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Detection of genetic relationships among some species belongs to genus *Malus* from mid Iraqi regions

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Abstract
RAPD-PCR method was used for systematic study and revealing the genetic relationships in *Malus* by using 7 apple cultivars from some mid Iraqi regions, DNA of fresh leaves was extracted using modified protocol of CTAB, 22 prechosen random decamer primers were applied to detect *Malus* genotypes. four primers gave reproducible and appeared polymorphism in the RAPD profile, a total 48 bands were produced out of which 36 bands were polymorphic, the results arise two main clusters, the first one included *Malus sylvestris* has unique amplified and discriminated from other *Malus* taxa, thus it can be a good molecular tool for taxa identification which separated at the similarity value of 0.48, and the second cluster contained two groups, one included *M. domestica*, and *M. domestica* var. ralls janet which appeared as closely related species with a stronger correlation at similarity range of 0.09, furthermore, it considered that the present study identifies reservoir of alleles that useful for breeding programs in parental crosses.

Key words: *Malus*, RAPD markers, cultivar, identification, genetic relationships, taxonomy

Introduction
The genus *Malus* (apple) has been considered as a one of the important fruit cultivated within Rosaceae family, also it has been characterized by broad diversity, the interspecific hybridization and breeding gene pools among this fruit groups and their wild relatives have probably have main roles in the evolution of the Rosaceae [1 , 2]. This has caused to produce individuals with intermediate phenotypes and genotypes characters [3], Hybridization activities has led to add or reduce taxa according to commercial roles, this might have caused to change the genetic identify and complicated taxonomic relationships within this family [4,5,6,7] Therefore, clearing relationships, taxonomy, and diversity is important for developing breeding strategies, conserving biodiversity, and improving breeding efficiency. Also understanding genetic variability in *Malus* is critical for characterizing germplasm, controlling genetic erosion and the registration of new cultivars [8 , 9]. Many genetic studies at Pomoideae like [10, 11, 12, 13, 14, 15, 16, 17, 18 ,19] mention that the *Malus* has been economic significance and large geographical distribution but the origin of it unclear.

References listed one species of *Malus* in flora of Syria, Palestine, Sinai, Iraq, Iran, and Turkey [20, 21, 22, 23], while[24] were mentioned 12 species of *Pyrus* in New Delhi flora belonged to Pomoideae subfamily.

Molecular evidence have become forceful tools to identify the hybridization and evolution processes, as well as provide a basic background knowledge to implement conservation genetics programmers, mutation rate or in dominance characteristics [3, 25 ,26]

The random amplified polymorphic DNA (RAPD) have been vastly used in DNA fingerprinting gene mapping as a reliable, fast, and simplest technique and to isolate phylogenetic relationships of many organisms taxonomy [27 , 28], and within many plant families as a molecular technique for cultivar identification [29 and 30].

Other molecular markers (amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), inter simple sequence repeat (ISSR) also have been used to analyzing the genetic diversity into *Malus* species such as [3, 11, 12, 13, 31, 32 , 33].

On the other hand chloroplast and mitochondrial DNA and minisatellite (M13) probes have also been used for DNA fingerprinting in several Rosaceae family [34 , 35].

The objectives of this study were investigated and identify the level of genetic diversity among apple cultivars, systematic analysis using RAPD technique as reliable molecular markers of these taxa.

Materials and Methods
1. Plants materials: Seven taxa of *Malus* genus were collected during April 2015 from different Iraqi regions included Salmanpak and Alsiafiyah south east Baghdad, (Baquba) Dyiala, and (AlHindia) Babel. All taxa present in table 1.

2. DNA extraction: Approximately 50 to 100 mg of young fresh leaf tissue put in 1.5 ml tube, after homogenizing the tissue using liquid nitrogen with a conical hand tissue grinder.

