

Morphological and Molecular Profiling of Twelve Native Grapevine Varieties From Crete and Thira Islands of Southern Greece: Insights Into Intra-varietal Diversity

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Abstract

As in the case with other countries with well-documented history in grape cultivation, the discrimination of Greek grapevine resources is an arduous and complicated task due to the use of numerous synonyms and homonyms, the evolution of many phenotypes within varieties and the doubtful origin of several Greek grapevine landraces. The aim of the present study was to present a comprehensive exploration and characterization of twelve autochthonous Greek grapevine cultivars grown in various regions of Crete and Thira islands of southern Greece, using both ampelographic traits and molecular markers. From 2018 to 2022, a total of 112 accessions from commercial vineyards were analyzed using 48 ampelographic characters developed by the International Organization of Vine and Wine (OIV) and 10 microsatellite loci (SSR). According to both methods, the results showed that: (a) nine of the twelve studied varieties appeared in a single cluster in the ampelography-based clustering with the exception of 'Vilana', 'Moschato Spinas' and 'Mandilaria' phenotypes that exhibited a relative significant intra-varietal variation, (b) the matrices produced from ampelographic data revealed a distance between the studied samples from Cretan and Thira vineyards and the reference samples from the national grapevine repository for 'Athiri' and 'Aidani lefko' varieties, (c) 'Dafni' exhibited a clear molecular-genetic distinction and was significantly separated from the other cultivars studied, (d) our data did not support the previously reported high similarity between the varieties 'Vilana' and 'Vidiano'. The combination of ampelographic description and molecular determination of the SSR profile proved to be effective for studying genetic diversity and identifying grapevine cultivars.

Keywords: grapevine, ampelography, Mediterranean, SSR markers, OIV descriptors

1. Introduction

Viticulture and winemaking have been essential to Greek culture since ancient times and are characterized mainly by small, family-owned vineyards thriving in diverse landscapes and climates. Under vine areas amount to nearly 70,000 hectares, the vast majority of which are covered with wine grapes that produce approximately 2.0 million hectoliters of wine per year (ELSTAT, 2022; International Organisation of Vine and Wine [OIV], 2024). According to Lazarakis (2018), the Greek vineyard is composed of eight wine regions, among which Crete (with 8,000 hectares of wine grapes) and the Aegean islands (primarily the island Thira, also known as Santorini, with 1,000 hectares of wine grapes) are considered to be of particular significance for the national wine production. Over the past few decades, Greece has made significant progress in the global wine market through the adoption of a branding and marketing strategy implemented by Greek winemakers in 2010. This strategy considers the creation of distinctive wines utilizing Greece's indigenous grape varieties as a critical factor in maintaining competitiveness and economic viability in international wine markets. Thus, conservation,

identification, and exploitation of autochthonous Greek wine grape varieties can be considered fundamental elements for the viability and competitiveness of the Greek wine sector.

Globally, climate change imposes to the viticulture and wine industry challenges and threats that are more severe in the Mediterranean Basin (Santillán, Garrote, Iglesias, & Sotes, 2020). In this context, the preservation, evaluation and exploitation of inter-varietal and intra-varietal variability related to important agronomic and technological grape traits has become one of the crucial adaptation strategies in a changing environment (Duchene, 2016; Baltazar, Castro, & Gonçalves, 2025). Contrary to these widely accepted facts, contemporary viticulture practices tend to reduce the number of grape varieties and clones cultivated, leading to a rapid decline in both inter- and intra-varietal genetic diversity (Gonçalves, Aubyn, & Martins, 2013). In Greece, this trend is further exacerbated by subsidized state programs for vineyard replanting, which are adopted without any provision for the preservation of the inter- and intra-varietal diversity commonly found in older vineyards of Greek autochthonous varieties. Currently, the preservation of indigenous grapevine genetic resources in Greece is achieved through the maintenance of one or a few accessions of each variety in field collections supervised by the Hellenic Agriculture Organization-DIMITRA and, consequently, the preservation of autochthonous Greek varieties is conducted mostly at the inter-varietal level. However, given that old autochthonous varieties demonstrate considerable diversity in quantitative traits, the preservation of a single or limited number of clones from an ancient grape variety is insufficient to ensure comprehensive conservation for future challenges and sustainable viticulture (Gonçalves & Martins, 2022).

