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Molecular Characterization and Discrimination among Grapevine Cultivars by RAPD Markers

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Abstract: This study was performed in order to investigate the possibility of using RAPD analysis in the molecular identification and discrimination among eight grapevine cultivars (*Vitis vinifera* L.), which vary in berry colour and shilling requirements. Among the 20 RAPD primers utilized in the initial screening, only 6 informative were selected due to their ability to produce polymorphic and unambiguous markers between these cultivars. The selected primers were OPA-3, OPA-8, OPS-19, OPQ-15, OPW-15 and OPE-7. These primers produced a total of 70 DNA fragments, of which 66 fragments (94.2%) were reproducible polymorphic amplified fragments, while 4 DNA fragments were conserved among the 8 cultivars. All the cultivars studied showed a unique banding pattern for the 8 primers used. The most effective primer was OPA-8, which discriminated all studied cultivars. The data obtained were used to identify cultivar specific markers and to estimate the relationships among the grapevine genotypes. The results indicated that 15 positive and 5 negative markers were detected. The marker fragments size ranged between 371 to 605 bps for the negative markers and from 182 to 1600 bps for the positive markers. The degree of the genetic similarity ranged from 82.5% to 42.3%. The dendrogram tree resulting from the UPGMA suggested the existence of grapevine cultivars with high similarity. The markers used in the present investigation proved to be quite powerful in detecting high polymorphism as well as distinguishing between the tested grapevine cultivars. The results of the present study indicate that RAPD-PCR is a reliable and very useful method for the identification and genetic analysis of grapevine cultivars.

Key words: Grapevine, *Vitis vinifera*, RAPD-PCR, dendrogram

INTRODUCTION

The grape is unique: not only because of its status as a major global horticultural crop but also for its ancient historical connections with human culture. Currently, *vitis vinifera* is among the most important plant species being cultivated on area of about 7.9 million ha with more than 10000 grape cultivars worldwide, and an annual production of approximately 58 million tons (FAO, 2004).

Accurate grapevine identification is necessary because of the global problem which has arisen as a result of the long history of cultivation and distribution of vegetative cutting from new cultivars that were wrongly identified and renamed. The spread across cultural boundaries has also increased the problem due to different countries or regions adopting different names for the same cultivar.

Traditional methods of discrimination and identification of grape varieties have relied on morphological characters whose expression is affected by developmental and environmental factors. In recent years, molecular markers have proved to be a valuable tool for genetic studies and cultivar characterization. The random amplified polymorphic DNA (RAPD) technique has several distinct advantages: the cost per reaction is low, only a small amount of plant material is required for DNA extraction and the method does not require any prior knowledge of the sequence of the genome (Karatash and Sabit Agaoglu 2008).

The RAPD technique has been used for many crop plants in recent years such as pepper (Kumar *et al.*, 2007), tomato (Rajput *et al.*, 2006), date palm (Javouhey *et al.*, 2000, Eshraghi *et al.*, 2006) and mints (Momeni *et al.*, 2006).

Such technique has been successfully used for genetic studies in grapevines (Martinez *et al.*, 2003, Benjak *et al.*, 2005 and Kocsis *et al.*, 2005) which increased the understanding of relatedness of cultivars and facilitated research in vitas genetics (Reisch, 2000).

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The aim of this study was to identify and discriminate grapevine cultivars which dominate in Assiut region of Egypt and to determine their genetic similarities based on RAPD–PCR analysis.

MATERLIALS AND METHODS

Plant Materials:

The present study was carried out during 2010 growing season on trees grown in the Farm of the Faculty of Agriculture, Assiut University, Assiut, Egypt. The study was conducted on eight grapevine cultivars which are dominating in Assiut region, namely: Thompson Seedless, Red Roomy, Palomino, Rish Baba, Ruby Seedless, Beauty Seedless, Superior and Flame Seedless. These cultivars varied in berry colour and shilling requirements as shown in Table 1.

Table 1: Berry color and shilling requirements of eight grapevine cultivars.

