



Project title:

**«Developing Identity ONYield, SOil and Site
– DIONYSOS»**

INTERREG V-A COOPERATION PROGRAMME: GREECE –BULGARIA 2014-2020"



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‘Selecting main local wine varieties and identifying their characteristics’

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DIONYSOS

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Introduction

The peculiarity of the geographical landscape of Greece led to the creation of isolated microenvironments, which contributed decisively to the appearance of a large number of indigenous grapevine varieties. Genetic identification of the grapevine varieties is an indispensable national priority in order to support and develop the wine sector. In the present work, different grapevine genotypes have been collected from the wider area of Soufli. The issue of synonymy and homonymy (This et al., 2004) may occur in this occasion. In order to accurately determine their identity it is necessary to use molecular markers, in order to be identified, while the technological characteristics of the wines produced by them, have been studied, leading to a preliminary evaluation.

The cultivation of the grapevine plants in ancient Greece was particularly widespread, not only in Athens and the Peloponnese, but on the islands, in Macedonia and Thrace as well. Especially in the region of east Macedonia/ Thrace, wine has a long history.

Nowadays the Region of Eastern Macedonia and Thrace is the smallest wine-growing region of Greece. The cultivated vineyards of the region correspond to the 2% of the total vineyard cultivation in Greece (Hellenic Statistical Authority, 2016). In addition, until recently viticulture has not been the main pillar for the local economy, with the result that wine production is limited and it is consumed mainly in a local level.

Since the 1950s, a small number of vineyards in the wider region have been eroded, as they did not have any economic interest. The process of vineyard erosion was stopped in the early 1990's, with the abandonment of tobacco cultivation, so farmers switched to the cultivation of the grapevine plant again.

The aim of this study is not to prove that some regions of the East Macedonia and Thrace have the ability to produce wine, but given the ease of grapevine plant for adaptation, wine can be produced even in the most adverse conditions. For example, in the most diverse microclimates wines could be produced with low yields, with superior quality features, thus they should be recognized and rewarded.

In conclusion, there is a need at central level and at the level of local government to build a strategy in order to make wines of high quality, competitive products.

Material and Methods

Study Area: The Terrain of the East Macedonia/ Thrace region is mostly mountainous, with mountain ranges extended into the sea. The territory is characterized by elongated mountainous ranges, plains to the coasts and large rivers flowing through the area. The climate is different from the rest of Greece, because of the north and northwest winds during winter. Moreover, the temperature goes down low in the mountain regions, the rain level is higher than the rest of the greek mainland, while the snow is much more and does not melt before mid-spring. In coastal areas, the climate is clearly milder, typically temperate with Mediterranean features, with warm and dry summer and during winter there are several rains.

Plant material: A total of 34 samples (Table 2) were collected from equal number of representative plants from Bellas vineyard (Alania area, Soufli) (Figure 1) and from the Traditional vineyard of "Stafilos" at the wider area of Soufli (Table 1) (Figure 2), Greece, reflecting the varietal diversity. It is important to mention that the samples were collected on the basis of the empirical identification. The collection of plant material took place on Friday 13th of July, 2018.

It should be mention that with the collaborating department of Hellenic Agricultural Organisation "Demeter" (HAO), leaf samples were collected from the Greek National Ampelographic Collection, and served as references for the comparison of the material from the wider area of Soufli. The Collection occupies about 70 acres at the Lykovrysi district in Attica, Greece, maintaining over 500 autochthonous accessions and representing the oldest and most important genetic grapevine reservoir of the country. The ampelographic and molecular characterization of the Collection is under way.

Row 1	Row 2	Row 3	Row 4	Row 5	Row 6	Row 7	Row 8	Row 9
Kiratsouda	Kiratsouda	Papadis	Ligaridia	Ligaridia	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Kiratsouda	Kiratsouda	Papadis	Ligaridia	Ligaridia	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Kiratsouda	Kiratsouda	Papadis	Ligaridia	Ligaridia	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Kiratsouda	Kiratsouda	Boulgaroudi	Ligaridia	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Kiratsouda	Kiratsouda	Boulgaroudi	Ligaridia	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Kiratsouda	Kiratsouda	Boulgaroudi	Ligaridia	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Nichato	Red Chavouzalís	Boulgaroudi	Ligaridia	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	LeukaFeggia	LeukaFeggia	AlponouraLeuki	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	LeukaFeggia	White Patiki	AlponouraLeuki	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	LeukaFeggia	White Patiki	Kolokithis	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	LeukaFeggia	White Patiki	Kolokithis	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	Sefka	White Patiki	Kolokithis	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	Sefka	White Patiki	Kolokithis	
Nichato	Red Chavouzalís	Boulgaroudi	Karnachalas	Bogiatjides	Sefka	White Patiki	Kolokithis	
Nichato	Korifia Rose	Boulgaroudi	Karnachalas	Bogiatjides	Sefka	White Patiki	Kolokithis	Siasla
Nichato	Korifia Rose	Boulgaroudi	Karnachalas	Bogiatjides	Sefka	White Patiki	Kolokithis	Siasla
Nichato	Korifia Rose	Boulgaroudi	Karnachalas	Bogiatjides	Sefka	White Patiki	Kolokithis	Siasla
Nichato	Korifia Rose	Boulgaroudi	3 Black Berries	Bogiatjides	Sefka	White Patiki	Kolokithis	Siasla
Nichato	Korifia Rose	Boulgaroudi	3 Black Berries	Bogiatjides	Sefka	White Patiki	kolokithis	Siasla
ROSE	Korifia Rose	Boulgaroudi	3 Black Berries	Bogiatjides	Sefka	White Patiki	Tsougianides 2	Siasla
	Korifia Rose	Boulgaroudi	3 Black Berries	Bogiatjides	Sefka	White Patiki	Tsougianides 2	Siasla
	Korifia Rose	Boulgaroudi	3 Black Berries	Bogiatjides	Sefka	Tsougianides 1	Tsougianides 2	Siasla
	Korifia Rose	Boulgaroudi	3 Black Berries	Bogiatjides	Sefka	Tsougianides 1	Tsougianides 2	Siasla
				Bogiatjides	Sefka	Tsougianides 1	Tsougianides 2	Savvatiano
				Bogiatjides	Sefka	Tsougianides 1	Tsougianides 2	Savvatiano
						Tsougianides 1	Tsougianides 2	Savvatiano
						Tsougianides 1	Tsougianides 2	Savvatiano
						Tsougianides 1	Tsougianides 1	Savvatiano
						Tsougianides 1	Tsougianides 1	Savvatiano