Total genomic DNA was isolated from fresh leaves using modified of cetylrimethyl ammonium bromide (CTAB) method of [36], the powder suspend in 2.5 ml of CTAB extraction buffer (1.4 M NaCl, 2% CTAB) and 5ml of B - mecaptoethanol. The suspension was mixed well, ad put incubated at 60ºC for 20 min to homogenate, followed by chloroform: isoamyl alcohol extraction (24:1), and precipitation with two volume of isopropanol at -20ºC, then bring down the sample formed after centrifugation for 5min, was washed with 1ml of 70% ethanol and 10m M of ammonium acetate, the DNA by TE buffer

(20m M EDTA, 0.1 M Tris- HCL p H= 8), for detection of the DNA samples that were electrophoresed in 1% agarose gel and stained with 0.5 mg/ ml ethidium bromide, with 1500 bp ladder (Sib Enzyme Ltd. Russia) with 100 v for 45 min visualized and photographed under a UV transilluminator [37].

3. Screening of PCR : Twenty two different 10mers RAPD primers were tested in this study (table 2) which supplied by Bioneer company were screened, four primers which had previously been shown indicated results of band patterns, multi master mix were used, the thermo profile for the PCR reaction was: 95°C for 5 minutes, then 35 cycles of 95°C for 30 sec, 37°C for 1 minute, and 72°C for 5 minute. Genotypes were visualized on 1% agarose gel, 1x TBE, 100 volts, for 55 minutes and scored as 1 or 0 based on presence or absence of a band, within the size range of 150-1800 base pairs (bp).

Table 1. The list of taxa under study

Seq	Scientific name	Common name	Collected region
1	*Malus pumila* Mill.	Golden delicious apple	Babel
2	*M. sylvestris* (L.) Mill.	Crab apple	Babel
3	*M. pumila* var *domestica* (Borkh.)C.K. Schneid	Fuji apple	Baghdad
4	*M. sieversii* (Ledeb.) Roem.	Kazakhstan apple	Baghdad
5	*M. domestica* Borkh.	Red delicious apple	Baghdad
6	*M. domestica* var *ralls janet*	Ralls jennet apple	Dyiala
7	*M. orientalis* Uglitz.	Uglitzk apple	Dyiala

Table 2. The sequences of twenty RAPD primers used in this study including those produced amplified

Primer name	Sequence (5´- 3´)
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-07	AAGTCCGCTC
OPA-09	GGGTAACGCC
OPA-11	CAATCGCCGT
OPA-13	CAGCACCCAC
OPB-01	GTTTCGCTCC
OPB-02	TGATCCCTGG
OPB-03	CATCCCCCTG
OPB-04	GGACTGGAGT
OPC-06	GAACGGACTC
OPC-12	TGTCATCCCC
OPC-13	AAGCCTCGTC
OPD-03	GTCGCCGTCA
OPG-01	CTACGGAGGA
OPG-02	GGCACTGAGG
OPK-14	CCCGCTACAC
OPZ-01	GAGCCCTCCA
OPZ-03	CAGCACCGCA
OPZ-04	AGGCTGTGCT

Table 3. Total number and size range of amplified bands obtained for each primer.

Primer name	Sequence (5´- 3´)	AN	Size range of bands(bp)	PM	%	MM	%
OPC-06	GAACGGACTC	12	250-1800	9	75	3	25
OPC-13	AAGCCTCGTC	8	150-1100	5	62.5	3	37.5
OPD-03	GTCGCCGTCA	10	250-1250	7	70	3	30
OPK-14	CCCGCTACAC	18	200-1500	15	83.3	3	16.6
	Total	48		36		12	

4. Data analysis: The NTSYS-pc statistical package version 2.1 used to analyze RAPD matrix. The data matrix was used to calculate the genetic similarity within and among taxa based on Jaccard's similarity coefficients, and a dendrogram displaying relationships among the 7 genotypes was constructed by the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Results

48 RAPD bands were produced using the 4 primers, 36 (75 %) of which were polymorphic. The apple was shown to exhibit high interspecific polymorphism due the nature of cross-pollination in that culture genotypes.

