PHYLOGENETIC RELATIONSHIPS AMONG (GRAPEVINE) *Vitis* TAXA BASED ON RANDOM AMPLIFICATION OF POLYMORPHIC DNA (RAPD-PCR) MARKERS

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ABSTRACT

Phylogentic relationships among seven genotypes of *Vitis* were analyzed by RAPD-PCR markers, DNA of fresh young leaves was extracted from each sample for RAPD analysis. Performed with 20 decamer primers selected six primers that showed clear results. A total sixty eight bands were produced out of which forty eight bands were polymorphic, The dendrogram reveals that grapevine taxa separated to main groups (A and B), the first group includes the most of taxa were *Vitis hissarica* Vass., *V. hissarica* subsp. *rechingeri*, *V. sylvestris* Fl. Bed., *V. vinifera* L. (Native), and *V. vinifera* var. *sativa* Beck., while group B contains 2 species were *V. coignetiae* Planch., and *V. berlandieri* Pulliatt. The taxa *V. hissarica* and *V. hissarica* subsp. *rechingeri* have been recorded closest genetic distance matrix among taxa under study with similarity range 0.11. The results of this study were offered an important data in taxonomy and may be can be a phylogenetic key to isolating the taxa. Moreover it is provide basic information for future genetic studies such as gene expression profiling.

Key words: Grapevine**,** hylogenetic, RAPD, genetic distance.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) belong to Vitaceae family, one of the anciently and momentous perennial crops in the global (Bodea *et al*, 2009; Nagaty *et al*, 2011). Townsend and Guest (1980) reported three main species in Iraq, and confirmed there were no wild species found in Iraq, because the genus extensively cultivated. *Vitis* occur in the tropical and subtropical regions (Soejima and Wen, 2006).

Classical procedures of identification, based on botanical, morphological, and ampelographic characters consider instability due to the influence of internal and external environment (Bodea *et al*, 2009). In addition, morphological method requires extensive field features and evaluation (Nagaty *et al*, 2011). Molecular

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markers have more advantages compering with morphological and biochemical markers (Kamali *et al*, 2010). The molecular systematic studies give us deeper light of genetic structures. Fingerprinting markers have been an impotence role for many purposes in molecular biology, such as analyzing of granitic diversity for classification of the cultivars and germplasm collections, and clearing the phylogenetic relationships among taxa, or closely related species. The identify sequence of nucleotides in the DNA of plants significantly increased our understanding of plant's families evolution, (Carlson and Holsinger, 2010).

Vitaceae has been considered a confusing and complicated taxonomic group with unclear genetic lines (Lombardi, 2000). The phylogeny of *Vitis* has been widely employed in the last decades, among them random amplified polymorphic DNA RAPD (Ye *et al*, 1998 ; Tamhankar *et al*, 2001 ; Bodea *et al*, 2009 ; Nagaty *et al*, 2011), amplified fragment length polymorphism AFLP (Cervera *et al*, 2000 ; Ergul *et al*, 2006), restriction fragment length polymorphism RFLP (Bourquin *et al*, 1993), inter simple sequence repeat ISSR (Sabir *et al*, 2009), simple sequence repeat SSR (Bowers *et al*, 1996 ; Sefc *et al*, 1999), and chloroplast of DNA cp (Trias-Blasi, 2012) also have been used to analyze the genetic diversity to identification in grapevine cultivars. RAPD technique has been widely used in DNA gene mapping isolation of phylogenetic relationships of many ranks, because low cost, tiny plants material, and its ability to reveal high degree of polymorphism it has successfully been applied as a molecular technique for cultivar identification within many plant families (Karataş and Ağaoğlu, 2010 ; Nagaty *et al*, 2011; Alanbari *et al*, 2014 ; Alanbari *et al*., 2015).

However rare data about the genetic diversity within grape species in Iraq. This study was attempted to investigate and identify of genetic diversity and relationships among grapevine cultivars, as well as systematic phylogenetic analysis of these taxa based on DNA profiling using RAPD technique can using as phylogenetic taxonomic key to isolate taxa .