Table 1: Lay out of the traditional vineyard “Stafilos” at the area of Soufli

Bag number	Variety	Num. of Samples	Row	Num. of Plant	Vineyard
1	Karnachalas	1	F17	P3	Bellas Vineyard
2	Karnachalas	2	F20	P2	Bellas Vineyard
3	Karnachalas	3	F21	P4	Bellas Vineyard
4	Mpougialamas	1	F8	P13	Bellas Vineyard
5	Mpougialamas	2	F8	P20	Bellas Vineyard
6	Mpougialamas	3	F8	P22	Bellas Vineyard
7	Nichato	1	F1	P5	Stafilos Vineyard
8	Nichato	2	F1	P7	Stafilos Vineyard
9	Kiratsouda	1	F1	P17	Stafilos Vineyard
10	Chavouzalis Red	1	F2	P9	Stafilos Vineyard
11	Korifia Rose	1	F2	P4	Stafilos Vineyard
12	Korifia Rose	2	F2	P5	Stafilos Vineyard
13	Voulgaroudi	1	F3	P10	Stafilos Vineyard
14	Boulgaroudi	2	F3	P12	Stafilos Vineyard
15	Pappadis	1	F3	P19	Stafilos Vineyard
16	Pappadis	2	F3	P20	Stafilos Vineyard
17	Ligaridia	1	F4	P3	Stafilos Vineyard
18	Ligaridia	2	F4	P5	Stafilos Vineyard
19	3 Black Berries	1	F4	P5	Stafilos Vineyard
20	3 Black Berries	2	F4	P1	Stafilos Vineyard
21	Bogiatjides	1	F5	P5	Stafilos Vineyard
22	Bogiatjides	2	F5	P8	Stafilos Vineyard
23	Sefka	1	F6	P6	Stafilos Vineyard
24	Sefka	2	F6	P9	Stafilos Vineyard
25	LeukaFeggia	1	F6	P21	Stafilos Vineyard
26	LeukaFeggia	2	F6	P22	Stafilos Vineyard
27	Tsougianides	1	F7	P1	Stafilos Vineyard
28	White Patiki		F7	P10	Stafilos Vineyard
29	White Patiki		F7	P11	Stafilos Vineyard
30	Tsougianides 2	1	F8	P1	Stafilos Vineyard
31	Kolokithis	1	F8	P8	Stafilos Vineyard
32	Kolokithis	2	F8	P9	Stafilos Vineyard
33	AlponouraLeuki	1	F8	P5	Stafilos Vineyard
34	AlponouraLeuki	2	F8	P6	Stafilos Vineyard

Table 2: Leaf Samples for DNA extraction

Sampling and processing of plant material

Leaf Sampling

Selected Greek, from the wider area of Soufli, indigenous and traditional grapevine varieties were analyzed. The collection of appropriate plant material were carried out in the wine-producing regions of the study of East Macedonia/ Thrace, therefore the results will reflect the diversity of both Greek vines in terms of varieties. The selection of the vineyards included to the research was carried out and thereafter the selection of vivid, healthy plants of the varieties (Figure 4).

In the present study, in order to find the suitable leaf type for the varieties of interest, 10 healthy leaves were collected from each grapevine plant. The leaf sampling took place early in the morning. The plant material was young leaves and placed at -20°C in polystyrene boxes and moved to the Agricultural university of Athens at the Oenology Laboratory.



Figure 1: Leaf samples from Bellas vineyards, (Bougialamas leaves- on the left, Karnachalas leaves- on the right)



Figure 2: Leaf samples collected from the Stafilos vineyards (AlponouraLeuki leaf samples)

Berry Sampling

Collection of the appropriate kilograms of grapes per different variety took place at the suitable technological maturity of the berries of each variety (1-2 baskets for micro-vinification). In the parcels the berry samples were collected randomly, always covering the whole vineyard. Grape berries were collected from the vine plants of the main shoots from different positions (shaded or unshaded) and from different positions of each grape bunch (top, middle, base).

At the end of sampling all baskets with the grape berry samples were shipped to the Laboratory of Oenology at the Agricultural University of Athens.



Figure 3: Labeling the grapevine plants, Stafilos vineyard



Figure 4: Selection of healthy grapevine plants, Stafilos vineyard

Genetic Analysis: Genomic DNA was isolated from young leaves using commercially available kits following manufacturer recommendations. Quality and integrity of the DNA was evaluated by electrophoresis in agarose gels (Figure 5) whereas its concentration was estimated by the use of a NanoDrop 2000 Spectrophotometer (Thermo-Fisher Scientific) (Figure 6).

The Polymerase Chain Reactions (PCRs) that were carried out using a set of seven microsatellites (VVS2, VVMD5, VVMD7, VVMD25, VVMD28, VvZAG67, and VvZAG79) as well as the subsequent analysis and the construction of dendrograms, followed the description of Merkouropoulos *et al.* (2015). It is worth to mention that four of the microsatellites that were used in this study, have already been incorporated in the catalogue published by the Organisation Internationale de la Vigne et du Vin (OIV) as molecular descriptors (OIV, 2009).

