AGRICULTURAL UNIVERSITY OF ATHENS
DEPARTMENT OF CROP SCIENCE
LABORATORY OF VITICULTURE

POSTGRADUATE PROGRAMM
TOP SECTORS & INNOVATIVE APPLICATIONS IN PRODUCTION
& MAINTENANCE OF FRUIT, VEGETABLE & FLORICULTURE SPECIES

MSc Thesis

Genetic Study of Cretan Vine Varieties of *Vitis vinifera* L by molecular markers

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Γενετική Μελέτη Κρητικών Ποικιλιών Αμπέλου *Vitis vinifera* L με μοριακούς δείκτες

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ABSTRACT

Local grapevine varieties, particularly in the latter half of the 20th century, have experienced abandonment due to the prevalent market preference for international grape varieties. This transition towards modern cultivars has led to a decline or even disappearance of indigenous local varieties. The presence of synonyms and homonyms further compounds the challenge of identifying minority cultivars.

For the conservation, characterization, and sustainable utilization of genetic resources of the Greek grapevine cultivars (*Vitis vinifera* L.) in breeding and cultivation, a comprehensive exploration of grapevine cultivars' germplasm is necessary, especially in a vineyard with such varietal richness, as is the Cretan one. The identification and discrimination of grapevine varieties cultivated in the vineyards of Crete is expected to yield insights of both botanical and archaeological significance.

This effort not only contributes to preserving the cultural and genetic heritage of these grapevine varieties, but also advances our understanding of their historical context. The discrimination of grapevine cultivars is a difficult task due to several challenging factors. These factors include the sheer abundance of grape varieties, their wide geographic distribution, and the presence of numerous synonyms for each variety. This complex endeavor forms the central focus of viticulture and ampelography, a specialized scientific field dedicated to the study of grapevine cultivation. Historically, the identification, differentiation, and categorization of grape varieties relied primarily on ampelographic methods.

Specifically, this approach involved analyzing the ampelographic characteristics of the various organs of the grapevine, observing how each variety behaved under cultivation conditions, and assessing its economic significance. These traditional methods formed the foundation for grapevine classification. It is worth noting that recent developments in the field, such as advancements in genetic analysis and DNA fingerprinting, have provided more precise means of identifying and classifying

grapevine varieties. These modern techniques supplement the traditional ampelographic descriptions and contribute to our understanding of grapevine diversity.

The aim of the present study was to investigate the genetic diversity of the most well-known indigenous Cretan varieties, found in productive vineyards of Crete, and more specifically of Prefecture of Heraklion. Different samples from these varieties were collected in order to examine the genetic variability that exists within these grapevine varieties using molecular markers. It should be noted that besides the research and genetic interest of the identification and discrimination of the different biotypes of the Cretan varieties studied, the results of the present study have a great viticultural interest since they could constitute the base for the implementation of clonal selection program, thus exploiting the most appropriate clones as well as the preservation and exploitation of precious germplasm.

Keywords: *Vitis vinifera* L., ampelographic discription, molecular markers, clones, biotypes

Γενετική Μελέτη Κρητικών Ποικιλιών Αμπέλου *Vitis vinifera* L. με μοριακούς δείκτες

ΠΜΣ Τομείς Αιχμής & Καινοτόμες Εφαρμογές στην Παραγωγή & Συντήρηση Οπωροκηπευτικών & Ανθοκομικών Ειδών
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ΠΕΡΙΛΗΨΗ

Οι τοπικές ποικιλίες αμπέλου, ιδιαίτερα στο δεύτερο μισό του 20^{ου} αιώνα, έχουν εγκαταλειφθεί λόγω της επικρατούσας προτίμησης της αγοράς για διεθνείς ποικιλίες αμπέλου. Αυτή η μετάβαση προς τις σύγχρονες ποικιλίες οδήγησε σε μείωση της καλλιέργειας ή ακόμα και εξαφάνιση των γηγενών τοπικών ποικιλιών. Η παρουσία συνωνύμων και ομώνυμων ενισχύει περαιτέρω την πρόκληση της ταυτοποίησης και διάκρισης αυτών των ποικιλιών.

Για τη διατήρηση, τον χαρακτηρισμό και τη αειφόρο χρήση των γενετικών πόρων των ελληνικών ποικιλιών αμπέλου (*Vitis vinifera* L.) στην αναπαραγωγή και την καλλιέργεια, είναι απαραίτητη η διεξοδική διερεύνηση του γονιδιώματος των ποικιλιών αμπέλου, ειδικά σε ένα αμπελώνα με ποικιλιακό πλούτο, όπως είναι αυτός της Κρήτης. Η διάκριση και ταυτοποίηση των ποικιλιών αμπέλου που βρίσκονται τους αμπελώνες της Κρήτης αναμένεται να αποδώσει γνώσεις τόσο βοτανικής όσο και αρχαιολογικής σημασίας.

Η προσπάθεια αυτή συμβάλλει όχι μόνο στη διατήρηση της πολιτιστικής και γενετικής κληρονομιάς αυτών των ποικιλιών αμπέλου, αλλά προάγει επίσης και την κατανόησή μας για το ιστορικό τους πλαίσιο. Η διάκριση μεταξύ των καλλιεργούμενων ποικιλιών αμπέλου είναι ένα δύσκολο έργο λόγω αρκετών παραγόντων. Αυτοί οι παράγοντες περιλαμβάνουν την αφθονία των ποικιλιών αμπέλου, την ευρεία γεωγραφική τους κατανομή και την παρουσία πολυάριθμων συνώνυμων για κάθε ποικιλία. Αυτή η πολύπλοκη προσπάθεια αποτελεί το επίκεντρο της αμπελουργίας, ενός εξειδικευμένου επιστημονικού πεδίου αφιερωμένου στη μελέτη της αμπελοκαλλιέργειας. Ιστορικά, η αναγνώριση, η διαφοροποίηση και η κατηγοριοποίηση των ποικιλιών αμπέλου βασίστηκαν κυρίως στις αμπελογραφικές μεθόδους.

Συγκεκριμένα, η προσέγγιση αυτή περιλάμβανε την ανάλυση των αμπελογραφικών χαρακτηριστικών των διαφόρων οργάνων του φυτού της αμπέλου, την παρατήρηση του τρόπου με τον οποίο συμπεριφέρεται κάθε ποικιλία υπό συνθήκες καλλιέργειας και την αξιολόγηση της οικονομικής τους σημασίας. Αυτές οι

παραδοσιακές μέθοδοι αποτέλεσαν το θεμέλιο για την ταξινόμηση των ποικιλιών αμπέλου. Αξίζει να σημειωθεί ότι οι πρόσφατες εξελίξεις στον τομέα αυτό, οι πρόοδοι στη γενετική ανάλυση καθώς και η εύκολη απομόνωση του DNA, έχουν παράξει πιο ακριβή και αντικειμενικά μέσα για τον εντοπισμό και την ταξινόμηση των ποικιλιών. Αυτές οι σύγχρονες τεχνικές συμπληρώνουν τις παραδοσιακές αμπελογραφικές περιγραφές και συμβάλλουν στην κατανόηση των ποικιλιών αμπέλου.

Σκοπός λοιπόν της παρούσας μελέτης ήταν να διερευνηθεί η γενετική ποικιλομορφία των πιο γνωστών γηγενών ποικιλιών της Κρήτης, που βρίσκονται σε παραγωγικούς αμπελώνες της Κρήτης, και ειδικότερα του Νομού Ηρακλείου. Συλλέχθηκαν διαφορετικά δείγματα από αυτές τις ποικιλίες για να εξεταστεί η γενετική παραλλακτικότητα που υπάρχει εντός αυτών των ποικιλιών με τη χρήση μοριακών δεικτών. Θα πρέπει να σημειωθεί ότι εκτός από το ερευνητικό ενδιαφέρον για τον εντοπισμό και τη διάκριση των διαφόρων βιοτύπων των μελετημένων Κρητικών ποικιλιών, τα αποτελέσματα της παρούσας μελέτης έχουν μεγάλο αμπελουργικό ενδιαφέρον, δεδομένου ότι θα μπορούσαν να αποτελέσουν τη βάση της εφαρμογής του προγράμματος κλωνικής επιλογής, αξιοποιώντας έτσι τους καταλληλότερους βιότυπους-κλώνους καθώς και τη διατήρηση και την εκμετάλλευση του πολύτιμου γενετικού υλικού τους.

Λέξεις κλειδιά: *Vitis vinifera* L., αμπελογραφική περιγραφή, μοριακοί δείκτες, κλώνος, βιότυπος

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ΕΞΕΤΑΣΤΙΚΗ ΕΠΙΤΡΟΠΗ

ΜΑΡΙΤΙΝΑ ΣΤΑΥΡΑΚΑΚΗ, Επίκουρη Καθηγήτρια ΓΠΑ
ΚΑΤΕΡΙΝΑ ΜΠΙΝΙΑΡΗ, Αναπληρώτρια Καθηγήτρια ΓΠΑ
ΙΩΑΝΝΗΣ ΠΑΠΑΔΑΚΗΣ, Αναπληρωτής Καθηγητής ΓΠΑ

To my family and Dimitris Kafetzopoulos

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I would like to dedicate this master's degree to my family and to a person who is not alive but who stood as a father to me in my journey in Biology.

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INTRODUCTION

Nowadays, grapevine (*Vitis vinifera* L. spp *sativa*) is regarded as one of the most valuable cultivated species in the world. (Alston et al., 2019) From ancient times, it seems to have had a central economic and cultural role in the Mediterranean basin and surpasses the Greco-Roman era (McGovern, 2007). Many geneticists, archaeobotanists and ampelographers concur for the origin of the domestication (cultivation) period of the wild progenitor (*Vitis vinifera* spp *sylvestris*), which has occurred during the Neolithic period, between 7000 and 6000 B.C.E., in the Near East, south of the Caucasus. (Myles et al., 2011, Riaz et al., 2018) More information about that is from chemical analyses of pottery vessels that suggest that wine had already been produced in the Caucasian region, 8000–6000 years ago (McGovern, 2007, McGovern, 2017). The domesticated grapevines apparently shifted southwards (Black and Caspian Sea, Mesopotamia) and later to Europe (Greece and southern Balkans) (Zohary et al., 2012, McGovern, 2019)

The Greek vineyard is considered one of the most ancient vineyard in the world, although small in size, it is precious, in history and in grapevine varieties. (Stavrakaki and Stavrakakis, 2017). More specifically, the Cretan vineyard, according to historical records, exhibits evidence of cultivation and winemaking by the Minoans in Crete, long before cultivated significantly in the rest of Greece (Marangou, 1991; McGovern, 2003). The Early Minoan IIB period, ca. 2200 B.C., was considered the highest in the crafting wine, with a characteristic example of the production of ‘retsina’ for the first time in the Greek region. (Logothetis, 1975) During this period also known as Protopalatian, there were more other grape wine varieties which are cultivated in Phaestos and they seem to be interesting, as they are different from those that have been found in the rest of Greece (Evans et al., 1925)

The origin of Cretan vine varieties and the evolution of viticulture is an element for different theories and reflections. According to the theory of Evans (1925), the Minoans were descendants of the Egyptians, and as a result, the cultivation of the vine was transferred to the Greek area through Minoan Crete (3000-2800 BC). Basic element of that time is considered to be the close commercial, economic and cultural relationship between the Egyptians and the ‘Kefti’ (as the Egyptians called the Cretans).

Nevertheless, some of recent genetic studies have shown that mitochondrial DNA of the Minoans is very different from the Egyptians, while it seems to have an important similarity with the populations of the Neolithic period. Based on these results, it was

concluded that Crete from the Neolithic period was a center and place of residence for many people who came from the Caucasus region, 9000 years ago, the period when the Caucasian vine was also cultivated. (Jeffery et al., 2013)

Taking into account the most recent research, it can be concluded that the cultivated indigenous vine varieties of Crete *(Vitis vinifera* spp *sativa)* originated either from wild grape populations (*Vitis vinifera* spp *sylvestris*) or from crosses with Caucasian grapevine varieties *(Vitis vinifera spp caucasica).* More specifically, the validity of this hypothesis is supported by the distinctive ampelographic characteristics observed in the Cretan grape varieties, most of which can be categorized under the eco-geographical groups known as *proles orientalis* and *proles pontica*. These distinctive characteristics further substantiate the claim that the Minoan civilization played a pivotal role in the dissemination and cultivation of these grape varieties across the broader region (Negrul, 1946).

The vast array of archaeological discoveries discovered across the entirety of Minoan Crete provides compelling evidence not only of the Minoan civilization's remarkable expertise in cultivating vines and producing wine but also of the presence of priceless grape varieties (Stavrakakis, 2019). These exceptional varieties gained widespread recognition in ancient Greece and western Europe, owing to the immense power and influence of the Minoan fleet (Figures 1, 2).

One might consider an alternative perspective on the ancient myth of Theseus and Ariadne, viewing it as a fictional tale. Under this interpretation, Theseus, captivated by the exceptional grapevine varieties and exquisite wines of Crete, not only coveted Ariadne as his prized possession but also desired to acquire the valuable grapevine cuttings (canes) held by King Minos. This intriguing speculation presents a possible origin narrative for the esteemed Cretan grapevine varieties, tracing their subsequent journey throughout Greece, the Mediterranean, and countless centuries. It sheds light on the intertwined relationship between these varieties and the renowned Cretan wines known as "Passos," "Thireos" (Cretan Theran wine), "Cretan Athirin," "Vinum Cretense," "Malvasia," as well as the diverse varietal wines enjoyed in present times.

Fig. 1. A stone basin for grape pressing, Archeological Museum of Heraklion, Crete

Fig. 2. Pithoi jars from Knossos Archeological Museum of Heraklion, Crete

CRETAN VARIETIES

According to Anagnostakis (2013) written sources documenting Cretan grape varieties such as Athiri, Vidiano, Dafni, Kotsifali, Liatiko, and others started appearing in the 12th-13th century. It can reasonably be infered that the majority of grapevine varieties were cultivated with the same or similar names as early as, or even before, the Hellenistic period. However, an interesting observation is that during the timeframe from the 7th century to the 11th-12th century, there are substantial gaps in the recorded nomenclature. There are several reasons for this, and as a result, the names of cultivated grapevine varieties remained unrecorded for extended periods, spanning centuries.

Pockocke's observations from 1773 suggest that the vineyards in Crete were believed to generate a rich diversity of 72 grape types or varieties. However, it becomes evident, even upon a casual inspection, that Pockocke's count encompassed cultivated grapevine variations that might have been denoted through numerous synonyms. Moving into the late 18th and early 19th centuries, Sieber's documentation in 1823 itemized a compilation of 37 grapevine varieties present in Crete. This inventory was subsequently endorsed by Heldreich in 1910, who independently recorded a comprehensive tally of 43 grapevine varieties. Shifting towards a later timeframe, Fragaki's report in 1969 asserted that there were 33 cultivated grapevine varieties thriving in Crete. These differing

accounts underscore the intricacies involved in precisely quantifying grapevine varieties, illustrating how differing perspectives and methodologies can yield disparate numerical outcomes.

If we disregard the element of time and the discrepancies in the number and names of grapevine varieties mentioned earlier, it's reasonable to assume that the variations can be attributed to certain factors. Specifically, Heldreich's records encompassed grapevine varieties from across the entirety of Crete, while Fragaki (1969) likely focused her research on the Heraklion Prefecture.

Furthermore, if we set aside concerns related to variations in spelling (such as "eftakoilo" vs. "eftakylo" or "frapsatiri" vs. "thrapsathiri") and variations influenced by the local dialect (like "plytho" vs. "plyto" or "moschato" vs. "mouskado"), we can identify a core set of 23 grapevine varieties that are common among the records. These common varieties likely serve as the basis for understanding and characterizing the grapevine diversity in Crete.

achladia / valaitis / vidiano / vouidomata /dafni /eftakoilo / kokkifali / kouminato aspro / kouroutachta /Ladikino / liatiko aspro / liatiko mavro / melissi / moschato / plyto / razaki aspro / razaki mavro / romeiko mavro / sarakino / siriki / frapsatiri / fraoula

Certainly, it's important to note that some of the oldest and most esteemed grapevine varieties in the Cretan vineyard, including Mandilaria, Athiri, and Vilana, were not recorded by Heldreich. This omission can be attributed to the fact that the data he collected originated from regions where these grapevine varieties were predominantly known by their local names and synonyms that were in common use during his time.

While comprehensive information regarding the ampelographic characteristics of leaves and bunches is limited, a comparative analysis of available data suggests the following associations:

a. The grapevine variety "Liatiko aspro" (white) most likely corresponds to Athiri, both of which are acknowledged as high-quality varieties.

b. The grapevine variety "Romeiko aspro" (white) is most likely synonymous with the grapevine variety Vilana, as both are known for their high productivity.

c. The grapevine variety "Gaidourades galano" (white) corresponds to Gaidouria (white).

d. The variety "Gaidourades mavro" (black), as described by Sieber (1823) with its large and dense bunches, blue-black skin, and juicy flesh that's compressed, probably corresponds to biotypes of the Mandilaria cultivar, known as thrapsa or kserothrapsa.

e. The grapevine cultivars Sarakino and Enstagarina are certainly of eastern origin, and their names are linked to the Arabs who conquered Crete in the 10th century. Both of these varieties are late-season table grapes, with Sarakino having large bunches and berries with a crimson skin color. Additionally, they can be preserved on the vines for an extended period.

f. In terms of the characteristics of the bunch and berries, the description provided corresponds to Hourmades, which is a biotype of the grapevine variety Akiki. It appears that several biotypes of the grapevine cultivar Akiki, or related varieties, were widespread during the Middle and Late Byzantine periods.

g. At a later date, the monk Agapios (secularly known as Athanassios Landos from Crete) mentions in his "Geoponikon" the grapevine variety "Poriko," a reddish table grape variety, which is certainly a synonym for Akiki Oporiko.

h. Lastly, the matching of grapevine varieties such as Razaki mavro (black) with Aitonichi mavro (black) Sokiano with Fokiano Daktilato with Aitonichi aspro (white) Zardani with Tsardana Tsiri with Tsilores and Dermatades with Dermatas appears to be a feasible task, according to Davidis (1982). Furthermore, grapevine varieties Kantinio (also known as Kantinia today) and Grompola seem to constitute clones of the grapevine variety Razaki.