MATERIALS AND METHODS

Plants materials: Fresh young healthy grape leaves of seven taxa of genus *Vitis* were *V. vinifera* L. (Native), *V. sylvestris* Fl. Bed., *V. hissarica* Vass., *V. coignetiae* Planch., *V. berlandieri* Pulliatt., including 1 subspecies *V. hissarica* subsp. *rechingeri*, and 1 variety *V. vinifera* var. *sativa* Beck collected during May 2015 from Diyala province. To identify the taxa we used the taxonomic keys that

published in flora, such as flora of Iran (Parsa, 1951), flora of Turkey (Davis, 1975), and flora of Iraq (Townsend and Guest, 1980).

DNA extraction: Approximately 50 to 100 mg of young fresh leaf tissue put in 1.5 ml tube, homogenized the tissue using liquid nitrogen with a conical hand tissue grinder. Genomic DNA was isolated from leaf samples using the procedure described by DNeasy Plant Mini Kit Protocol (Bioneer, Korea).

Screening of PCR: A total of twenty different 10mers RAPD primers were tested in this study (Table 1) supplied by Bioneer company were screened, six primers which had been shown indicated results of band patterns, multi master mix were used, the thermos profile for the PCR reaction was: 95 °C for 5 minutes, then 35 cycles of 95 °C for 30 seconds, 37 °C for 1 minute, and 72 °C for 5 minutes. Genotypes were visualized on 1% agarose gel, 1x TBE, stained with 0.5 mg ml^{-1} ethidium bromide, with 1500 bp ladder (SibEnzyme Ltd. Russia) and 100 v for 45 min visualized and photographed under a UV transilluminator.

Data analysis: The amplified bands were scored as 1 or 0 based on presence or absence of a band, within the size range between 110-1150 base pairs (bp). RAPD matrix analyzed by NTSYS-pc statistical package version 2.1. The data matrix was used to calculate the genetic similarity within 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).

Table 1. The sequences of twenty RAPD primers used in study including those produced amplified

Primer name	Sequence (5´- 3´)	Primer name	Sequence (5´- 3´)
OPA-01	CAGGCCCTTC	OPC-13	AAGCCTCGTC
OPA-02	TGCCGAGCTG	OPF-08	GGGATATCGG
OPA-03	AGTCAGCCAC	OPF-14	TGCTGCAGGT
OPA-13	CAGCACCCAC	OPG-01	CTACGGAGGA
OPA-18	AGGTGACCGT	OPG-02	GGCACTGAGG
OPB-01	GTTTCGCTCC	OPM-04	GGCGGTTGTC
OPB-02	TGATCCCTGG	OPM-15	GACCTACCAC
OPB-03	CATCCCCCTG	OPO-07	CAGCACTGAC
OPC-06	GAACGGACTC	OPZ-01	GAGCCCTCCA
OPC-12	TGTCATCCCC	OPZ-03	CAGCACCGCA

RESULTS AND DISCUSSION

68 amplified RAPD bands outcome from this study ranging from 110 bp to 1.15 kb in size were shown from the 7 grape genotypes. Due large numbers of polymorphism, we can predict that a strong relationship among genotype under study. The number of RAPD bands varied from 7 (primer OPM-04) to 17 (primer OPO-07). Forty eight polymorphic bands were outcome (Table 2), (Figure 1).

Table 2. The 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	%
OPA-18	AGGTGACCGT	12	110- 910	8	66.7	4	33.3
OPF-08	GGGATATCGG	10	200- 700	7	70	3	30
OPF-14	TGCTGCAGGT	10	150- 1090	5	50	5	50
OPM-04	GGCGGTTGTC	7	250- 650	4	57.1	3	42.9
OPM-15	GACCTACCAC	12	195- 800	11	91.7	1	8.3
OPO-07	CAGCACTGAC	17	155-1150	13	76.5	4	23.5
	Total	68		48		20	

AN= amplification number; PM= Polymorphic bands; MM= Monomorphic bands, %PM= PM/ANx100, % MM= MM/AN x100

According to Trias-Blasi *et al* (2012) the phylogentic studies of *Vitis* were unresolved. The numerous bands based on each primer depends on sequence of primer and extent of variation in specific genotype (Chan and Sun, 1997 ; Shukla *et al*, 2006 ; Shiran, 2007).