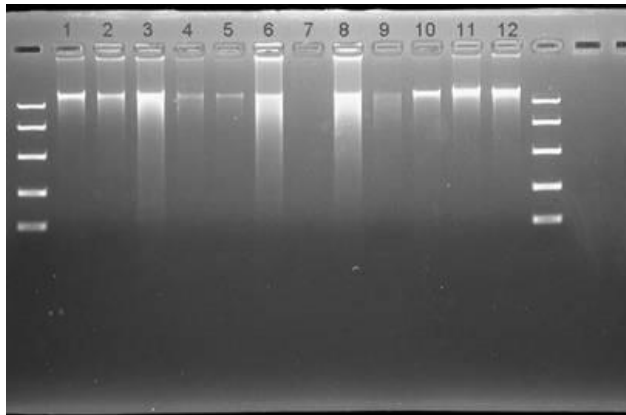


Figure 5: DNA evaluation by electrophoresis in agarose gel (position/ well 1- DNA Karnachalas, well 2- Bougialamas, well 3- Pappadis, well 4- Bogiatjides, well 5- Ligaridia, well 6- Ligaridia, well 8- White Patiki, well 9- Kiratsouda, well 10- Sefka, well 11- LeukaFeggia, well 12- marker DNA)



Figure 6: NanoDrop 2000 Spectrophotometer (Thermo-Fisher Scientific)



Figure 7: PCR thermocycler, Bio-Rad

Vinification process

White varieties: White Patiki

The grapes from the white variety Patiki, was hand harvested during early hours and they were then transferred to the experimental winery of the Agricultural University of Athens where they were put in the fridge during the night. In addition, 200 berries were frozen at -20°C for further analyses (precursor aromatic compounds). The following morning, they were destemmed, crashed and pressed. Potassium metabisulfite (7g/hL) was added to the juice and two hours later pectolytic enzymes were added as well. The juice was then chilled to 4° C and 24 hours later it was racked to 5L capacity plastic bottles. At this stage, the sugar content (Brix) of the juice was determined and also classical analyses were performed (pH and total acidity) and YAN (OIV). The must was then inoculated and the alcoholic fermentation began. A couple of days later, additions of yeast nutrients (Springferm) was made. Brix measurements were performed daily.

When the alcoholic fermentation was successfully completed, the wine was racked, chilled to 4°C and 8g/hL of Potassium Metabisulfite was added. The wines were bottled and kept to a constant temperature of 10°C.

Classical analyses (Brix, pH, Total Acidity, Volatile Acidity, % Alcoholic Content, Reducing Sugars), the determination of Total YAN (according the OIV guidelines) was carried upon the completion of the alcoholic fermentation. The browning rate was also determined (Singleton and Kramling, 1976; Sioumis, *et al.*, 2006) and the chromatic characteristics by measuring the absorbance at 420nm as well (OIV).

Red Varieties: Karnachalas, Bougialamas, Voulgaridia, Ligaridia, Bogiatzides, Papadis

The grapes from each red variety were hand harvested during early hours and they were then transferred to the experimental winery of the Agricultural University of Athens where they were put in the fridge during the night in separate bins. In addition, 200 berries were frozen at -20°C for further analyses (Iland, *et al.*, 2004). The following morning, they were destemmed, crashed and cold pre-fermentation maceration for 24hours was performed at 5°C. An addition of Potassium metabisulfite (7g/hL) was done. At this stage, the sugar content (Brix) was determined and classical analyses were performed (pH and total acidity), the chromatic characteristics and YAN (OIV). They were then inoculated and the alcoholic fermentation began. Three times a day there was the break of the cap in order to submerge the skins. Additions of yeast nutrients (Springferm) was made. Brix measurement was performed daily. Six days after the end of alcoholic fermentation and when the color intensity started to be stable, the skins were separated from the must and were pressed. After the completion of the alcoholic fermentation, the malolactic fermentation occurred and as soon as it was completed, the wines were racked and an addition of 8g/hL of Potassium Metabisulfite was made. The wines were bottled and kept at room temperature.

Classical analyses (Brix, pH, Total Acidity, Volatile Acidity, % Alcoholic Content, Reducing Sugars), the determination of Malic acid Total YAN and the chromatic characteristics (according the OIV guidelines) were carried upon the completion of the alcoholic and malolactic fermentation.

In addition, the following analyses were carried in the finished wines:

- Chemical age, Anthocyanes' Ionization Degree %, Total Anthocyanes, Chromatic characteristics, Total phenolics, Colour components in the presence of SO₂ (Somers et al, 1974, 1977)
- Tannins determination – BSA (Harbertson et al, 2002)
- Tannins determination – MCP (Sarneckis et al, 2006; AWRI, 2009)
- Total phenolics Folin-Ciocalteu (Waterman & Mole, 1994)

In both red and white wines, the following analyses are in the process of determination:

- Organic acids
- Anthocyanes using HPLC (Kallithraka S., et al., 2005)
- Volatile compounds using GC-MS

In both red and white grape varieties, the following analyses will be performed:

- Anthocyanes and total phenolics (Iland P., et al., 2004)
- Extractability of anthocyanins and tannins and the phenolic potential – Glories (Ribéreau-Gayon, P., et al., 2000)

Moreover, spontaneous vinifications took place, of the red grapevine varieties Bougialamas and Karnachalas, in smaller plastic bottles in order to isolate the indigenous yeasts.



Figure 8: Baskets of Bougialamas grapevine berries



Figure 9: Destemmed berries by hand



Figure 10: Destemmetionprocess of the Bougialamas grape berries



Figure 11: Pressing process of the skins after separating the skins from the must

Sensory analysis

The Sensory Analysis Tests of wines produced from the grapevine varieties coming from Thrace took place in the laboratory of Oenology at the Agricultural University of Athens. The Sensory session took place in the morning (from 11:00 a.m. to 15:00 p.m.). The samples were presented to the panel blind and coded. The panel was comprised of people recruited from members of the laboratory or external tasting specialists. In order to measure the traceability, each panel member wrote down his results on a tasting sheet (Table 3) containing the date and the assessors identification.