These insights provide valuable historical context regarding the diversity of grapevine varieties in Crete and their connections to various regions and historical periods.

In the context of this study, the term "Cretan varieties" refers to grapevine cultivars that have historical documentation or reports of being cultivated since at least the 12th-13th century. This classification is irrespective of their geographical origin or specific region of cultivation. This group encompasses a range of grapevine varieties. It includes indigenous varieties that are native to the Cretan region such as Athiri, Dafni, , Mantilaria, Kotsifali, Vidiano, Plyto, Vilana, Liatiko, and Tachtas. Additionally, it involves varieties originating from the eastern regions like Lakidino, as well as those from the western areas like Tsardana and Romeiko. Despite their origins, these varieties have been cultivated for centuries in Crete and, in some instances, exclusively within the region. There are additional grapevine varieties that originated in Crete but have since been introduced and cultivated in other viticultural regions of Greece under different names. For instance, names like Katsano, Diminitis, and Plantani are used for these varieties in other regions. Some Cretan grapevine varieties were also transferred to the

Ionian Islands and Cyclades, where they were grouped under the general name "Kritiko." This name is accompanied by "aspro" or "mavro" to distinguish between white and black-skinned berries, respectively. Interestingly, there were never any grapevine varieties cultivated in Crete under the name "Kritiko," regardless of berry color (Stavrakaki and Stavrakakis, 2017).

Through comparative ampelographic analysis, it has been revealed that the name "Kritiko aspro" corresponds to the Dafni and Plyto varieties cultivated in the Cyclades. However, Plyto takes on a different name in the southern Peloponnese, where it is known as Kitrinovaria and/or Asprovaria. These cases highlight the complexity of grapevine variety names and the challenges in accurately identifying and classifying them across different regions (Stavrakaki and Stavrakakis. 2017).

The Mandilaria variety, and occasionally Ladikino, were transferred to the Peloponnese region, particularly during the period of prominence for Cretan Malvasia (14th-18th century). In the Peloponnese, Ladikino was labeled as "Thrapsa," deriving from "thrapseros," an adjective signifying fertility and productivity. Mandilaria was cultivated under the name "Vaftra" in the Cyclades and assumed various other "aliases" in different regions. Notably, the term "thrapsa" or "thrapses" was employed by the Cretans to denote their most productive grapevine varieties, whether white (Vilana, Plyto), black (Mantilaria, Ladikino), or possibly clones of these varieties (e.g., thrapsathiri: productive Athiri) (Stavrakaki and Stavrakakis. 2017).

Conversely, the Liatiko grapevine variety, known as "Diminitis," migrated to the Dodecanese region, where it continues to be cultivated, particularly in Rhodes. Over time, it becomes evident that numerous grapevine varieties present in Cretan vineyards were originally introduced to Crete from viticultural regions to the east. Subsequently, during the later periods, particularly the 17th-18th centuries and the 20th century, various grapevine varieties reached Crete from western regions. This historical movement of grapevine varieties underscores the intricate interplay of viticulture and cultural exchange over the centuries.

VITICULTURE IN CRETE

From ancient times to the present day, the cultivation of grapevines in Crete has had a profound impact on Cretan culture, socio-economic development, and the intertwining of tradition and religious beliefs. However, when compared to viticultural practices in other regions of Greece, the cultivation of grapevines in Crete has maintained distinctive characteristics. Notably, the worship of Dionysus/Bacchus as the protector of vine growers was notably absent in ancient Cretan practices. Similarly, the veneration of St. Tryphon, the patron saint of vine growers in Eastern Orthodox tradition, was and remains relatively limited in Crete, despite the spread of Christianity (Koufos et al., 2020).

Viticultural practices in Crete have also shaped the myths and traditions surrounding vine and wine in unique ways compared to other parts of Greece. For instance, while mainland Greek mythology often depicts wild boars as the primary threat to vineyards, in Crete, it was male goats ("tragoi") that posed a greater threat. The extent of devastation caused by these goats on vineyards was so significant that during the Cretan Resistance period (1941-1945), the secret password "male goats in the vineyards" used by freedom fighters, such as those in Anogia, indicated an imminent attack by the Nazi forces. This exemplifies the tangible and symbolic significance of viticulture in Cretan history and society (Stavrakaki and Stavrakakis. 2017).

Over the course of history, the vineyards, olive groves, Minoan civilization landmarks, and the traditional stone wine presses have consistently formed integral components of Crete's rural scenery (Figure 3). These elements have collectively contributed to shaping the distinct character of the island's countryside. The presence of grapes, raisins, wine, tsikoudia (raki), olives, and olive oil has been a constant and ubiquitous feature in the Cretan diet, deeply ingrained in the everyday culinary practices and cultural traditions of the region.

Numerous factors have had a profound impact on the Cretan vineyard's structure, composition, and grapevine varieties. Two standout factors are the introduction of the Soultanina grapevine variety and the appearance of Phylloxera, a peculiar aphid species. Soultanina swiftly altered the grapevine landscape in Crete, particularly in the Heraklion Prefecture, as it quickly spread across more than 30,000 hectares of vineyards. Meanwhile, the arrival of Phylloxera in the Messara Plain vineyards in 1978, coupled with vineyard grubbing policies, played a significant role in reducing cultivated land and

reshaping the varietal composition. Additionally, the importation of foreign grape varieties, primarily of French origin, influenced the synthesis of grape varieties. An accurate analysis of surface area allocation per variety category sheds light on the significance of various Cretan grapevine varieties. In recent replanting endeavors post-Phylloxera, the cultivation of Kotsifali, especially in Heraklion, and the resurgence of "forgotten varieties" like Thrapsathiri, Vidiano, and Moschato Mazas/Spinas expanded, while the cultivation of Liatiko, Soultanina, and Razaki grapevine varieties decreased.

Fig. 3. Tachtas, Old vineyard, Megali Vrysi, Crete

DETAILS OF VITICULTURE

According to data provided by OIV in 2017, worldwide vine cultivation spans an extensive 7.427.900 hectares. However, this area of agriculture has witnessed a noteworthy decline from the 1980s, when it peaked at approximately 102 million hectares. More specifically the table below shows the evolution of cultivated areas of the world vineyard from 2008 to 2017 (Table 1) (Stavrakakis, 2019)

Table 1. Evolution of cultivated areas of the world vineyard (OIV, 2017)

Year	Area (100 hectares)
2017	7428
2016	7463,9
2015	7504,2
2014	7553,9
2013	77372

2012	7516,3
2011	7547
2010	7645
2009	7639
2008	7732

As for the Greek vineyards, which date back to antiquity, today they extend to approximately 108,400 hectares (Stavrakakis, 2019). During the last century, this area of viticulture has undergone remarkable changes affected by economic, political, and social changes, diseases, epidemics as well as by the impact force of climate change. The following table shows the evolution of cultivated areas of wine varieties during the five years 2013-2017 for different regions of Greece (Table 2). As we can see over the years, even in our country, the cultivation of vines has started to become smaller and smaller.

Table 2. Evolution of cultivated areas in hectares of wine varieties during the five years 2013-2017 (Ministry of Rural Development and Food)

Region	**2013**	**2014**	**2015**	**2016**	**2017**
Eastern Macedonian- Thrace	2026,1	2038,3	2042,9	2018,7	2093,4
Central Macedonian	4747,5	4764,4	4680,4	4593,2	416,5
Western Macedonian	2544,1	2510,9	2443,3	2485,4	232,5
Epirus	732,4	759,6	762,1	763,3	847,8
Thessaly	4220,9	4159,0	4023,6	3948,1	3763,0
Ionian Islands	2716,7	2734,2	2756,9	2744,4	3061,8
Western Greece	8913,1	8905,1	8814,5	8720,0	8803,6
Central Greece	7148,0	7110,0	7043,0	6825,3	6894,0
Peloponnese	11078,3	10946,5	10739,9	10342,6	9857,6
Attica	6493,7	6400,1	6312,9	6132,2	5972,8
North Aegean	3090,4	3072,7	2992,6	2827,5	2768,9
South Aegean	3838,4	3849,0	3825,4	3812,1	3907,0
Crete	7780,2	7769,9	7574,7	7557,3	7522,3
Total	**65330,2**	**65020,2**	**64012,9**	**62773,4**	**61984,4**

Table 3 also presents some data for some of the Cretan varieties in order to note the sharp decline concerning in native Cretan varieties. We could consider this happens because of the problems we mentioned previously but also because of the winegrowers' preference for foreign varieties.

Table 3. Cultivation area per Cretan variety (hectares) (Ministry of Rural Development and Food)

Cretan Varieties	**2015**	**2018-21**
Vilana	599	164,6
Vidiano	98	91,5
Dafni	-	5,0
Kotsifali	1,366	281,0
Liatiko	2,672	94,0
Kotsifoliatiko	-	9,2
Mandilaria	277	82,5
Plyto	-	8,5

CLIMATE CHANGE AND CRETAN VINEYARD

Climate change, known as CC, is any change in the state of the climate that persists over a long period of time and is considered one of the major environmental concerns facing humanity in the 21st century.

Climate change can have significant effects on vineyards around the world, including those in Crete, Greece. This is also one of the reasons why this problem will be analyzed in more detail in this work. Vineyards are sensitive to changes in temperature, precipitation patterns, and extreme weather events, all of which are influenced by climate change. Here are some ways in which climate change can impact Cretan vineyards. Climate change is already having significant impacts on Cretan vineyards, and these effects are expected to continue and intensify in the coming years. Crete, like many other wine-producing regions around the world, is facing a range of challenges due to shifting climate patterns. Here are some specific impacts of climate change on Cretan vineyards:

Temperature Increases: One of the most noticeable impacts of climate change in Crete is the rise in temperatures. Warmer temperatures can lead to earlier bud break and flowering in grapevines. While this might seem beneficial at first, it can also result in a shorter growing season, reduced acidity in grapes, and changes in the flavor profile of the wines produced. In some cases, extreme heat can even cause vine stress, which can harm the vines and reduce yields

Altered Rainfall Patterns: Changes in precipitation patterns are occurring in many regions, including Crete. Extended droughts and irregular rainfall can stress grapevines, leading to reduced yields and smaller grapes. Conversely, intense rainfall events can increase the risk of soil erosion and damage to the vineyards.

Water Scarcity: Drier conditions and increased evaporation due to higher temperatures can lead to water scarcity in vineyard regions. Irrigation is crucial for vineyards in arid climates like Crete, and water shortages can significantly impact vine health and grape quality.

Pest and Disease Pressure: Climate change can affect the prevalence and distribution of pests and diseases that affect grapevines. Warmer temperatures can create more favorable conditions for certain pests, such as grapevine moths, and diseases like downy mildew and powdery mildew. This can result in increased pesticide use and higher production costs for vineyard managers.

Extreme Weather Events: Crete is susceptible to extreme weather events, including heavy storms, hail, and wildfires. These events can damage vines, grapes, and infrastructure in the vineyards, leading to financial losses and reduced wine production.

Shifts in Grape Ripening: Climate change can cause grapes to ripen earlier than usual, which can disrupt traditional harvest schedules. This can be challenging for winemakers who rely on specific grape maturity levels for their wine styles.

Varietal Suitability: The suitability of grape varieties currently grown in Crete may change due to shifting climate conditions. Some traditional grape varieties may become less viable, while others that were less common in the region may become more suitable. Winemakers may need to adapt their grape choices accordingly (Koufos et al., 2020).

Fig. 4. Abandoned vineyard, Panagia Heraklion, Crete

To address these challenges, vineyard managers and winemakers in Crete are adopting various adaptation strategies:

- Changing Grape Varieties: Some vineyards are experimenting with new grape varieties that are better adapted to warmer and drier conditions.
- Improved Irrigation Practices: Sustainable irrigation practices, such as drip irrigation, can help conserve water resources and ensure adequate moisture for the vines.
- Integrated Pest Management: Integrated approaches to pest and disease management can help reduce reliance on chemical pesticides.
- Soil Management: Enhancing soil health and structure can improve the vine's resilience to changing climate conditions.
- Research and Innovation: Collaboration with research institutions and adopting innovative technologies can help vineyard managers stay ahead of climate-related challenges.

The impacts of climate change on Cretan vineyards highlight the urgency of addressing climate change on a global scale. Sustainable and climate-resilient practices are essential to ensure the continued success of Cretan wine production and the preservation of its unique terroir and grape varieties. (Koufos et al., 2020)

DISTINCTION AND CLASSIFICATION

The efforts to distinguish and classify cultivated grape varieties have a long history dating back to ancient times. In Greek antiquity, grape varieties were distinguished based on various criteria, including geographical origin and grape

characteristics. These early distinctions laid the foundation for the systematic classification of grape varieties in European viticulture.

The first systematic systems for distinguishing and classifying grape varieties in European viticulture were based on the comparative description of the morphological characteristics of all the individual parts of the grapevine, including the trunks and other organs. These early classification systems formed the basis of classical viticulture, which is the scientific study and cultivation of grapes. These efforts to categorize and differentiate grape varieties were essential for understanding the diverse range of grapes and their suitability for different winemaking purposes.

As time has passed, advancements in grapevine genetics, ampelography (the study of grapevines and grape varieties), and modern viticultural techniques have provided more precise methods for distinguishing and classifying grape varieties. DNA analysis and molecular markers, for example, have revolutionized the ability to identify and confirm grapevine varieties, making the classification more accurate and reliable.

Overall, the efforts to distinguish and classify grape varieties have evolved over the centuries, combining traditional methods with modern science to provide a comprehensive understanding of the vast diversity of grape varieties cultivated in viticulture (Krimbas, 1938).

Modern viticulture encompasses several key objectives beyond the categorization and differentiation of vine species and varieties. It involves the comprehensive examination of factors, both environmental and cultural, that influence the outward characteristics of grapevines. This includes the economic and cultural assessment of their qualitative and quantitative attributes for their practical use in grape production.

In the realm of viticulture, especially within the context of productive grape farming, it is of paramount importance to accurately identify grape varieties. This necessity has prompted the active engagement of numerous individuals, including winegrowers and experts in systematic classification, to ascertain and confirm the identities of grapevine varieties.

Numerous systems have been developed for the description and categorization of grapevine varieties. These systems are rooted in diverse criteria, including the morphological features of vine organs (morphological classification), measurements of these features (morphometric classification), various stages in the annual grapevine growth cycle (phenological classification), the geographical regions where varieties originate and are distributed (geographical classification), and their classification based

on shared physical traits (phenotypic classification). This rich array of classification systems underscores the multifaceted nature of grapevine diversity and classification efforts in viticulture.

In the realm of oenological methodology, three key approaches are employed as outlined by Davidis (1982). These approaches include oenological description, comparative oenology, and experimental oenology.

Oenological Description: The primary objective of oenological description is to differentiate and categorize grape varieties and species. This is achieved by scrutinizing and evaluating the outward characteristics, specifically the morphology, of the grapevine's various components. These visible traits serve as the basis for identifying and classifying different grape varieties.

Comparative Oenology: Within oenological methodology, comparative viticulture tackles specific challenges related to the nomenclature of grape varieties across different regions. It delves into the intricacies of synonyms, where the same grape variety may carry different names in various locations. Furthermore, it delves into the investigation of the clonal composition within grape populations, shedding light on genetic variations and relationships among cultivated grapevine varieties.

Experimental Oenology: A crucial facet of this methodology is experimental oenology, which involves conducting controlled experiments to enhance our understanding of the winemaking process. It explores various factors such as fermentation techniques, aging procedures, and other winemaking practices to optimize wine quality and characteristics.

In summary, oenological methodology combines these three approaches oenological description, comparative oenology, and experimental oenology to advance our knowledge of grapevine diversity, tackle naming discrepancies, and improve the winemaking process. This comprehensive approach plays a vital role in the world of viticulture and enology. Experimental viticulture focuses on addressing and resolving issues related to the origins of grapevine varieties, employing a combination of genetic analysis, phytogeographical methods, and historical data. It distinguishes itself from classical botanical description not only in its objectives but also in its content, particularly concerning grapevine varieties. In botanical terms, the concept of "variety" refers to a specific systematic entity that encompasses a population of individuals, including those in their wild form. These individuals are capable of freely interbreeding with each other and typically originate from natural propagation, displaying discernible differences.

Experimental viticulture, however, approaches the concept of variety within grapevines with a distinct purpose. It seeks to trace the origins of grape varieties, a task that involves exploring their genetic makeup, geographical distribution, and historical context. This field of study delves into the unique characteristics and histories of grapevine varieties, differentiating itself from traditional botanical descriptions. In the realm of production viticulture, winegrowers employ the term "grape variety" to refer to a population of grapevine individuals that share roughly similar growth and production characteristics. This term is widely used by those involved in the cultivation of grapes and the production of wine.

However, when it comes to official records and adherence to the International Code of Nomenclature for Cultivated Plants, a more specific term is utilized. In the Vine Register and in accordance with this international code, the term "cultivated vine variety" is preferred. This term, often abbreviated as "cultivar" or "cv," is employed to denote grapevine varieties in a more systematic and standardized manner. It helps ensure consistency and precision in the identification and documentation of grapevine varieties, particularly for official and regulatory purposes within the viticultural industry.

A cultivated grape variety, as defined by Davidis (1982), is a group of grapevine individuals deliberately chosen for specific desirable traits. These individuals typically exhibit a reasonably consistent level of similarity in terms of their physical appearance, viticultural behavior, and productivity. These characteristics are relatively stable over time. Cultivated grape varieties are typically the result of controlled breeding and propagation efforts, often involving more than one parent plant or clone. These varieties are typically propagated through methods such as grafting or cutting, as opposed to being grown from seeds. Over time, the shared characteristics of these grapevines have led to the establishment of a common name for the variety, reflecting its distinct attributes and qualities within the world of viticulture and winemaking.