The number of RAPD bands varied from 8 (the primer OPC-13) to 18 (the primers OPK-14) (table 3). Thirty six polymorphic bands were obtained, Some representative polymorphisms revealed by RAPD primers were presented in table 3, The dendrogram shown the genetic relationships among the 7 apple genotypes (Figure 2) showed that apple taxa were essentially divided into 2 main clusters, The two main clusters separated at the similarity value of 0.48, the 1st (cluster I) contained two groups with similarity value 0.40 which separated into 2 clades, (IA) containing *M. pumila*, and the second clade (IB) that separated to 2 subclades with value similarity of 0.23, (IB1) divided to 2 groups 2 taxa isolated from *M. pumila* var *domestica* with 0.21 similarity value, were *M. domestica*, and *M. domestica* var *ralls janet* with showed highest similarity range among taxa under study which was 0.09, while (IB2) including 2 taxa were *M. sieversii* and *M. orientalis* with 0.15 similarity, cluster (cluster II) consisted of *Malus sylvestris* according to the dendrogram linkage joining rule were more distantly related and separated from the (IA) group.

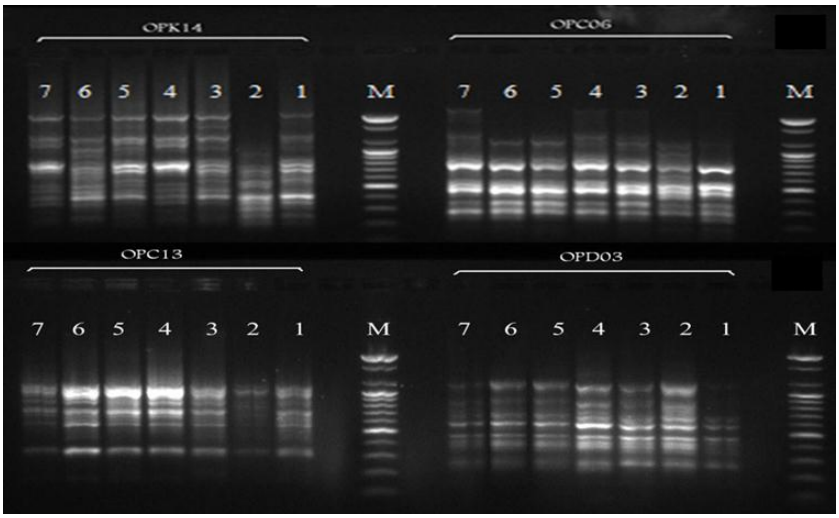

Figure 1. Spectrum of DNA amplification products of 7 taxa of genus Malus (table 1), molecular weight marker 1500-bp DNA Ladder

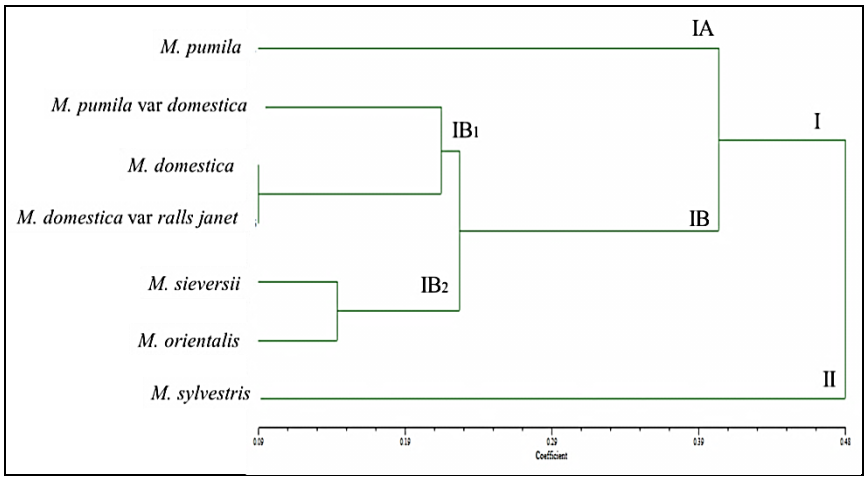

Figure 2. Dendrogram showing genetic relationships among 7 taxa of Apple.

Discussion

The fingerprinting analysis with RAPDs showed a high genetic diversity among taxa under study, generally we can predict that a strong relationship within taxa characterized by high level of polymorphism due to vegetative propagation. One of

the reasons that led to high level of polymorphism in fruit tree species they appear due to the high somatic mutation rates of different traits associated with sequences repetitive and constitutes an improvement over the basic genotype [33]. Moreover the difference between reproducible bands based by each primer depends on the sequence of primer used and extent of variation in specific genotype [38, 39, 40].

