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Genetic study of grape cultivars belonging to the muscat family by random amplified polymorphic DNA markers

by

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Summary: Eleven decamer primers of arbitrary nucleotide sequence were used to amplify genomic DNA through the polymerase chain reaction (PCR-RAPD) in order to identify and discriminate between 14 grape cultivars (types or synonyms) belonging to the muscat family. Over 115 reproducible polymorphic fragments were generated by this method. On the basis of these fragments the degree of genetic similarity was calculated and the dendrogram of the 14 cultivars was established. The results indicate that there is genetic variation among the cultivars of the muscat family with values of the genetic similarity ranging from 0.666 to 1.00. On the basis of the observed bands it was possible to identify and discriminate between the cultivars studied except for Moschato aspro and Moscudi which were found to be identical.

Key words: dendrogram, muscat grape cultivars, PCR, RAPD, similarity index, Vitis vinifera.

Introduction

The group of "Muscat grapes" ("Moschoudia" in Greece) includes some very interesting cultivars grown all over Europe. The most important are Muscat de Frontignan, Moschato aspro (production of dessert wines), Muscat of Alexandria (a multipurpose variety) and Muscat Hamburg (a table grape variety). The characteristic muscat flavor is the common feature in all of them.

In Greece more than 12 grape cultivars (types or synonyms) belong to this group. The most important is the native Moschato aspro (or Moschato of Samos). This ancient Greek variety was probably brought to France by the Romans (Davidis 1967 and Galet 1979) and was called Muscat de Frontignan or Muscat blanc à petits grains. Other cultivars (types or synonyms) grown in Greece are Moscudi, Moschostaphilo, Moschato chondro, Moschato mavro, Moschato of Spinas, Moschato of Masas, Moschato of Corfou, Moschato of Limnos and, of course, Muscat of Alexandria and Muscat Hamburg.

Krimbas (1943) has described the cultivars Moschato aspro and Moschostaphilo and reported the "types" that were cultivated in Crete, Corfou, Peloponnesus and Samos; Guillon (1896) has described the cultivar Muscat blanc de Grece and Rovasenda (1888) has reported on Moschato bianco. Davidis (1967) described the cultivar Moschato of Samos and mentioned the Moschoudi, Moschostaphilo, Moschato aspro and Muscat de Frontignan as synonyms while Galet (1979) described the cultivar Muscat blanc à petits grains and mentioned Muscat de Frontignan and Muscuti to be synonyms.

The aim of this study was to identify and to discriminate cultivars of the muscat group and to determine the genetic similarities by using the RAPD-PCR analysis. This method, based on random amplified polymorphic DNA obtained by polymerase chain reaction analysis, allows the direct comparisons of the genetic material of grape cultivars. DNA molecular markers have been used successfully to reveal genetic variation among and within grape cultivars (Bowers *et al.* 1993; Büscher *et al.* 1993; Collins and Symons 1993; Jean-Jaques *et al.* 1993; Grando *et al.* 1995; Moreno *et al.* 1995; Biniari *et al.* 1996; Stavrakakis *et al.* 1997).

Material and methods

Grapevine material: Fourteen cultivars, types and synonyms of the muscat group, grown in Greece were chosen for identification (Tab. 1). Two of them, Muscat Reine des Vignes and Muscat Ottonel, are hybrids but were included in the study for comparison reasons.

DNA extraction: Grapevine DNA was extracted from young and fully expanded leaves according to Thomas *et al.* (1993) with minor modifications. 1 g of leaves from individual vines was frozen in liquid nitrogen and ground to a fine powder, thawed and resuspended in 12.5 ml buffer A [0.25 M NaCl, 0.2 M TRIS-Cl (pH 8.0), 50 mM EDTA, 0.1 v/v 2-mercaptoethanol, 2.5 % w/v polyvinyl-pyrrolidone (MW 40,000)]. A crude nuclei pellet was obtained by centrifugation at 7,000 *g* for 10 min at 4 °C. The pellet was resuspended in 2.5 ml of extraction buffer B [0.5 M NaCl, 0.2 M TRIS-Cl (pH 8.0), 50 mM EDTA, 1 % v/v 2-mercaptoethanol, 2.5 % w/v polyvinyl-pyrrolidone, 3 % sarkosyl, 20 % ethanol] and incubated at 37 °C for 45 min. An equal volume of chloroform/isoamyl alcohol (24:1) was then added and the phases were separated by centrifugation at 14,000 rpm for 15 min.

