Genetic study of grape cultivars used for the production of Malvasia wine by RAPDs

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Summary

Malvasia, the famous Greek wine, that has been internationally known since the 14th century was probably made by the use of several grape cultivars. Main cultivars were (or among these cultivars) Monevasia, Athiri, Thrapsathiri, Aidani, Trifera, Glykerithra, Ladikino, Tactas etc which are characterized by their peculiar light aroma.

Ten decamer primers of arbitrary nucleotide sequence were used to amplify genomic DNA through the polymerase chain reaction (RAPD- PCR) in order to identify and discriminate between fifteen grape cultivars grown in different areas in Greece and seven of the Malvasia group grown mainly in Italy, which probably have been used for the production of Malvasia wine. More than 105 reproducible polymorphic fragments were generated by this method. On the basis of these fragments the degree of genetic similarity was calculated and the dedrogram of the 22 cultivars was established. The results indicated that there is genetic variation among the above cultivars, as the degree of genetic similarity detected electrophoretically ranging from 0.56 to 0.88, except for the cv Malvasia del chianti and Malvasia lunga which were found to be identical. A comparatively high degree of genetic similarity was shown by the following cultivars pairs Monevasia-Athiri (0.88), Monevasia-Thrapsathiri (0.79), Ladikino- Thrapsa (0.85), Athiri -Thrapsathiri (0.79), Aidani- Malvasia del chianti and Malvasia lunga (0.85), Malvasia nera -Malvasia lazio (0.79), Malvasia lazio- Plyto (0.85) and Malvasia di Candia-Thrapsathiri (0.79). A comparatively low degree of genetic similarity was found between the cv Monevasia with all the cultivars of Malvasia group.

Introduction

Several ideas have been proposed (KRIMBAS 1943; LOGOTHETIS 1965) concerning the origin and the place of the first production of Malvasia wine. According to one proposal the Malvasia wine (vinum de Malvasia) was first made in Monevasia of Peloponnese, mainly by the use of the grape cultivar Monevasia and maybe other varieties as well as Trifera, Thrapsa, Glykerithra, Goustolidi etc. Another proposal consider as the place of the first production of Malvasia wine was the region of Malevisi in Grete by the use of the grape cultivars Athiri, Trapsathiri, Thrapsa, Ladikino, Tactas, Liatiko and Monevasia. Crete has been the main center of production of Malvasia wine since 14th century. This wine was exported from Rethimnon and Iraclion (Candia), both Cretan ports. KRIMBAS (1943) suggested that Malvasia wine originated from the famous wine 'Cretan Athyris' which was mentioned by Ptochoprodromos. A third proposal consider that Malvasia wine was first made in the island of Chios where the famous ancient wine 'Ariousios' was also produced.

The prevailing propose is that Malvasia wine was first produced in the Monevasia area at the beginning of the 13th century mainly from the grape cultivars Monevasia Trifera, Thrapsa etc. Later Venetians brought either the method of vinification and/or the grape cultivars to Crete where they kept the name of Malvasia (or other names such as Monevasie, Malevasie, Malvesie, Malvoisie, Malvazia). For historical, economical and social reasons the Cretan Malvasia wine dominated for centuries the international markets.

Malvasia was a desert, aromatic wine produced mainly by white wine grape cultivars. The reddish or brown color was due to its boiling, which was a usual practice for transportation. Probably among the grape cultivars which were used were the white cultivars Monevasia, Athiri, Thrapsathiri, Trifera, Glykadi (Asproudi), Tactas and the red cultivars Thrapsa, Ladikino and Liatiko. All these cultivars (except cv Thrapsa) are characterized by a peculiar light aroma.

It is not exactly known when the import of the Greek grape cultivars in the countries of Western Europe started. Although in Greece there was and still is only one variety with the name Monevasia, in Western Europe a great number of cultivars with the general name Malvasia (or Malvazia, Malvatica, Malvoisie) are mentioned (ROVASENDA 1887; POULIAT 1888; MOLON 1906; VIALA et VERMOREL 1904). In this group there are cultivars, the name of which indicates a Greek origin such as Malvasia Candia (or M. di Candia, M. bianca di Candia), Malvasia nera di candia (or M.nera, Malvosie noire

musquee), Malvasia greca, malvasia di Zante, Malvasia aspri and the cultivars Malvasia bianca di Piemonte, M. lipari, M. lunga, M. del Cianti, M. aromatica, M. rojia, M. bianca di trani, M. candida etc. It is highly possible that most of the above cultivars originated from local cultivars which were renamed in Malvasia irrespective of whether they had the aromatic character or not.