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Figure 12: Performing Sensory analysis Tests for the red wines

Name					
Samples					
Visual evaluation					
Color intensity (lower- medium- higher) Scale from 1 to 10					
Hue (lower- medium- higher) Scale from 1 to 10					
Olfactor evaluation					
Smell intensity (lower- medium- higher) Scale from 1 to 10					
Red Fruits (lower- medium- higher) Scale from 1 to 10					
Veggie aromas (lower- medium- higher) Scale from 1 to 10					
Spiciness (lower- medium- higher) Scale from 1 to 10					
Taste Evaluation					
Taste Balance (lower- medium- higher) Scale from 1 to 10					
Bitterness (lower- medium- higher) Scale from 1 to 10					
Astringency (lower- medium- higher) Scale from 1 to 10					
Acidity (lower- medium- higher) Scale from 1 to 10					

Table 3: Sensory sheet for the red wines

Results

Oenological evaluation

The oenological evaluation of the seven grapevine varieties has been conducted by carrying out the basic wine parameters, such as pH, alcoholic title, total acidity, volatile acidity according to the Compendium of International Methods of Wine and Musts (2018). (Table 4)

A total of 34 samples were collected from representative plants located at Bellas vineyard (Alania area, Soufli) and the Traditional vineyard of "Stafilos" in the wider area of Soufli, Greece, reflecting the varietal diversity.

However, only seven varieties, of which 6 red Karnachalas, Bougialamas, Voulgaroudi, Ligaridi, Bogiatjides, Pappadis and one white White Patiki, were vinified at the experimental winery of the Oenology Lab of Agricultural University of Athens. The preliminary results indicated that Karachalas and Pappadis wines scored the highest alcoholic strength of 13,5 and 13,6 respectively. Although, the highest Total Acidity was measured in Voulgaroudi and Bogiatjides wine (Table 4).

Furthermore, it is to be noted that Bougialamas wine is characterized by high concentration of Total Anthocyanins, lower value to that of Ligaridia wine but still higher than the other wines, while the lower value was scored by Bogiatjides wine (Table 13). As well as, Bougialamas was found to have the largest concentration of total phenolics among all the wines (Table 14). Moreover, according to the two methods for tannin determination, Methyl cellulose precipitable (MCP) (Sarneckis et al., 2009) and Bovine serum albumin (BSA) (Habertson et al., 2002) Bougialamas (1096 mg/L (MCP) and 246 mg/L (BSA)), Voulgaroudi (1142 mg/L (MCP), 104 mg/L (BSA) and Ligaridia (959 mg/L (MCP) and 124 mg/L (BSA)) showed the highest tannin concentration (Figure 15 and 16).

In addition, the higher value of color intensity was found to Ligaridia with 16.98, while the lowest among all, was found to Voulgaroudi wine with 0.64.

Finally, the white wine produced from White Patiki variety is characterized by a good value of Total acidity, and with a decent alcohol strength (Table 4). According to the browning test (Sioumis et al., 2006) the wine presented a low rate alteration factor $k = 0.0058$, consequently it is not prompt to oxidation (Table 19). Practically, this wine would not develop brown color easily.

Variety	pH	Tot. Acid. (Tartaric acid g/L)	Vol. Acid. (Acetic acid g/L)	Alcohol ethanol (%)	Res. Sugars (g/ L)	Color intensity	Hue
Karnachalas	3.589	5.32	0.402	13.5	1.79	1.72	1.05
Bougialamas	3.888	5.17	0.36	11.2	2.32	14.18	0.73
Voulgaroudi	3.341	6.07	0.468	12.3	1.46	0.64	1.51
Ligaridia	3.870	4.95	0.681	12	2.92	16.98	1.28
Bogiatjides	3.663	5.32	0.213	12.3	1.63	3.60	0.82
Pappadis	3.870	4.95	0.426	13.6	1.83	3.47	0.78
White Patiki	3.539	3.83	0.468	12	0.79	-	-