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The aqueous layer was collected and 0.54 volume of cold isopropanol (-20 °C) was added to precipitate the DNA. The DNA was fished and resuspended in 300 µl TE (10 mM Tris-HCl, pH 7.4, 1 mM EDTA) containing 15 µg·ml^{-1} RNase A and incubated for 15 min at 37 °C. Protein was removed by the addition of a half volume of 7.5 M ammonium acetate, followed by centrifugation and the DNA in the supernatant was precipitated with a 0.25 of cold isopropanol; ca. 120 µg DNA per g FW was obtained.

Amplification conditions: For RAPD analysis the protocol reported by WILLIAMS *et al.* (1990) was followed with minor modifications. Amplification reactions were performed in volumes of 25 µl containing 60 ng of genomic DNA, 10 mM TRIS-Cl pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 % Triton X-100, 200 M each of dATP, dGTP, dCTP, dTTP, 50 ng primer and 1 unit of Taq DNA polymerase (Biometra). The surface was covered with 30 µl of mineral oil (Sigma). Eleven random decamer oligonucleotides were used as primers for the amplification of RAPD sequences (Tab. 1) Primers OPF5, OPF8, OPF9, OPF13, OPF15, OPF18 and OPF20 were obtained from Operon Technologies, Inc. (Alemeda, Calif., USA) while primers 1224, 1225, 1226 and 1227 were obtained from IBBM (University of Crete, Greece).

Amplification was performed in a Perkin Elmer DNA Thermal Cycler 480. After 5 min at 94 °C, 34 cycles of PCR were performed, (1 min at 94 °C, 1 min at 44 °C, 2 min at 72 °C) followed by 10 min at 72 °C for extension.

Gel electrophoresis: Aliquots of the RAPD products were analysed in 1.4 % agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate and 1mM EDTA, pH 8). After staining in ethidium bromide (1µg·ml^{-1}) the gels were photographed on a Gel Doc 1000 (Biorad). All of the reactions were repeated at least twice with independently isolated genomic DNA as templates.

The electrophoretically detected degree of genetic similarity between each pair of cultivars studied (Tab. 2) was calculated using the NTSYS-pc package 1.8 developed by ROHFL (Exeter Software, New York, USA).

Results and Discussion

Eleven single, arbitrary 10-mer oligonucleotide primers were used to amplify genomic DNA from 14 grape cultivars. Each primer provided at least two polymorphic bands. 118 reproducible polymorphic fragments were generated by this method. The primers OPF 5, OPF 9, OPF 13, 1225 and 1227 proved much more useful in differentiating cultivars as they generated more polymorphic DNA fragments (Tab. 1). Examples of RAPD patterns amplified with primers OPF 5, OPF 13 and 1225 are shown in Fig. 1.

As expected, there was genetic variation among the cultivars studied. Moschato aspro, Moscudi, Moschato of Corfou, Moschato of Spinas, Moschato of Masas and Muscat de Frontignan were grouped in a single branch of the tree while Moschostaphilo and Moschato mavro were grouped in a different branch (Fig. 2). The very high degree of genetic similarity (0.957) between Moschato aspro and Muscat de Frontignan (only 4 out of 87 bands were not common) may indicate that these cultivars originated from a common stock (Tab. 2). Due to mutation the patterns are not completely identical. The same holds true for the cultivars Moschato aspro and Moschato of Corfu, Moschato of Spinas and Moschato of Masas. The identical patterns between the cultivars Moschato aspro and Moscudi supports the conception that they are synonyms (DAVIDIS 1967; GALET 1979). On the other hand the very low degree of genetic similarity between cv. Moschato aspro and cvs Moschostaphilo and

