (Schiliro *et al*.(2001) and sawazaki *et al*. (2002)). RAPD is used to identify pear species and cultivars (Sharifani and Jackson (2000), Kim *et al*. (2005), and Lisek and Rozpara (2010)).

Amplified fragment length polymorphism (AFLP) technique is the most novel method in constructing DNA finger printing in the world. In AFLP, as RAPD technique, sequencing information is not required, also the PCR technique is fast, but the major advantage of the AFLP technique is the large number of polymorphisms that the method generates. Maughan *et al*. (1996) found that AFLP produced more polymorphic loci per primer than SSRs and RAPD in their study of Soybean diversity. Similarly, Nakajima *et al*. (1998) found that AFLP marker produced an average four times many bands per reaction compared in their analysis of Daucus diversity. AFLP remains the powerful and reliable technique (Tignon and Kettman, 2000), and yields reproducible patterns of bands and the number of fragments amplified is

sufficient for larger scale analysis (Broothaerst *et al*, 2000). Also, AFLP technique is used to study the genetic diversity of pear (Monte-Corvo *et al*. (2002), Pan *et al*. (2002), and Shenghua *et al*. (2002)).

The aim of the present study was to estimate the genetic relatedness among pear wild types and local cultivars native to Syria based on the genetic distances obtained by RAPD and AFLP markers and try to gain an understanding of the genetic relationship among pear cultivars and the species *Pyrus syriaca* Boiss .

MATERIAL AND METHODS

This investigation was carried out in the plant biotechnology researches laboratory at the faculty of agriculture - Cairo University, Giza, Egypt.

Plant material:

Plant materials that used in this investigation were listed in Table 1. The material included 3 wild type genotypes of *pyrus syriaca* Boiss that are distributed in different altitudes and soil types and grow in different environmental regions. These wild types acquire good tolerance for biotic and abiotic stresses. Three local Syrian pears cultivars, all of them were collected from the germplasm of the agricultural scientific research center in Sweida at the south of Syria, which located on 1500 m altitude were also included in the study. In addition, Licontei cultivar, which is the main commercial cultivar in Egypt that distributes in the north of Egypt, was also examined. All genotypes were grafted on Pyrus communis in the nursery in Al- Nobarieah, about 100 Km north of Cairo, to provide source of leaves for DNA extraction.

Table (1)
List of pear cultivars and wild type genotypes used in the study.

Code number	Cultivars and wild types	Climatic region	Blooming time	Maturity time	End use
1	Meskawi (Local Cultivar)	South Syria	1-10 April	July	Cultivar
2	P. syriaca Boiss (Wild type 1)	South Syria	1-10 April	September	Seedling rootstock
3	Abu satel (Local Cultivar)	South Syria	1-10 April	September	Cultivar
4	P. syriaca Boiss (Wild type 2)	South Syria	20 March-1 April	July	Seedling rootstock
5	P. syriaca Boiss (Wild type 3)	South Syria	1-10 April	September	Seedling rootstock

Table (1) (cont.)

Code number	Cultivars and wild types	Climatic region	Blooming time	Maturity time	End use
6	Romi (Local Cultivar)	South Syria	20-30 April	September	Cultivar
7	Licontei (P.communis x P.pyrifolia)	North Egypt	20 March-1 April	September	Cultivar

Genomic DNA extraction and purification:

Extraction of total DNA was performed using CTAB protocol according to Porebski *et al*. (1997). To remove RNA contamination, RNase A (sigma Co., USA) were added to the DNA solution and incubated at 37o C for half an hour. The extracted DNA was deproteinized by adding proteinase K (sigma Co, USA) and incubating at 37o C for 2 hours.

Estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

Random amplified polymorphic DNA (RAPD):

A set of 32 random 10-mer primers (Invetrogene life technology and Operon technology) was used in the detection of polymorphism among the seven pear genotypes (Table 2). The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 4 mM MgCl2, 0.3 mM dNTPs, 60 P mol primer, 2 U Go Taq DNA polymerase and 25 ng template DNA.PCR amplification was performed in a T-gradient thermal cycler (Biometra; T Gradient). Programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94o C. Each cycle consisted of a denaturation step at 94o C for 1 min, an annealing step at 35o C for 2 min, and an extension step at 72o C for two min, followed by extension cycle for 7 min at 72o C in the final cycle. The amplification products were resolved by electrophoresis in a 1.4% agarose gel containing ethidium bromide (0.5µg/ ml) in 1x TBE buffer at 90 Volts for 2 hours. Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0 respectively.