Table 4: Basic wine parameters

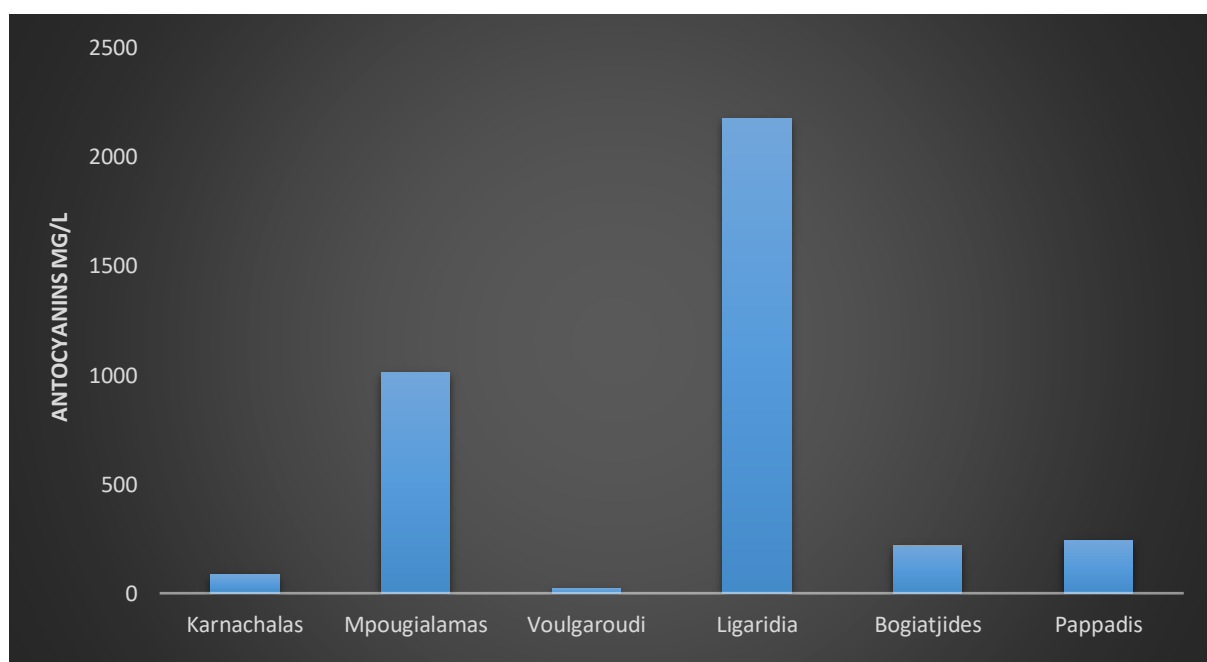


Figure 13: Total Anthocyanins in red wines mg/L

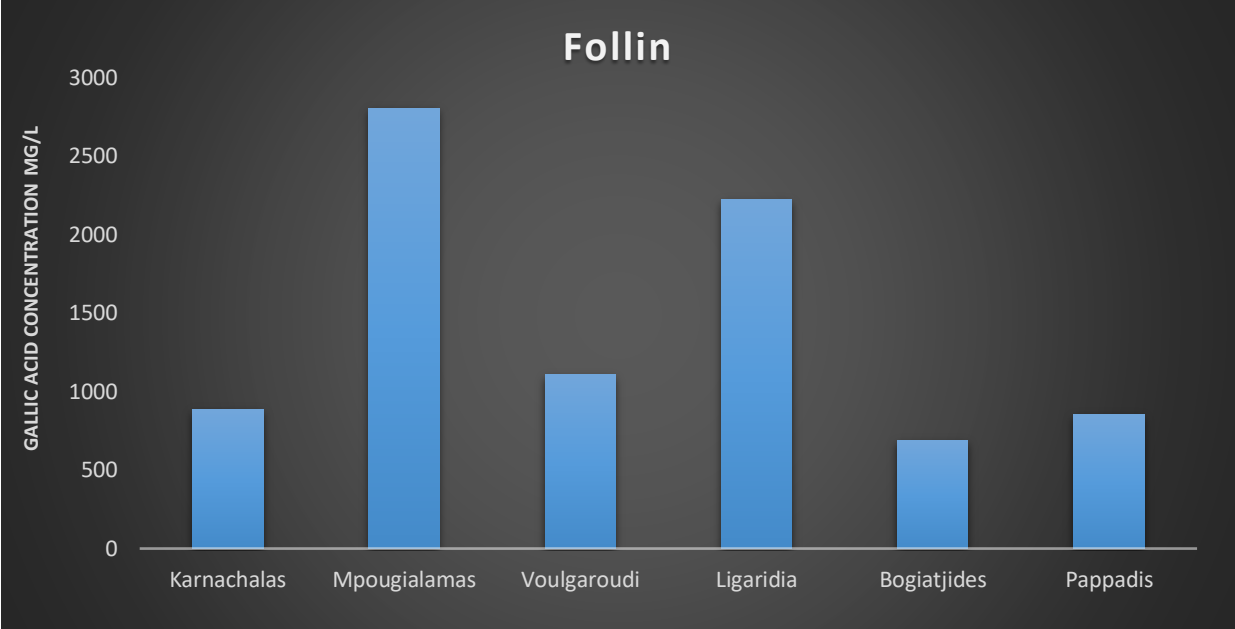


Figure 14: Total phenolics (FolinCiocalteu) expressed in mg Gallic acid/ L of wine

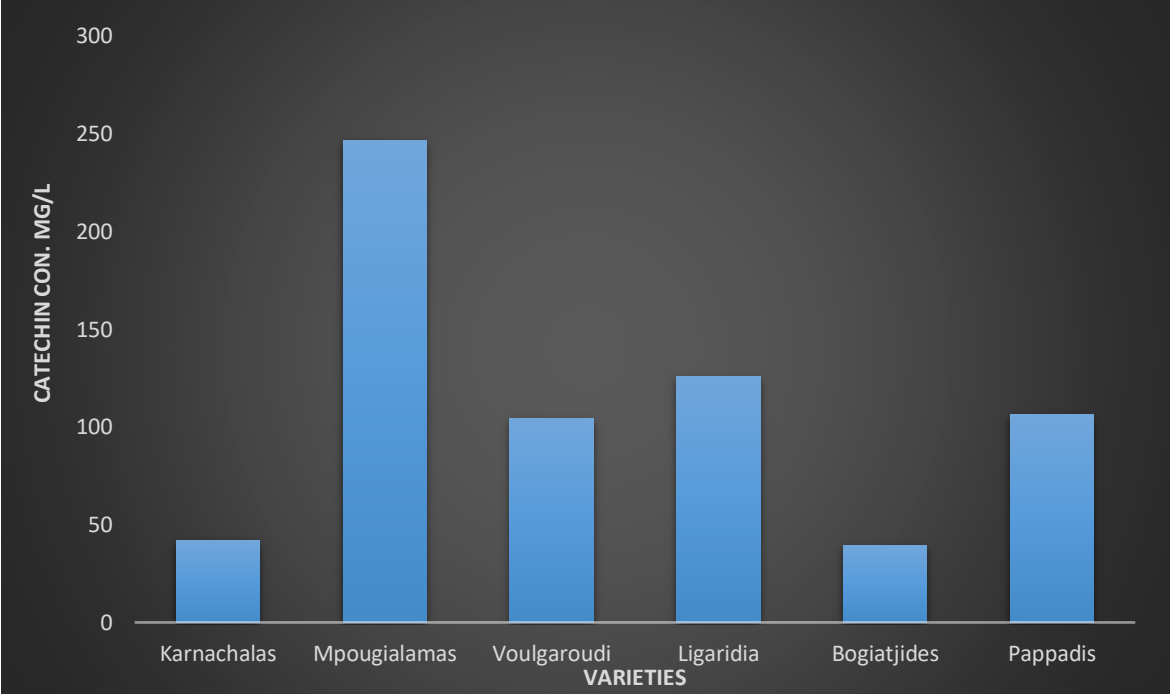


Figure 15: Tannin Determination with the method Bovine Serum Albumin (BSA) expressed in concentration of mg catechin/L of wine