The aim of this study was to identify and to discriminate 22 grape cultivars probably, involved in the production of the Malvasia wine and to determine their genetic similarities based on 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 (GRANDO at al 1995; MORENO at al 1995; BINIARI at al 1996; STAVRAKAKIS at al 1997; STAVRAKAKIS and BINIARI 1998)

Materials and methods

G r a p e v i n e m a t e r i a l : Fifteen Greek wine grape cultivars (*Vitis vinifera* L.) grown in different areas of Greece and seven grape cultivars of Malvasia group grown in Italy were chosen for genetic study, during the period 1999-2001 (Tab. 1).

D N A e x t r a c t i o n : 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-merca-ptoethanol, 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 added, 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 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 0.54 volume 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 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 μM each of dATP, dGTP, dCTP, dTTP, 50 ng primer and 1 unit of Taq DNA polymerase (Qiagen). Ten random decamer oligonoucleodides were used (Table 1) 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 44^{o}C, 2 min at 72^{O}C) followed by a 10 min at 72^{o}C for extension.

Gel electrophoresis: Aliquots of the RAPD products were analysed in 2% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate and 1mM EDTA, pH 8). After staining in ethidium bromide (1μg.l^{-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.3) was calculated using the NTSYS-pc package 1.8 developed by ROHLF (Exeter Software, New York, USA).

Results and Discussion

Ten single, arbitrary 10-mer oligonucleotide primers were used to amplify genomic DNA from 22 grape cultivars. Each primer provided at least 3 polymorphic bands. More than 125 reproducible polymorphic fragments were generated by this method. The primers OPF-05, OPM-01, OPM-18, 1225 and 1226 proved very useful in differentiating the cultivars studied as they generated many polymorphic DNA fragments (Table 2). Examples of RAPD patterns amplified with primers 1224, OPM-02 and OPM-18 are shown in Fig. 2 (a,b,c).

As expected, in general, there was genetic variation among the cultivars studied. It is interesting that Greek grape cultivars Thrapsa, Ladikino, Vilana, Glykerithra, Liatiko and Dafni were grouped in a single branch of the tree while Malvasia aromatica, Malvasia nera

and Malvasia istria were grouped in a different branch (Fig 1). The same holds for the cultivars Aidani, Malvasia del Chianti and Malvasia lunga. The identical patterns between the cultivars Malvasia del Chianti and Malvasia lunga suggested that, at least for the primers studied, these are synonyms. Another interesting finding was the comparatively lower degree of genetic similarity between the grape cultivar Monemvasia and the group of Malvasia cultivars which varies from 0.65 – 0.72, even though Malvasia comes from the name of the area of Monemvasia and suggested a common origin of these cultivars.

The relatively high genetic similarity between the cultivar Monemvasia and the cultivars Athiri, Thrapsathiri, Tryfera, Glykadi and Malvasia di candia (0.88, 0.79, 0.79, 0.75 and 0.72 respectively) may indicate that these cultivars originated from a common stock by natural hybridisation; most Greek grape cultivars are simple or multi-hybrids (LOUKAS et al 1982). There are several aspects concerning the geographic origin of these cultivars. In any case there is a differentiation between the initial geographic origin of these cultivars and their modern centres of cultivation. For example while cv Monemvasia was reported (LOGOTHETIS 1965) to have originated in the area of Southern Peoloponnese now-a-days the centre of cultivation is the island of Paros. The same holds for the cultivar Athiri. This ancient Greek variety which, was mainly cultivated in Crete since the 12th century (KRIMPAS 1943) today can be found in the Cyclades islands, Dodekannisos Island of Rhodes) and other viticultural areas in Greece, including Macedonian, while in Crete only a few tens of hectares were occupied by Athiri.

The group of Malvasia cultivars

Another interesting finding was the relatively high degree of genetic similarity detected electrophoretically between the cultivars of Malvasia group which varied from 0.60 (Malvazia di candia – Malvasia istria) to 1 (Malvasia del Chianti – Malvasia lunga). In contrast, in the Greek cultivars the values of genetic similarity (Tab 3) ranged from 0.85 for the most closely related cultivars (Aidani- Malvasia del Chianti – Malvasia lunga) to 0.56 for the most distant related ones (Glykadi -Malvasia istria).

Athiri and Malvasia di Candia grouped to a single branch of the tree (Fig 1) supporting the hypothesis (KRIMBAS 1943; LOGOTHETIS 1965; VLACHOS 1986) that the cv Malvasia di Candia and maybe other Malvasia cultivars of Western Europe could be of Cretan origin as Candia was the name given by the Venetians to the city of Heraklion. These

two cultivars have similarities in ampelographical and morphological characters (MOLON 1906; KRIMBAS 1943; LOGOTHETIS 1965).