A significant part of the Greek autochthonous grapevine varieties is cultivated for centuries in Crete and Thira islands of the southern Aegean Archipelagos. Some of the oldest and most esteemed grapevine varieties in the Cretan and Thira vineyard include the red-skinned ‘Kotsifali’, ‘Mandilaria’, ‘Mavrotragano’ and ‘Liatiko’ and the white-skinned ‘Assyrtiko’, ‘Athiri’, ‘Vilana’, ‘Vidiano’, ‘Dafni’, ‘Aidani lefko’, ‘Moschato Spinis’ and ‘Plyto’ (Stavarakaki & Stavrakakis, 2017).

‘Kotsifali’ is an early to midseason- ripening, thin-skinned variety that is mainly cultivated in the eastern part of Crete. ‘Kotsifali’ has no synonyms, and according to a report of Avramidou et al. (2023) the variety exhibited a robust phylogenetic signal with unambiguous morphological and genetic distinction from other grape cultivars. To deepen the inherently pale color of ‘Kotsifali’ wine, the cultivar usually blends with ‘Mandilaria’ (synonyms ‘Mandilari’, ‘Mandilaros’). ‘Mandilaria’ is cultivated across Greece’s viticultural regions under different local names (e.g., ‘Koundoura’ in Attica, ‘Dombrena’ in Viotia and ‘Amorgiano’ in the island of Amorgos). ‘Mandilaria’ is genetically closely related to a number of unique cultivars, namely ‘Agianniotiko’, ‘Thrapsa’, ‘Kotselina’, ‘Pappoudes’, ‘Mouchtaro’, ‘Mavro Kymis’, and ‘Mavro Spetson’ (Stavarakaki & Stavrakakis, 2017). The variety ‘Liatiko’ takes its name from the Greek word for July since ‘Liatiko’ grapes mature during the first days of the month. Ampelographic descriptions and molecular techniques provide evidences that ‘Liatiko’ is a distinct variety, completely different from ‘Aleatiko’, ‘Kotsifoliatiko’, and ‘Mavroliatis’ (Stavarakaki & Biniari, 2017). The rather low-yielding, dark-skinned cultivar ‘Mavrotragano’ was facing extinction for many years, but nowadays plantings are on the rise and a number of producers release single-varietal or blended ‘Mavrotragano’ wines. Mavrotragano’s intricate flavor profile has drawn comparisons to the esteemed Piedmontese grape, ‘Nebbiolo’.

‘Vilana’, the primary white variety of Crete, is used to produce wines of Protected Designation of Origin (PDO), as well as Protected Geographical Indication (PGI) Cretan wines. Using the molecular marker approach (Merkouropoulos et al., 2015) revealed a high degree of genetic relationship between ‘Vilana’ and ‘Asprouda Patron’ (variety cultivated in the Peloponnese area). ‘Vidiano’ is an old, indigenous variety, which is also mentioned as ‘Avidiano’ (Merkouropoulos, 2023). In the past decades the variety was sparsely cultivated, with growers only recently increasing their plantings, since ‘Vidiano’ is now widely regarded as one of the finest white cultivars on the island. ‘Vidiano’ shares a close genetic relationship with ‘Lagorathi’ cultivar and to a lesser extent with ‘Vilana’ and ‘Thrapsothiri’ (Biniari & Stavrakakis, 2007). ‘Dafni’ or ‘Daphni’ or ‘Dafnia’ is a late ripening variety with high yields. Lacombe et al. (2013) suggested that ‘Dafni’ could have been emerged from the physical hybridization of the cultivars ‘Ferral’ (‘Prunesta rosso violacea’) and ‘Syriki’ (‘Assouad karech’). ‘Moschato Spinis’ an extremely rare, indigenous grape variety is originated from the west part of Crete. A recent report note that ‘Moschato Spinis’ and ‘Moschato Samou’ (synonym ‘Moschato aspro’) are closely related genotypes, originating from a common progenitor (Avramidou et al. 2023). ‘Plyto’ variety was also saved on the brink of extinction by local growers. Observations produced from ampelographic-based classifications link ‘Plyto’ with the cultivars ‘Kritiko aspro’, ‘Platani’, ‘Katsano’, ‘Petrolanos’ ‘Ploto’, ‘Kitrinovaria’, and ‘Asprovaria’ (Stavarakaki & Stavrakakis, 2017). The white grape cultivar ‘Athiri’ (synonyms ‘Asprathiri’, ‘Athiri lefko’) is grown all over the Cyclades and Dodecanese islands, perfectly adapted to the arid and hot Aegean