Grapevine clones are indeed sets of individuals that have been propagated from a single parent plant, typically from a bud or an "eye," and selected for specific desirable characteristics. These characteristics can include attributes such as disease resistance, fruit quality, yield, or adaptability to specific growing conditions. The genetic and phenotypic variation within grapevine varieties can be significant, and this variation can make it challenging to maintain consistency in vineyards and wine production. By selecting and propagating specific clones, grape growers and winemakers can ensure a

higher degree of uniformity in their vineyards, which can be crucial for maintaining the quality and characteristics of a particular grape variety.

The work of organizations like the International Organisation of Vine and Wine (OIV) in establishing standards for clone selection and description helps in maintaining consistency and genetic stability within the grapevine industry. These standards can be important for the wine industry, as they contribute to the production of wines with consistent flavor profiles and characteristics, which is essential for branding and quality control.

In the past, when describing vineyards, people would often pick a grape variety sample rather arbitrarily from a population (called a holotype). However, in modern viticulture, the approach has evolved. Now, the sample chosen is carefully selected to represent the full range of physical characteristics found in the population of a specific grape variety at a particular growing location (referred to as a median sample). This variation in characteristics is influenced by the environment and growing conditions.

The transition from cultivating grape varieties with high genetic diversity, which can encompass a mix of different individual vines, to focusing on specific clones of grape varieties has become a critical development in modern and efficient viticulture.

In summary, the way we describe and select grapevine samples has changed. Instead of arbitrary choices, we now aim for samples that truly reflect the range of characteristics influenced by the environment. Additionally, the shift towards cultivating specific grapevine clones has significantly impacted modern viticulture, enhancing its productivity and precision.

AMPELOGRAPHIC DESCRIPTION

Describing different vine species and varieties in viticulture can be a challenging task. This difficulty arises from the need to define specific objectives and decide which characteristics to focus on when describing them. Moreover, the wide range of variations within vine species and varieties, along with the presence of multiple clones within cultivated *vinifera* varieties, further complicates the work of winegrowers. It underscores the importance of establishing a universally accepted system for describing vines.

In an effort to address this challenge, the International Organization of Vine and Wine (OIV), the International Union for the Protection of New Varieties of Plants (UPOV), and the International Commission for the Conservation of Biodiversity

(formerly known as the International Plant Genetic Resources Institute, IPGRI) collaborated to establish a Common Code of Vintage Descriptions (CVD) for identifying, distinguishing, and classifying vine species and varieties. This code was adopted in 1997. The International Code of Appellation (C.A.P.) is quite comprehensive, containing over 140 different characteristics. More than 24 of these characteristics are specifically used for describing the vine aspects of grapevine species and varieties. In its latest version, the OIV has expanded on this by including additional characteristics related to the vine's grapevine features, its resistance to diseases, isoenzyme systems, and molecular markers (as of OIV 2009).

In this code, each characteristic is linked to a unique OIV number, and its description is provided using specific terms that correspond to these designated numbers, which range from 1 to 9. This approach allows for the digitization of individual wine characteristics, making it feasible to integrate them into a software system, regardless of the specific nature of the characteristics being described. Some of the characters of OIV are given in the table.

Table 4. Some viticultural characters of the viticultural description code according to OIV (2009)

Code OIV	Viticultural Character	Biological Scale
001	Young shoot: Shoot tip type	3: closed, 5: moderately open, 7: open
003	Young shoot: Intensity of anthocyanin coloration on the prostrate hairs at the shoot tip	1: absent 3: weak 5: moderate 7: very strong 9: strong
004	Young shoot: Density of erect hairs at the shoot tip	1: absent 3: rare 5: moderate 7: dense 9: very dense
011	Shoot: Erect hair density on nodes	1: absent 3: rare 5: moderate 7: dense 9: very dense
016	Shoot: Number of consecutive tendrils	1: intermittent, 2: continuou
051	Young leaf: The colour of the upper surface of the blade (4th leaf)	1: green, 2: green with copper spots, 3: yellow 4: yellow with copper spots 5: yellow copper, 6: copper, 7: red
065	Mature leaf: The size of the blade	1: very small 3: small 5: medium 7: large 9: very large
067	Mature leaf: Shape of the blade	1: wedge-shaped, 2: heart-shaped, 3: pentagonal 4: circular, 5: kidney-shaped

068	Mature leaf: Number of lobs	1: none, 2: three, 3: five, 4: seven, 5: more than seven
072	Mature leaf: Shrinkage on the blade	1: absent, 9:presence
073	Mature leaf: Undulation between main and lateral veins on blade	1: absent 2: at the halfway point 3: everywhere
075	Mature leaf: Blistering on the upper surface of the blade	1: absent 3: weak 5: moderate 7: very strong 9: strong
080	Mature leaf: Shape of petiole sinus	1: U 2:V
085	Mature leaf: Density of erect hairs between the main veins on the lower surface of the blade	1: absent 3: rare 5: moderate 7: dense 9: very dense

BIOCHEMICAL METHODS

Biochemical methods have proven valuable for genetic marking, discrimination, and identification of various plant species, including grapevines. One notable technique involves separating proteins into their distinct isozyme forms using appropriate electrophoretic methods. This approach has also been employed in the study of grapevines. In fact, the application of this method to vines dates back to 1976 when Wolfe utilized grape juice as the electrophoretic material. Subsequently, in 1982, Stavrakakis extended the method's application by using pollen as the electrophoretic material.

By employing electrophoresis, the technique involves separating proteins based on their isozyme profiles. This process yields unique patterns that aid in distinguishing genetic variations and identifying different grapevine varieties. The pioneering work of Wolfe and the subsequent refinement by Stavrakakis highlight the significance of these biochemical methods in uncovering genetic insights within the grapevine species. Pollen serves as an advantageous choice due to several factors, including its straightforward extraction process and the reliability of electrophoretic patterns it offers. This reliability comes without some of the drawbacks associated with other materials, like its haploid nature and the challenges tied to collection and preservation. Despite these benefits, there are certain considerations to keep in mind.

For the successful implementation of this method, two conditions must be met, which simultaneously set limitations on its utility. The first prerequisite involves the

absence of substantial genetic diversity within cultivated grapevine varieties. Simultaneously, there should be evident genetic differentiation between these various grapevine varieties. The second requirement pertains to the stability of the electrophoretic zones within the studied tissues. These conditions are pivotal as they define the method's efficacy and applicability. The genetic landscape and the stability of electrophoretic zones stand as crucial factors that can impact the accuracy and interpretation of the results obtained through this technique.

To put it differently, the discussion revolves around both quantitative and qualitative alterations evident in the electrophoretic patterns as an individual grows and develops, or based on the particular stage and circumstances of tissue maturation. These changes are pivotal factors that constrain the method's application. Qualitative shifts pertain to the vanishing of specific electrophoretic bands or the emergence of new ones. On the other hand, quantitative shifts relate to variations in the extent and rapidity of the allozyme reaction, manifesting as differences in the intensity of the electrophoretic bands' appearance. The predicament posed by these quantitative and qualitative changes can be addressed by resorting to pollen as the genetic material for electrophoresis. The utilization of pollen helps bypass these challenges. Biochemical methods have found extensive application in the identification of various plant species, grapevines included. Enzyme polymorphisms, in particular, have been harnessed to investigate the genetic diversity present within and among Greek grape varieties. Researchers (Loukas et al., 1983; Stavrakakis and Loukas, 1983; Stavrakakis and Loukas, 1985, 1990, and 1991), have employed enzyme polymorphisms to delve into the genetic makeup of these grape varieties. By utilizing enzyme polymorphisms, these studies aimed to discern the variations in enzymatic profiles within the grapevine species. This approach enabled researchers to gain insights into the genetic differences existing both among different Greek grape varieties and within individual varieties. The methodology's effectiveness in uncovering genetic diversity underscores its significance in the realm of grapevine research and genetic analysis.

Certainly, as technology has progressed, the use of enzyme polymorphisms has encountered notable limitations. One primary reason for this limitation is the inherent susceptibility of proteins, which serve as markers and are generated through gene translation, to a range of influences. Notably, these influences encompass factors like the developmental stage of the plant.

The dynamic nature of protein expression throughout a plant's lifecycle poses challenges for using enzyme polymorphisms as consistent markers. Various developmental stages can cause fluctuations in protein expression levels, rendering them less reliable as markers for genetic analysis. This drawback has prompted researchers to seek more stable and accurate alternatives as technology has evolved. Despite its limitations, the application of enzyme polymorphisms played a foundational role in advancing our understanding of genetic diversity within grape varieties.

MOLECULAR METHODS

The remarkable diversity and global distribution of *Vitis vinifera* L. owe their origins to processes such as sexual reproduction, vegetative propagation, and somatic mutation. Understanding the source of grapevine biodiversity is crucial for comprehending its evolutionary trajectory and biological characteristics. Furthermore, this knowledge serves as a valuable resource for genetic enhancement, particularly when paired with molecular markers (Crespan, 2010).

When compared to traditional morphological descriptions, DNA-based molecular markers offer distinct advantages. These markers are less susceptible to environmental influences or developmental stages. Morphological traits, like those used in ampelographic and ampelometric methods, can be influenced by factors such as development and the environment. This can lead to uncertain attributions and potentially compromised results. In contrast, DNA-based methods remain unaffected by environmental conditions (Sefc et al., 2000; Carimi et al., 2010; Trosphin et al., 2015; Stajner et al., 2023).

While the identification of clones has traditionally relied on visual traits, the expression of these traits can be influenced by a variety of factors. This uncertainty in attributions can be mitigated by employing DNA-based methods, which provide more stable and accurate results. As a result, molecular markers have emerged as a powerful tool in understanding grapevine diversity, facilitating genetic improvements, and enhancing our insights into the evolution of *Vitis vinifera* L.

Molecular characterization has emerged as a preferred method for quantifying variation within germplasm samples (Emanuelli et al., 2013). In the 1990s, the introduction of microsatellite markers, also known as Simple Sequence Repeat (SSR) markers, brought forth a wealth of new insights into issues like synonyms and homonyms

ambiguities (Cipriani, 2008; Crespan, 2010). Microsatellites, prized for their polymorphism, reproducibility, and co-dominant nature, have risen to prominence as the markers of choice for consolidating, standardizing, and exchanging information related to grapevine genetic resources (This et al., 2004; Agar et al., 2012)

Numerous microsatellites have been developed for the *Vitis* genus, catering to a diverse range of applications such as mapping, genotyping, and breeding. (Thomas et al., 1993; Bowers, 1996; Scott et al., 2000) The use of microsatellites has contributed to enhancing our understanding of genetic diversity, facilitating more precise characterization of grapevine varieties, and enabling advancements in grapevine research and breeding efforts.

The genetic makeup of cultivated grapevines has been shaped by human selection, a phenomenon well-documented in research (Aradhya et al., 2003). This configuration can be likened to a complex pedigree due to the multitude of intricate higher-order pedigree relationships that exist (Myles et al., 2011). The presence of unknown connections between different grape cultivars adds complexity to the study of genetic structure, potentially leading to the overestimation of probable subpopulation numbers (referred to as "K") when using conventional methods (Pritchard et al., 2007).

Despite this complexity, there has been a dearth of comprehensive studies involving a wide-ranging sampling of Greek genetic resources. Such studies are essential to gain a comprehensive understanding of the intricate higher pedigree relationships among these resources. A hierarchical approach becomes imperative in order to delve deeper into the intricate relationships within the *Vitis* germplasm. This approach would allow researchers to untangle the web of relationships and better grasp the underlying genetic structure of these grape varieties. Numerous endeavors worldwide have focused on the molecular characterization of *Vitis* germplasm.

Microsatellites have proven invaluable in unraveling the genetic intricacies of numerous plant species, including grapevines. Over the recent years, a slew of researchers (Bowers, 1996, Sefc et al., 1998; Sefc et al., 1999; This et al., 2004; Merdinoglu et al., 2005) have spearheaded the development of microsatellite markers. The pioneering work of Thomas et al. (1993) unveiled the utility of repetitive DNA in identifying grapevine varieties. Subsequent research illuminated the co-dominant Mendelian inheritance pattern of microsatellite alleles in grapevines, enabling the confirmation of putative parentage through the scrutiny of their microsatellite profiles (Sefc et al., 2001).

Simultaneously, researchers find immense interest in exploring genetic connections, such as parentage, and constructing familial lineage among varieties. Microsatellites emerge as a valuable instrument in advancing these endeavors (Thomas et al, 1994; Bowers, 1997; Sefc et al., 1998; Bowers et al., 1999; Boursiquot et al., 2009).

Due to the remarkable sensitivity of microsatellite analysis, discovering two identical genotypes in distinct individuals serves as evidence that these individuals share the same variety. Consequently, microsatellite analysis not only aids in confirming the identity of a known variety but also facilitates the identification of an unknown variety by comparing its genotype with documented genotypes in established databases.

The high precision of microsatellite analysis enables the detection of matching genotypes in two distinct individuals, serving as evidence that these individuals share the same variety. Consequently, microsatellite analysis proves valuable not only in ascertaining variety identity but also in identifying unknown varieties through genotype comparisons with established databases. Furthermore, it facilitates the confirmation and differentiation of synonyms, as noted by Sefc et al. (2001), extending its capability beyond vine varieties according to Botta et al. (1995). Additionally, the Commission, having received input from stakeholders, confirms the previously stated conclusions in the absence of further comments. It is emphasized that the application of plant protection products derived from this analysis should be restricted to the production of the specific product, as outlined in 2013.

In Italy, lots of researchers (Zulimi et al., 2002; Cipriani et al., 2010; Crespan, 2010; Emanueli et al., 2013; Alba et al., 2014) successfully employed microsatellite markers to characterize Italian *Vitis* cultivars. A similar microsatellite analysis was carried out for Sicilian grapevine cultivars (Carimi et al., 2010). Moving to Anatolia, the genetic diversity of wild grapes was explored by Vouillamoz et al. (2006), while SSR markers were used to estimate the genetic diversity of *Vitis* cultivars from Eastern Turkey (Eyduran, et al., 2015). Microsatellites were employed for fingerprinting homonym grapevines in South-East Turkey (Karatas et al., 2007). In Iran, Fatahi et al. (2003) and Doulati-Baneh et al. (2013) delved into the genetic diversity of *Vitis* cultivars using SSR markers. Armenian, Georgian, (Frare et al., 2010) and Algerian *Vitis* cultivars were also identified utilizing SSR markers (Laiadi et al., 2009).

The assessment of microsatellite variability in grapevine cultivars across different European regions, including Greece, was undertaken by Sefc et al. (2000). Notably, Lefort and Roubelakis-Angelakis (2000) made significant strides in molecularly

identifying Greek *Vitis vinifera* L. cultivars through microsatellite profiling. These authors continued their genetic comparison of Greek *Vitis* cultivars using microsatellite markers. (Lefort et al., 2002; Anagnostopoulos, 2003; Avayanneli, 2005; Stavrakaki, 2008) A comprehensive approach involving ampelographic descriptions, oenological traits, and SSR markers was applied by Merkouropoulos et al. (2015) to study native Greek grapevine varieties.

Nonetheless, it's evident that there is limited data available for indigenous material from Crete, a historically significant viticulture region in Greece. Further research in this region would undoubtedly contribute to a more comprehensive understanding of the local grapevine genetic diversity and its historical context.

A worldwide initiative has been launched to actively gather, conserve, and assess historical grapevine cultivars and clones. This effort also involves the establishment of organized collections to safeguard these invaluable genetic resources.

PURPOSE OF WORK

The aim of the present study was to investigate the genetic diversity of the most well-known indigenous Cretan varieties, found in productive vineyards of Crete, and more specifically of Prefecture of Heraklion. Different samples from these varieties were collected in order to examine the genetic variability that exists within these grapevine varieties. It should be noted that besides the research and genetic interest of the identification and discrimination of the different biotypes of the Cretan varieties studied, the results of the present study have a great viticultural interest since they could constitute the base of the implementation of clonal selection program, thus exploiting the most appropriate clones as well as the preservation and exploitation of precious germplasm.

MATERIALS AND METHODS

PLANT MATERIAL

Fifty-three samples (53) collected from twenty-eight (28) natively inferred grapevine cultivars (*Vitis vinifera* L.) from Crete, in Greece, sourced from 4 different collection sites across the prefecture of Heraklion, on the island of Crete, were selected for this study (Figure 5).

The details for studied varieties, like their special characters and the areas from where they were collected are presented in the Table 5. The Greek grapevine varieties' authentic names are presented both in the Greek alphabet and in their ISO 843 transliteration. This transliteration standard, known as "Information and documentation – Conversion of Greek characters into Latin characters" (ISO 843:1997 [E]), was established by ISO in Geneva, Switzerland (Lefort et al., 2001).