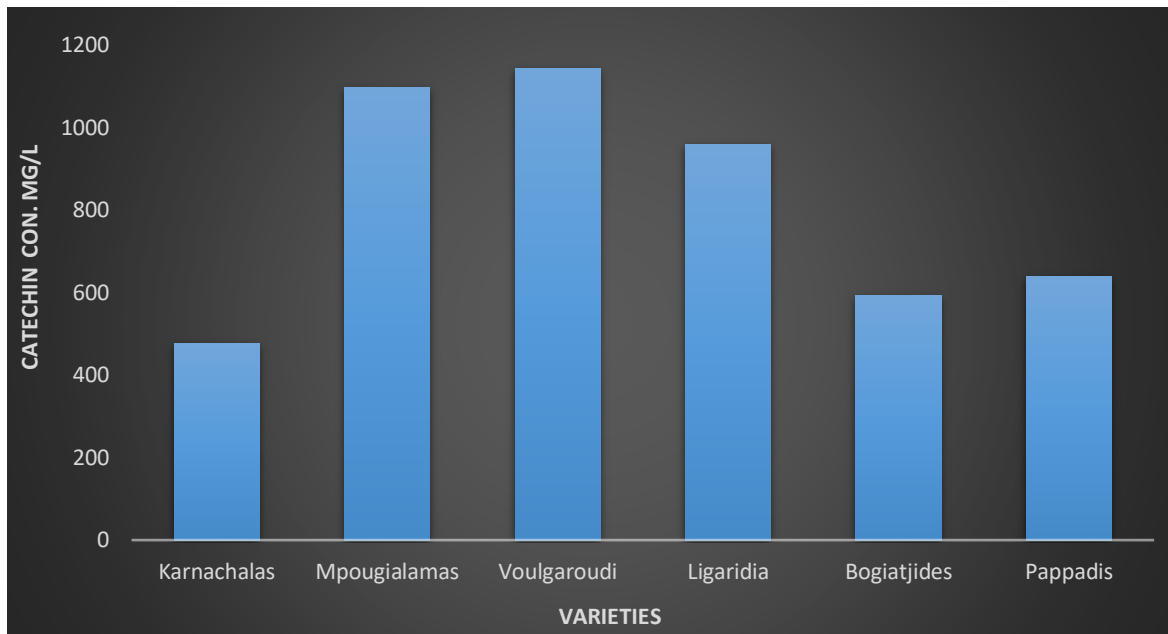


Figure 16: Tannin Determination with the method Methyl CelulosePrecipitable (MCP) expressed in concentration of mg catechin/L of wine