climate; recently, numerous vineyards have been planted in Lakonia (south Peloponnese) and Halkidiki (Macedonia). Besides the polyclonal nature of the cultivar, meaning that the name ‘Athiri’ represents a diverse range of accessions that are genetically distant (Bibi, Gonias, & Doulis, 2020), it is well documented that there is a high degree of genetic similarity between ‘Athiri’ and ‘Thrapsathiri’ which are considered closely related cultivars (Krimbas, 1943; Biniari & Stavrakakis, 2007). One of the emblematic cultivars in the Mediterranean basin, ‘Assyrtiko’ (also written ‘Assyrtico’ or ‘Asyrtiko’) is originated from Santorini and due to its high plasticity and adaptation potential, ‘Assyrtiko’ is continually expanding to many Greek wine regions, as well as, in Italy, Australia, Cyprus, South Africa, and the USA. DNA profiling conducted in 2011 revealed a parent-offspring connection between ‘Assyrtiko’ and the Aegean cultivars ‘Gaidouria’ and ‘Platani’ (Myles et al., 2011). A blending partner of ‘Assyrtiko’ is the early-ripening, lower in sugar and acidity, cultivar ‘Aidani lefko’ (synonyms ‘Aidani’, ‘Adani’, ‘Aedano Leyko’, ‘Aidani Blanc’, ‘Aspraidano’, and ‘Moschaidano’ (Merkouropoulos, 2023)). According to Biniari and Stavrakakis (2007) the red cultivar ‘Aidani mavro’ is a berry’s color mutation of ‘Aidani lefko’ rather than a distinct variety.

Traditionally, ampelographic studies have played a fundamental role in defining the identity of grapevine varieties and the relationships among them by relying on the grapevine’s vegetative and reproductive traits, as established by the early research of Krimbas (1943), Logothetis and Vlachos (1966), and Galet (1979). Nowadays, according to OIV the established system for describing and characterizing *Vitis* species and varieties includes 151 morphological and agronomic descriptors of which 48 are usually used. During the last decades, in addition to the ampelographic traits, the advent of molecular markers (especially Simple Sequence Repeat markers - SSR) offered a reliable and reproducible methodology to understand the origin and the diversity, and also to identify *Vitis* genetic resources (Thomas, Matsumoto, Cain, & Scott, 1993; Lefort & Roubelakis-Angelakis, 2001; Lacombe et al., 2013; De Michele et al., 2019; Tsivelikas et al., 2022). Currently, the initial recommendation for using six SSR loci has been updated to nine SSR markers in 2012 in order to assess the genetic diversity and to estimate the relationships between and among grapevine genotypes (Maul et al., 2012).

Considering the importance for the Greek wine sector of the pool of autochthonous Greek grapevine varieties and the preservation of their intra-varietal variability, we aimed to locate and study accessions of 12 autochthonous *V. vinifera* cultivars in Crete and Thira islands. To achieve this objective a combined approach encompassing both ampelographic description and molecular analysis has been employed to characterize 126 grapevine genotypes (including accessions from the Greek national grapevine repository). Our final goal was to establish the first multi-accession repository of the aforementioned 12 cultivars in Greece.