Table (2)

Nucleotide sequences of 32 (10-mer) primers used in RAPD – PCR marker

Primer name	Sequence (5'-3')	Primer name	Sequence (5'-3')
P23	TGC CGA GCT G	P43	GTG AGG CGT C
P25	AGG GGT CTT G	OPA-01	CAG GCC CTT C

Table (2) (cont.)

Primer name	Sequence (5'-3')	Primer name	Sequence (5'-3')
P27	GGT GAC GCA G	OPA-02	TGC CGA GCT G
P28	TTC CCC CGC T	OPA-03	AGT CAG CAA C
P30	TTT GCC CGG A	OPA-04	AAT CGG GCT G
P31	GTG ATC GCA G	OPA-05	AGG GGT CTT G
P32	TCG GCG ATA G	OPA-06	GGT CCC TGA C
P33	TCT GTG CTG G	OPA-07	GAA ACG GGT G
P34	GAC CGC TTG T	OPA-08	GTG AAT CGC G
P35	AGG TGA CCG T	OPA-09	GGG TAA CGC C
P36	CTC ACC GTC C	OPA-10	GTG AAT CGC G
P37	GAC GGA TCA G	OPA-11	CAA TCG CCG T
P39	GGA CTG GAG T	OPA-12	TCG GCG ATA G
P40	CCT TGA CGC A	OPA-13	CAG CAC GCA C
P41	TTC CCC CGC T	OPA-14	TCT GTG CTG G
P42	AGG GAA GGA G	OPA-15	TTC CGA ACC C

Amplified Fragment Length Polymorphism (AFLP):

AFLP analysis was performed using the GIBCO BRL system I (Cat.No.10544) according to the manufacturer's protocol. Ten primer combinations between EcoRI primer plus three 3' extension bases and MseI primer plus three 3' extension bases (E-AAC / M-CAG, E-AAG / M-CAC, E-AAC / M-CAG, E-AAC / M-CTA, E-AAG / M-CTC, E-AAC/M-CTG, E-ACA / M-CAT, E-ACA / M-CAA, E-ACG / M-CTA, and E-ACG / M-CTT), were used to selectively amplify the DNA fragments that matches the primer- extension sequence.

The thermocycling profile was consisted of one cycle at 94o C for 30 second, 65o C for 30 second, and 72o C for 60 second followed by a decrease in the annealing temperature each cycle 0.7o C during 12 cycles that gave a touch down phase of 13 cycles. Then, twenty-four cycles were performed at 94o C for 30 second, 56o C for 30 second, and 72o C for 60 second. An extension step at 72o C for 5 min was performed. After PCR, 2 µl of form amide dye were added to 4 µl of the PCR products. Samples were denatured at 94o C for 5 min and immediately placed on ice. The polyacrylamide denaturing sequencing gel preparation and electrophoresis, sequencing gel system was used.

The gel was pre-run at 60 W. To achieve a gel surface temperature of approximately 50o C, then 6 µl of each denatured sample, were loaded into the respectively well. At completion of loading, running of the gel was

performed into the silver staining step.

DNA silver staining system from Promega- Company was used according to the manufacturer manual.

The gel plate was placed on a white light box in the dark (or with safe light), the APC film (Promega, USA) was positioned on the gel with its glossy side facing the gel. The film was exposed for 60 to 120 seconds, depending on the gel background and the intensity of the white light. The film was developed manually by soaking in 1x Kodak GBX Developer for 3 minutes in 1x Kodak GBX Fixer. A final washing step for 1 minute was performed in water.

Data analysis:

The banding patterns generated by RAPD and AFLP markers were compared to determine the genetic relatedness of the seven pear genotypes. The amplified fragments were scored either as present (1) or absent (0). Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Jaccard coefficient (Jaccard, 1908). Dendrograms were clustered by cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA) on the basis of RAPD and AFLP data.

