Territoires du vin
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Patrimoine et valorisation des territoires de la vigne et du vin

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Introduction

1 Viticulture in northern Greece is dated in the Neolithic era. Recent archaeological activities in northern Greece (Dikili Tash location at Philippi, Macedonia, Greece) unearthed charred pips and skins dated to 2400 BC, thereby establishing the region of eastern Macedonia as the oldest site in Europe for which there are strong indications for wine production and consumption (Valamoti *et al.*, 2007; Valamoti, 2015). The archaeological site is located within the greater Mount Pangaion area, which was one of the earliest places of worship of Dionysus, the ancient Greek god of grapes, winemaking, wine, festivity, and ritual madness (Wikipedia, 2016). In many occasions ancient

rituals are still met in modern customs (Visaltis, 2016) demonstrating the continuity of cultural elements through the passing millennia.

2 Ismarikos, Maronios, Mendaios, Acanthios, Skionios and Vivlinos were famous wines from the regions of ancient Macedonia and Thrace, whereas the Lemnios, Lesvios, and Thassios wines were from the respective islands of the northern Aegean (Τράιου, 1993). In all these occasions the name of each wine signified its place of origin, which is the forerunner of contemporary VQPRD practice: the wines are identified and marked with geographical names and appellations (Τράιου, 1993). The viticulture in Greece continued both in Byzantine times as well as during the Ottoman occupation (13th- 19th century). Ottoman tax records listed in viticulture and wine production at Kozani district (in 1528) and Thassos island (Καλινδέρη, 1974; Χιόνης, 2016, respectively). Wine from Thassos island was merchandised in Constantinople during the 16th and 17th century (Χιόνης, 2016). Over time wine production became the driving force of viticulture in northern Greece utilizing local grape varieties until the "phylloxera plague" came up early in the 20th century (Νικολάου, 2008) threatening to set an end on the long viticulture tradition. The use of American rootstocks, however, ensured grapevine cultivation.

3 During the 20th century, the combination of intensive production systems that prevailed in global agriculture supporting the cultivation of a limited number of improved and highly productive international varieties or hybrids, and also a strategic turn in favour to tourism and urban development rather than agricultural production, resulted in dramatic reduction or even disappearance of a large number of local cultivars from current farming systems (Σαμαράς & Ματθαίου, 2006; Hammer & Teklu, 2008 and references therein).

4 In recent years, modern family based wineries have been established aiming to revive local viticulture to the contemporary standards and qualities partly relying on the cultivation of the numerous local varieties. This trend reflects the intention to invest in the plant genetic legacy that represents the long standing wine tradition, aiming to produce innovative products. Local varieties have excellent adaptation to the soil and climatic conditions of the local microenvironment, thus support environmentally friendly, low-input agricultural practices that contribute to the sustainability of natural resources,

while they deliver optimum quality results comparable to the international varieties (Σταυρακάκης, 2003).

5 In the recent past, studies on morphological and molecular characterization of the local varieties have been performed leading to the development of the Greek *Vitis* Database (Lefort and Roubelakis, 2001; http://gvd.biology.uoc.gr/gvd/). The current study aims to assess genetic diversity of wine producing local varieties of northern Greece. Genetic relationships were assessed by the application of 10 SSR markers, including the six highly polymorphic SSRs molecular markers that have been suggested as molecular descriptors by the OIV (OIV, 2009). The main outcome of this is the construction of a genetic database, which will constitute the comparative framework that can be used to authenticate the local grapevine varieties and contribute to further development of the wine industry, while the use of the OIV set of molecular markers enables comparative studies with international varieties. Moreover, the results could provide knowledge and valuable tools for future breeding activities.

Materials and methods

6 Young leaves were collected at the beginning of the growing season and were stored at -80^{0}C. Later, isolation of genomic DNA was performed using the 'NucleoSpin Plant II' kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. DNA concentration of all isolates was measured using a NanoDrop-1000 (Thermo Scientific, Wilmington, DE, USA) spectrophotometer at 260 and 280 nm ultraviolet lengths, whereas the integrity of all DNA samples was estimated by gel electrophoresis in 0.8 % agarose gels. Samples were then diluted to 20 ng/μl working concentration.