Figure 5. Collection sites of indigenous inferred *Vitis vinifera* L. cultivars from Crete, Greece. Megali Vrysi, Agios Thomas, Moroni, Plouti, Asites

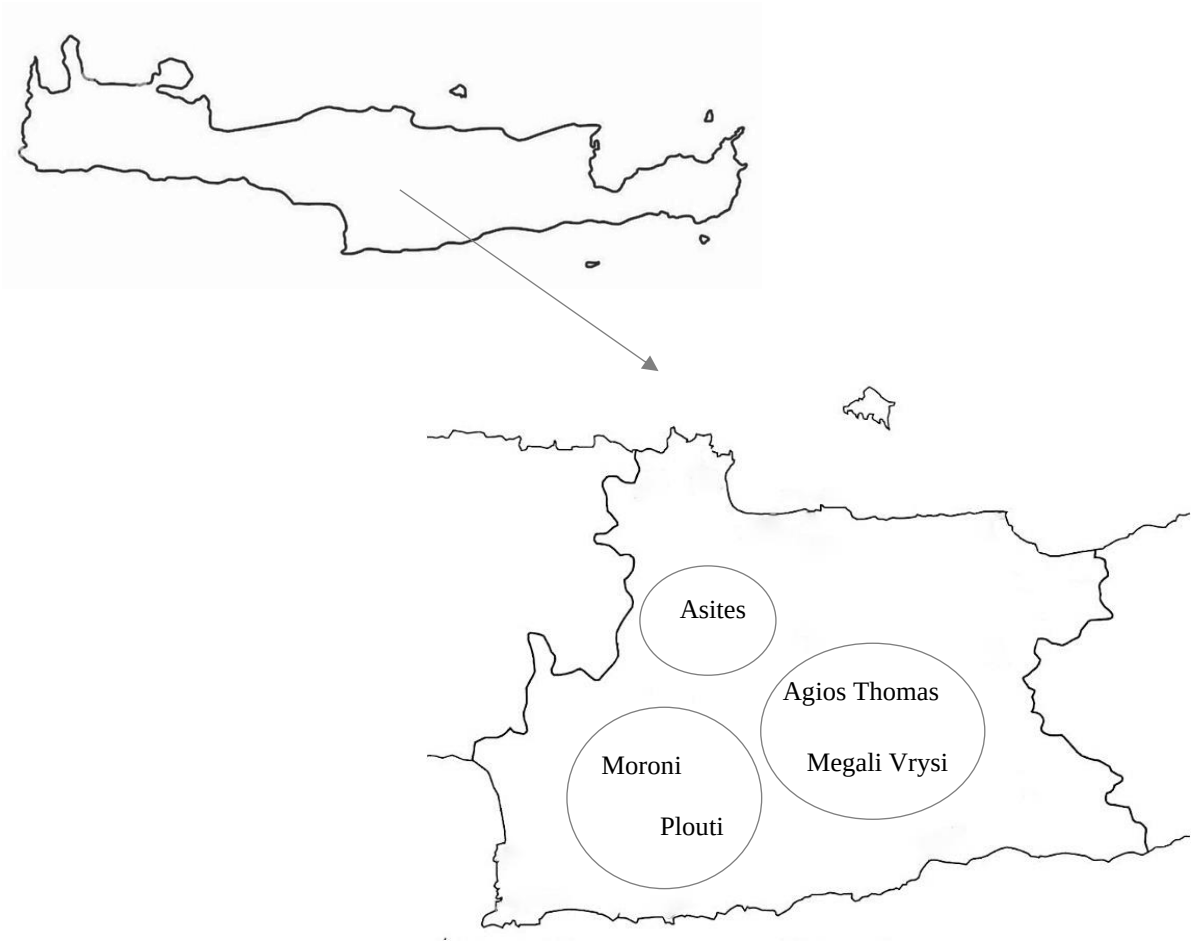

Table 5. The Greek name, the transliteration of the Greek name, the uses, the berry color, the number of individuals per cultivar, and the collection site of the 28 inferred *Vitis vinifera* L. cultivars

a/a	Greek Name	Name[1]	Use[2]	Berry Color[3]	Collection
1	Βηλάνα	Vilana	W	B	E1, E8, E20, V4
2	Βιδιανό	Vidiano	W	B	E2, E9, E15, E21, V23
3	Κοτσιφάλι	Kotsifali	W	N	E3, E10, E16, E22, V5
4	Λιάτικο	Liatiko	W	N	E4, E17, E23, V6
5	Κοτσιφολιάτικο	Kotsifoliatiko	W	N	E5, E25
6	Μανδηλαριά	Mandilaria	W	N	E6, E12, E18, E26, V14
7	Ταχτάς	Tachtas	W	B	E7, E14, E19, E27, E28
8	Δαφνί	Dafni	W	B	E11, V28
9	Πλυτό	Plyto	W	B	E13, V15
10	Ρωμαίκο	Romeiko	W	N	E29, V32
11	Τσαρδάνα	Tsardana	W	N	V2
12	Αθήρι	Athiri	W	B	V3
13	Μαλαγουζιά	Malagouzia	W	B	V9
14	Μοσχάτο Άσπρο	Moschato Aspro	W	B	V33
15	Λημνιό	Limnio	W	N	V40
16	Λημνιώνα	Limniona	W	N	V41
17	Πλατάνι	Platani	W	B	V52
18	Μπεγλέρι	Begleri	W	B	V61
19	Λακιδινό	Lakidino	W	N	V73
20	Εφτάκοιλο	Eftakoilo	W	N	V77
21	Λαγόρθι	Lagorthi	W	B	V85
22	Γαϊδουριά	Gaidouria	W	B	V88
23	Κατσανό	Katsano	W	B	V90
24	Αυγουλάτο Κρήτης	Avgoulato Crete	W	B	V94
25	Ασπρομανδηλαριά	Aspromandilaria	W	N	V91
26	Τσαούσι	Tsaousi	W	B	V100
27	Κρεβατίνα	Krevatina	W	Rs	E31
28	Ασύρτικο	Assyrtiko	W	B	V22

[1.] Transliteration of the Greek name, ISO 843–1997 transliteration scheme

[2.] Wine (W)

[3.] Blanc/white (B) rose/pink (Rs) rouge, bleu, noir/red, blue, black (N)

The experimental material utilized consisted of the young leaves from the primary shoots of the vines. Initially, an ampelographic description was conducted, followed by a macroscopic assessment of the health status of the vine. This assessment was performed both throughout the vegetation period and during the complete maturation of the crop. The goal was to identify and designate healthy and characteristic vines for each variety, which were subsequently chosen for further evaluation.

The age of the leaves has a notable impact on both the quality and quantity of extracted DNA. Through pertinent assessments and by consulting existing literature, it was determined that the optimal time for leaf collection is during the phase of swift shoot growth (Lodhi et al, 1994; Biniari, 2000). More than five samples were procured from each vine, with each sample stored in separate bags. Immediately following collection, the samples were swiftly placed in a portable dry-ice refrigerator (approximately -80°C), and subsequently relocated and stored in a deep-freeze refrigerator set at -80°C. This careful handling of samples helps preserve the integrity of the genetic material for subsequent analyses.

CRETAN VARIETIES

VILANA

Vilana stands as an ancient white grapevine cultivar exclusively grown within the vineyards of eastern Crete, likely since the 14th century or even earlier. Heraklion serves as its primary cultivation hub, while certain regions of Lasithi also embrace its growth. Vilana holds its roots firmly within the Cretan vineyard and is believed to have played a role in the production of Cretan Malvasia. Logothetis suggests that the name "Vilana" is derived from the dative singular "villano," a Latin term meaning "rustic." (Logothetis, 1965)

It's plausible that this name was bestowed upon the existing cultivar by the Venetians, either due to its resilience against dry and hot climatic conditions or due to its wines' quality, which might have been perceived as lesser compared to more distinguished grape varieties like Athiri and Liatiko. Vilana belongs to the "Asproudia" group, and its ampelographic characteristics closely align with Asproudi Patron, a variety found in Achaia. This alignment leads to the hypothesis that Vilana may have been known

by another name before the Venetian occupation of Crete, possibly being referred to as "Asproudi."

Vilana emerges as the predominant white cultivar in Crete, occupying an impressive 650 hectares. It stands out as one of the few Greek varieties that bears no synonyms. As the main white grapevine variety of Crete, Vilana is recommended for cultivation within the region (Stavrakaki and Stavrakakis, 2017).

Ampelographic Description

Shoot tip: open, yellow green with red margin, downy.
Young leaves: yellow green, with bronze-red areas, cobwebby on blade's upper surface, downy to felty on lower one.
Veins: green, cobwebby on leaf's upper surface, downy on lower one.
Young shoot: semi-erect to horizontal, ribbed, glabrous, and locally cobwebby, green on ventral side, green with reddish streaks on dorsal one.
Nodes: green with red pigmentation, glabrous.

Mature leaf: medium to large, circular to pentagonal, 5-lobed with V- or U-shaped, medium deep, closed and often naked petiolar sinus of overlapping edges. Superior lateral sinuses deep to very deep, U-shaped, closed, with overlapping edges. Inferior lateral sinuses medium deep, U-shaped, open. Leaf blade medium thick to thick, slightly crimped and bullate, twisted, involute, deep-green, glabrous, and locally cobwebby on upper surface, brown-green and downy on lower one. Main veins yellow-green, glabrous, and locally cobwebby on blade's upper surface, yellow-green and cobwebby on lower one. Teeth unequal, in 2 series, both sides convex, small to medium. Petiole short, slightly shorter than middle vein's length, thick, green-red, cobwebby. Tendrils intermittent, yellow-green, cobwebby, forked, and ternate, short to medium.
Bunch: medium to large, cylindrical -conical, with 1-2 wings, medium dense to dense. Peduncle short to medium, green, difficult to detach. Berry small to medium, globose, easy to detach from pedicel skin almost thin, yellow to yellow-white, medium to high bloom slightly firm, sweet, juicy pulp, colorless. Pedicel short, green. Seeds 1-3 (usually 2) per berry, small, with short and thick beak.
Cone: brown to red brown with darker nodes, ribbed; circular to elliptic cross section.
(Stavrakakis, 2021)

Cultivation Profile

Vilana grapevines exhibit a vigorous to very vigorous growth pattern, yielding 2 clusters per cane, usually emerging from the 4th to 6th node (rarely on the 3rd). This variety is characterized by a mid-early budding phase and mid-late ripening cycle. In modern vineyards, bilateral cordon training is implemented using vertical shoot positioned (VSP) trellis systems, accompanied by 2-node spur pruning. However, in instances of particularly vigorous vines, a recommended technique involves cane pruning to 5-node canes. For older rainfed vineyards, head training and 2-node spur pruning remain the norm.

No documented incompatibilities exist with established rootstocks. Vilana thrives when grafted onto rootstocks like 110R, 140Ru, 1103P, and 41B. The density of canopies, which can heighten the vines' vulnerability to fungal diseases and excessive production, can be managed by opting for lower-vigor rootstocks (such as SO4), coupled with canopy management techniques such as shoot thinning and topping.

Vilana prefers elevated northeastern regions characterized by sandy, calcareous, gravelly, and infertile soils. Cooler temperatures during the maturation phase also contribute to its success. In regions with heavy and fertile soils in lower elevations, where irrigation and fertilization practices are commonplace, production levels can often exceed 25 tons per hectare. This figure significantly surpasses the authorized production limit of 12 tons per hectare designated for Protected Designation of Origin (PDO) wines.

Vilana displays a moderate resistance to downy mildew while being susceptible to drought, powdery mildew, and Botrytis bunch rot. Grapes of the Vilana variety are utilized in the production of PDO Peza wines, characterized by their vibrant greenish-yellow color. These wines boast unique aromas encompassing herbs, citrus, and yellow fruit, culminating in a harmonious and richly delightful character. Vilana is also blended with Thrapsathiri grapes to craft PDO Sitia wines. Moreover, Vilana is authorized for the production of all of Crete's Protected Geographical Indication (PGI) Regional and District white wines.

Must composition typically includes sugar levels ranging from 190 to 210 g/L, total acidity (as tartaric acid) spanning 7.5 to 9.5 g/L, and pH levels between 3.6 and 3.8.



VIDIANO

Vidiano is a notable grapevine cultivar with a rich history on the island of Crete. It has been cultivated exclusively in Crete since at least the 13th or 14th century, although its origins may even date back further (Stavrakaki and Stavrakakis, 2017). Vidiano is considered one of the grape varieties that were used in the production of Cretan Malvasia, a renowned Cretan wine. The exact origin of Vidiano is uncertain, but it is believed to have undergone polyclonal synthesis, meaning it has been developed through the natural crossbreeding of different grape varieties. Genetically, Vidiano is closely related to the Lagorthi cultivar, and to a lesser extent, to the Thrapsathiri and Vilana varieties (Biniari and Stavrakakis, 2007). These relationships were identified in a lot of studies (Biniari and Stavrakakis, 2012; Bibi, et al., 2020; Avramidou et al., 2023.)

The name "Vidiano" is thought to have derived from the village of Avdou, located in the Heraklion region of Crete. This suggests a connection between the grapevine cultivar and the specific geographical area where it was cultivated. Vidiano has gained recognition and popularity in recent years for its quality in producing white wines. It is known for its aromatic profile, which typically includes notes of stone fruits, citrus, and floral elements. The grape variety is now considered one of the prominent indigenous grape cultivars of Crete and has contributed to the unique viticultural heritage of the island.

The name "Vidiano" is believed to have a connection to the village of Avdou, which is thought to have been named after the Prophet Avdiu (also known as Obadiah or Abdias). It is said that a monastery was founded in his name at the location of Lines.

Following this premise, a sequence is proposed to explain the evolution of the cultivar's name: Avdiu / Avdou / Avdano / Avidiano [the variety's first name] / Vidiano (Bibi, et al., 2020).

Based on this line of reasoning, it can be reasonably inferred that Vidiano was likely cultivated under a different name in the Heraklion and Lassithi Prefectures. From there, it is believed that the grapevine was introduced to Rethymnon by revolutionists or refugees from Avdou during the 14th century. This information is further supported by the presence of a small village called Avdanites in Mylopotamos.

Historically, Vidiano was grown sporadically in old vineyards that had various grape varieties. It was often misidentified or confused with other white cultivars such as Thrapsathiri and Vilana. However, in recent times, Vidiano has earned its reputation as one of the finest white wine cultivars in Crete. As a result, the cultivation of Vidiano in mono-varietal vineyards, particularly in the Heraklion Prefecture, has significantly increased (Stavrakaki and Stavrakakis, 2017).

Ampelographic Description

Shoot tip: open, white-green with red margin, downy.

Young leaves: yellow-green, with bronze areas, cobwebby to downy on leaf’s upper surface, felty on lower one.

Veins: green, cobwebby on blade’s upper surface, downy on lower one.

Young shoot: erect, fairly striate, cobwebby, green on ventral side, green with reddish streaks on dorsal one.

Nodes: green on ventral side, green with reddish streaks on dorsal one, cobwebby.

Mature leaf: medium, pentagonal to wedge- shaped, 5-lobed with V- or U-shaped, medium deep and closed, petiolar.sinus of almost overlap - ping edges. Superior and inferior lateral sinuses shallow to medium deep, V-shaped, almost closed. Petiolar junction red. Leaf blade thick, fairly bulupper surface, white-green. and downy on lower one. Main veins yellow-green, glabrous, and locally cobwebby on blade’sfipper surface, yellow-green and red at the base (bristly) on lower one. Teeth unequal, in 3 series, small to medium, combination between both sides convex (the longer ones) and both sides straight (the shorter ones). Petiole medium, almost equal to middle vein’s length, thick, green-red, cobwebby. Tendrils intermittent, green, cobwebby, usually ternate, medium.

Bunch: medium to large, cylindrical or conical-cy-lindrical, dense to medium dense. Peduncle short to medium, green with lignification (base only), difficult to detach. Berry

small to medium, obovoid to elliptic, difficult to detach from pedicel; skin medium thin, yellow-gold, low bloom (almost tiny); slightly firm, very sweet, and juicy pulp with thin flavor, colorless. Pedicel short, green. Seeds 1-3 (usually 2) per berry, small to medium, with very short beak.

Cane: red to brown-red to yellow-brown with darker nodes, fairly striate; elliptic cross section (Stavrakakis M., 2021).

Cultivation profile

This grape variety is characterized by its strong growth and moderate but inconsistent productivity. Typically, it produces one cluster per cane, which develops on the 4th or 5th node. It buds and ripens in the middle to early stages. In modern vineyards, this variety is trained using bilateral cordon on vertical shoot positioned (VSP) trellis systems. The vines are pruned to 2-3 node spurs. However, older vineyards, which rely on rainfall for irrigation and have their own rootstocks, adopt a head-training method. In this case, the vines are pruned to 2-node spurs. When dealing with highly vigorous vineyards, regardless of the training system employed, it is recommended to use cane pruning for balanced wine production. This involves pruning 5-8 node canes or utilizing a mixed approach with 2-node renewal spurs and 5-8 node canes per arm (Stavrakaki and Stavrakakis, 2017).

Vidiano, a grape variety similar to Thrapsathiri and Vilana, requires careful selection of grafting capes from authentic and identified Vidiano vines. There have been no reported incompatibilities with known rootstocks. It thrives when grafted onto rootstocks 11OR, 140Ru, or 41B. To prevent shedding, it is advisable to choose rootstocks with lower vigor. While Vidiano exhibits fair resistance to drought, it is highly susceptible to hot, strong winds. It demonstrates considerable resilience against downy mildew but is vulnerable to powdery mildew, leafroll virus diseases, and grape moths (Lobesia botrana). It grows well in various soil types but shows a preference for light, calcareous clayey-calcareous, and gravelly soils, particularly in hilly and cooler regions.

Climate change, specifically rising temperatures, is expected to have a severe impact on vineyards located at lower altitudes. However, in vineyards at higher elevations, Vidiano displayed remarkable resistance to hot weather conditions and retained its aromatic character, unlike Sauvignon blanc, which faced challenges during the 2015-2016 period in the Cretan vineyard.

Vidiano is susceptible to poor fruit set if a cold weather spell occurs during bloom, leading to low yields of only 25%. Single-varietal Vidiano wines exhibit a brilliant green and lemon color, showcasing rich fruit flavors and a complex bouquet of citrus aromas such as bergamot, along with hints of pear, banana, and melon. Even at an alcohol level of approximately 13%, Vidiano wines maintain their fresh acidity. Typically, Vidiano must is blended with other Cretan varieties like Plyto, Vilana, and Thrapsathiri, as well as foreign varieties such as Sauvignon blanc and Chardonnay.

In addition to its role in the production of white wines, Vidiano is also involved in the creation of regional and district wines, including PGI Chania, PGI Heraklion, and PGI Rethymnon.

When it comes to the must of Vidiano, it typically contains sugar levels ranging from 210 to 230 grams per liter. The total acidity, represented by tartaric and acetic acid, falls within the range of 6.5 to 7.5 grams per liter. The pH level of Vidiano must is around 3.2 to 3.3. These parameters contribute to the overall character and quality of the resulting wines.