The expected heterozygosity of the polymorphic loci (Hav(p)) was calculated as follows: $B = np/(np + nnp)$ where B is the fraction of polymorphic loci, np is the number of polymorphic loci and nnp is the number of non-polymorphic loci. Then $Hav(p) = B^* (\sum Hp/np)$. The marker index (MI) calculated from the formula:

$MI = Hav(p) * E$, where E is the effective multiplex ratio and calculated from the formula: $E = nB$ (Powell *et al.*, 1996) where nB is the number of polymorphic loci. The software's used through this study were Microsoft EXCEL, SPSS, Bioprofil- Bio- 1D, and Gene stat.

RESULTS AND DISCUSSION

Polymorphism detected by RAPD markers:

As a result of reactions carried out with thirty two primers 286 DNA fragments were obtained, 53 fragments of them were monomorphic. Thus, 233 fragments were polymorphic (81.6%) among the seven genotypes. However, when the Licontei was excluded, the 32 primers revealed 281bands from which 222 were polymorphic (79%) among the Syrian pear genotypes (Table 3). There was not a single primer (out of the 32 studied) which could differentiate clearly between all the genotypes (Figure1). Oliveira *et al.* (1999) reported that RAPD techniques revealed high

polymorphism percentage (91%) with a total of 358 bands,327 of them polymorphic when compared different pear cultivars derived from different species. The different primers revealed different levels of polymorphisms among the seven pear genotypes. The differences in the degree of polymorphism depending on RAPD primer used, and RAPD primers should undergo precise selection if a higher degree of polymorphism is to be produced (Lisek and Rozpara, 2010). The highest number of amplified DNA fragments was 14 with OpA05, while the lowest number was 4 with the primer OpA15 with an average number 8.94. The number of polymorphic amplicons per primer ranged from 2 (primer OpA15) to 12 (primer OpA05and OpA11) with an average number 7.28. The best primers producing the highest number of reproducible polymorphic amplicons were P25, P30, P33, P37, OpA04, OpA05, OpA09, OpA11,OpA12 and OpA14. Teng *et al*. (2002a) stated that primers OpA09 and OpA12 produced the highest number of polymorphic amplicons, which is in agreement with our results.

Table (3)
Polymorphism Detected by RAPD Markers. Values between brackets related to the Syrian pear genotypes

RAPD primer	Total number of amplicons	Polymorphic amplicons	Percentage of polymorphism
23	5	4	80
25	8	7	87.5
27	12	8	66.67
29	8	7	87.5
30	7	7	100
31	13	11	84.6
32	12	10	83.33
33	10	9	90
34	12	10	83.33
35	13	11	84.6
36	10	7	70
37	11	10	91
39	7	6	85.7
40	8	7	87.5
41	5	5	100
42	6	3	50
43	7	6	85.7
opA01	9	5	55.5
opA02	10	8	80

Table (3) (cont.)

RAPD primer	Total number of amplicons	Polymorphic amplicons	Percentage of polymorphism
opA03	7	3	42.85
opA04	10	9	90
opA05	14	12	85.7
opA06	8	6	75
opA07	7	5	71.43
opA08	7	5	71.43
opA09	11	10	91
opA10	8	6	75
opA11	13	12	92.3
opA12	8	8	100
opA13	9	7	77.77
opA14	7	7	100
opA15	4	2	50
32 Primers	286 (281)	233 (222)	80.63 (79)