7 Polymerase Chain Reactions (PCRs) were performed in a volume of 20 μL including 30 ng genomic DNA, 200 mM dNTPs, 40 pmol primers, 2 μL 10X KAPATaq DNA Polymerase buffer, and 1 U KAPATaq DNA Polymerase (KapaBiosystems, Cape Town, South Africa). The following 10 pairs of primers were used: VVS2, VrZAG62, VrZAG67, VrSZAG79, VVMD5, VVMD7, VVMD27, VVMD28, VVMD32, and VVMD25. Forward primers were 5'-end fluorescently labeled either with FAM, HEX, ROX or TAMRA. PCR amplifications were performed in a MasterCycler (Eppendorf, Hamburg, Germany) as follows: an initial step of 5

min at 95°C, followed by 35 cycles, each one including 15 s at 95°C for denaturation, 15 s at 52° to 56°C (depending on the primer) for annealing, and 10 s at 72°C for elongation; a 5 min step at 72°C was programmed as a final extension. PCR fragments were separated using capillary electrophoresis in a 3730xl DNA Analyzer (Applied Biosystems, USA). Data analysis, sizing and genotyping were performed using the GeneMapper (version 4.0) software. The comparative study of the results was performed in the GenAlex platform. Dendrogram was constructed using the MEGA4 program.

Results and discussion

8 Genotyping of the Greek local varieties is of crucial importance for the Greek wine industry because it will provide the basis of the study of the local varieties backing up their oenological potential and the production of healthy plant material that could be used for the establishment of new vineyards contributing to agricultural development. Further, genotyping the Greek vineyard could be used for authentication or identification of varieties that are currently in cultivation in the country. It is estimated that there are about 300 local Greek varieties. A large number of them, including red, white, table and wine producing varieties, as well as varieties for raisin production, are conserved and evaluated in the *ex-situ* ampelographic collection of the GGB. Most of these varieties have been described ampelographically (Stanpakas, 2011).

9 In the current study, a total of 53 varieties has been analysed with the use of 10 SSRs: samples from 12 varieties were collected from the GGB (indicated with a single asterisk in Table 1 and in Figure 1), 11 were generously donated from Dr Erica Maul of Julius Kühn-Institut (indicated with two asterisks in Table 1 and in Figure 1), whereas the remaining 30 samples were collected from different regions of northern Greece in order to identify them molecularly- designation of the latter ones is based almost exclusively on the empirical ampelographic capability of the donor grapevine grower.

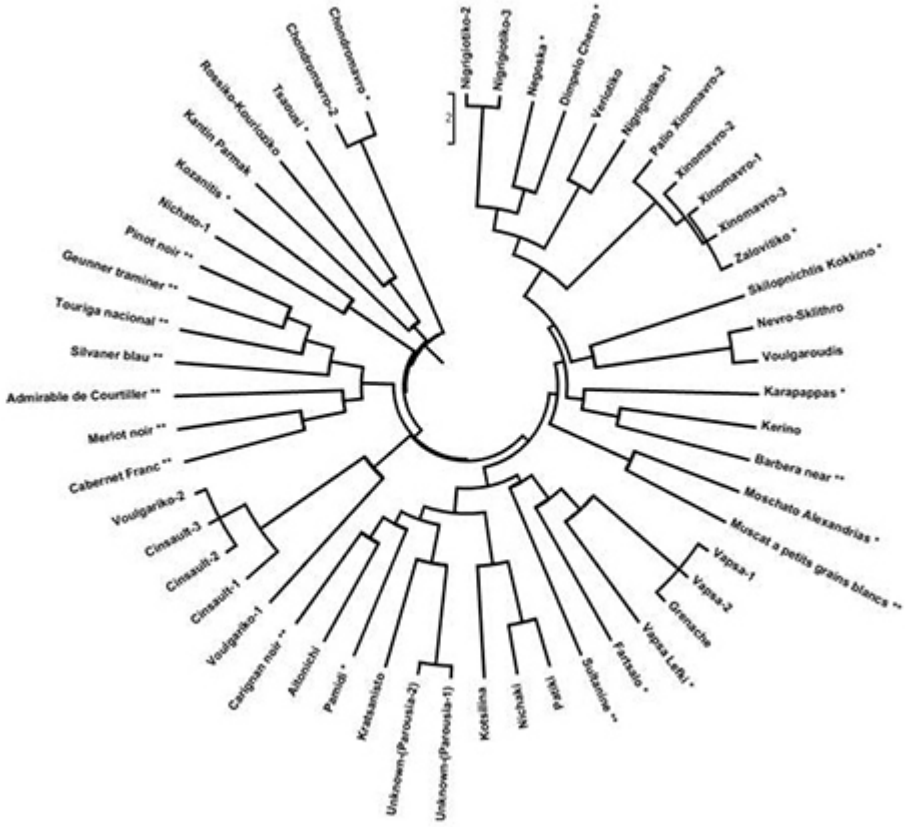
Table 1: names and collection areas of the 53 grapevine varieties analysed in the current study