The genetic diversity detected between the Malvasia cultivars in Western Europe and in the present study indicates that during the period 1500-1700 aC, a number of clones of Athiri and others varieties such as Thrapsathiri, Thrapsa, Tryfera, Aidani lefko, Glykerithra, Glykadi, Monevmasia, mainly from Crete and the Cyklades, were transported to Western Europe under the name Malvasia (or Malvazia, Monevasie, Malevasie, Malvesie, Malvoisie) where, as it usually happens, they were hybridised with local varieties. Cultivar Thrapsa was selected due to its exeptional capacity and productivity.

Table 1: Cultivars studied and berry color

Code	**Cultivars**	**Berry color**
1	**Athiri**	(W)
2	**Thrapsathri**	(W)
3	**Thrapsa**	(B)
4	**Aidani**	(W)
5	**Glykadi**	(W)
6	**Agrioglykadi**	(W)
7	**Glykerithra**	(W)
8	**Tryfera**	(W)
9	**Plyto**	(W)
10	**Liatiko**	(B)
11	**Dafni**	(W)
12	**Vidiano**	(W)
13	**Vilana**	(W)
14	**Ladikino**	(B)
15	**Monemvasia**	(W)
16	**Malvasia di candia**	(W)
17	**Malvasia chianti**	(W)
18	**Malvasia lunga**	(W)
19	**Malvasia aromatica**	(W)
20	**Malvasia istria**	(W)
21	**Malvasia lazio**	(W)

22	**Malvasia nera**	(B)

Table 2: Primers were used, nucleotide sequence and total number of fragments amplified

Primer's Code	Sequence 5'-3'	Number of bands
1224	CAGGCCCTTC	8
1225	AGGTGACCGT	11
1226	CGCAGGATGG	11
1227	GTGTGCCCCA	10
OPM-01	GTTGGTGGCT	14
OPM-02	ACAACGCCTC	9
OPM-05	GGGAACGTGT	3
OPM-18	CACCATCCGT	20
OPF-03	CCTGATCACC	7
OPF-05	CCGAATTCCC	14

Table 3: Genetic similarity among the grape cultivars studied.

	ATHIRI	THRAPSATHIRI	THRAPSA	AIDANI	GLYKADI	AGRIOGLYKADI	GLYKERITHRA	TRYFERA	PLYTO	LIATIKO	DAFNI	VIDIANO	VILANA	LADIKINO	MONEMVASIA	M.CANDIA	M.CHIANTI	M.LUNGA	M.AROMATICA	M.ISTRIA	M.LAZIO	M.NERA
ATHIRI	1,00																					
THRAPSATHIRI	0,79	1,00																				
THRAPSA	0,66	0,66	1,00																			
AIDANI	0,70	0,67	0,71	1,00																		
GLYKADI	0,70	0,75	0,62	0,70	1,00																	
AGRIOGLYKADI	0,65	0,67	0,59	0,67	0,68	1,00																
GLYKERITHRA	0,65	0,74	0,75	0,72	0,66	0,76	1,00															
TRYFERA	0,79	0,74	0,64	0,65	0,66	0,72	0,72	1,00														
PLYTO	0,72	0,72	0,75	0,74	0,68	0,74	0,76	0,72	1,00													
LIATIKO	0,68	0,75	0,69	0,68	0,60	0,73	0,80	0,71	0,73	1,00												
DAFNI	0,71	0,71	0,69	0,64	0,60	0,66	0,73	0,68	0,68	0,79	1,00											
VIDIANO	0,64	0,75	0,62	0,71	0,69	0,68	0,73	0,61	0,71	0,67	0,72	1,00										
VILANA	0,55	0,65	0,78	0,62	0,54	0,65	0,79	0,62	0,67	0,68	0,68	0,70	1,00									
LADIKINO	0,65	0,69	0,85	0,67	0,63	0,65	0,76	0,67	0,74	0,73	0,75	0,68	0,76	1,00								
MONEMVASIA	0,88	0,79	0,59	0,67	0,75	0,69	0,67	0,79	0,69	0,75	0,68	0,66	0,58	0,67	1,00							
M.CANDIA	0,67	0,79	0,70	0,62	0,75	0,65	0,72	0,67	0,67	0,75	0,68	0,73	0,67	0,69	0,72	1,00						
M.CHIANTI	0,66	0,66	0,72	0,85	0,69	0,63	0,68	0,63	0,68	0,74	0,69	0,69	0,66	0,73	0,66	0,73	1,00					
M.LUNGA	0,66	0,66	0,72	0,85	0,69	0,63	0,68	0,63	0,68	0,74	0,69	0,69	0,66	0,73	0,66	0,73	1,00	1,00				
M.AROMATICA	0,63	0,73	0,62	0,61	0,65	0,59	0,59	0,68	0,61	0,67	0,62	0,60	0,66	0,61	0,68	0,68	0,67	0,67	1,00			
M.ISTRIA	0,67	0,69	0,68	0,65	0,56	0,62	0,65	0,69	0,72	0,66	0,61	0,61	0,65	0,69	0,65	0,60	0,66	0,66	0,68	1,00		
M.LAZIO	0,70	0,73	0,69	0,71	0,72	0,75	0,75	0,73	0,85	0,76	0,65	0,69	0,66	0,68	0,70	0,73	0,72	0,72	0,69	0,75	1,00	
M.NERA	0,63	0,73	0,60	0,61	0,67	0,73	0,73	0,68	0,68	0,74	0,67	0,67	0,70	0,70	0,68	0,70	0,67	0,67	0,76	0,68	0,79	1,00