LIATIKO

Liatiko stands as one of the oldest red-wine, polyclonal cultivars within the Cretan vineyard, along with Mandilaria. Its existence dates back to the 3rd (or potentially 2nd) century C.E. The wine produced from this cultivar was referred to as "*liatikos oenos,*" and its renown has closely paralleled that of Cretan Malvasia since the 13th century. The name "liatiko" is believed to have its origins in the Greek word for July (Ioulios → Iouliatiko → liatiko), denoting the fact that the cultivar matures in the initial weeks of

that month. In the Cretan context, "liatiko" is synonymous with "early maturation." Often, any fruit that ripens early is referred to as "liatiko." Interestingly, the term "Liatika" is also used to describe young boys who exhibit early signs of puberty, such as the growth of a mustache (Stavrakaki and Stavrakakis, 2017).

Ampelographic descriptions and molecular techniques have substantiated that Liatiko is a distinct cultivar, separate from Aleatiko as well as the cultivar bearing the scientifically questionable name "Kotsifoliatiko," an obscure French grape variety introduced to Crete during the 1980s amidst the phylloxera crisis. Liatiko is highly recommended for cultivation within Crete, where it has established itself as the most prevalent grape variety on the island. Additionally, it has received authorization for cultivation in the viticultural regions of Central Greece and Macedonia (Stavrakaki and Biniari, 2017).

Ampelographic Description

Shoot tip: open, yellow green with rose margin, felty.
Young leaves: yellow green, with bronze- red areas, cobwebby on blade's upper surface, downy to felty on lower one.
Veins: yellow-green, cobwebby on leaf's upper surface, green and downy on lower one.
Young shoot: semi-erect to horizontal, ribbed glabrous, green on ventral side, green with red, dish streaks on dorsal one.
Nodes: cobwebby, green on shoot's ventral side, green red on dorsal one.
Mature leaf: medium, pentagonal to slightly wedge-shaped, 5-lobed with V-shaped, closed, and deep petiolar sinus. Superior and inferior lateral sinuses medium deep to deep, closed, U-shaped, with tooth at base. Leaf blade medium thick, slightly bullate and crimped, involute, glabrous and locally cobwebby, deep green on up-per surface, brown-green and downy on lower one. Main veins yellow green to reddish at base, glabrous and locally cobwebby on blade's upper surface, cobwebby to downy on lower one. Teeth unequal, both sides straight, pointed, small. Petiole medium, equal to middle vein's length, thick, green-red, glabrous, and locally cobwebby. Tendrils intermittent, green-rose, glabrous and locally cobwebby, forked, and ternate, short to medium.
Bunch: medium, cylindrical-conical, dense. Peduncle short to medium, thick, green with me-dium lignification, easy to detach. Berry small to medium, globose, easy to detach from pedicel skin thin, red to red-violet, uniform, high bloom slightly firm and juicy pulp,

sweet to very sweet, colorless. Pedicel short, green. Seeds 2-3 per berry, medium, with short and thin beak.

Cone: yellow brown to brown-red with numerous freckles, slightly ribbed circular cross section (Stavrakakis, 2021).

Cultivation Profile

Liatiko is characterized as a vigorous and highly productive grapevine variety, typically bearing 2 clusters per cane, located between the 3rd and 6th nodes. Both blind and lateral buds exhibit fruitfulness. This grape variety is known for its early bud burst and very early maturation, marking it as Greece's earliest indigenous grapevine. In younger vineyards, bilateral cordon training is employed on vertical shoot positioned (VSP) trellis systems, coupled with spur pruning to 2-3 node spurs. In contrast, older vineyards opt for head training and spur pruning to 2-node spurs.

Notably, no recorded incompatibilities exist with established rootstocks. Liatiko thrives when grafted onto rootstocks such as 110R, 140Ru, and 1103P. Adverse cool weather during the bloom phase can lead to shot berries. To mitigate poor fruit set, shot berries, and lack of color, it is advisable to avoid virus-infected plants. Liatiko exhibits a preference for deep clayey-calcareous soils. It demonstrates resilience against drought and hot weather conditions, showcasing moderate resistance to downy mildew. However, it is notably susceptible to powdery mildew, Botrytis bunch rot, leafroll virus diseases, and Erinose (*Eriophyes vitis*) (Stavrakaki and Stavrakakis, 2017).

The berries of Liatiko are characterized by their low anthocyanin content (less than that of Kotsifali), elevated sugar levels, and a range from low to moderate acidity. Wines produced from Liatiko grapes may not possess intense coloration and can have a tendency to oxidize rapidly. However, these wines are distinguished by their distinctive aromas, a freshness ranging from moderate to high acidity, and soft tannins (Stavrakaki and Stavrakakis, 2017).

Liatiko finds application in both dry wines (PDO Dafnes) and sweet varietal wines crafted from sun-dried grapes (PDO Dafnes). Frequently, it is blended with the Mandilaria grape variety to create sweet red wines in eastern Crete (PDO Sitia). Furthermore, Liatiko grapes contribute to the production of all of Crete's PGI wines (Chania, Heraklion, Lasithi, Rethymnon). Must composition typically includes around

220-240 g/L of sugar, total acidity (as tartaric acid) ranging from 4.4 to 6.5 g/L, and pH levels spanning 3.3 to 3.9.



KOTSIFALI

Kotsifali is considered the most important red grapevine cultivar of the Cretan vineyard. Together with Liatiko, they constitute Crete's best-known indigenous, red cultivars. It has been cultivated mainly in central-eastern Crete for a very long time and possibly as far back as the 13th century although there are no data confirming that last premise. It is often said that its wines are typical of the "Mediterranean style". After the phylloxera crisis, it has been the wine variety most planted in Crete, occupying an overall 1,500 ha in surface area.

It was mentioned by Viala and Vermorel (1909) as Kotsifali or Kotsiphali and described ampelographically by Krimbas (1943).

Kotsifali is recommended for the viticultural areas of Crete, the Cyclades, and the island of Ikaria. Unlike other Cretan cultivars, Kotsifali has no synonyms, and its name derives from "kotsyfi", the Greek word for "blackbird" (*Turdus merula*). A cultivar known as Kaniskadiano is cultivated in western Crete and is identical (clone) to Kotsifali. The name Kaniskadiano may be a derivative of the word "kaniski", the diminutive form of the ancient Greek word κάνεον (kaneon: basket). In the past, Cretan tradition decreed that the gifts given a newly married couple include grapes of Kotsifali placed in a receptacle such as the "kaniski" (Stavrakaki and Stavrakakis, 2017).

Ampelographic Description

Shoot tip: open, white green with red margin, downy.
Young leaves: yellow green to green-red, cobwebby to downy on blade's upper surface, downy to felty on lower one. yellow-green, cobwebby on leaf's upper surface, green and downy on lower one.
Young shoot: almost erect, slightly striate, gla-brous and locally cobwebby, green on ventral side, green with reddish streaks on dorsal one.
Nodes: glabrous, green on shoot's ventral side, green red on dorsal one.
Mature leaf: medium to large, circular to pen-tagonal, 5-lobed (rarely 7-lobed) with U-shaped, deep and open petiolar sinus, with 1-2 teeth. Su-perior lateral sinuses deep to very deep, closed, U-shaped. Inferior lateral sinuses deep, closed, U-shaped. Leaf blade medium thick, slightly bul-late and twisted, often involute, glabrous, deep green on upper surface, green-brown and cob-webby to downy on lower one. Main veins green to reddish at base, glabrous and locally cobwebby on blade's upper surface, pubescent (bristly) on lower one. Teeth unequal, both sides convex (the longer ones), hooked (the shorter ones), small. Petiole short, shorter than middle vein's length, thick, green-red, glabrous, and locally cobwebby. Tendrils intermittent, green-red, glabrous and locally cobwebby, forked and rarely ternate, short.
Bunch: medium, conical to cylindrical-conical, dense, with small, green berries. Peduncle short, thick, green with good lignification, difficult to detach. Berry small to medium, elliptic, easy to de-tach from pedicel; skin medium thin, red-violet to red black, not uniform, high bloom; slightly firm and juicy pulp, very sweet, colorless, slightly sour. Pedicel short, green. Seeds 1-3 (usually 1) per berry, medium with short and thin beak.
Cone: yellow brown to brown-red with numerous lenticels, slightly striate circular cross section (Stavrakakis, 2021).

Cultivation Profile

Kotsifali stands as a vigorous and remarkably productive wine grape variety, typically bearing 2 clusters per cane, occasionally 3, emerging between the 3rd and 5th nodes. Both blind and lateral buds exhibit fertility. This variety buds in the mid-late period and ripens in the mid-early stage. Younger vineyards adopt bilateral cordon training on vertical shoot positioned (VSP) trellis systems, with spur pruning maintained at 2-3 node

spurs. Conversely, older minimally irrigated vineyards adopt head training, coupled with spur pruning to 2-node spurs.

Incompatibility concerns with established rootstocks have not been recorded. Kotsifali thrives when grafted onto rootstocks like 110R, 140Ru, 1103P, and 41B. During the bloom phase, cooler weather can result in shot berries. To prevent compromised fruit set, shot berries, and reduced color quality, it is advisable to steer clear of virus-infected plants. Kotsifali thrives in deep clayey-calcareous soils, as well as gravelly and even drought-prone soils on hills and elevated slopes. This variety demonstrates resilience against drought and hot weather conditions, showcasing a moderate level of resistance to downy mildew. Conversely, it is sensitive to powdery mildew, Botrytis bunch rot, leafroll virus diseases, and Erinose (*Eriophyes vitis*). Notably, a distinctive symptom of littleleaf disease is observed near the shoot's edge during the initial stages of maturation, likely resulting from natural factors or viral infections (Stavrakaki and Stavrakakis, 2017).

The berries of Kotsifali are characterized by their low anthocyanin content (ranging between 600 and 640 mg/kg berries), elevated sugar levels, and a lack of acidity. Wines produced from Kotsifali grapes are notable for their elevated alcohol content, intense aromas, soft texture, minimal acidity, and inherent color instability. Renowned Crete PDO wines like Archanes and Peza are crafted through the co-maceration of Kotsifali and Mandilari grapes. Contemporary winemaking practices often involve blending Kotsifali with Syrah. Additionally, Kotsifali plays a role in Crete's PGI wines (Stavrakaki and Stavrakakis, 2017).

The varietal wine of Kotsifali is marked by its ruby hue with subtle garnet undertones. The aroma is intense and enduring, punctuated by notes of red fruit, sweet spices, and a touch of leather. Must composition typically range from 230 to 250 g/L of sugar, acidity (as tartaric acid) between 3.8 and 5.5 g/L, and pH levels spanning 3.4 to 3.7.

MANDILARIA

Mandilaria is deeply rooted in the history of the Cretan vineyard, boasting an ancient and diverse origin. Its wide distribution across Greece has resulted in numerous synonyms, with several unique cultivars like Agianniotiko, Thrapsa, Kotselina, Pappoudes, Mouchtaro, Mavro (Black) Kymis, and Mavro (Black) Spetson either being clones of Mandilaria or closely related genetically. This pattern is common, where as a variety expands, new names emerge, often referencing its place of origin or the characteristics of its bunches and berries (Stavrakaki and Stavrakakis, 2017).

Among its various synonyms, Mandilaros and Mandilari are prevalent in Crete, Amorgiano alludes to the island of Amorgos in the south Aegean, Dombrena or Doubraina mavri (black) refers to the village Dovrena in Viotia, and Kountoura or Koundoura mavri (black) signifies areas like Attica and the island of Evvoia. The name "Koundoura" derives from the Turkish word "kundura," which means a woman's slipper. This is due to the appearance of the vines after winter pruning, notably in places like Spata (Attica) and the island of Evvoia. A similar reference is observed with the term "Koundoura aspri (white)," which is a key synonym for the white cultivar Savvatiano in Attica and Evvoia (Koukkidis, 1960).

According to Krimbas (1943), the name "Mandilaria" most likely stems from the shape of a slipper that the bunch resembles when the wings of the primary bunch develop. The name's origin can also be traced to the Cretan idiomatic word "mantila," which refers to a square piece of cloth or a cloth napkin, or "mantiles," which pertains to the head covers worn by Cretan men. Both interpretations hold merit: historically, laborers in Crete would employ the "mantila" to wrap their midday meal, which usually comprised simple fare like bread and olives.

The term "mantila" also had practical uses during harvest season, as farm laborers utilized it to wrap freshly harvested grapes. The sizable and substantial bunches of Mandilaria grapes would entirely occupy the cloth square, providing a fitting reason for the name "Mandilaria" to be bestowed upon the grape variety (Stavrakaki and Stavrakakis, 2017).

Mandilaria is suggested for cultivation across seven of Greece's eleven viticultural regions: the Peloponnese, Central Greece, Thessaly, Northern Aegean, the Cyclades, the Dodecanese, and Crete.

Ampelographic Description

Shoot tip: open, green with red margin, downy to felty.
Young leaves: yellow green, with red-bronze areas, cobwebby on leaf's upper surface, downy to felty on lower one.
Veins: green, cobwebby on leaf's upper surface, downy on lower one.
Young shoot: semi-erect to horizontal, glabrous, or slightly cobwebby, smooth to slightly ribbed, green on ventral side, green with red streaks on dorsal one.
Nodes: glabrous or slightly cobwebby, green on shoot's ventral side, red-violet on dorsal one.
Mature leaf: medium, circular to pentagonal, 5-lobed, with V-shaped, closed petiolar sinus of slightly overlapping edges. Superior lateral sinuses medium deep, V-shaped. Inferior lateral sinuses, shallow, open, V-shaped. Leaf blade medium thick, soft, slightly bullate, crimped and wavy, involute, glabrous, deep-green on upper surface, brown-green and downy to felty on lower one. Main veins green to yellow-green, cobwebby on blade's upper surface, yellow-green, cob-webby to downy and locally (on veins' junction) pubescent (bristly) on lower one. Teeth unequal, numerous, both sides convex, small to medium. Petiole short, equal to or slightly shorter than middle vein's length, thick, green-red, cobwebby. Tendrils intermittent, yellow-green, pubescent (bristly) and locally cobwebby, forked and ternate, medium, thick.
Bunch: medium to large, conical-cylindrical, of-ten with wings, dense to very dense, with shot berries. Peduncle very short, thick, green-rose, with good lignification, difficult to detach. Berry medium to large, globose to obloid, difficult to de-tach from pedicel; medium thick, blue-black skin; tiny, uniform, high to very high bloom slightly firm and medium juicy pulp, sweet to slight sour (or with a sub-acid taste), colorless. Pedicel short, green. Seeds medium, 2-3 per berry, medium to large, with thick beak.
Cone: yellow-brown to brown with darker brown nodes, slightly ribbed; circular to elliptic cross section (Stavrakakis, 2021).

Cultivation Profile

This red wine variety is known for its vigorous growth and high productivity. It typically produces 1 to 2 clusters per cane, situated on the 3rd and 4th nodes. Both the blind and lateral buds can temporarily bear fruit. This variety buds late and ripens late as

well. In newer vineyards, it's trained using a bilateral cordon system on vertical shoot positioned trellises (VSP), while older vineyards tend to be head-trained. In either case, vines are pruned to 2-3 node spurs to strike a balance between canopy and production. Winter pruning involves two spurs per arm, with 2-3 nodes each, aiming to avoid overly vigorous canopy growth. No issues have been reported with grafting onto various rootstocks, including 1103P, 110R, 140Ru, and 41B.

The quality of the resulting wines isn't solely determined by climate, soil conditions, and viticultural practices but also by the specific clones cultivated. The Pariano Mandilari clone is particularly esteemed. Optimal growth sites are dry and infertile, or warm with low altitudes to aid sugar accumulation. In these conditions, Mandilaria produces high-quality wines. However, at higher altitudes and in fertile soils, it tends to overproduce, resulting in lower sugar content, delayed ripening, and susceptibility to *Oidium* and *Botrytis* infections.

Mandilaria thrives in dry conditions and displays moderate resistance to powdery mildew. Nevertheless, it's vulnerable to *Botrytis* bunch rot and downy mildew. Its deep color makes it one of Greece's most intensely colored varieties, second only to Vertzami and Mavrodafni. The thick-skinned berries are rich in anthocyanins (900-1,100 mg/kg) and total phenols (1,900-2,100 mg/kg). The must has low sugar content, relatively high total acidity, and a moderately low pH.

Mandilaria's vibrant color makes it a suitable partner for blending with other grapes. For instance, it's often blended with Kotsifali grapes to enhance color, tannin levels, alcohol content, and produce remarkable wines like PDO Archanes and PDO Peza. Additionally, Mandilaria grapes can be mixed with other varieties, including white ones like Monemvasia, to create wines such as the red PDO Paros. This unique blend comprises 80% Monemvasia and 20% Mandilaria.

When made into a single varietal wine (PDO Rhodes), Mandilaria exhibits moderate acidity, a light palate, and subtle aromas and flavors. The wine boasts a lively cherry pink hue and abundant aromas of red fruits like raspberries and cherries, with delicate hints of licorice and candy. It's refreshingly crisp on the palate and maintains a firm texture. Must characteristics include sugar levels of 180-200 g/L, total acidity (tartaric acid) ranging from 5.8-7.8 g/L, and a pH of 3.2-3.3.

DAFNI

Dafni stands out as a unique type of white wine grape. Its origins trace back to the beautiful island of Crete, where it has likely been cultivated since the 15th century or even earlier. Remarkably, Dafni shares its mysterious history with other grape varieties native to Crete. Intriguingly, records from the 6th (or 7th) to the 11th century reveal little about its name or existence, leaving a gap in its historical narrative.

The name "Dafni" finds its roots in either the verdant shade of its leaves or the captivating essence that emanates from its must. Both of these attributes bear a striking resemblance to the renowned bay laurel (*Laurus nobilis*) (Stavrakaki and Stavrakakis, 2017).