RAPD technique shown some bands were incomparably amplified in single taxon such as *Malus sylvestris* the bands in 200 bp- 1.35 kb, and these bands have significance interest in optimal management of germplasm collections, in addition they provide the identification of cultivars, duplicates, and verify possible pollen or seeds contamination during conservation activities [41].

The outcome of RAPD- PCR analysis and index matrix based on all DNA bands that isolated by four primes observed the strongest homogeneity between *M. domestica*, and *M. domestica* var *ralls janet*, so it can be considered as a reservoir of alleles useful for breeding , due divergent genotypes may has a reliable breeding value [42], or has substitution rates and high levels of gene rearrangements, furthermore the origin of the cultivated species *M. x domestica* is an urgent taxonomic problem for apple breeding[13].

ccording to US apple association the *M. pumila* var *domestica* progeny from *M. domestica*, and *M. domestica* var ralls janet. As noted by our results that species *M. sieversii* and *M. orientalis* were assumed to be ancestors of the domestic species, these apple are sweet and large [43]. This assumption was supported by RFLP analysis [44] However, some authors assume *M. sylvestris* and *M. pumila* may also be ancestors of the domestic apple [31 , 45].

Results showed that the *M. sylvestris* is genetically close to the *M. orientalis* and *M. sieversii* respectively, but there are genetic distance between in *M. sylvestris* and *M. sieversii*, this outcome against with [13] when he found the smallest genetic distance was between the *M. sylvestris* and *M. sieversii.*

Indeed when the new data are associate with existing botanical and molecular data it will be a clearer picture, Current study suggests fruit color and pattern vary considerably, and fruits sizes indicating a taxa, this result agreed with [46].

Moreover, our results deviate in part from those of another study [29 , 47] they regarding the identity of taxon but also by environmental differences such us geographic location,

The *Malus sylvestris* indicated its unique banding form over the rest taxa, the rustles ensured it's characterized by specific genotype, this lower genetic variability in this commercial hybrid compering with another taxa which is has narrower origin of hybrid and genetic erosion because intensive breeding [48].

Actually hybridization, introgression, and migration with in species, notably out crossing and open-pollinated these character genetically a great diversity of phenotypes, most of them being intermediate patterns [27].

Conclusion

The result indicate a high degree of correlation among studied taxa according reliable method of analysis that used in the present study provided an effective tool to understand evaluating germplasm material, or gene flow in order to identify the species that could be further evaluated.

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الكشف عن العلاقات الوراثية بين بعض مراتب التفاح التابعة للجنس *Malus* من بعض مناطق وسط العراق

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الملخص

تم استخدام طريقة RAPD-PCR لدراسة العلاقات الوراثية والتطورية في التفاح باستخدام 7 من مراتب التفاح المزروعة في وسط العراق، تم استخلاص الحامض النووي من الأوراق الطازجة باستخدام بروتوكول معدل لطريقة CTAB ، تمت تجربة 22 من البواديء العشوائية المؤلفة من عشرة قواعد نتروجينية للكشف عن الأنماط الوراثية. أربعة من البواديء فقط هي التي أظهرت تعدد الأشكال في صور الهلام الناتجة من التضخيم، والتي لوحظ فيها ما مجموعه 48 حزمة منها 36 حزمة متعددة الأشكال، اظهرت النتائج مجموعتين اساسية فقد تميز النوع *Malus sylvestris* الذي أظهر حزم فريدة امكن من خلالها التمييز بينه وبين الانواع الاخرى المدروسة، وبالتالي فإنه يمكن أن تكون أداة جزيئية جيدة لتحديد الأصناف التي انفصلت عن النوع اعلاه بقيمة تشابه 0.48، اما المجموعة الثانية فتضمنت مجموعتين احداهما تحوي النوعين *M. domestica* و *M. domestica* var. ralls janet الذين اظهرا اكبر تشابه وراثي في نطاق التشابه بقيمة 0.09، علاوة على ذلك فان الدراسة الحالية شخصت مستودع الجينات المفيد لبرامج التربية في التضريبات الأبوية.