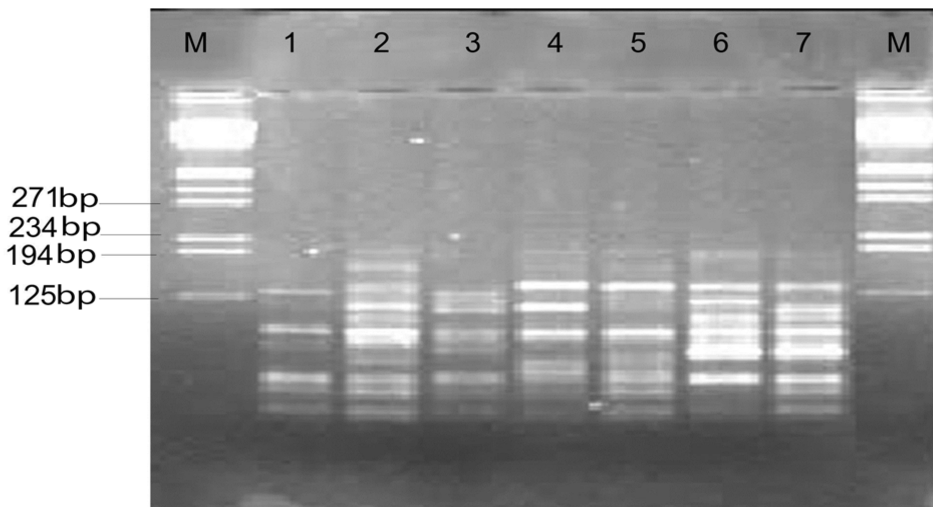

Figure (1): RAPD profile of the seven pear genotypes amplified with RAPD primer 33. M: Lambda Hind III DNA Molecular marker. Lanes 1 through 7 refer to pear genotypes: Meskawi, W.T1, Abu-Satel, W.T2, W.T3, Romi, and Licontei.

Polymorphism detected by Amplified fragment Length Polymorphism (AFLP):

In this study, the ten primer combinations used in the AFLP analysis revealed 1473 amplicons including 1362 polymorphic amplicons (92.5%) with the seven pear genotypes (Figure 2). However, when Licontei was

excluded, 1407 fragments were amplified from which 1254 were polymorphic (87.7%). Shenghua *et al*. (2002) obtained high polymorphism percentage (79.2%) between the ten pear cultivars were studied. The sizes of AFLP fragments ranged from 1300 to 100 bp, and the polymorphic fragments were distributed across the entire size range. The number of amplicons produced by the different primer combinations ranged from 105 (E-AAC/M-CTG) to 195 (E-ACA/M-CAA) and the average number of scorable bands per gel was 147(Table 4). The level of polymorphism percentage ranged from 80.1% to 98.8% in primer combinations (E-ACG/M-CTT) and (E-AAG/M-CAC), respectively. These results represent one of the most important advantages of the AFLP technique. Hence, this type of markers provides wide range coverage of the genome (Krauss, 1999).

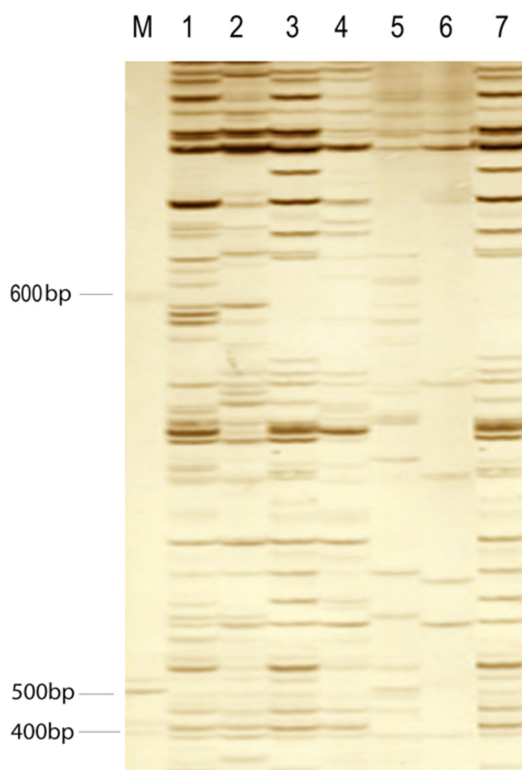

Figure (2): Profiles of the seven pear genotypes using the Primer combination E-AAC/M-CTA M: DNA MW marker (100bp ladder). Lanes 1 through 7 refer to pear genotypes: Meskawi,W.T1, Abu-Satel, W.T2, W.T3, Romi, and Licontei.

Table (4)
Polymorphism detected by AFLP marker. Values between brackets related to the Syrian pear genotypes

Primer combination	Total number of amplicons	Polymorphism number	% polymorphism
E-AAC/M-CAA	139	136	97.84
E-AAG/M-CAC	170	168	98.82
E-AAC/M-CAG	124	120	96.7
E-AAC/M-CTA	132	122	92.4
E-AAG/M-CTC	162	160	98.8
E-AAC/M-CTG	105	102	97.1
E-ACA/M-CAT	171	147	85.96
E-ACA/M-CAA	195	179	91.8
E-ACG/M-CTA	134	115	85.82
E-ACG/M-CTT	141	113	80.1
	1473(1407)	1362(1254)	92.5(87.7)

Genetic Relationship as Revealed by RAPD Data:

The level of genetic similarity between the seven pear genotypes according to Jaccard coefficient (Jaccard, 1908) was performed (Table 5). The highest genetic similarity was between W.T1 with W.T3 (62%), while the lowest genetic similarity was to between Meskawi with W.T2 (40%). However, the average of GS was 53.4%. Licontei genotype revealed the highest genetic similarity with Romi genotype, which was 61%. While revealed the lowest genetic similarity with W.T2 genotype (0.49). Teng *et al*. (2002b) obtained a useful genetic similarity values from RAPD data during the evaluation of 92 pear accessions derived from East Asia.