2. Materials and Methods

2.1 Time Frame and Plant Material

The study was conducted during four consecutive seasons (2019-2022) in vines from 30 commercial vineyards on Crete and Thira (Santorini) islands in the southern Aegean Archipelagos. Twenty-three vineyards were located in the Cretan prefectures of Chania, Rethymno, Heraklion, and Lassithi, whereas the remaining seven were located in Thira (Figure 1). Based on the guidance of local vine growers, several vines corresponding to candidate accessions of 12 autochthonous wine-grape cultivars ‘Kotsifali’, ‘Mandilaria’, ‘Mavrotragano’ and ‘Liatiko’ (red-skinned) and ‘Assyrtiko’, ‘Athiri’, ‘Vilana’, ‘Vidiano’, ‘Dafni’, ‘Aidani lefko’, ‘Moschato Spinias’ and ‘Plyto’ (white-skinned) were spotted. Therefore, each identified vine corresponded to one potential accession of the respective cultivar. The varieties studied, the number of spotted vines (accessions), and also the areas from where the samples were collected are presented in detail in Table 1. It should be noted that grapevine material from each of the 12 cultivars was collected from vines conserved in the Greek national grapevine repository of the Hellenic Agricultural Organization-DIMITRA (Lykovryssi, Athens; geographic longitude 23°46’554”E, geographic latitude 38°04’189”N). These vines were considered as reference samples for each variety. The spotted vines in the Cretan vineyards were either bush- or vertical-trained and grafted to various rootstocks whereas those in the Thira vineyards were own-rooted and trained to the highly specialized local training system of ‘kouloura’ (Xyrafis, Gambetta, & Biniari, 2023). Cultivation practices followed the local patterns of vineyard management at each collection site.

After spotting the candidate vines, initial screening was performed by visual assessment of their growth and health status. Only vines with acceptable shoot vigor and no visual symptoms of disease or physiological disorders were selected for further study. A total of 262 vines were further examined using PCR assays for the presence of seven viruses as described in Doupis et al. (2024). A set of 112 healthy vines were subjected to ampelographic description and SSR analysis as candidate accessions.