Cultivar	Berry colour	Chilling requirements
Thompson Seedless	White	Low
Red Roomy	Red	Medium
Palomino	White	High
Rish Baba	White	Low
Ruby Seedless	Black red	Medium
Beauty Seedless	Black red	Low
Superior	White	Medium
Flame Seedless	Black red	Low

Molecular Analysis:

Young leaves were collected from 5 trees for each cultivar and immediately frozen in liquid nitrogen and stored at -80^{o}c. DNA was extracted from the leaf samples following the protocol for minipreps by using CTAB (Dellaportta *et.al*, 1983). For RAPD analysis, the method described by Williams *et.al*, (1990) was used to optimize the RAPD conditions.

Twenty different primers among the stocks available in our laboratory were tested (obtained from Operon technologies, Almeda, CA, USA) on samples of the cultivars. Primers that produce reproducible, polymorphic bands were used to amplify the rest of the cultivars. Six 10-mer primers which were found to be polymorphic were used to generate the RAPD markers. The PCR reaction conditions were optimized and reaction mixtures (50 µl total volume) consisted of 10X PCR buffer, $MgCl_2$ (50 mM), $dNTP_s$ (2 mM), primer (5 µl), template DNA (10 ng/µl), Taq DNA polymerase (5 units). DNA amplification was carried out for 45 cycles in Perkin Elemar G. thermalcycler. Amplified products were size-separated by electrophoresis in 1.5% agarose gel and visualized by ultraviolet illumination after staining with ethidium bromide.

Data Analysis:

RAPD assays were repeated twice for each primer and only clear bands were scored, with particular attention to sharp bands. Faint ones were ignored.

The data of the primers were used to estimate genetic similarity on the basis of the number of shared amplification products (Nei and Li, 1979). The equation used was: No. of shared amplification products = 2 X (No. of common bands between any two lanes) / (total No. of bands in the same two lanes).

Genetic relationship among the genotypes was estimated with the dendrogram constructed using DICE computer package to estimate the pairwise differences matrix and plot the phonogram among genotypes. Unique bands detected in a particular genotype but not in others were used as positive DNA markers. The absence of a common band for a given genotype was referred to as a negative specific marker.

RESULTS AND DISCUSSON

(1) Degree of Polymorphism:

The RAPD profiles with the six primers are shown in Figure 1. In order to investigate the genetic diversity of the eight grapevine cultivars 20 RAPD primer were utilized in the initial screening. As a consequence, 6 informative were selected due to their ability to produce polymorphic and unambiguous markers between these cultivars. The primers revealed total number of 70 amplified fragments (Table 2). Of the 70 bands obtained at the end of RAPD analysis, 66 were determined as polymorphic Amplified fragments size ranged from 141 to1600 bps and the number of bands per primer varied from 5 (OPA-5) to 24 (OPA-8). The six primers