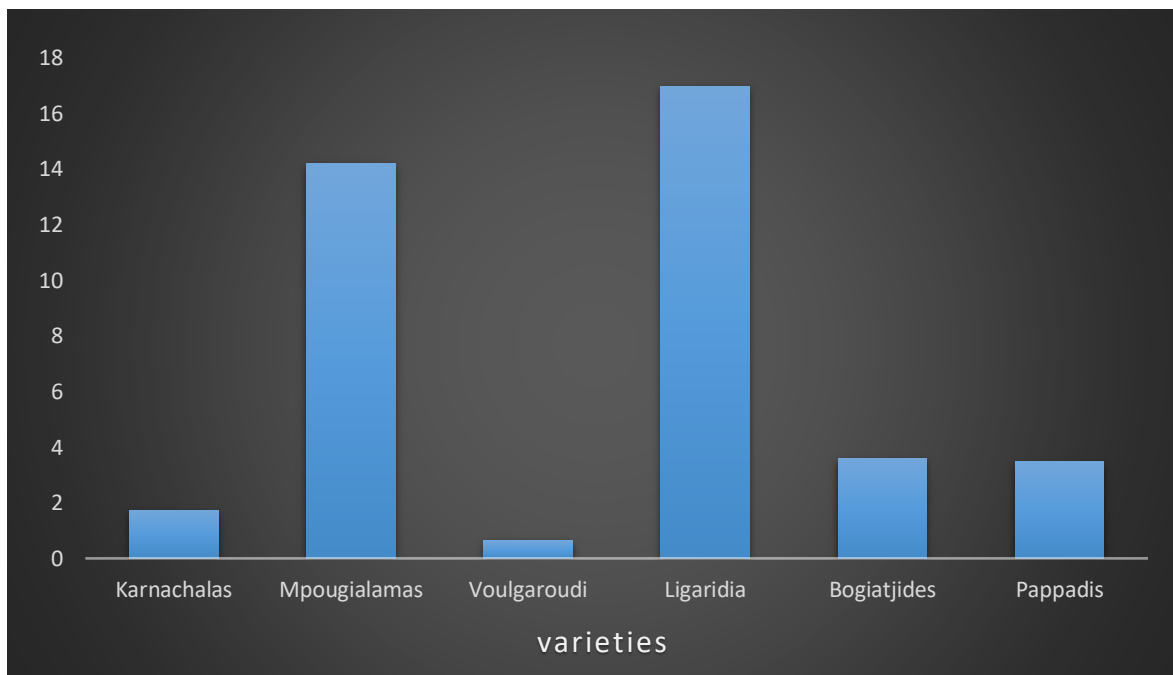


Figure 17: Red Wine Color Intensity

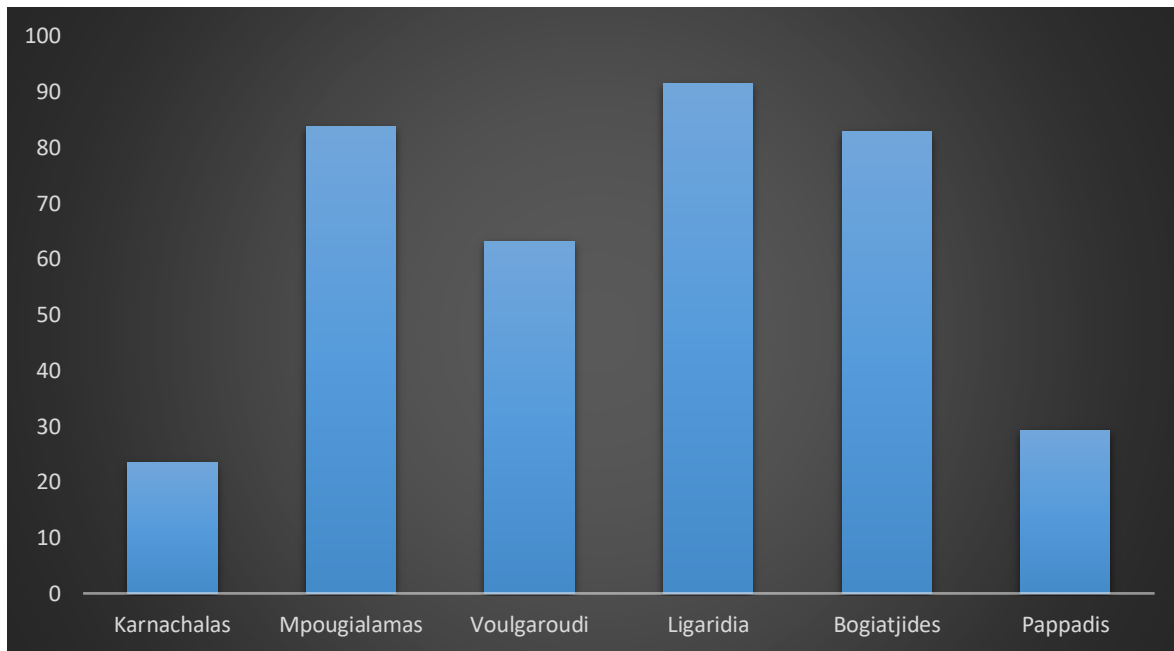


Figure 18: OD 280

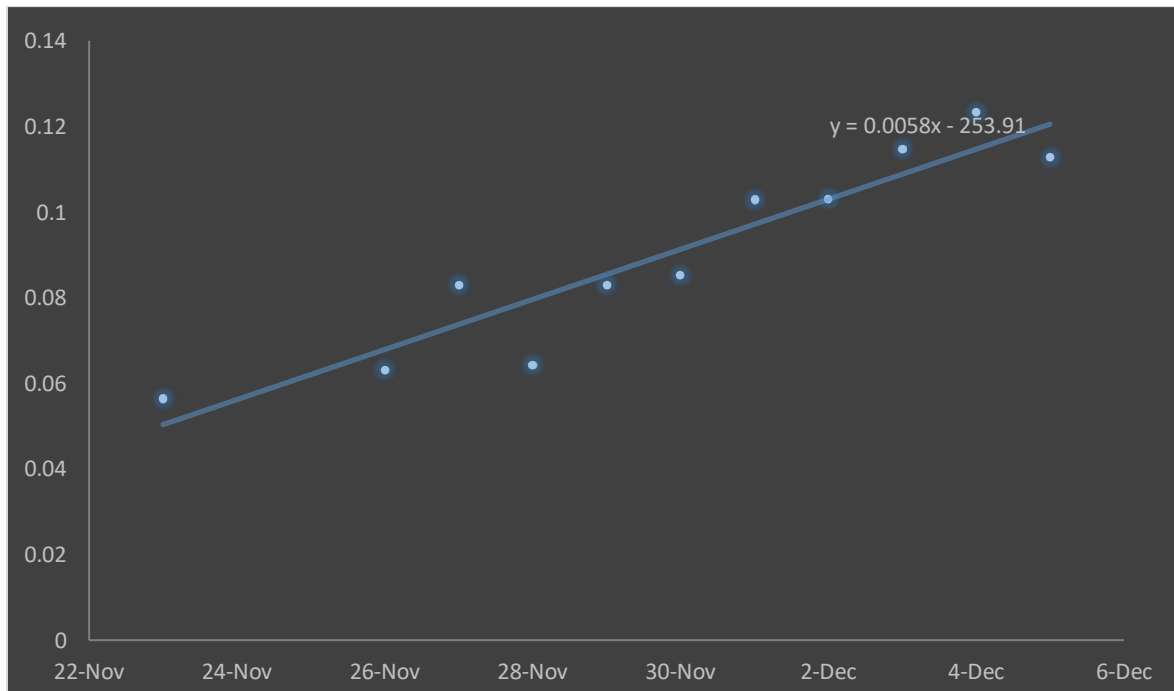


Figure 19: The regression line obtained from the graphical representation of A420 values versus time, for the white wine White Patiki

Must and Grape analysis at the red grapevine varieties (Oct. 2019)

All grapes from the different grape varieties were harvested at their optimum technical maturation. The higher brix values, at the grapes, were recorded at the red varieties Karnachalas and Ligaridia (Table 5) which can be correlated to the highest values of alcoholic strength at the produced wines (Table 4). Moreover, at Bougialamas and Ligaridiamust recorded the highest values of colour intensity (table 5), consequently the produced wines of those varieties had the highest values of colour intensity (Fig. 17).