Fig. 1: Dendrogram showing the relationship among the grape cultivars studied.

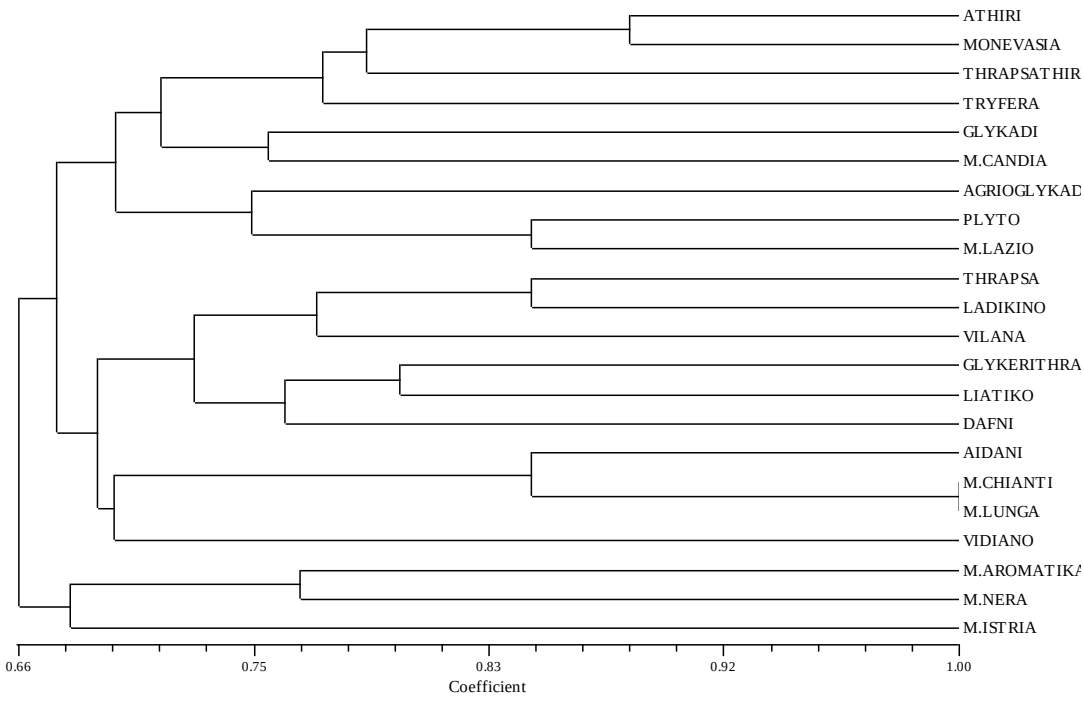
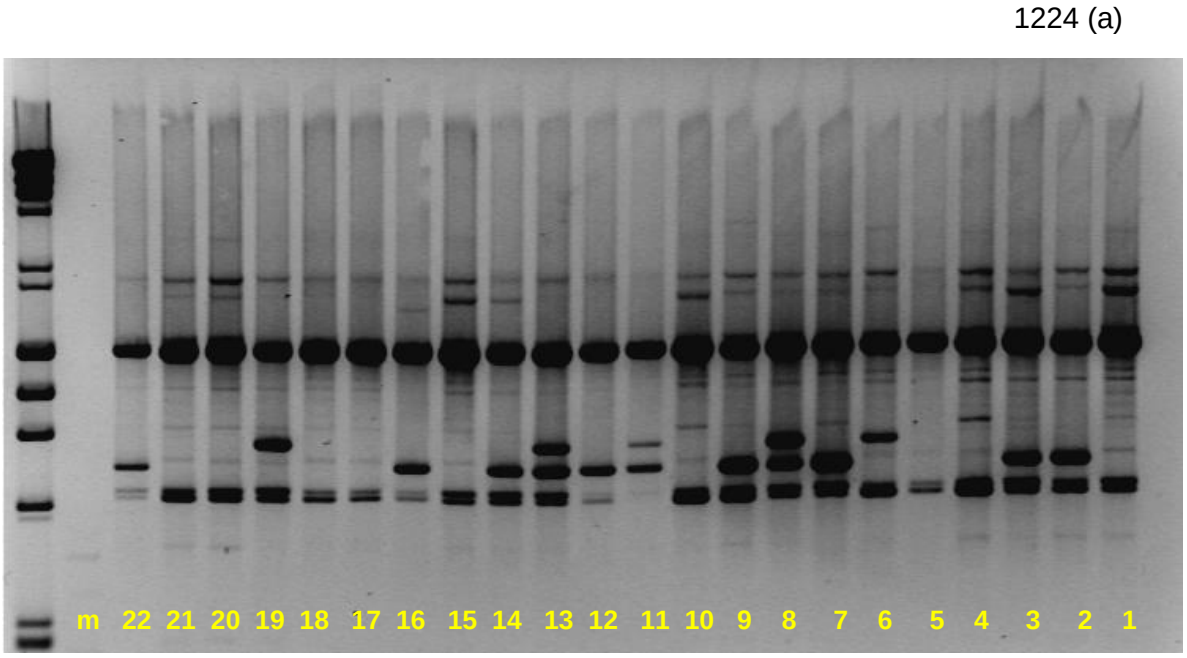