Table 1

Cultivars, sampling areas and synthetic desoxyribonucleotides used as primers for amplification of grape cultivar DNA

Cultivar	Sampling area	Primer code	Nucleotide sequence (5' to 3')	Total number of fragments amplified	Cultivar code
Moschato aspro	(a),(b)	1224	CAGGCCCTTC	8	E1
Moscudi	(a)	1225	AGGTGACCGT	11	E2
Moschostaphilo	(a)	1226	CGCAGGATGG	9	E3
Muscat Frontignan	(a)	1227	GTGTGCCCCA	21	E4
Moschato Corfou	(a),(c)	OPF5	CCGAATTCCC	22	E5
Muscat Alexandria	(a),(c)	OPF8	GGGATATCGG	9	E6
Moschato Limnos	(a),(d)	OPF9	CCAAGCTTCC	13	E7
Moschato chondro	(a)	OPF13	GGCTGCAGAA	11	E8
Muscat Hamburg	(a)	OPF15	CCAGTACTCC	3	E9
Moschato mavro	(a)	OPF18	TTCCCGGGTT	4	E10
Moschato Masas	(c),(e)	OPF20	GGTCTAGAGG	7	E11
Moschato Spinas	(c),(e)				E12
M.Reine des Vignes	(a)				E14
Muscat ottonel	(a)				E15

(a): Institute of Vine, NAGREF, Athens, (b): Vineyards of Peloponnesus, (c): Institute of Vine, NAGREF Iraklion, Crete, (d): Vineyards at the island of Limnos, (e): Vineyards near Chanea, Crete.

Table 2

Genetic similarity values (x 1000) of 14 grape cultivars

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	M. Aspro	---													
2	Moscudi	1000	---												
3	Moschostaphilo	661	661	---											
4	M. Frontignan	957	957	686	---										
5	M. Corfou	923	923	652	949	---									
6	M. Alexandria	779	779	711	822	771	---								
7	M. Limnos	779	779	711	805	771	932	---							
8	M. Chondro	711	711	694	754	703	830	796	---						
9	M. Hamburg	711	711	677	737	703	864	813	762	---					
10	M. Mavro	720	720	737	745	745	703	720	720	686	---				
11	M. Masas	932	932	677	957	923	813	796	762	745	788	---			
12	M. Spinas	906	906	669	932	898	805	788	754	720	745	940	---		
13	M. Reine Vignes	754	754	669	796	779	737	737	788	720	728	805	762	---	
14	M. Ottonel	686	686	686	728	694	754	737	737	703	728	737	711	762	---