	Variety name	Collection area		Variety name	Collection area
1)	Cinsault-1	Western Macedonia	28)	Nigrigiotiko-3	Western Macedonia
2)	Palio Xinomavro-2	Western Macedonia	29)	Voulgaroudis	Thrace
3)	Grenache	Western Macedonia	30)	Nichaki	Thrace
4)	Veriotiko	Western Macedonia	31)	Negoska *	Greek Gene Bank (GGB)
5)	Kerino	Central Macedonia	32)	Pamidi *	Greek Gene Bank (GGB)
6)	Vapsa-1	Central Macedonia	33)	Fartsalo *	Greek Gene Bank (GGB)
7)	Voulgariko-1	Central Macedonia	34)	Zalovitiko *	Greek Gene Bank (GGB)
8)	Kratsanisto	Central Macedonia	35)	Karapappas *	Greek Gene Bank (GGB)
9)	Cinsault-2	Central Macedonia	36)	Skilopnichtis Kokkino *	Greek Gene Bank (GGB)
10)	Vapsa-2	Central Macedonia	37)	Chondromavro *	Greek Gene Bank (GGB)
11)	Kotsilina	Ithaka island	38)	Moschato Alexandrias *	Greek Gene Bank (GGB)
12)	Aitonichi	Ithaka island	39)	Tsaousi *	Greek Gene Bank (GGB)
13)	Cinsault-3	Thrace	40)	Vapsa Lefki *	Greek Gene Bank (GGB)
14)	Kantin Parmak	Thrace	41)	Dimpelo Cherno *	Greek Gene Bank (GGB)
15)	Rossiko-Kourioziko	Eastern Macedonia	42)	Kozanitis *	Greek Gene Bank (GGB)
16)	Unknown-Parousia-1	Eastern Macedonia	43)	Admirable de Courtiller **	Julius Kühn-Institut
17)	Unknown-Parousia-2	Eastern Macedonia	44)	Barbera near **	Julius Kühn-Institut
18)	Patiki	Eastern Macedonia	45)	Cabernet Franc **	Julius Kühn-Institut
19)	Xinomavro-1	Western Macedonia	46)	Carignan noir **	Julius Kühn-Institut
20)	Nigrigiotiko-1	Western Macedonia	47)	Geunner traminer **	Julius Kühn-Institut
21)	Voulgariko-2	Western Macedonia	48)	Merlot noir **	Julius Kühn-Institut
22)	Chondromavro-2	Western Macedonia	49)	Muscat a petits grains blancs **	Julius Kühn-Institut

23)	Xinomavro-2	Western Macedonia	50)	Pinot noir **	Julius Kühn-Institut
24)	Nevro-Sklithro	Western Macedonia	51)	Silvaner blau **	Julius Kühn-Institut
25)	Nichato-1	Western Macedonia	52)	Sultanine **	Julius Kühn-Institut
26)	Xinomavro-3	Western Macedonia	53)	Touriga nacional **	Julius Kühn-Institut
27)	Nigrigiotiko-2	Western Macedonia		---------- ----------	---------- ----------

10 Varieties with one asterisk (*) are maintained in the GGB (Greek Gene Bank); varieties with two asterisks (**) have been donated from the Julius Kühn-Institut; varieties with no asterisk have been collected from various areas of northern Greece.