The dendrogram reveals of genetic linkages among the seven *Vitis* genotypes (Figure 2) shown that grapes taxa were mainly separated into two basic groups with a similarity value at 0.44, the first (group A) consisted of five *Vitis* taxa, it had divided to three clusters, *V. sylvestris* separated from other four taxa with similarity value 0.2, while *V. vinifera* and *V. vinifera* var. *sativa* which separated by value similarity at 0.15, but *V. hissarica* and *V. hissarica* subsp. *rechingeri* have been recorded closest genetic distance matrix among taxa under study with similarity range 0.11, (group B) contained two taxa were distinct genotype were *V. coignetiae,* and *V. berlandieri* that separated at the similarity value at 0.32. These two taxa isolated from other genotype. The general similarity ratio were found between 0.11-0.44. This results were associated with Sabir (2009) but disagreed with other researchers were they reported the similarity ratio were 0.3-0.9 in Turkish grape cultivars (Ergul *et al*, 2002 ; Karataş, 2005 ; Ağaoğlu, *et al*, 2006 ;

Karataş and Ağaoğlu, 2010) may be this ascribable to strong relationship among the taxa under study. Different authors agreed that genetic diversity was attributed by two factors, the geographic origin of varieties, morphological characters and the somatic mutations (Ulanovsky, 2002 ; Ergul *et al*, 2002 ; Sabir, 2009 ; Karataş and Ağaoğlu, 2010).

Our DNA finger printing study was shown no environment's power or biotic conditions effecting on genetic diversity among grapevine genotype, this is match with obtained by Nagaty (2011), and his results confirmed the two cultivars showed high similarity because they had the same fruit characters, but speciation pressures may be caused 4% differences, this result agreed with our results that observed the strongest homogeneity between *V. hissarica* and *V. hissarica* subsp. *rechingeri* Although the seven taxa were collected from the same farm but they have different genetic profile during the time (Nagaty, 2011) as *V. coignetiae*, and *V. berlandieri* isolated from other genotype. So it could be considered as a reservoir of alleles useful for breeding, because the divergent genotypes may had a reliable breeding value (Gwanama *et al*, 2000), or had substitution averages and high levels of gene rearrangements.

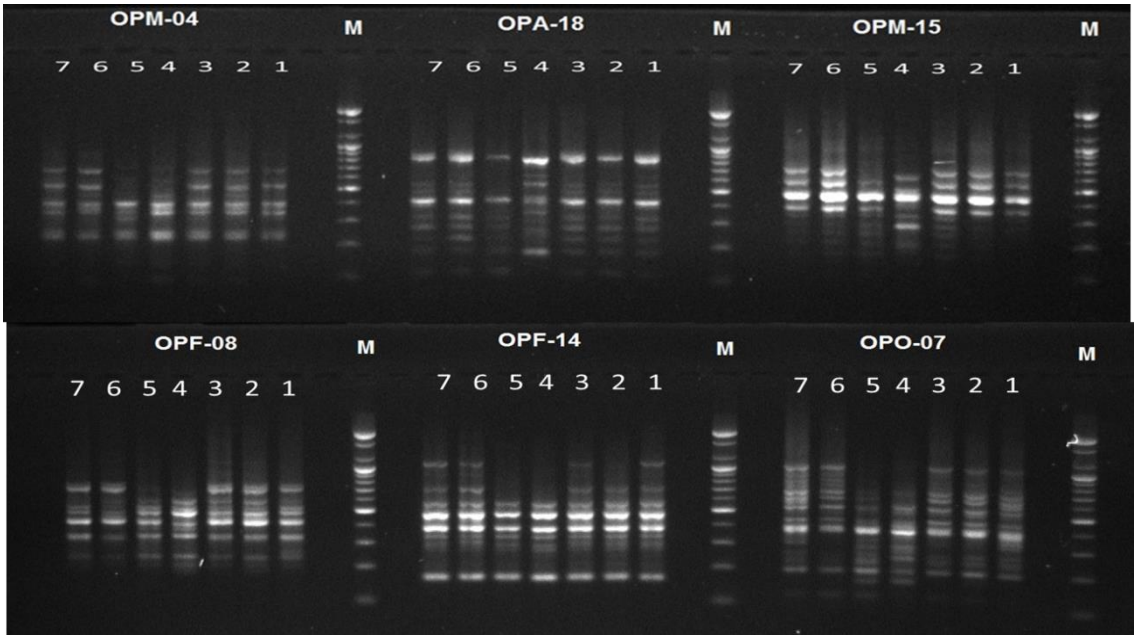

Fig. 1. RAPD profile obtained with 6 primers. M-Marker1500-100; 1- *V. sylvestris*; 2- *V. vinifera var. sativa*; 3- *V. vinifera*; 4- *V. coignetiae*; 5- *V. berlandieri*; 6- *V. hissarica*; 7- *V. hissarica subsp. rechingeri*