According to Iland method (Iland et al., 2004)Ligaridia and Bougialamas grapevine variety, provide grape berries with the highest concentrations of total phenolics and total anthocyanins in their berries during harvest (Fig 20 and 21)

Variety	Berry volume (mL)	Brix	Tot. Acid. (g/L)	pH	Color Intensity	Hue	YAN A/ BA
Karnachalas	2.8	21.5	4.1	3.5	0.35	2.20	157(29/128)
Bougialamas	2.4	21.1	3.8	5	3.14	2.04	145 (15/ 130)
Voulgaridia	2.4	20.6	4.0	3.5	0.46	1.96	88 (12/ 76)
Ligaridia	0.7	22.9	3.7	3.9	11.5	1.02	168 (4/ 164)
Bogiatjides	3.6	21.9	2.8	3.8	0.6	1.52	154 (25/ 129)
Papadis	3.3	23.1	4.1	3.7	0.39	1.74	80 (9/ 71)
White Patiki	3.1	21.5	5.86	3.5	-	-	97(10/87)

Table 5: Conventional must analysis in grapevine varieties from Soufli (Oct. 20018)

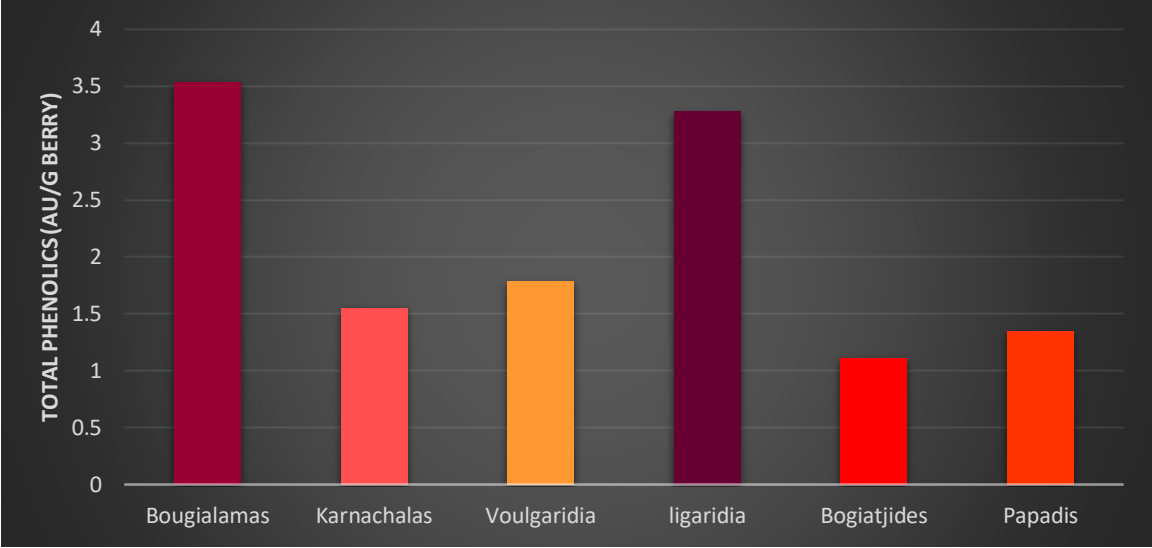


Figure 20: Total Phenolics expressed in AU/ g of berry

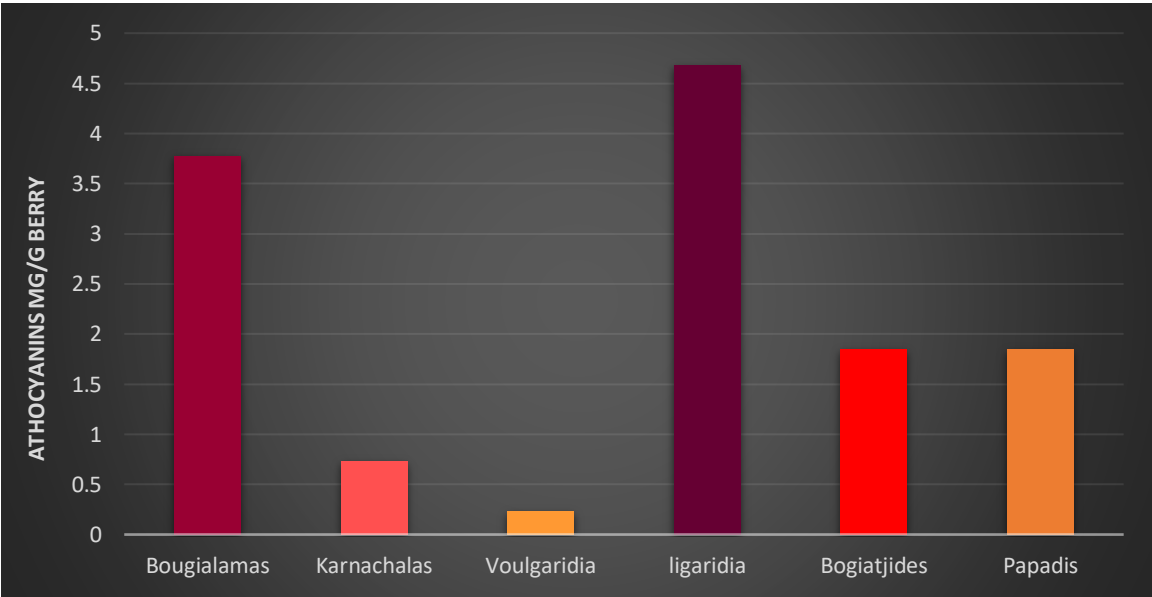


Figure 21: Anthocyanins expressed in mg/ g berry

Conclusion

This can be characterized as an outstanding project, because it provides an holistic description of the vine. It combines the molecular identification of the grapevine varieties and the oenological evaluation of the wines produced by them. It must be noted that the name of each sample is being accepted as it has been given at the collection point. However, once the Genetic Identification will be completed, we will be able to cross-check the uniqueness of the variety and the name.

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