Figure 1 : dendrogram based on 10 SSRs showing the genetic relationship among the 53 grapevine varieties analysed in the current study



11 Varieties with one asterisk (*) are maintained in the GGB; varieties with two asterisks (**) have been donated from the Julius Kühn-

Institut; varieties with no asterisk have been collected from various areas of northern Greece.

12 Studying the constructed dedrogram, the following genetic relationships are revealed:

1. Variety ‘Chondromavro’-2, which was collected from western Macedonia, has been grouped in the clade with the variety ‘Chondromavro’ maintained in the GGB. This demonstrates the importance to possess a genetic database that could be used to identify by comparison the varieties possessing high genetic relationship to the varieties maintained in the ampelographic collections.
2. Variety ‘Nevro’ (or ‘Sklithro’) collected from the Pelekanos region of the Kozani district (western Macedonia) has been grouped with the ‘Voulgaroudis’ variety that has been collected from the Soufli area (Thrace), revealing a case of synonymy (various designations attributed to a single genotype). Further analysis with more SSRs is needed in this occasion.
3. A potential case of homonymy (the same designation attributed to different genotypes) was also revealed: ‘Nigrigiotiko’-1 has been grouped in the same clade with ‘Veriotiko’, whereas ‘Nigrigiotiko’-2 and ‘Nigrigiotiko’-3 have been grouped in a different clade.
4. ‘Xinomavro’ related varieties have been grouped in the same clade with ‘Zalovitiko’ confirming the ampelographic closeness between them.
5. Three ‘Cinsault’ samples (‘Cinsault’-1, -2, -3) have been grouped in the same clade with the ‘Voulgariko’-2 variety.
6. Two Unknown varieties from the Parousia Estate (‘Unknown-Parousia’-1 and ‘Unknown-Parousia’-2) that had been collected from the slopes of Mount Pangaion (eastern Macedonia) have been found to be loosely related to the ‘Kratsanisto’ that has been collected from the Livadi area on the western slopes of Mount Olympus (southern parts of central Macedonia). Since ‘Kratsanisto’ is a novel designation, further study is needed.
7. Interestingly, the majority (7/11) of the international varieties that were used as references, have been grouped together in one clade distinctively separated from the Greek local varieties.

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English

Grapevine cultivation and wine production occur in Greece since antiquity. Modern wineries have focused on the cultivation of international varieties; however, a marked trend to the revival of the local grapevine varieties has been noted in the last two decades underlying the confidence and the intention to invest on the local plant resources aiming to produce unique products. This brings up the urgent necessity to genetically identify the local varieties that constitute the Greek vineyard so as they will be available for further development. Simple-Sequence Repeats (SSRs) molecular markers, including those proposed by the International Organization of Vine and Wine (OIV) as molecular descriptors, have been used to genotype: i) 12 wine producing varieties maintained in the *ex-situ* ampelographic collection of the Greek Gene Bank (GGB), ii) 30 varieties that have been collected from various sites of northern Greece in order to verify and confirm their identity, and iii) 11 international varieties that have been used as references. The final dendrogram displays the genetic closeness between the analysed varieties, bringing up cases of synonymies and homonymies.

Mots-clés
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Georgios Merkouropoulos
Institute of Plant Breeding and Genetic Resources, HAO-DEMETER, Greece

Photini Mylona
Institute of Plant Breeding and Genetic Resources, HAO-DEMETER, Greece