The ‘vine of Pafsanias’ and the group of grape cultivars ‘Mavroudia’ of the vineyard of Peloponnese.

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Abstract: This study presents the results regarding the genetic diversity of grape cultivars belonging to Mavroudia group (*Vitis vinifera* L.spp. *sativa* D.C.) grown in the vineyard of Peloponnese and the Pafsanias vine (*Vitis vinifera* L.spp. *silvestris* Gmel.) using the molecular method RAPDs-PCR. The cultivars studied were Agiorgitiko, Mavroudi 1, Mavroudi 2, Karvouniaris, Mavrostafylo psilorogo, Mavrostyfo, Mavro kalavryton, Mavreli, Mavro tsoupoto, Mavro koryfisio Mavro kolliniatiko, Mavro voulgariko, Agiomavritiko, Karambraimis while for the RAPD-PCR analysis 6 primers were used which produced more than 120 electrophoretic bands. The study of the degree of the genetic similarity for every possible pair of the cultivars studied showed a strong genetic differentiation between the grape cultivars and the Pafsanias’ vine.

Abstract: Στην παρούσα ερευνητική εργασία επιχειρήθηκε η γενετική μελέτη της ομάδας των ποικιλιών με το γενικό όνομα Μαυρούδια (*Vitis vinifera* L.spp. *sativa* D.C.) που καλλιεργούνται στην Πελοπόννησο και του «κλήματος του Παυσανία» (*Vitis vinifera* L.spp. *silvestris* Gmel.) με τη μέθοδο RAPD-PCR. Μελετήθηκαν οι ποικιλίες Αγιωργίτικο, Μαυρούδι, Καρβουνιάρης, Μαυρόστυφο, Μαυροστάφυλο ψιλόρογο, Μαύρο Καλαβρύτων, Μαυρέλι, Μαύρο τσουπωτό, Μαύρο κορυφίσιο, Μαύρο κολλινιάτικο, Αγιομαυρίτικο, Καραμπραίμης, Μαυρούδι Βουλγαρίας. Για τη RAPD- PCR ανάλυση χρησιμοποιήθηκαν 6 εκκινητές (περισσότερες από 120 ζώνες) που αποδείχθηκαν ιδιαίτερα πολυμορφικοί και αποτελεσματικοί για τη διάκριση των ποικιλιών που μελετήθηκαν. Έτσι, κάθε εκκινητής παρουσίασε διαφορετικό βαθμό πολυμορφισμού και με βάση τις ηλεκτροφορητικές ζώνες που καταγράφηκαν υπολογίστηκε ο βαθμός γενετικής ομοιότητας για κάθε δυνατό ζεύγος των ποικιλιών που μελετήθηκαν και σχηματίστηκε το σχετικό δενδρόγραμμα. Διαπιστώθηκε ότι οι ποικιλίες vinifera διέφεραν τόσο μεταξύ τους όσο και με το κλήμα Παυσανία, ενώ ο βαθμός γενετικής ομοιότητας ήταν περίπου ίδιος και στις δύο περιπτώσεις (0,70).

Keywords: *Vitis vinifera* L., grapevine, RAPD-PCR, molecular markers, genetic similarity

Introduction

With conventional criterion the color of the berries and under the general name “Mavroudia”, there are many greek grapevine varieties (*Vitis vinifera* L.) mentioned, among which many of the most important varieties of the greek vineyard, such as

Agiorgitiko, Xinomavro etc. Usually, the name Mavroudi or Mavro is followed by a toponym (for example Nemea, Naoussa, Spetses etc.) or by an adjective associated with the size and texture of the berries (Mavroudi chondro, Mavroudi psilo, Mavro tragano etc.). The number of the cultivars belonging to the group "Mavroudia" is extremely high. In the National catalogue, more than 50 grapevine cultivars of Mavroudia are mentioned, followed by an even greater number of synonyms. In the past, the use of toponyms (Nemea, Naoussa), which were associated with the production of high quality greek wines, created many problems in the identification of the wines which resulted in the establishment of the official names Agiorgitiko instead of Mavro Nemeas and Xinomavro instead of Mavro Naoussas. Ampelographic descriptions have been conducted for most of the cultivars of the group in question (Krimbas 1943,44,49, Davidis 1967, Logothetis and Vlachos 1966, Vlachos 1986). This work involved the genetic study of certain grapevine cultivars (*Vitis vinifera* L. *sativa* D.C.) of the group "Mavroudia" grown in the Peloponnesian vineyard and the determination of the genetic relationship not only among themselves but also among the vine of Pafsania, a very old vine located in the viticultural area near Kalavrita and is considered to belong to the wild vine (*Vitis vinifera* L. spp. s*ilvestris* Cmel.) and is ranked in the female types (Θ1, Θ2), with characteristically small bunches and with small black berries (Logothetis 1962). The identification and discrimination of the above cultivars was done using the molecular method RAPD-PCR, which has proven to be very effective in similar research papers (Biniari 2000, Bowers et al 1996, Stavrakakis et al 1997, Stavrakakis and Biniari 1998, Meneghetti et al 2011.)