Contrary to assumptions, there exists inadequate evidence to substantiate the theory that the variety and its name find their origins within a specific geographical region or community, whether it be the Peloponnese's Lakonia, the captivating island of Crete, or the historic expanse of Attica. In recent times, the cultivation area for Dafni in Crete has undergone a gradual expansion; however, it remains confined to a meager extent, barely exceeding 0.5 hectares. Recommendations for its cultivation are centered on Crete, with authorization extending to the Cyclades islands. A more recent proposition posits that Dafni might arise from the union of Ferral (Prunesta rosso violacea) and Syriki (Assouad karech) through physical hybridization (Lacombe et al., 2013).

Nevertheless, the lack of a comprehensive ampelographic depiction for the Dafni cultivar leaves lingering uncertainties regarding the authentic identity of the studied Dafni specimens. Remarkably, "Dafnia" stands as the sole synonym for the Dafni cultivar.

Ampelographic Description

Shoot tip: open, yellow green to green with red margin, cobwebby to downy.
Young leaves: yellow-green with bronze areas, glabrous on both sides.
Veins: green, cobwebby on leaf's upper surface, pubescent (bristly) on lower one.
Young shoot: semi-erect, glabrous, smooth to slightly ribbed, green on ventral side, green with reddish streaks on dorsal one.
Nodes: green on ventral side, green with reddish pigmentation on dorsal one, glabrous.
Mature leaf: medium, pentagonal to wedge-shaped, 5-lobed with open U- or lyre-shaped petiolar sinus. Superior lateral sinuses, shallow to medium deep, V-shaped. Inferior sinuses shallow, open, V-shaped. Leaf blade almost thin, slightly wavy (twisted), bullate, glabrous on both sides, green to deep green on upper surface, light green on lower one. Main veins green, glabrous on blade's upper surface, yellow-green, pubescent (bristly) on lower one. Teeth unequal, in 2 series, both sides straight, small to medium, with thorn (pickle). Petiole short to medium, slightly shorter than or equal to middle vein's length, thick, green with green-red base, glabrous and locally pubescent (bristly). Tendrils intermittent, green, glabrous, forked, long.
Bunch: large to very large, conical, often winged, or double, medium dense to fairly loose. Peduncle medium to large, long, green with sufficient lignification, often with tendril, easy to detach. Berry medium to large, globose to obloid, easy to de-tach from pedicel skin medium thick with light bloom, green to yellow-green slightly firm and juicy pulp, medium sweet, colorless, with thin but characteristic flavor. Pedicel short and green. Seeds medium, 3-4 (usually 4) per berry, with conical beak.
Cone: brown with darker brown nodes, ribbed, often of late and insufficient lignification; circular cross section (Stavrakakis, 2021).

Cultivation profile

This grapevine variety exhibits a robust to highly vigorous growth pattern, contributing significantly to its impressive productivity. Each cane of this variety typically bears a single cluster of grapes, a feature commonly observed on the 4th or 5th node, often on 5th node.

Manifesting both early-budding tendencies and a unique ripening behavior spanning from mid-late to late in the growing season, this variety encompasses a distinctive temporal aspect in its development (Stavrakaki and Stavrakakis, 2017). In the traditional multi-varietal vineyards of Crete, the practice of head training is prevalent. This method encourages the central stem of the grapevine to ascend vertically, accompanied by lateral branches or canes extending outward. This framework fosters an open canopy arrangement, supporting improved air circulation and optimum sunlight exposure critical elements for grapevine health. Contemporary vineyards embrace an evolved approach, adopting bilateral cordon training that aligns with vertical shoot positioned (VSP) trellis systems. The utilization of either spur pruning or cane pruning in these vineyards serves to strike a harmonious equilibrium between fruit production and vegetative growth. For more robust biotypes and enhanced outcomes, the application of the lyre (Y) system proves advantageous. This system enhances canopy growth and optimizes fruit exposure to sunlight, promoting an ideal environment for grape development (Stavrakaki and Stavrakakis, 2017).

To augment maturation and mitigate the risk of Botrytis bunch rot, leaf removal comes into play as an effective strategy. Selectively removing leaves improves the maturation process and reduces susceptibility to fungal infections.

The irrigation strategy merits keen attention, as improper water application can lead to compact bunches, uneven berry coloration, and delayed ripening. A cautious approach to irrigation is vital for optimal grape development (Stavrakaki and Stavrakakis, 2017).

It's important to note that this variety shows compatibility with known rootstocks. It thrives particularly well when grafted onto rootstocks 110R, 140Ru, and 1103P, especially in hilly plantings or regions with limited soil depth or fertility. The variety demonstrates moderate resistance to drought, reasonable resistance to powdery mildew and downy mildew, and susceptibility to Botrytis bunch rot (Stavrakaki and Stavrakakis, 2017).

The must extracted from Dafni grapes serves as the foundation for crafting a distinct varietal wine with a hint of laurel fragrance. This wine exhibits a moderate to low alcohol and acidity content, coupled with a delightful flavor profile. Often blended with Vilana, the varietal wine emanating from Dafni grapes features a whitish-yellow hue and a profound, enduring aroma that unequivocally signals its rich and balanced nature. Must analysis reveals sugar content ranging from 190 to 210 g/L, total acidity (as tartaric acid) between 4.5 and 5.5 g/L, and a pH level of 3.8 to 4.0.

PLYTO

Originating from the island of Crete, the enigmatic grapevine known as "Plyto" has deep roots dating back to the 14th century. It is an ancient white grape cultivar that has remained intricately entwined with the island's history and viticulture. The grapes of Plyto were once harmoniously mingled with other varietals, contributing to the creation of the esteemed Cretan Malvasia wine, celebrated for its excellence (Stavrakaki and Stavrakakis, 2017).

Subsequently, specific biotypes (or genetic duplicates) of Plyto underwent transplantation, assuming the identity of Kritiko aspro (with a white hue) in the Cyclades region. In a parallel endeavor, Plyto was introduced as Ploto on the island of Kythira, and as Kitrinovaria (or Asprovaria) in the Peloponnese. Plyto represents a diverse range resulting from polyclonal synthesis, encompassing an intriguing assemblage of cultivars, which are either closely interlinked (Platani, Katsano, Petrolanos) or indistinguishable (Ploto, Kitrinovaria, Asprovaria).

Krimbas (1943) was the initial author to delineate the variation between Plyto and Ploto. These two variants exhibit slight variations in their ampelographic traits. Notably, Ploto grapes display a conical bunch shape, while Plyto grapes do not. In terms of berry shape, Ploto grapes tend to be obloid, whereas Plyto grapes differ in this aspect. Additionally, the number of seeds diverges between the two, with Ploto grapes commonly having around 4 seeds, unlike Plyto grapes. The cultivar Pluto, once cultivated in Kerkyra (Corfu), (Viala et al., 1909) however, it has since disappeared. In the region of Heraklion Prefecture, the grape variety Plyto was referred to as Plytho. (Fragaki, 1969)

The term "Plyto" finds its likely origin in the Greek verbs "plyno" or "pleno," both of which denote the action of washing. (Babiniotis, 2009) The Greek verb "plyno" and its related forms such as "pleo" (to sail), "plotos" (floating), and "ploutos" (wealth) encompass concepts associated with water abundance. This concept can be linked to the ample and watery berries of the Plyto grape variety. Moreover, these terms underscore notions of brilliance and clarity, much like the essence of being "washed" (as in "plyno plymenos"). This progression is reflected in the transformation from "plyno" to "ploutos" to "plouto" to "plytho" and eventually to "plyto." Plyto grape variety has gained recognition and approval for cultivation in various regions. Initially recommended for viticultural zones in Crete, it has also received authorization for cultivation in Kythira and within the Cyclades. Notably, the name "Plyto" was later altered to "Ploto."

Ampelographic Description

Shoot tip: open, green with red margin downy

Young leaves: green, with bronze areas, cobwebby to downy on blade's upper surface, felty on lower one.

Veins: green, cobwebby to downy on leaf's upper surface, white-green and downy to felty on lower one.

Young shoot: semi-erect to horizontal, striate, glabrous and locally cobwebby, green on ventral side, green with reddish streaks on dorsal one

Nodes: green with red pigmentation, glabrous, and locally cobwebby.

Mature leaf: medium to large, pentagonal, 5-lobed with V-shaped, medium deep, closed petiolar sinus of overlapping edges. Superior lateral sinuses deep, U-shaped, almost closed, with overlapping edges. Inferior lateral sinuses medium deep to shallow, U-shaped, open. Leaf blade thick, crimped and bullate, twisted, deep-green, glabrous, and

locally cobwebby on upper sur-face, brown-green and downy on lower one. Main veins yellow-green, glabrous, locally cobwebby on blade's upper surface, pubescent (bristly and velvety) on lower one. Teeth unequal, both sides convex (the longer ones), both sides straight (the shorter ones). Petiole short to medium, far shorter than middle vein's length, thick, green-red, cobwebby. Tendrils intermittent, green-red, cobwebby, medium, forked, and ternate.

Bunch: large, cylindrical-conical, with 1-2 wings, dense to very dense. Peduncle very short, green, difficult to detach. Berry medium to large, globose to obovoid, difficult to detach from pedicel; skin almost thick, yellow to yellow-gold, medium to high bloom, with brown lenticels; slightly firm, sweet, and very juicy pulp, colorless. Pedicel short, green. Seeds 2-3 per berry, medium, con-vex, with large beak.

Cane: yellow-brown with darker nodes, striate; circular to elliptic cross section (Stavrakakis, 2021).

Cultivation profile

Plyto is characterized as a vigorously growing to very vigorous grape variety, displaying high productivity. Typically, it yields 1-2 clusters per cane, commonly on the 4th and 5th nodes, occasionally on the 3rd node. Both blind and lateral buds exhibit fertility. This variety boasts an early bud burst and early maturation, occurring roughly two weeks ahead of Vilana. In modern vineyards, bilateral cordon training is employed with vertical shoot positioned (VSP) trellis systems, adopting spur pruning to 1-2 node spurs. Conversely, older rain-fed vineyards utilize head-training and spur pruning to 2-node spurs. (Stavrakaki and Stavrakakis, 2017)

No reported incompatibilities exist with established rootstocks. Plyto thrives when grafted onto rootstocks such as 110R, 140Ru, and 41B. It flourishes in higher elevated regions, characterized by calcareous, gravelly, moderately fertile (even potentially drought-prone) soils, and cooler summer temperatures. Nonetheless, it is vulnerable to hot weather conditions. Preventive measures should be taken against sunburn, particularly for bunches situated high on the vine, which could be exposed to excessive sunlight. Training systems like open T or open Y are not recommended due to this susceptibility.

Regarding disease resistance, Plyto exhibits notable resistance to powdery and downy mildew, while displaying moderate susceptibility to Eutypa dieback. However, it

is highly susceptible to Botrytis bunch rot and the grape berry moth (Lobesia botrana), attributed to the density of its clusters. The grapes of Plyto find application in crafting single-varietal wines characterized by their straw-pale yellow hue, complex aroma with both intensity and quality, and a well-balanced flavor profile (Stavrakaki and Stavrakakis, 2017).

For the production of Crete's Regional wines (PGI Crete) and District wines (PGI Heraklion, PGI Lasithi), Plyto has received authorization. Must composition typically ranges around 200-210 g/L of sugar, total acidity (as tartaric acid) within 5.5-7.5 g/L, and pH levels between 3.5 and 3.6.

TACHTAS

Tachtas is an ancient indigenous grape variety that has been exclusively cultivated in the Cretan vineyard, likely since the 16th century or even earlier. Its name's origin is believed to derive from the Turkish word "tahta" (wood), possibly due to the woodiness of its canes, or from the Greek word "tachtarisma" (dandling), stemming from the Turkish "tahtiri". (Babiniotis, 2009) This could allude to the resemblance between Tachtas' plump grape clusters and the endearing plumpness of babies. Interestingly, Tachtas grapes were once used in the production of the renowned Cretan Malvasia wine. (Stavrakaki and Stavrakakis, 2017)

Historically, Tachtas was extensively cultivated for table grapes until the late 1950s, primarily because of the desirable qualities of its berries, which bore a resemblance to those of the Razaki variety. This resemblance led to instances of Tachtas cuttings being mistakenly transferred to other regions of Greece as Razaki. A significant

portion of the Tachtas harvest was also earmarked for raisin production. During the drying process, the grape bunches were immersed in an alkaline solution due to the size and thickness of the berries. Following treatment with sulfur dioxide (SO_2), the resulting raisins took on a light golden hue and were termed "elemes" (or "elernedes"), suggesting a product of superior quality. The Tachtas cultivar itself was known as Kourou-Tachtas, with "kourou" meaning "dried" in Turkish. (Stavrakaki and Stavrakakis, 2017) In the present day, Tachtas grapes are utilized for winemaking purposes. The aromatic nature of the berries suggests the possibility of producing a quality varietal wine from Tachtas, given certain conditions.

Ampelographic Description

Shoot tip: open, white-green with red margin, downy.
Young leaves: yellow-green, with bronze-red areas, glabrous and locally cobwebby on blade's upper surface, downy on lower one
Veins: green, glabrous on leaf's upper surface, cobwebby on lower one.
Young shoot: semi-erect, ribbed, glabrous and locally cobwebby, green on ventral side, green with reddish streaks on dorsal one.
Nodes: glabrous, green on shoot's ventral side, green-red on dorsal one.
Mature leaf: medium, pentagonal to wedge-shaped, 5-lobed with V-shaped, medium deep, often closed petiolar sinus of overlapping edges. Superior lateral sinuses medium deep, V- or U-shaped, almost closed. Inferior lateral sinuses shallow, V- or U-shaped, open. Petiolar junction red. Leaf blade medium thick to thick, bullate and crimped, deep-green glabrous and locally cobwebby on upper surface, brown-green and downy on lower one. Main veins green to yellow-green, red at base, glabrous and locally cobwebby on blade's upper surface, pubescent (velvety) on lower one. Teeth unequal, medium, both sides straight (the longer ones), hooked (the shorter ones). Petiole medium, slightly shorter than middle vein's length, thick, green-red, glabrous, and locally cobwebby. Tendrils intermittent, green-rose, cobwebby, forked and ternate, long to very long.
Bunch: medium to large (depending on biotype), cylindrical-conical, medium dense to dense. Peduncle short, green with lignification (base only), difficult to detach. Berry large, ovoid, difficult to detach from pedicel skin thick, rich in tannins, yellow-green to yellow-gold, with medium bloom; very firm, sweet, and medium juicy pulp, colorless,

with thin flavor. Pedicel short, green. Seeds 2-4 (usually 2) per berry, with short and pointed beak.

Cane: brown-red with darker nodes, fairly striate; circular cross section (Stavrakakis, 2021).

Cultivation Profile

Tachtas is characterized as a vigorous and productive grape variety, typically yielding 1 cluster per cane on the 4^{th} or 5^{th} node. This variety exhibits a late-budding and mid-late-ripening pattern. Notably, often fertile blind buds, especially lateral ones, contribute to its growth, and the lateral shoots bear numerous small bunches. In modern vineyards, Tachtas is trained using bilateral cordon on vertical shoot positioned (VSP) trellis systems, adopting spur pruning with 2-node spurs. Conversely, older rainfed vineyards employ head training, often featuring taller trunks due to the sizable clusters, and spur pruning to 2-node spurs (Stavrakakis, 2019).

Tachtas thrives in elevated regions with calcareous, gravelly, and moderately fertile soils. These conditions, coupled with cooler summer temperatures, foster the production of high-quality grapes with distinct aromas. No recorded incompatibilities exist with established rootstocks. It performs well when grafted onto rootstocks like 110R, 420A, and 41B.

It demonstrates moderate resistance to downy mildew and susceptibility to various conditions including drought, hot weather, windy environments, powdery mildew, *Botrytis* bunch rot, and Erinose (*Eriophyes vitis*). Furthermore, it is highly susceptible to leafroll virus diseases (Stavrakakis, 2019).

The must produced from Tachtas grapes can be utilized in a variety of Protected Geographical Indication (PGI) wines crafted in Crete. The composition of must typically includes sugar levels ranging from 210 to 230 g/L, total acidity (as tartaric acid) within 4.5 to 5.8 g/L, and pH levels between 4.6 and 4.8.

DNA EXTRACTION

The extraction of DNA from vine leaves poses significant difficulties due to the presence of polyphenols and polysaccharides in the cells. To overcome these difficulties, various methods were initially applied although DNA was successfully isolated, the yield and purity were not adequate.

The procedure for isolation was executed following the protocol outlined by Thomas et al. (1993), with certain modifications introduced by Biniari (2000).

For DNA extraction process was utilized 1g of young leaf tissue per vine sample, which was grinded with liquid nitrogen and then was homogenized in 12,5 mL of Buffer solution 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 procured through centrifugation at 7000 rpm for 10 minutes at 4^{o}C which was redissolved in 2,5 mL of Buffer solution 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 (MW 40.000), 3% sarcosyl, 20% ethanol] and was incubated at 37^{o}C for 45 minutes with regular shaking. Afterwards an equal amount (2,5 mL) of chloroform/isoamyl alcohol (24:1) was swiftly blended in through a quick vortex and the layers were separated through centrifugation at 12,000 rpm for 20 minutes. The aqueous phase was harvested and mixed with 1,6 mL of isopropanol (-20^{o}C) to cause the DNA to precipitate. Under these circumstances, DNA molecules create complexes which are precipitated and then they are isolated. This obtained DNA pellet was dissolved in 300 μL TE (10 mM TRIS-Cl (pH 7,4), 1 mM EDTA), 5 μL RNase A (DNase I free) concentration of 1 μg/ μL was added 1 and it was incubated at 37^{o}C for 20 minutes. Finally, 150 μL ammonium acetate (7,5 M) was added followed by separation by centrifugation (14,000 rpm for 10 minutes) at room temperature and transfer of the supernatant to a new tube where the DNA was precipitated with 1,6 mL of isopropanol (-20^{o}C). The DNA that appeared after slow shaking was resuspended in 200 μL of TE.