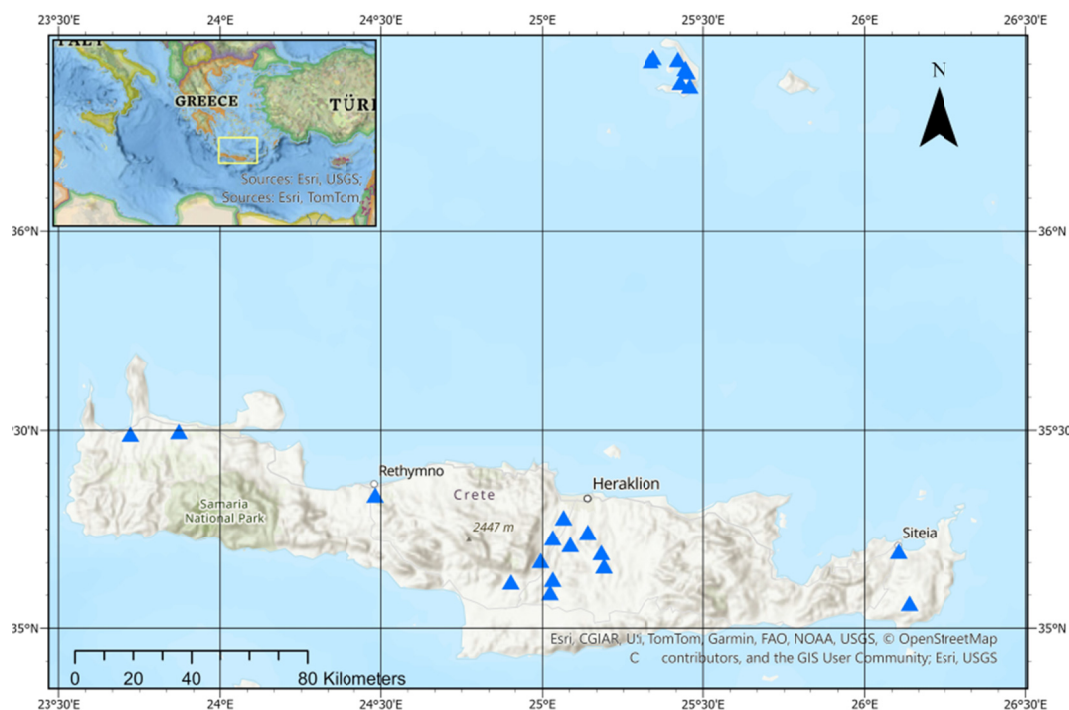


Figure 1. Map of Crete and Thira islands with the locations of the 30 vineyards assessed in this study marked with a triangle

2.2 Ampelographic Description and Data Analysis

Ampelographic description was conducted for a minimum of three years on each of the vines ultimately selected based on their vigor and health status (including the reference vines from the Greek national repository collection). For each growth season and vine, a set of 48 descriptors (Table 2) was evaluated at specific growth stages in accordance with the OIV (2009) guidelines for the ampelographic characterization of grapevine varieties and *Vitis* species.

For each vine, a minimum of 10 specimens of young shoots, mature leaves, developed shoots, grape clusters, and dormant canes were characterized according to the set of 48 descriptors. To assess berry characteristics, 20 berries were extracted from the middle section of 10 representative clusters per vine. Sampling took place *in-situ*, following OIV instructions, while in the case of certain OIV descriptors (e.g., pH, acid and sugar content of the must, berry weight), samples were directly transferred to the laboratory for further measurements by means of the appropriate equipment.

The mode values recorded for each descriptor across the years of the study were used for cluster and Principal Component Analysis (PCA). To conduct these analyses, specialized treatment of the data was necessary when inputting them into the corresponding algorithms, as the data were predominantly categorical or ordered-categorical (ordinal or nominal) (Table 2) rather than continuous-scale data, which are required for standard PCA. The categorization of ampelographic descriptors according to their data type is presented in Table 2. For PCA, descriptors with zero variance have been excluded from the analysis. Although both cluster analysis and PCA are frequently employed in ampelographic studies, the treatment of ampelographic data according to their data type is seldom documented (García-Muñoz, Muñoz-Organero, de Andrés, & Cabello, 2011). Data analysis and graph design were performed using R software (R Core Team, 2024). The ‘princals’ function of the ‘Gifi’ package was employed for PCA analysis (Mair & De Leeuw, 2022). The computation of the distance matrix in cluster analysis was performed utilizing the ‘daisy’ function of the ‘cluster’ package (Maechler, Rousseeuw, Struyf, Hubert, & Hornik, 2024). By default ‘daisy’ computes Gower’s distance (Gower, 1971) for mixed data types. A hierarchical dendrogram was generated based on the matrix of Gower distances.

Table 1. Variety name, sampling area and number of independent plants collected (#). In parenthesis the number of viruses-free plants characterized by ampelographic and molecular approaches