Material and Methods

Grapevine material

As mentioned above, fourteen grapevine cultivars grown in Peloponnese under the general name 'Mavroudia' belonging to *Vitis vinifera* L. *sativa* D.C. and the Pafsanias vine belonging to *Vitis vinifera* L. spp. s*ilvestris* Cmel., were chosen for genetic study and identification (Tab. 1). Leaf material of these cultivars was obtained from the grape germplasm collection of NAGREF in Athens, from the collection of Agriculture University of Athens, as well as from different grapevine areas in Peloponnese.

Table 1: Cultivars' code and studied cultivars.

Cultivar code	Cultivar
N1	Pafsanias vine
N2	Agiorgitiko
N3	Mavroudi 1
N4	Mavroudi 2
N5	Karvouniaris
N6	Mavrostafylo psilorogo
N7	Mavrostyfo
N8	Mavro kalavryton
N9	Mavreli
N10	Mavro tsoupoto
N11	Mavro koryfisio
N12	Mavro kolliniatiko

N13	Mavro voulgariko
N14	Agiomavritiko
N15	Karambraimis

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 were frozen in liquid nitrogen and ground to a fine powder, thawed and resuspended in 12,5 ml of 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 rpm for 10 min at 4 ^{o}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^{o}C for 45 min. An equal volume of chloroform/isoamyl alcohol (24:1) was then mixed in and the phases were separated by centrifugation at 14.000 rpm for 15 min. The aqueous layer was collected and 0.54 volume of frozen isopropanol (-20 ^{o}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 ^{o}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 MgCl2, 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 (Qiagen). Six random decamer oligonucleotides were used (Tab. 2) as primers for the amplification of RAPD sequences. Amplification was performed in a Perkin Elmer DNA Thermal Cycler 9600. After 5 min at 94^{o}C, 34 cycles of PCR were performed, (1 min at the 94^{o}C, 1 min at the 44^{o}C, 2 min at the 72^{o}C) followed by a 10 min at 72^{o}C for extension.

Table 2: Synthetic nucleotides used as primers for RAPDs analysis and number of bands.

Primer Code	**Nucleotide sequence** **5′→3′**	**Total number of fragments amplified**
1225	AGGTGACCGT	15
OPF-04	GGTGATCAGG	21

OPF-05	CCGAATTCCC	26
OPM-01	GTTGGTGGCT	21
OPM-04	GGCGGTTGTC	18
OPM-06	CTGGGCAACT	21

Gel electrophoresis – Data analysis

Aliquots of the RAPD products were analyzed in 2% 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 degree of genetic similarity (I) detected electrophoretically between each pair of the cultivars studied (Tab.3) was calculated using the SM coefficient and using the NTSYS-pc package 1.8 developed by Rohlf (Exeter Software, New York, USA) and the appropriate dendrogram was generated (Fig.2).