CALCULATION OF DNA CONCENTRATION

The concentration of DNA was determined with spectrophotometer (Nano Drop ND-1000 Microvolume spectrophotometer, Thermos Fisher Scientific, Waltham, Massachusetts, USA), at 260 and 280 nm, the amount of DNA needed was 1μL. The ratio

of absorbance at 260 nm to absorbance at 280 nm indicates the purity of the DNA. DNA concentrations and purity, as measured by the NanoDrop spectrophotometer, are shown in Table 6.

Table 6. DNA concentration of the samples studied.

a/a	Code	Name	DNA purity (260/280 nm)	DNA concentration (ng/μL)
1	E1	Vilana	2,02	566,2
2	E2	Vidiano	1,974	141,6
3	E3	Kotsifali	1,967	259,25
4	E4	Liatiko	1,901	403,7
5	E5	Kotsifoliatiko	1,914	387,5
6	E6	Mandilaria	1,962	360,7
7	E7	Tachtas	1,979	300,45
8	E8	Vilana	1,921	372,65
9	E9	Vidiamo	1,897	318,2
10	E10	Kotsifali	1,967	251,75
11	E11	Dafni	2,098	1156,35
12	E12	Mandilaria	1,992	320,75
13	E13	Plyto	2,081	780,15
14	E14	Tachtas	1,938	197,85
15	E15	Vidiano	1,932	302,2
16	E16	Kotsifali	1,898	442,5
17	E17	Liatiko	1,984	671,95
18	E18	Mandilaria	2,032	736,55
19	E19	Tachtas	1,916	372,0
20	E20	Vilana	1,937	284,95
21	E21	Vidiano	1,919	394,85
22	E22	Kotsifali	1,962	252,95
23	E23	Liatiko	1,937	358,35
24	E24	Thrapsathiri	1,953	284,85
25	E25	Kotsifoliatiko	1,983	217,55
26	E26	Mandilaria	1,99	108,45
27	E27	Tachtas 1	1,923	365,75
28	E28	Tachtas 2	1,956	306,4
29	E29	Romeiko	1,983	235,2
30	E31	Krevatina	1,929	394,75
31	E33	Mandilaria-Dask	1,908	455,4

32	V2	Tsardana	1,951	192,65
33	V3	Athiri	1,992	543,25
34	V4	Vilana	1,984	80,45
35	V5	Kotsifali	1,989	207,5
36	V9	Malagousia	1,954	341,45
37	V14	Mandilaria	1,882	376,7
38	V15	Plyto	2,034	286,3
39	V23	Vidiano	1,975	186,7
40	V28	Dafni	1,985	291,8
41	V32	Romeiko	1,989	115,1
42	V33	Mosxato lefko	2,062	154,65
43	V40	Limnio	1,904	352,05
44	V41	Limniona	1,919	142,3
45	V52	Platani	1,685	152,75
46	V73	Ladikino	1,895	91,0
47	V77	Eftakoilo	1,876	853,65
48	V85	Lagorthi	2,033	181,35
49	V90	Katsano	1,998	74,4
50	V91	Asprmandilaria	1,977	87,6
51	V94	Avgoulato Crete	1,967	230,1
52	V22	Assyrtiko	1,912	640,7
53	V100	Tsaousi	1,967	125,8

SSRs

Oligonucleotides sourced from Operon Technologies Inc. Europe, MGW – Biotech AG were employed as primers for the amplification of genomic DNA across all strains. Throughout the study, a series of primers were assessed, leading to the ultimate selection of five specific ones. The primers used for the analysis as well as their sequence are shown in Table 7.

Table 7. Primers used, sequence of bases, hybridization temperature and size of amplified products (base pairs) of each primer.

Primer	**Primer type**	**Primer Sequence**	**Annealing temperature (°C)**	**Length (bp)**	**Size range (bp)**
VVS2	Forward (5'→3')	CAGCCCGTAAATGTATCCATC	53.4	21	123-161
	Reverse (5'→3')	AAATTCAAAATTCTAATTCAACTGG	52.1	25	
VVMD5	Forward (5'→3')	CTAGAGCTACGCCAATCCAA	56.0	20	222-268
	Reverse (5'→3')	TATACCAAAAATCATATTCCTAAA	56.0	24	
VVMD7	Forward (5'→3')	AGAGTTGCGGAGAACAGGAT	52.0	20	231-265
	Reverse (5'→3')	CGAACCTTCACACGCTTGAT	52.0	20	
VVMD28	Forward (5'→3')	AACAATTCAATGAAAAGAGAGAGAGAGA	56	28	221-279
	Reverse (5'→3')	TCATCAATTTCGTATCTCTATTTGCTG	56	27	
VVMD36	Forward (5'→3')	TAAAATAATAATAGGGGGACACGGG	56	25	244-315
	Reverse (5'→3')	GCAACTGTAAAGGTAAGACACAGTCC	56	26	

The VVS2 primer was designed, developed, and characterized by Thomas et al. (1993), VVMD5, VVMD7 primers from Bowers et al. (1996) and primers VVMD28, VVMD36 from Bowers et al. (1999).

PCR

The preparation of the reaction was carried out at a temperature of 4°C. The final volume of the mixture was set at 10 μL. In Eppendorf tube, the foundational mixture was prepared in the following sequence: deionized and sterile water, followed by the 1x buffer

solution containing 50 mM KCI, 10 mM Tris-HCl (pH: 8,7 at 20°C), 15 mM $MgCl_2$, and $(NH_4)_2SO_4$. Subsequently, 0,2 µL of each deoxynucleotide (dATP, dTTP, dGTP, dCTP) was introduced, along with 1µL, 0,5µL respectively of each primer (forward and reverse), and 1 unit *Taq* DNA polymerase (Qiagen). Forward primers were 5'-end labelled with a fluorescent dye (FAM, NED, PET or VET). This set of components formed the reaction mixture used in the experiment.

The mixture was mixed and after centrifugation (14,000 rpm for 1 min at room temperature), aliquoted into 0.2 mL eppendorf tubes for the heat exchanger 1µL of strain DNA was added to each sample, then vortexed and centrifuged at 14,000 rpm for 1 min at room temperature. Immediately after, the samples were placed in the heat exchanger (PCR) at a temperature of 94°C.

PCR amplifications were performed in a Perkin Elmer DNA Thermal Cycler 9600 as follows: 1 cycle of 5 min at 94 °C, 35 cycles of 1 min at 94 °C, 1 min at 52–56 °C (depending on the primer), 1.5 min at 72 °C, and 1 cycle of 30 min at 72 °C.

During the initial phase of the cycle, the DNA extracted from the sample undergoes decomposition as the reaction temperature is raised to 94 °C. This process effectively eliminates complementary DNA clones. In the subsequent step, the temperature is lowered (adjusted based on the hybridization temperature of the SSR initiators), facilitating the hybridization of initiators with the DNA sequence. Since the initiators consist of distinct and non-complementary sequences, they do not hybridize with each other but specifically with the complementary DNA sequences. In the third and concluding step, occurring at 72 °C, the synthesis of complementary DNA clones takes place. This synthesis is facilitated by the enzyme DNA polymerase, enabling the construction of DNA in a 5' to 3' direction. Following the procedure, the samples are preserved by storing them in the refrigerator at 4°C.

ELECTROPHORESIS

Electrophoresis was employed to separate the amplified products, with 20 µL taken from each sample, utilizing a 2% agarose gel. The electrophoresis buffer solution of choice was TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.00), and ethidium bromide was used for DNA staining. The concentration of ethidium bromide was 0.5 µg/mL, both on the gel and in the buffer solution. The gel itself had dimensions of 20 cm x 15 cm and was 10 mm thick. Electrophoresis was carried out at a constant 120 V for a duration of 4

hours. To capture the results, the electrophoresis results were photographed using a Gel Doc 1000 (Biorad) equipment and the resulting images were stored on a computer for further analysis and documentation. PCR fragments were also separated using capillary electrophoresis. For the loading buffer, 13 L of deionized formamide with 0,5 L of GeneScan-500 (LIZ) size standard (Applied Biosystems, USA) were added to 1,0 L of each amplification sample. The samples were first denaturated at 94◦C for 5 min and then separated by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer (Applied Biosystems, USA). Data analysis, sizing and genotyping were performed using the GeneMapper v4.0 software (APPLIED BIOSYSTEMS, USA).

STATISTICAL ANALYSIS

For the statistical analysis of the molecular data, the method UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was used with one dissimilarity/distance coefficient. In order to present the genetic relationships between the cultivars, DIST (Average Taxonomic Distance) coefficient was used, as implemented in the NTSYS-pc package 2.1 developed by Rohlf (Exeter Software, New York, USA, 1993). The bigger the value of the coefficient for 2 samples i and j, the bigger the distance between them, as calculated with the following equation:

$$E_{ij} = \sqrt{\Sigma_k \frac{1}{n}\left(X_{ki} - X_{kj}\right)^2}$$

Based on the above coefficient, dissimilarity matrices were created from which the data was taken in order to perform a cluster analysis, using as mentioned above the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. This method is widely used in molecular studies. Based on this data, the tree plots (dendrograms) were created which depict the existence of genetic distances of the studied cultivars.

RESULTS

For the analysis of the degree of genetic distance among the samples studied, coefficient Distance (DIST) was used, as mentioned before, and the method UPGMA. This method is quite often used in studies involving the use of molecular markers. This particular method uses directly the distance coefficient and the grouping starts from the samples that are the closest to each other and then moves to the rest of the samples. Separate statistical analysis was carried out for each primer, so that the degree of polymorphism of each primer could be determined, in other words, to what extent each primer can distinguish the samples studied.

In this study, five primer combinations were used for genetic characterisation and identification of the studied grapevine cultivars. The genetic data were used to graphically represent the genetic distances among and within the samples originating from the studied cultivars. Figures 6, 7, 8, 9 and 10 present the dendrograms that were generated from each primer separately, namely primers VVS2, ssrVrZAG62, ssrVrZAG79, VVMD7 and VVMD25, respectively. The results of the statistical analysis for all samples studied and with the use of all primers are shown in Figure 11.

In Figures 6,7, 8 and 9 and primers VVS2, ssrVrZAG62, ssrVrZAG79 and VVMD7, the samples are grouped in 4 major clusters, in which most Cretan varieties are grouped, with no genetic distance being found in most of these sub-groups. This most likely confirms the common origin of Cretan varieties but the primers separately do not exhibit a high degree of polymorphism.

Primer VVMD25 (Figure 10) seems to be more polymorphic, at least for the samples studied, since it groups the samples in many sub-groups, and samples coming from the same variety but from different collections, are grouped together (i.e. all samples of Vidiano are grouped together, all samples of Mandilaria are grouped together etc.)

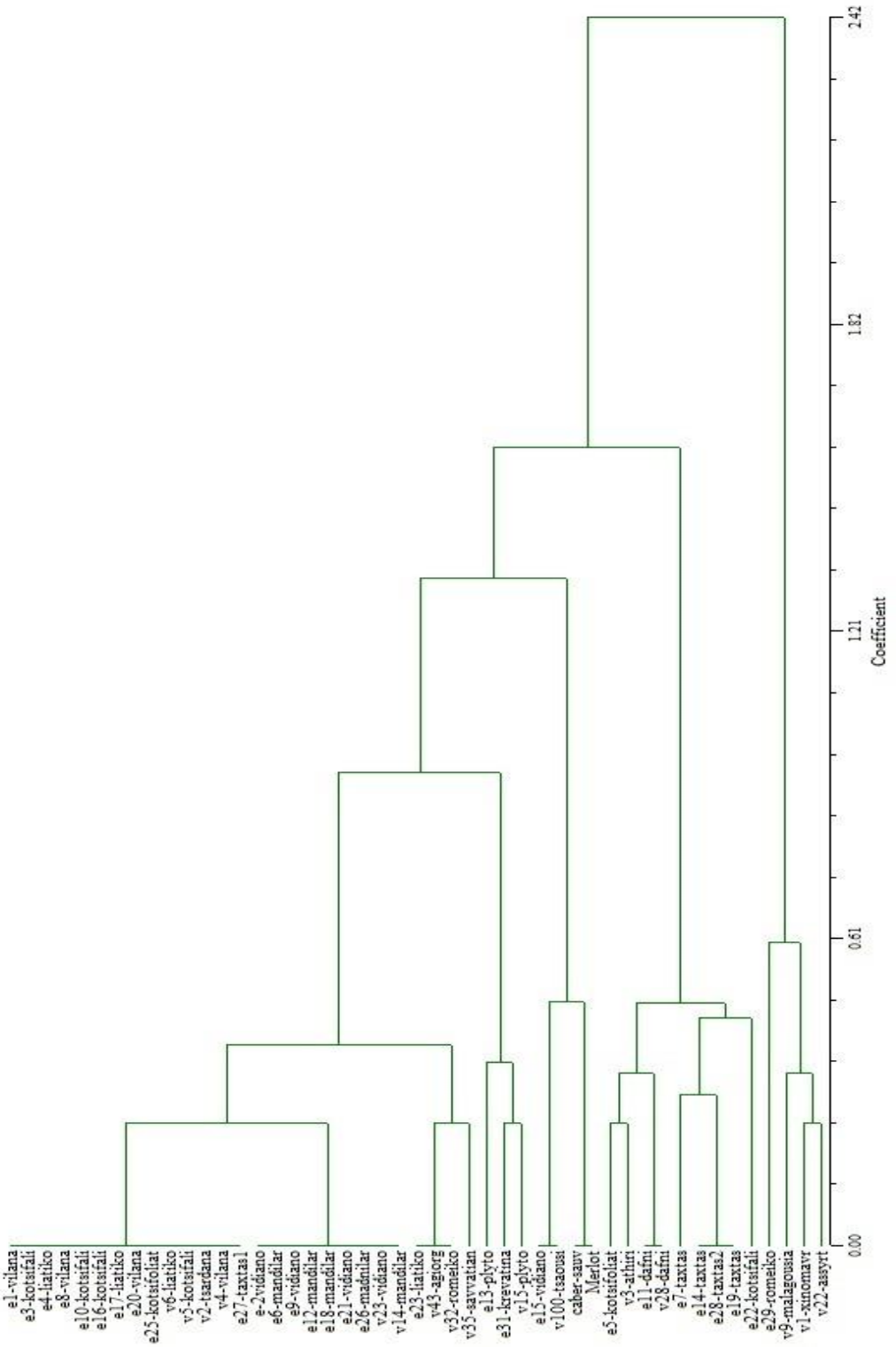

Fig. 6. Dendrogram of the results of the cultivars studied in relation to genetic distance coefficient DIST with SSR marker VVS2.

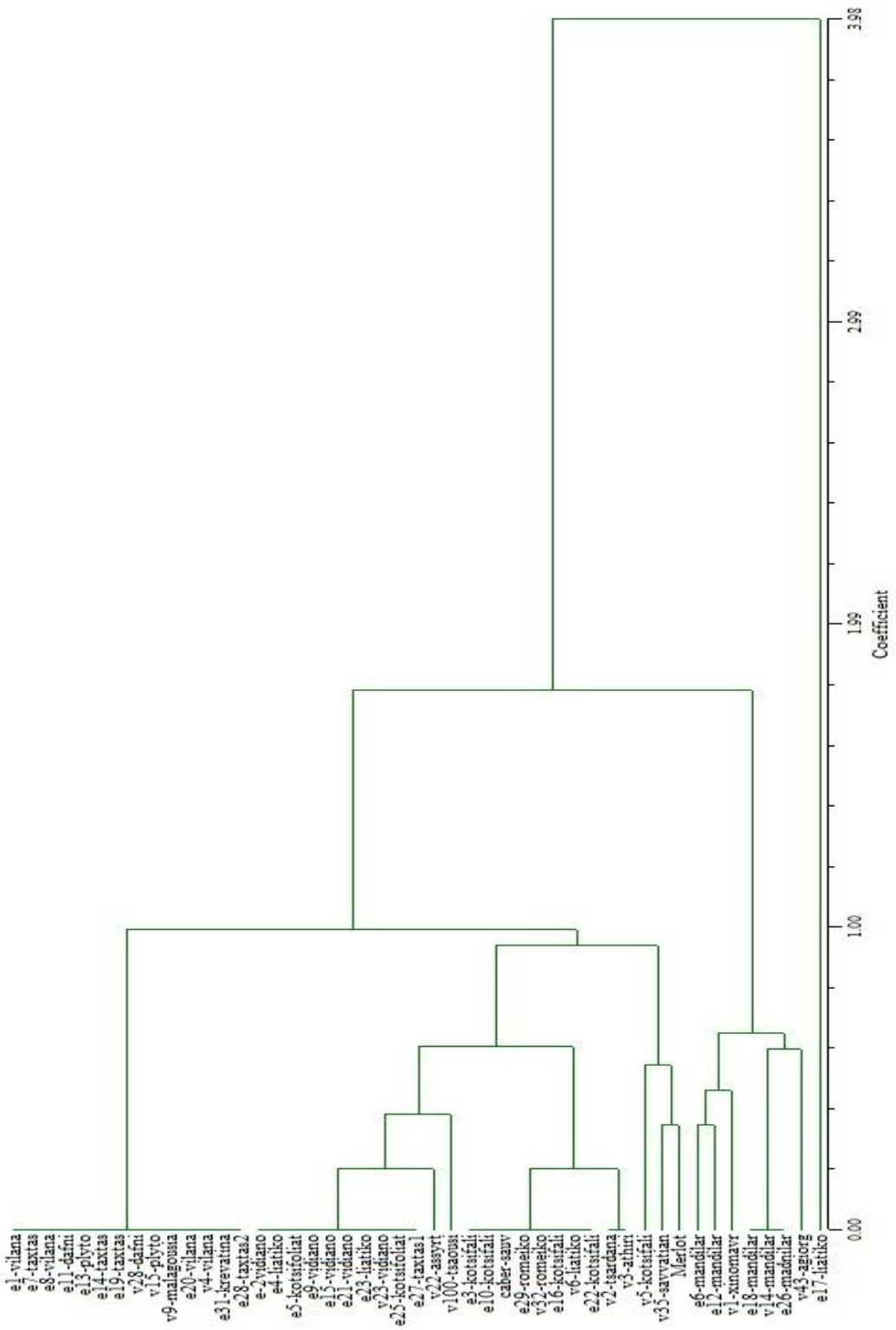

Fig. 7. Dendrogram of the results of the cultivars studied in relation to genetic distance coefficient DIST with SSR marker ssrVrZAG62.