Variety	#	Collection site/Sample code	Variety	#	Collection site/Sample code
Aidani lefko	30 (13)	Thira-Marmari/ai.g.No	Mandilaria	8 (3)	Crete-Katsampas/mn.e.No; Crete-Panorama/mn.h.No; Thira-Gaidouri/mn.c.No
Assyrtiko	67 (31)	Thira-Gaidouri/as.c.No; Thira-Imerovigli/as.d.No; Thira-Thirassia/as.m.No; Thira-Viologikos/as.o.No	Mavrotragano	10 (4)	Crete-Archanes/mv.a.No; Thira-Potamiotissa/mv.j.No
Athiri	20 (8)	Crete-Archanes/at.a.No; Crete-Katsampas/at.e.No; Thira-Potamiotissa/at.j.No	Moschato Spinas	20 (9)	Crete-Archanes/mu.a.No; Crete-Katsampas/mu.e.No
Dafni	6 (2)	Crete-Katsampas/da.e.No; Crete-Dafnes/da.l.No	Plyto	11 (4)	Crete-Alagni/pl.n.No
Kotsifali	32 (12)	Crete-Douli/ko.b.No; Crete-Pirgou/ko.i.No; Crete/Sitia/ko.k.No; Crete/Peza/ko.n.No	Vidiano	24 (11)	Crete-Zaros/vd.p.No
Liatiko	6 (1)	Crete/Ziros/li.q.No	Vilana	28 (14)	Crete-Douli/vl.b.No; Crete-Sitia/vl.k.No; Crete-Peza/vl.n.No

2.3 Microsatellite Analysis

In the 2022 growth season and in each collection site, healthy leaves received from the tip of the shoots (1st and 3rd upper nodes), from each candidate accession were collected, immediately frozen in liquid nitrogen, and stored at -20 °C until further treatment. The same procedure was followed for the corresponding reference vines preserved in the Greek national grapevine repository for each of the studied varieties. Genomic DNA was extracted from 100-130 mg of the frozen tissue using the commercially available NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) according to manufacturer's instructions. The integrity of the extracted genomic DNA was checked by agarose gel electrophoresis, while the concentration was estimated by using a Quawell spectrophotometer (Q3000 UV-Vis Spectrophotometer, Quawell Technology Inc., San Jose, CA, USA).

Polymerase Chain Reactions (PCRs) were performed in a volume of 20 µL using 25-30 ng genomic DNA as a template, 200 mM dNTPs, 40 pmol primers, 2 µL 10X KAPA Taq DNA Polymerase buffer, and 1 U KAPA Taq DNA Polymerase (KapaBiosystems, Cape Town, South Africa). Ten pairs of primers were used: VVS2 (Thomas & Scott, 1993), VVMD5 and VVMD7 (Bowers, Dangl, Vignani, & Meredith, 1996), VVMD25, VVMD27, VVMD28, and VVMD32 (Bowers, Dangl, & Meredith, 1999), and VrZAG62, VrZAG67, and VrZAG79 (Sefc, Regner, Turetschek, Glössl, & Steinkellner, 1999). VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 loci have been accepted as the base core set of SSR descriptors adopted by OIV and the European Vitis Database (<http://www.eu-vitis.de/index.php>), and are considered mandatory to be used in molecular characterization studies in *Vitis* species. The other four SSRs (VVMD25, VVMD28, VVMD32 and VrZAG67) were also preferred in this study, as they were frequently included in previous studies for assessing the genetic diversity among grapevine genotypes (Margaryan, Melyan, Röckel, Töpfer, & Maul, 2021). Forward primers were 5'-end fluorescently labeled with different fluorophores: FAM, HEX, ROX and TAMRA. Primers were custom labeled according to each dye's absorption and emission wavelength and length of the amplified product to avoid overlapping during electrophoresis. PCR amplifications were performed in a 96-well MiniAmp Thermal Cycler (Applied Biosystems, CA, USA) as follows: 1 cycle (95 °C, 2 min), 35 cycles (95 °C, 15 s; 52 to 60 °C (depending on the primers), 15 s; 72 °C, 10 s) and 1 cycle (72 °C, 20 min). PCR fragments were separated using capillary electrophoresis in a 3730 × 1 DNA Analyzer (Applied Biosystems, CA, USA). Data analysis, sizing and genotyping were performed using the GeneMapper software (version 4.0). GenAEx 6.5 program (Peakall & Smouse, 2006) was used for statistical analysis. Dendrograms were constructed using the MEGA4 program (Tamura, Dudley, Nei, & Kumar, 2007).