Table (5)
Genetic similarity matrices computed according to Jaccard coefficient from RAPD data.

	Meskawi	W.T1	Abu-Satel	W.T2	W.T3	Romi	Licontei
Meskawi	1						
W.T1	0.51	1					
Abu-Satel	0.58	0.55	1				
W.T2	0.41	0.61	0.50	1			
W.T3	0.49	0.62	0.51	0.59	1		
Romi	0.44	0.54	0.54	0.52	0.51	1	
Licontei	0.50	0.58	0.51	0.49	0.51	0.61	1

Genetic Relationships as Revealed by AFLP Data:

The similarity level among the seven pear genotypes according to Jaccard coefficient ranged from 54% to 23% between Abu-Satel and W.T2, and Meskawi and Licontei, respectively (Table 6). Licontei revealed the highest genetic similarity with Romi (31%), while the lowest relationship was between Licontei and Meskawi (23%). The genetic similarity between Syrian pear genotypes ranged from 54% to 30% between Abu-Satel and W.T2, and Romi with Meskawi, respectively. Shenghua *et al*. (2002) compared between European and Asian pear species using AFLP, and they found that the highest genetic similarity was between P. bretschneideri and P. pyrifolia (0.849), while the lowest similarity was between p. comunis and p. betulaefolia.

Table (6)
Genetic similarity matrices computed according to Jaccard coefficient from AFLP data.

	Meskawi	W.T1	Abu-Satel	W.T2	W.T3	Romi	Licontei
Meskawi	1						
W.T1	0.38	1					
Abu-Satel	0.33	0.48	1				
W.T2	0.31	0.49	0.54	1			
W.T3	0.35	0.46	0.51	0.50	1		
Romi	0.30	0.40	0.49	0.48	0.46	1	
Licontei	0.23	0.25	0.27	0.25	0.26	0.31	1

Cluster Analysis as Revealed by RAPD Data:

The dendrogram developed based on Jaccard dissimilarity matrix revealed the genetic relationship among the seven pear genotypes with different linkage distance shown in Figure (3). The dendrogram grouped the seven pear genotypes into three clusters. The first cluster contains two genotypes “Licontei and Romi” which have the same linkage distance, while the second cluster contains the three wild type genotypes which formed two subclusters; one containing only one genotype” W.T2” and the other contains W.T1 and W.T3 (which revealed the highest genetic relationship), then Meskawi and Abu-Satel formed the third cluster.

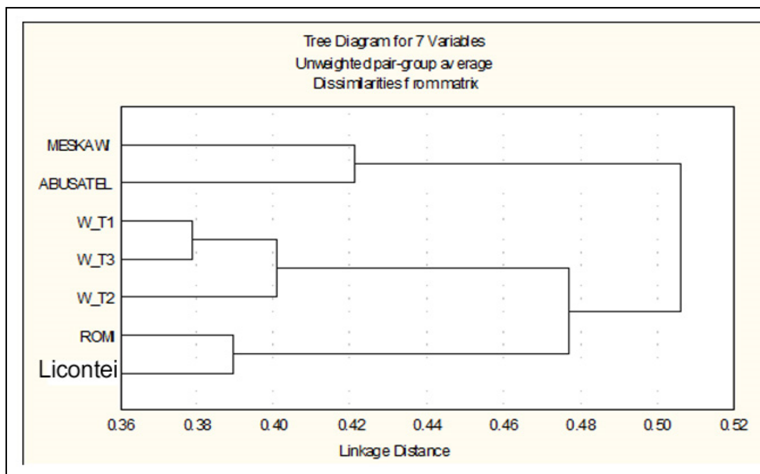

Figure (3): Cluster analysis as revealed by RAPD data

Cluster Analysis as Revealed by AFLP Data:

The dendrogram based on Jaccard coefficient showed the linkage distance between the seven pear genotypes, the seven pear genotypes grouped into three clusters where Licontei genotype formed a separate cluster due to its parents (P.communis and P.pyrifolia), while Meskawi genotype formed the second cluster (Figure 4). The third cluster divided into two subclusters, one containing only one genotype (Romi), and the other subcluster formed two groups, one containing one genotype (W.T1) and the second group subdivided into two subgroups, one containing only one genotype (W.T3), while the two genotypes " Abu-Satel and W.T2" fill in the second subgroup indicating the highest genetic relationship (closely related).

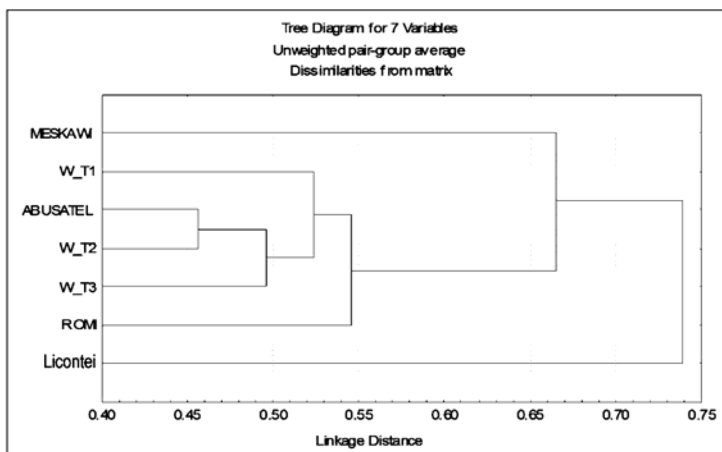

Figure (4): Cluster analysis as revealed by AFLP data

Genotype identification by unique DNA markers:

Unique DNA markers obtained by RAPD and AFLPs markers were used in the present study to characterize the seven pear genotypes. Two types of unique markers should be observed; the amplified bands were present in one genotype and absent in all other investigated genotypes (positive unique markers), and the amplified bands were present in all investigated genotypes except on (negative unique markers).

The seven pear genotypes were characterized by 37 positive and 30 negative unique RAPD markers. Lecontei genotype " derived from the hybridization between *P. communis x P. pyrifolia*" was characterized by 13 Unique RAPD markers (10 positive and 3 negative). In Syrian pear genotypes, Meskawi characterized by 13 unique markers (1 positive and 12 negative), while WT2 was characterized by 6 unique markers, which was the lowest number of unique RAPD markers. Certain primers such as primer OpA09 characterized four pear genotypes by 4 unique RAPD markers. Teng *et al*. (2002b) reported that OpA09 characterized 42% of Chinese white and sand pear cultivars by unique RAPD marker.

The ten AFLP primer combinations characterized the seven pear genotypes by a total of 227 unique AFLP markers (155 positive and 72 negative) with an average of 22.7 markers for each primer combination. The highest number of unique AFLP genotype markers (54 unique markers including 21 positive and 33 negative) characterizes Lecontei genotype. Within the Syrian genotypes, Meskawi genotype characterized by the highest number of unique markers which were 39 (10 positive and 29 negative), while Abu-Satel genotype characterized by the lowest number of unique markers which were 20 (18 positive and 2 negative). Tignon and Kettmann (2000) reported that a selection of two primer combinations was sufficient to identify 28 apple cultivars; this set characterized each cultivar by unique positive or negative markers. In addition, Boritzki *et al*. (2000) showed that AFLP technique were able to differentiate sweet sherry cultivars by unique positive banding patterns for each cultivar.

Comparison among the efficiency of RAPD and AFLP markers in the pear genome analysis:

The average of heterozygosity (Hav(p)), the effective multiplex ratio (E), and the marker index (MI) were computed for each marker type (Table 7).