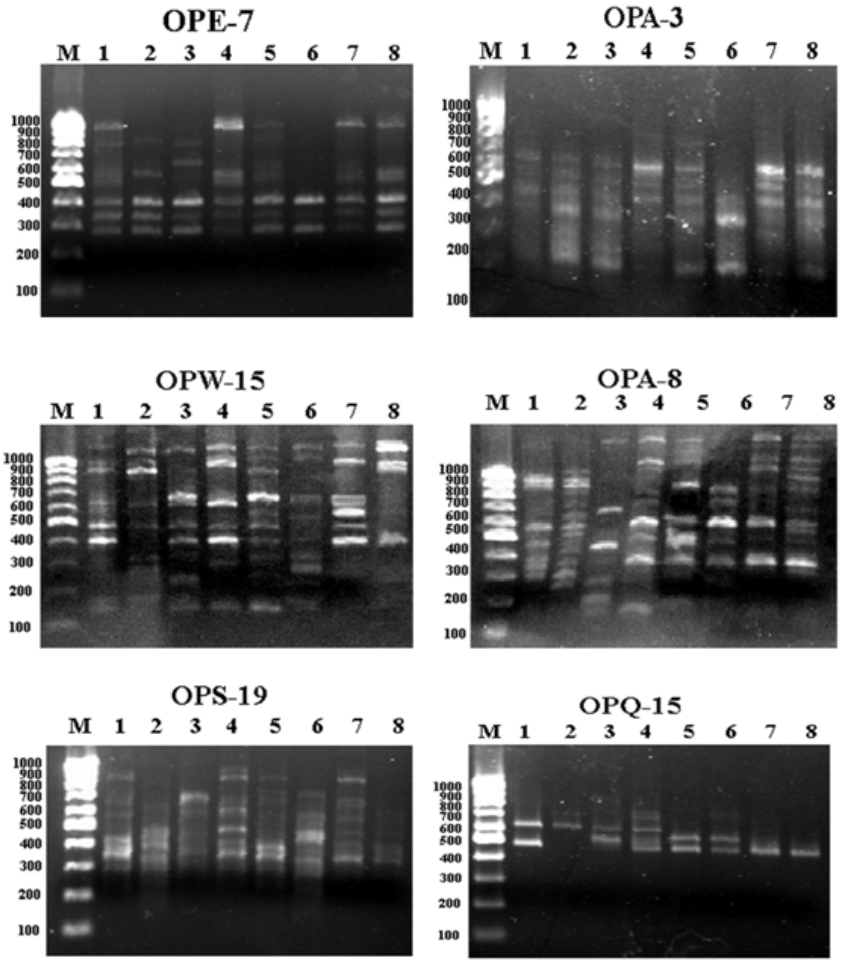

Fig. 1: Agarose gel electrophoresis of RAPD profiles in eight grapevine cultivars (1-8) generated by 6 RAPD primers. Where (1): Thompson Seedless, (2): Red Roomy, (3): Palomino, (4): Rish Baba, (5): Ruby Seedless, (6): Beauty Seedless, (7): Superior and (8): Flame Seedless.

Table 2: Details of six selected 10-mer primers and corresponding numbers of RAPD DNA markers (polymorphic and monomorphic).

Primer	Sequence	Band size (bp)	No. of amplified bands	Monomorphic bands		Polymorphic bands	
				No.	%	No.	%
OPA-3	AGTCAGCCAC	605-189	7	1	14.2	6	85.7
OPA-8	GTGACGTAGG	1600-182	24	0	0	24	100
OPS-19	GAGTCAGCAG	965-338	10	1	10	9	90
OPQ-15	GGGTAACGTG	700-437	5	0	0	5	100
OPW-15	ACACCGGACC	1255-141	16	1	6.2	15	93.7
OPE-7	AGATGCAGCC	1003-277	8	1	12.5	7	87.5
Total			70	4	5.7	66	94.2

discriminated all of the studied cultivars and the eight cultivars showed a unique banding pattern. The most effective primer was OPA-8, which produced 24 bands. The primer gave ratios of polymorphic bands: 100% for OPA-3 and OPQ-15, 93.7% for OPW-15, 90% for OPS-15, 87.5% for OPE-7 and 85.7% for OPA-3 primer. The highest ratio of polymorphism was 100 % (OPA-8, OPQ-15) while the lowest ratio was 85.7 % (OPA-3). The 6 primers used in the present study produced very high degree of polymorphism. These primers could be recognized to be the most appropriate primers for studies related to genetic diversity of cultivars. The suitability of RAPD technique for genetic diversity studies and germplasm evaluation has been reported by several authors (Kocsis *et al.*, 2005; Bodea *et al.*, 2009 and Maia *et al.*, 2009).

(2) Specific DNA Markers:

Unique DNA Fragments with different sizes were detected in particular genotype but not in the others. The presence or absence of such DNA fragments in a particular genotype could be used as positive or negative specific DNA markers for such genotype and might be helpful in genotype identification and discrimination. In the present investigation 15 positive and 5 negative markers were detected by the tested primers (Table 3). The marker fragments size ranged from182 to1600 bps for the positive markers and from 371 to 605 bps for the negative markers.

In Rish Baba cultivar, six positive markers (182, 1600, OPA-8; 965, OPS-19; 660, 700, OPQ-15 and 294 OPW-15) were detected. Four positive markers (261, 425, 650 and 700; OPA-8) were observed in Red Roomy cultivar. In addition three positive (219, OPA-8; 233, OPW-15 and 644, OPE-7) and two negative (593, 371; OPA-8) markers were observed in Palomino cultivar. One positive (1255, OPW-15) and one negative marker was detected in Flame Seedless cultivar. Meanwhile, no unique band was recorded in Thompson Seedless and Superior cultivars.