Table 2. Ampelographic characteristics based on the OIV descriptors list (OIV, 2009) and their categorization according to data type

Code	Description	Category	Code	Description	Category
001	Young shoot: opening of the shoot tip	ordinal	151	Flower: sexual organs	nominal
003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip	ordinal	155	Shoot: fertility of basal buds (buds 1-3)	ordinal
004	Young shoot: density of prostrate hairs on tip	ordinal	202	Bunch: length (peduncle excluded)	ordinal
006	Shoot: attitude (before tying)	ordinal	204	Bunch: density	ordinal
007	Shoot: color of the dorsal side of internodes	ordinal	206	Bunch: length of peduncle of primary bunch	ordinal
008	Shoot: color of the ventral side of internodes	ordinal	208	Bunch: shape	nominal
016	Shoot: number of consecutive tendrils	nominal	209	Bunch: number of wings of the primary bunch	ordinal
051	Young leaf: color of upper side of blade (4th leaf)	nominal	220	Berry: length	ordinal
053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)	ordinal	221	Berry: width	ordinal
067	Mature leaf: shape of blade	nominal	223	Berry: shape	nominal
068	Mature leaf: number of lobes	ordinal	225	Berry: color of skin	ordinal
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	ordinal	231	Berry: intensity of the anthocyanin coloration of flesh	ordinal
072	Mature leaf: goffering of blade	ordinal	235	Berry: firmness of flesh	nominal
074	Mature leaf: profile of blade in cross section	nominal	236	Berry: particularity of flavor	nominal
075	Mature leaf: blistering of upper side of blade	ordinal	241	Berry: formation of seeds	ordinal
076	Mature leaf: shape of teeth	nominal	301	Time of bud burst	ordinal
079	Mature leaf: degree of opening/overlapping of petiole sinus	ordinal	303	Time of beginning of berry ripening (veraison)	ordinal
080	Mature leaf: shape of base of petiole sinus	nominal	351	Vigor of shoot growth	ordinal
081-1	Mature leaf: teeth in the petiole sinus	nominal	502	Bunch: weight of a single bunch	ordinal
081-2	Mature leaf: petiole sinus base limited by vein	ordinal	503	Berry: single berry weight	ordinal
083-2	Mature leaf: teeth in the upper lateral sinuses	nominal	504	Yield per m ²	ordinal
084	Mature leaf: density of prostrate hairs between main veins on lower side of blade	ordinal	505	Sugar content of must	ordinal
087	Mature leaf: density of erect hairs on main veins on lower side of blade	ordinal	506	Total acid content of must	ordinal
094	Mature leaf: depth of upper lateral sinuses	ordinal	508	must specific pH	ordinal

3. Results and Discussion

Upon application of the Principal Components Analysis (PCA) algorithm to the dataset of OIV descriptors, 13 components (PCs) with eigenvalues exceeding 1 were computed, adhering to Kaiser's criterion of eigenvalues greater than 1 (Kaiser, 1960) as an estimation of component importance. These components accounted for approximately 80% of the total variation among the diverse cultivars (Figure 2).