The average of expected heterozygosity Hav(p) for polymorphic markers was used to evaluate the efficiency of different marker systems for polymorphism detection. RAPD technique revealed high Hav(p) (0.189) then AFLP (0.152).The obtained results in this investigation were agreed

with those of Powell *et al*. (1996); they reported RAPD revealed higher Hav(p) in the comparison with AFLP. Monte-Corvo *et al*. (2002) found that the expected heterozygosity between RAPD, AFLP and ISSR was very similar (0.52-0.53-0.54, respectively). Marker index (MI) for each assay was calculated based on the experimental data (Table 4). AFLP technique revealed the higher value of marker index (207.04) compared with RAPD technique, due to the high effective multiplex ratio of AFLP (1362). RAPD assay was (MI= 47.18).

Table (7)
Expected heterozygosity for polymorphic products Hav(p), effective multiplex ratio (E), and the marker index (MI) of each marker type used.

Marker type	Hav(p)	E	MI
RAPD	0.189	233	44.37
AFLP	0.152	1362	207.04

The correlation coefficient of AFLP with RAPD was (0.945), and that might be due to the similarity of nature of both markers where they were amplified in the same mechanism with some differences. Monte - Corvo *et al*. (2002) estimated the correlation between RAPD, AFLP and ISSR by comparing the similarity matrices of the different markers in pear. The ISSR and AFLP data were more correlated (r = 0.64) than RAPD and ISSR, especially RAPD and AFLP (r = 0.54) which is disagreed with our achieved results.

RAPD and AFLP are dominant markers, but AFLP is more complicated than RAPD. AFLP requires competent users with experience in molecular biology techniques. Therefore, it could be concluded that different markers differ in their ability to differentiate individuals, the mechanism of detecting polymorphism, genome coverage, and the ease of application. Therefore, they could be complementary to each other depending on technical availability. RAPD and AFLP molecular markers represent significant tools to study the relationships and genetic diversity among wild types and cultivated species. Hence, the two molecular markers used in this investigation were useful to identify the different closely related pear genotypes and gave rich source of genetic information and the comprehensive unique markers for the studied genotypes that provided basic knowledge for future breeding and conservation programs.

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العلاقات الوراثية بين بعض الطرز الوراثية للكمثرى باستخدام الواسمات الجزيئية RAPD و AFLP

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الملخص

تم دراسة التباين الوراثي بين سبعة طرز وراثية من جنس الكمثرى (3 طرز وراثية تتبع النوع *Pyrus syriaca* Boiss وثلاثة أصناف محلية سورية وصنف الـ Licontei الذي يزرع في مصر) باستخدام 32 بادئ RAPD و 10 بادئات AFLP. لقد كان مستوى التباين الوراثي الذي نتج عن تقنيتي الـ RAPD و AFLP 81.47% و92.5% على التوالي. أظهرت تقنيتا الـ RAPD وAFLP درجات قرابة وراثية مختلفة بين الطرز الوراثية السبع، حيث كانت أعلى نسبة تشابه وراثي بين التركيبين الوراثيين W.T1 وW.T3 في تقنية الـ RAPD (62%)، فيما كانت 54% في تقنية AFLP بين التركيبين الوراثيين Abu-Satel و W.T2. لقد ميز التحليل العنقودي الناتج عن التقنيتين بين صنف Licontei وبين الطرز الوراثية السورية، وقد أظهر التحليل العنقودي لتقنية الـ RAPD قرابة بين الصنف Licontei والصنف Romi ، فيما عزلت تقنية AFLP الصنف Licontei بمجموعة مستقلة. كما ميزت تقنيتا الـ RAPD وAFLP الطرز الوراثية المدروسة بعدد كبير من الواسمات الفريدة، حيث أظهرت 67 و 227 واسماً فريداً على التوالي. تم تمييز الصنف Licontei بأعلى عدد من الواسمات الفريدة (70 واسماً) يليه الصنف Romi الذي تميز بـ 50 واسماً فريداً، فيما أظهر الصنف Abu-Satel أقل عدد من الواسمات الفريدة (31). أظهرت تقنية RAPD أعلى نسبة تغاير وراثي (0.189)، فيما أعطت تقنية AFLP أعلى دليل مؤشر (207.4). وقد كان معامل الارتباط بين تقنيتي RAPD و AFLP مرتفعاً (0.945). وتشير هذه الدراسة إلى كفاءة تقنيتي RAPD و AFLP في تعريف طرز الكمثرى المختلفة وذات القرابة العالية فيما بينها.

الكلمات المفتاحية: القرابة الوراثية، كمثرى، واسمه AFLP، واسمه RAPD