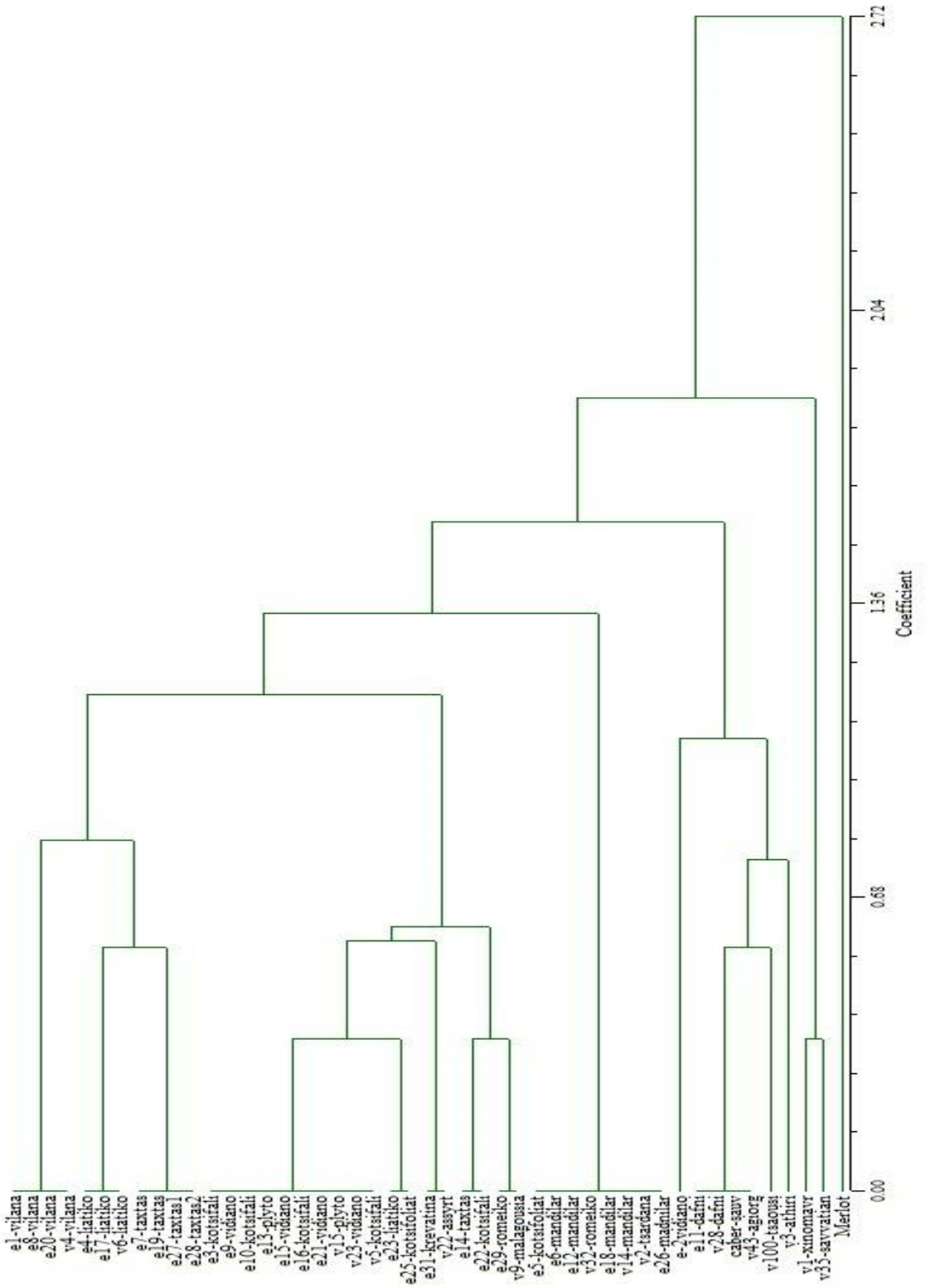

Fig. 8. Dendrogram of the results of the cultivars studied in relation to genetic distance coefficient DIST with SSR marker ssrVrZAG79.

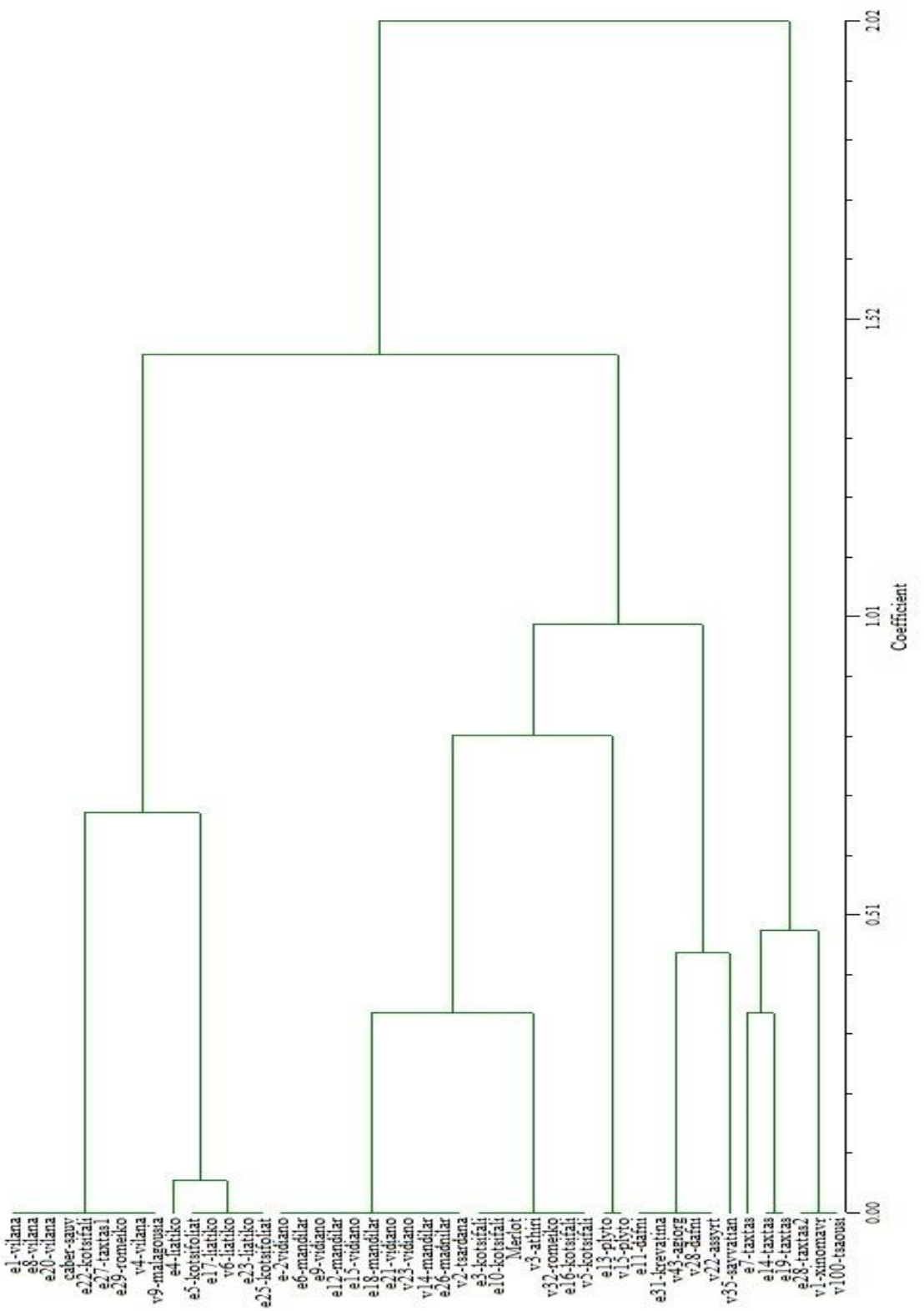

Fig. 9. Dendrogram of the results of the cultivars studied in relation to genetic distance coefficient DIST with SSR marker VVMD7.

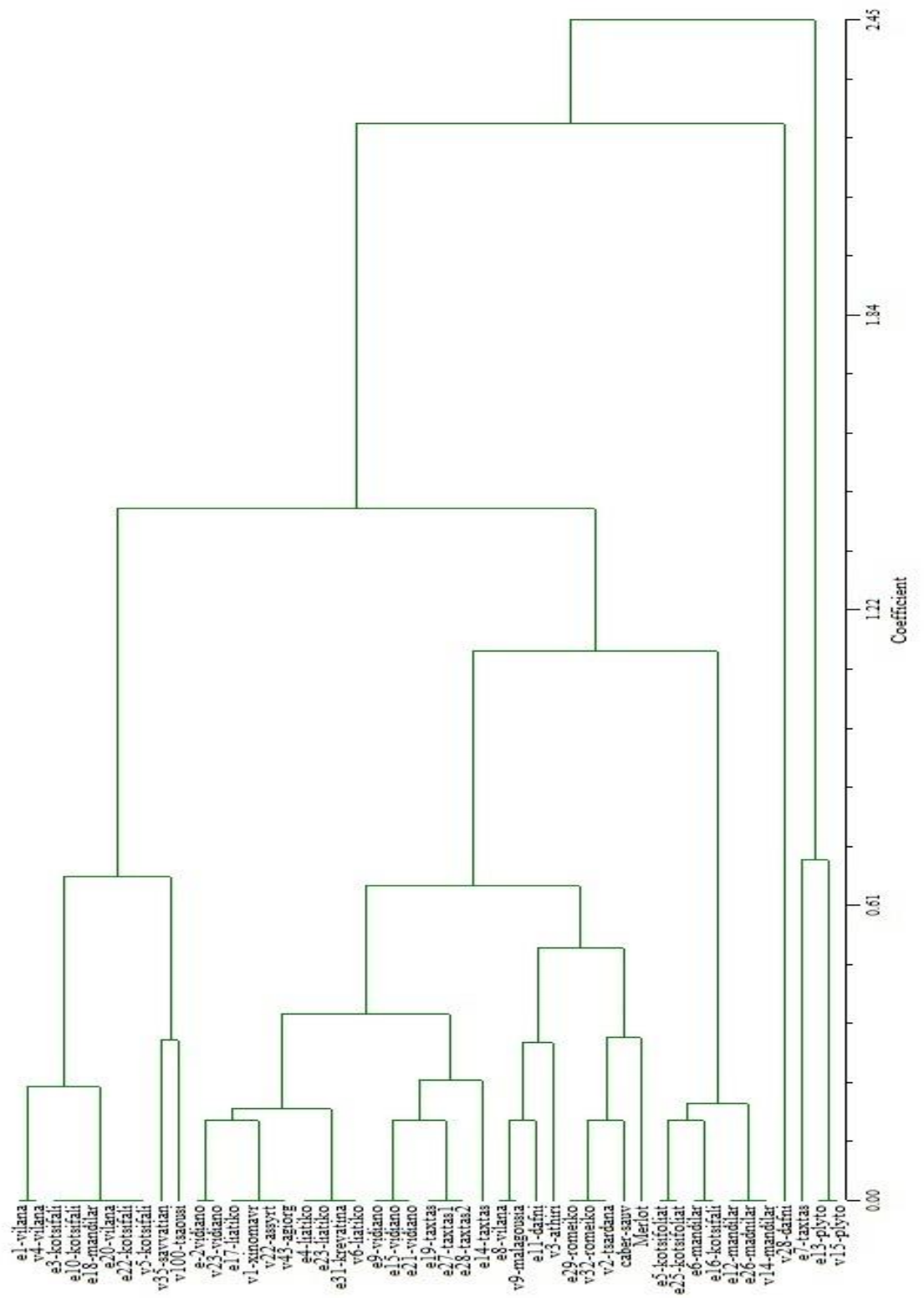

Fig. 10. Dendrogram of the results of the cultivars studied in relation to genetic distance coefficient DIST with SSR marker VVMD25.

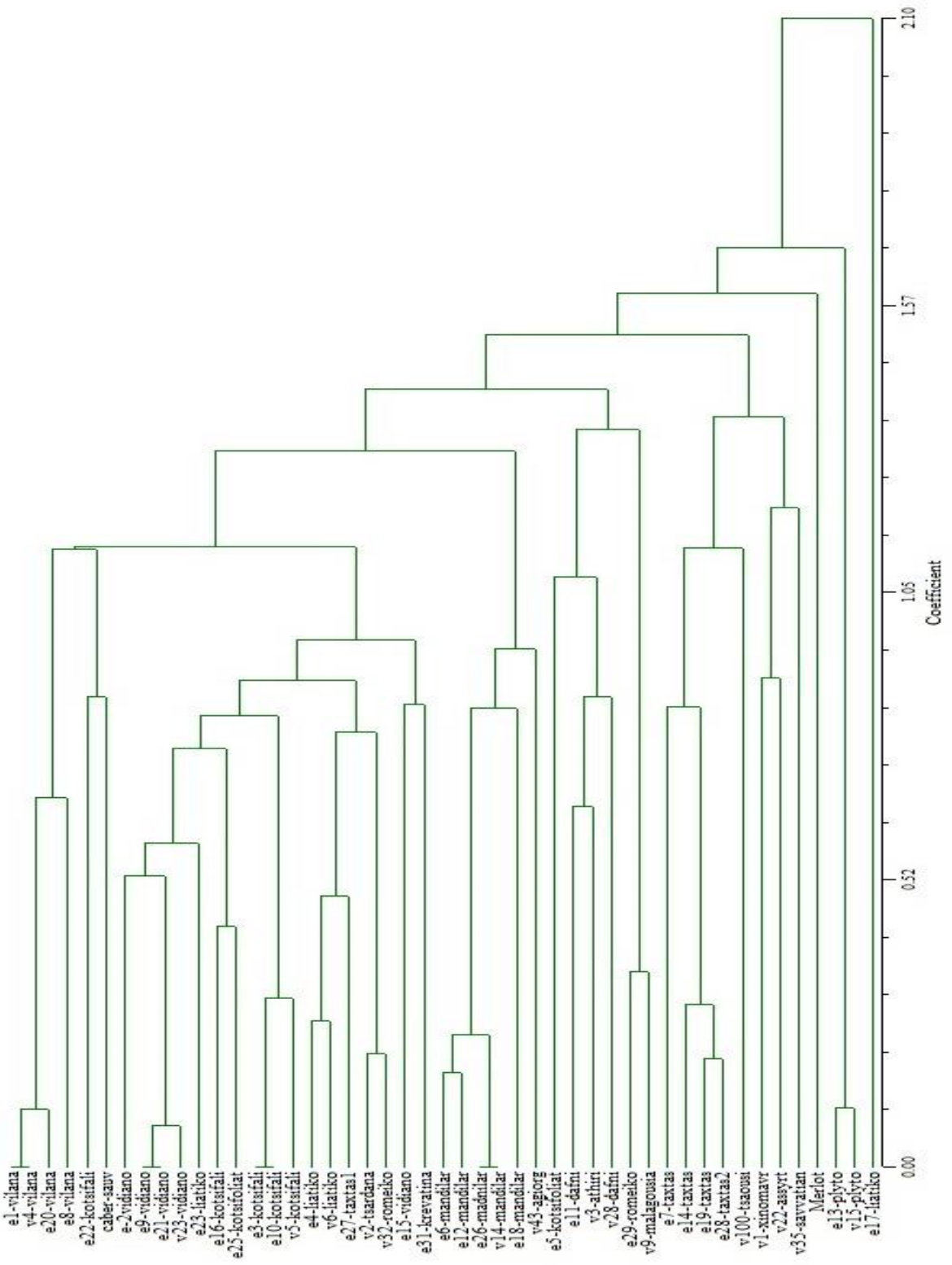

Fig. 11. Dendrogram of the results of the cultivars studied in relation to genetic distance coefficient DIST with the use of all primers.

According to Figure 11, and with the use of allo five primers, the samples studied are grouped in many clusters, meaning that this primer is quite polymorphic and is able to discriminate many of the samples studied. More specifically, the different samples from grapevine cultivars (Vidiano, Vilana, Kotsifali, Mandilaria, etc) are grouped together under each cultivar, confirming that the different samples of each variety are the same variety, but there is a small genetic distance among the samples, indicating the polyclonal nature of Greek varieties.

The overall results show that there is genetic variability both between and within the varieties, since the different samples from the same variety exhibited small genetic differences.

The different samples of the most important Creatan varieties are grouped together in the same cluster of the dendrogram, and the small genetic distance that they exhibit could very well be expressed through different qualitative and quantitative characteristics. At the same time, the grouping of most indigenous Cretan varieties in one cluster suggests their common origin and could also explain their high adaptability over the years in the conditions that prevail in the Cretan vineyard.

CONCLUSIONS

The study of the genetic diversity both between and among grapevine cultivars is a demanding task, especially when it comes to old grapevine cultivars which have been in cultivation for hundreds of years, as is the case of the Cretan varieties. Especially in view of climate change, it is of high importance to properly identify the different biotypes that exist within the indigenous varieties, to be able to highlight the biotypes that are more adapted to the new shaped conditions and can produce viticultural products of high quality.

Molecular methods, such as microsatellites that were used in the present study, constitute a powerful tool for the identification and discrimination of grapevine cultivars. The results originating from molecular methods are characterized by high accuracy and they can be exploited to further improve viticultural practice. The ampelographic description continues to be necessary for the study of grapevine cultivars. The most effective approach is the combination of the ampelographic description with the use of molecular methods in order to achieve a more comprehensive approach which will provide a solution to important identification issues of Greek grapevine cultivars.

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