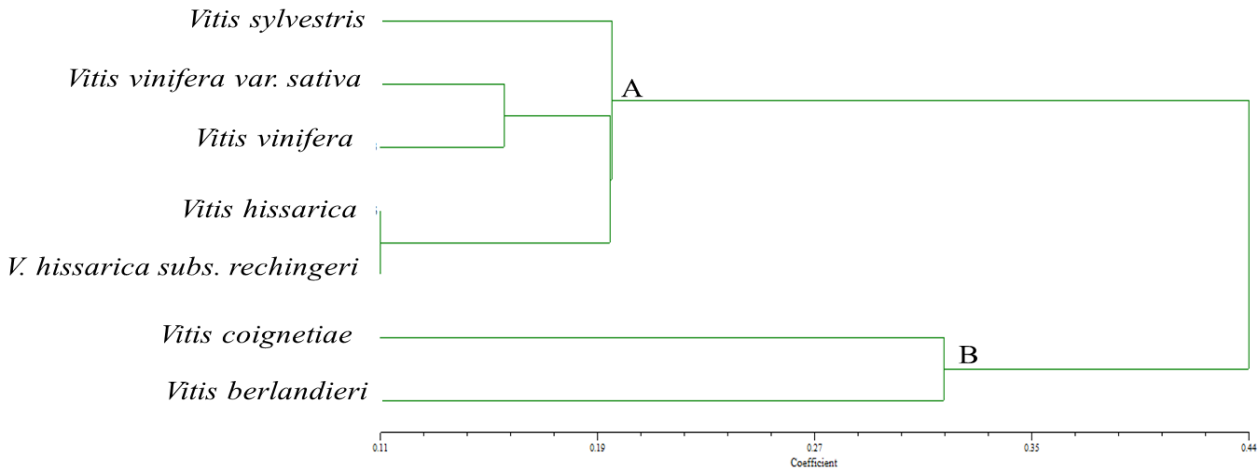

Fig. 2. Dendrogram of the7 *Vitis* taxa tested

However, from this study we can recognize that the new data were combined with existing botanical and molecular data it will be significant potential for genetic mapping, and identification in grapevine taxa. Moreover it considering a phylogenetic key can be useful tool in systematic studies to isolate taxa as well as an effective tool to understand evaluating gene flow in order to identify the taxa that could be further evaluated. These results indicate to interesting and high degree of correlation among grapevine taxa.

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العلاقة الوراثية بين مراتب جنس العنب *Vitis* اعتماداً على علامات التضاعف العشوائي للدنا

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المستخلص

العلاقة الوراثية بين 7 اشكال وراثية لجنس العنب تم تحليلها بواسطة تقنية تفاعل البلمرة التسلسلي العشوائي، واستخلص الدنا من اوراق يانعة طرية لكل عينة لتحليل التضاعفات العشوائية. تم العمل بواسطة 20 بادئات (ذات عشر قواعد ناتروجينية) منها فقط 6 بوادئ اوضحت نتائج ملموسة. كانت 48 حزمة من اصل 68 حزمة متباينة الاشكال. اظهر الشكل ان مراتب العنب انفصلت الى مجموعتين اساسية (أ و ب) واحتوت المجموعة الاولى اغلب المراتب تحت الدراسة وكانت المراتب هي:

Vitis hissarica Vass., *V. hissarica* subsp. *rechingeri*, *V. sylvestris* Fl. Bed., *V. vinifera* L.(Native), *V. vinifera* var. *sativa* Beck., بينما انفردت المجموعة الثانية برتبتين من العنب وهما *V. hissarica* و*V. hissarica subsp. rechingeri* . كانت اقرب معدل للعلاقة الوراثية هي 0.11 . وفرت هذه الدراسة بيانات مهمة للتصنيف ويمكن اعتبارها مفتاح تصنيفي لعزل المراتب النباتية كما انها وفرت معلومات اساسية للدراسات الوراثية المستقبلية للتعبير الجيني.

الكلمات المفتاحية: العنب، التقارب الوراثي، تقنية تفاعل البلمرة التسلسلي العشوائي، المسافة الوراثية.