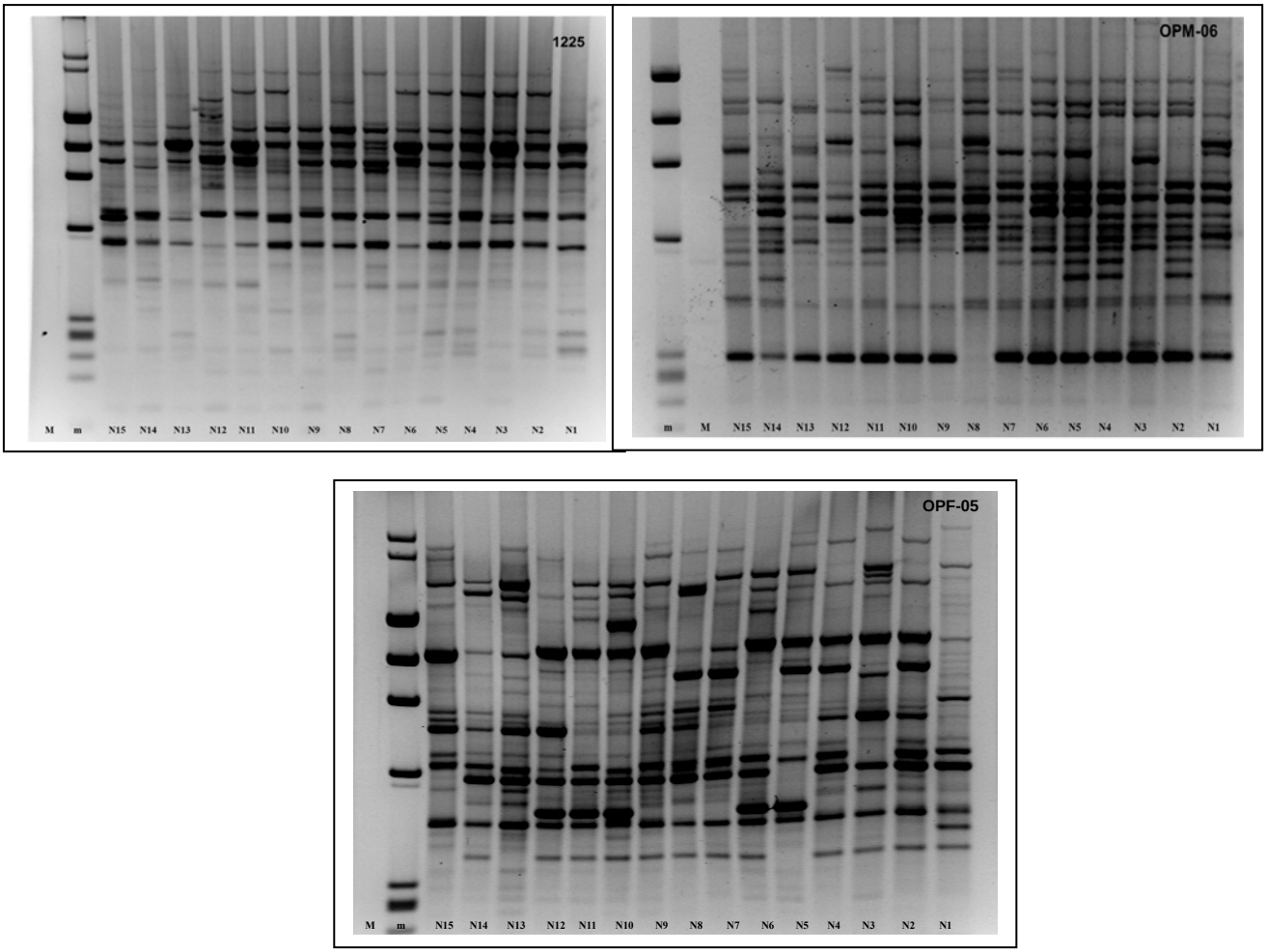

Figure 1: RAPD patterns obtained using primers 1225, OPM-06 and OPF-05 for all cultivars studied.

Table 3: Genetic similarity values between the cultivars studied as determined from RAPD analysis.

	Pafsanias vine	Agiorgitiko	Mavroudi 1	Mavroudi 2	Karvouniaris	Mavrostafylo psilorogo	Mavrostyfo	Mavro Kalavryton	Mavreli	Mavro Tsoupoto	Mavro Koryfisio	Mavro Kolliniatiko	Mavro Voulgariko	Agiomavritiko	Karabraimis
Pafsanias vine	1.00														
Agiorgitiko	0.72	1.00													
Mavroudi 1	0.66	0.70	1.00												
Mavroudi 2	0.72	1.00	0.70	1.00											
Karvouniaris	0.68	0.78	0.67	0.78	1.00										
Mavrostafylo psilorogo	0.61	0.73	0.67	0.73	0.77	1.00									
Mavrostyfo	0.79	0.72	0.66	0.72	0.73	0.70	1.00								
Mavro Kalavryton	0.73	0.71	0.69	0.71	0.70	0.62	0.76	1.00							
Mavreli	0.70	0.70	0.63	0.70	0.70	0.66	0.80	0.71	1.00						
Mavro Tsoupoto	0.65	0.68	0.67	0.68	0.69	0.65	0.66	0.62	0.68	1.00					
Mavro Koryfisio	0.61	0.66	0.65	0.66	0.72	0.88	0.66	0.64	0.63	0.62	1.00				
Mavro Kolliniatiko	0.68	0.68	0.59	0.68	0.65	0.69	0.71	0.69	0.71	0.62	0.70	1.00			
Mavro Voulgariko	0.67	0.69	0.66	0.69	0.65	0.65	0.69	0.68	0.72	0.65	0.70	0.66	1.00		
Agiomavritiko	0.76	0.71	0.72	0.71	0.67	0.67	0.73	0.69	0.73	0.67	0.70	0.64	0.70	1.00	
Karabraimis	0.72	0.69	0.63	0.69	0.70	0.66	0.74	0.68	0.69	0.61	0.68	0.68	0.77	0.76	1.00