Table 3: DNA specific markers in eight grapevine cultivars; based on RAPD analysis data.

Primer / Cultivar	OPA-3		OPA-8		OPS-19		OPQ-15		OPW-15		OPE-7		Total	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Thompson Seedless													-	-
Red Roomy			796 650 425 261										4	-
Palomino			219	593 371					233		644		3	2
Rish Baba			1600 182		965		700 660		294				6	-
Ruby Seedless			1247										1	-
Beauty Seedless		605										399	-	2
Superior													-	-
Flame Seedless									1255			409	1	1
Total	-	1	8	2	1	-	2	-	3	-	1	2	15	5

(3) Genetic Similarity:

According to the similarity matrix of the 8 grapevine genotypes combinations (Table 4), the highest similarity (82.5%) was found between Superior and Flame seedless cultivars while the lowest similarity (42.3%) was between Beauty seedless and Palomino cultivars.

The markers used in the present investigation proved to be quite powerful in detecting high polymorphism as well as distinguishing the tested grapevine cultivars. Figure 2 revealed the dendrogram tree of the eight grapevine cultivars resulting from the UPGMA of values presented in Table 4. Cluster analysis by UPGAM suggests the existence of groups with higher similarities. In the dendrogram the eight cultivars formed two main groups. Five cultivars were clustered together in the first main group three of which (Flame Seedless, Superior, Rish Baba) were clustered in the first sub group and two (Thompson Seedless, Ruby Seedless) were in the second sub group. In the second main group the cultivars Red Roomy, Palomino and Beauty Seedless were clustered together.

Table 4: Genetic similarity values calculated from the total DNA fragments amplified from eight grapevine cultivars using six random primers.

Cultivar	1	2	3	4	5	6	7	8
1- Thompson Seedless	--							
2- Red Roomy	0.703	--						
3- Palomino	0.571	0.551	--					
4- Rish Baba	0.676	0.575	0.522	--				
5- Ruby Seedless	0.767	0.557	0.618	0.684	--			
6- Beauty Seedless	0.456	0.508	0.423	0.476	0.581	--		
7- Superior	0.677	0.563	0.533	0.789	0.686	0.556	--	
8- Flame Seedless	0.636	0.556	0.459	0.667	0.704	0.545	0.825	--

In the present investigation, the high level of polymorphism detected using RAPD analysis and the determination of DNA markers, suggested that RAPD approach showed considerable potential for grapevine cultivars identification, discrimination and explaining the interrelationships between cultivars of grapevine as

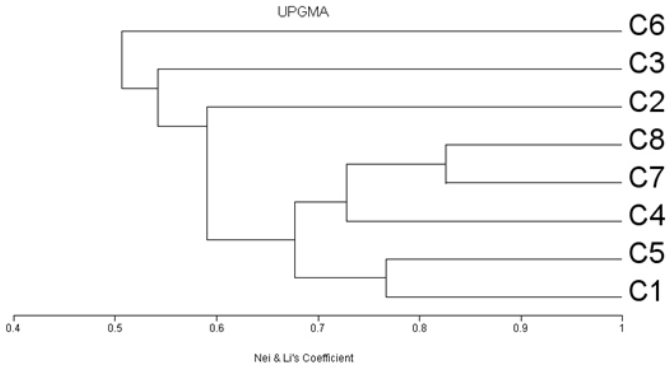

Fig. 2: Dendrogram demonstrating the relationships among the eight grapevine cultivars based on data recorded from polymorphism of RAPD markers. Where (C1): Thompson Seedless, (C2): Red Roomy, (C3): Palomino, (C4): Rish Baba, (C5): Ruby Seedless, (C6): Beauty Seedless, (C7): Superior and (C8): Flame Seedless.

well as useful selection tool in any breeding programs. Similar conclusion was obtained in different grapevine genotypes by Kim *et al.* (2002), Aras *et al.* (2005), Solouki *et al.* (2007), Salayeva *et al.* (2010) and Butiuc-Keul *et al.* (2010).

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