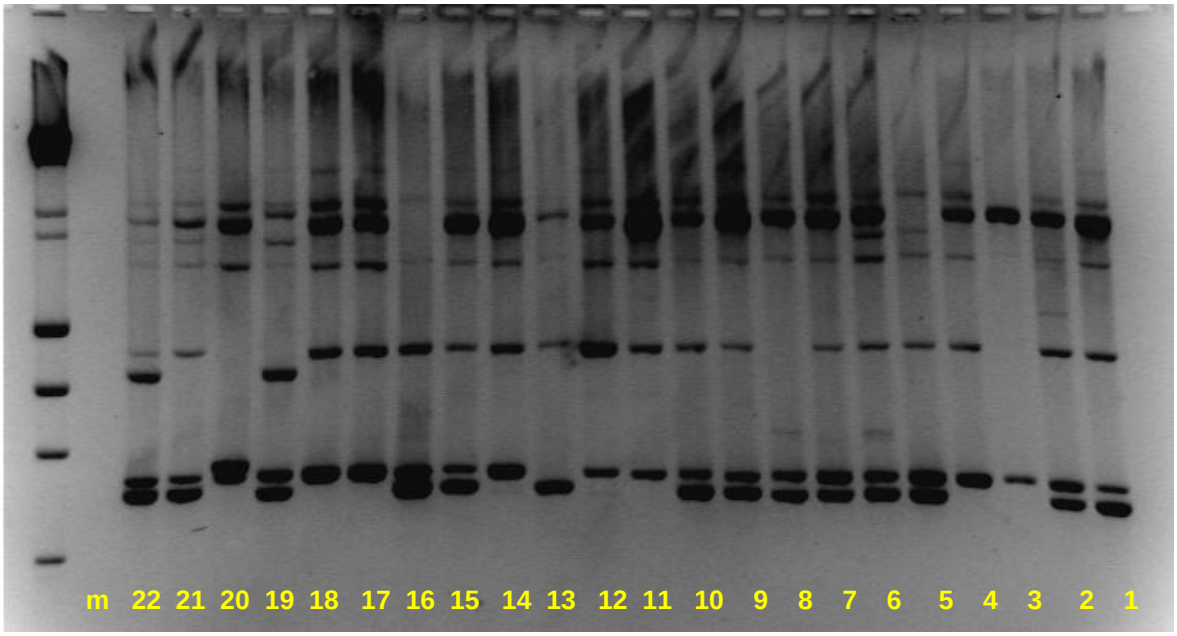


Fig. 2: Amplification patterns of polymorphic DNA from grape cultivars generated by primers 1224 (a), OPM-02 (b) and OPM-18 (c).



OPM-02 (b)



OPM-18 (c)