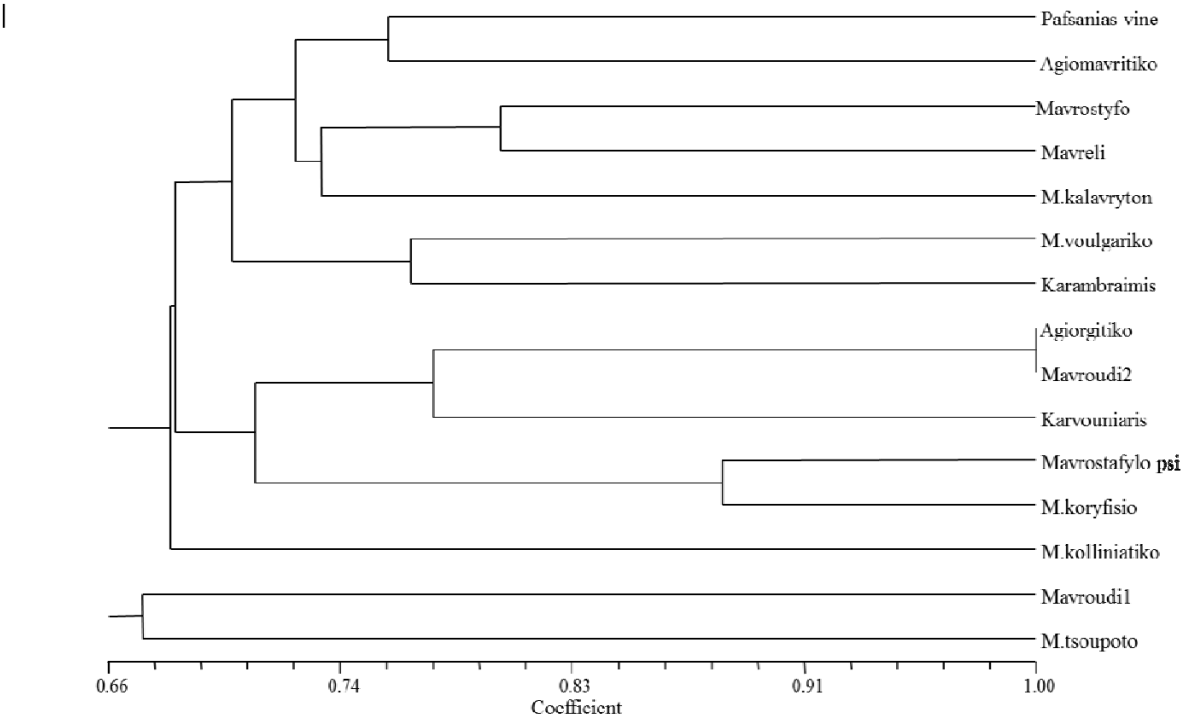

Figure 2: Dendrogram based on 122 RAPDs amplification products showing the relationship among the cultivars studied.

Results and Discussion

From the statistical analysis of the results, the degree of genetic similarity for each pair of the cultivars studied was determined (Table 3) and the corresponding dendrogram was generated (Figure 2). The analysis of the data reveals the following:
a) the low degree of genetic similarity (I=0,61-0,79) between the Pafsania vine and the grape cultivars studied does not support the notion that the Pafsania vine constitutes the original variety from which the others derived through mutations. The Pafsania vine showed the highest degree of genetic similarity with the cultivar Mavrostyfo (I=0,79) and then with the cultivar Agiomavritiko (I=0,76). The grapevine cultivar Mavrostyfo is grown mainly in the eastern Peloponnese while the sample from the cultivar Agiomavritiko (which is preserved at the grape germplasm collection) was obtained from Thessaly. The degree of genetic similarity between the cultivars Mavrostyfo and Agiomavritiko is equally low.
b) the low degree of genetic similarity between every possible pair of the cultivars studied shows that they are different cultivars, while the absence of common electrophoretic bands support the notion that the above cultivars are simple or multiple hybrids of other cultivars and thus, they didn't derive from one parent cultivar through mutations (Loukas et al. 1983).
The above results confirm those of the ampelographic description and those of the biochemical methods (Stavrakakis 1990). Further research is required as far as the origin of the vine of Pafsania is concerned.

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