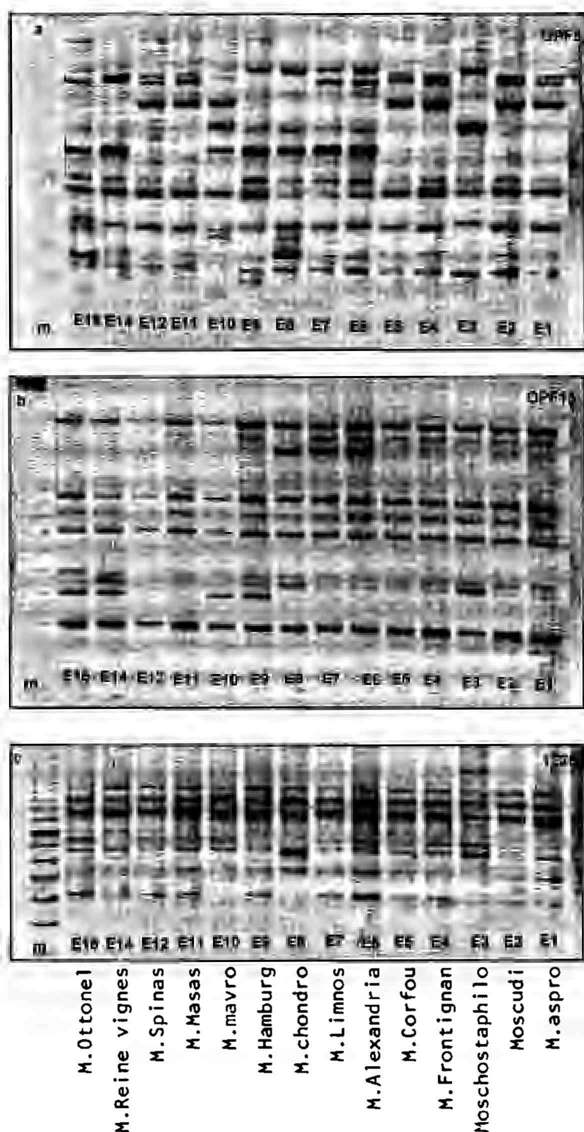

Fig. 1: Amplification patterns of polymorphic DNA from fourteen grape cultivars by OPF5 (**a**), OPF13 (**b**) and 1225 (**c**). m: 100-bp molecular weight ladder (Pharmacia Biotech).

Moschato mavro (0.661 and 0.720, respectively) indicates that they are different cultivars.

Muscat of Alexandria and Moschato of Limnos showed a very high degree of the genetic similarity (0.932) indicating that they are closely related cultivars. Probably the Moschato of Limnos is a mutation of Muscat of Alexandria.

On the other hand, Muscat Hamburg seems to be more related to Moschato chondro (0.762) than to Moschato mavro (0.686) even though Moschato mavro has been mentioned to be a synonym of Muscat Hamburg (DAVIDIS 1967). In any case the degree of genetic similarity between all the above cultivars is quite low indicating that they are different cultivars.

The relative high genetic similarity between Muscat Reine des Vignes and Muscat Ottonel (0.762) is surprising since both are hybrids of different cultivars (Muscat Reine des vignes is a cross between Souvenir de la Reine Elisabeth and Perle of Csaba; Muscat Ottonel probably is a cross between Chasselas and Muscat de Saumur).

On the basis of the RAPD profiles and the resulting similarity indices and the dendrograms it can be concluded that the muscat cultivars Moschato aspro, Muscat de Frontignan, Moscudi, Moschato of Corfu, Moschato of Masas and Moschato of Spinas consist of a separate group and that they are closely related cultivars originating from a common progenitor probably by accumulation of mutations. All other muscat cultivars of our analysis are different with a relatively high degree of genetic similarity.

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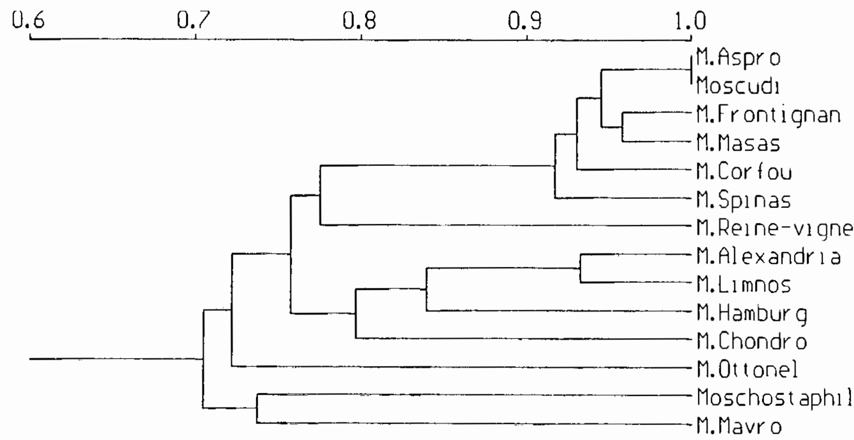

Fig. 2: Dendrogram based on 118 RAPD amplification products showing the relationship among the grape cultivars studied.

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