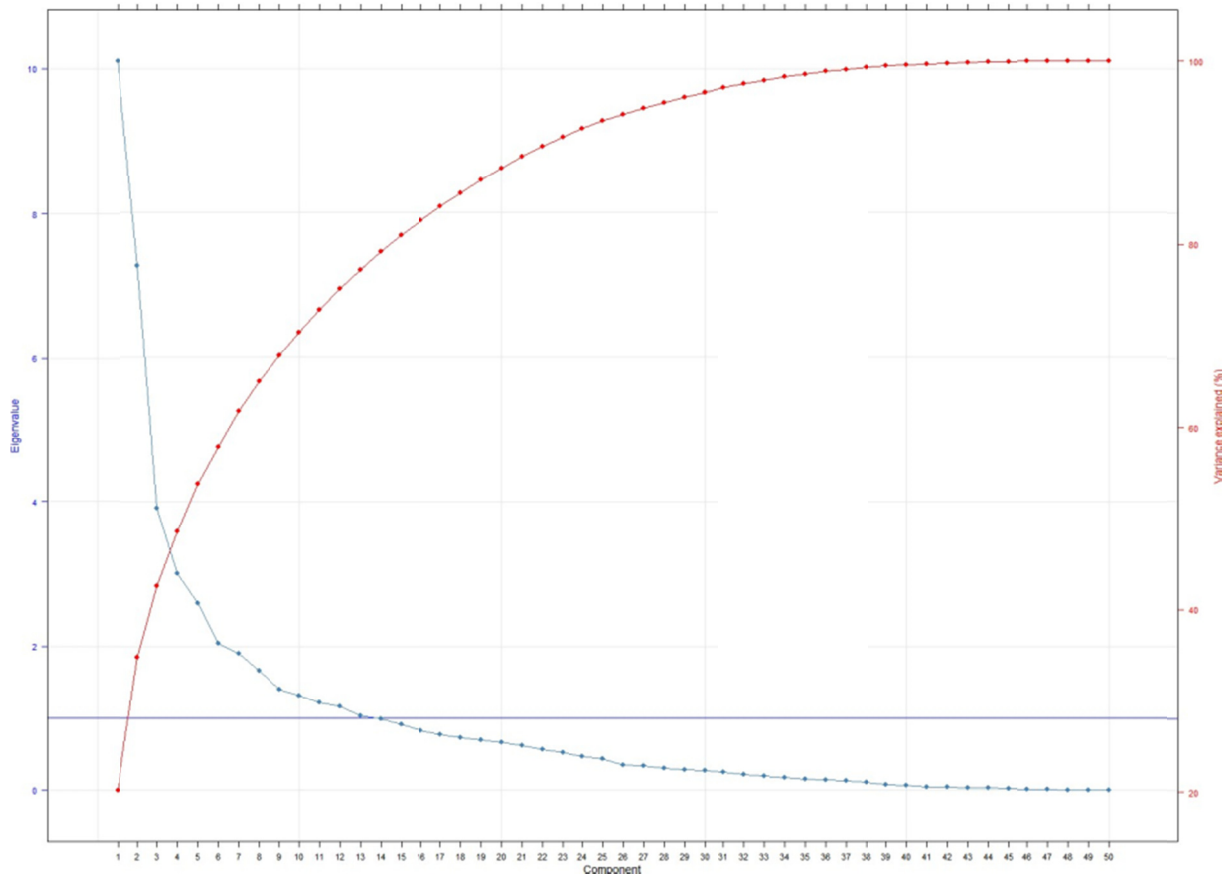


Figure 2. Scree plot of principal components, eigenvalues and percentage of total variance explained according to the PCA

It should be noted that for a highly efficient PCA most of the variance must be represented by a small number of PCs, the first two of them, preferably. As it can be seen in Figure 2, this was not the case in our study since 13 PCs are necessary to represent a significant part of the total inertia. This is a common finding among similar studies (Lamine et al., 2014; Biniari & Stavrakaki, 2018; Stavrakaki, Bouza, & Biniari, 2020; Tsivelikas et al., 2022), indicating that PCA along with other multivariate methods for dimensionality reduction (Garcia-Muñoz, Muñoz-Organero, de Andrés, & Cabello, 2011) may not be as effective as expected in analyzing ampelographic data. Despite these shortcomings, PCA can still assist analysis and interpretation of coded ampelographic data because not only extracts the important information from a data set but also expresses similarity patterns of observations by reflecting the structure of variables and observations (Abdi & Williams, 2010). Principal Coordinate Analysis (PCoA) provides an alternative method to study coded ampelographic data (Merheb et al., 2024) because the ordination of the observations in the component’s space is computed on a distance matrix instead of raw data. However, interpreting the ordination of observations in PCoA is somewhat more difficult.

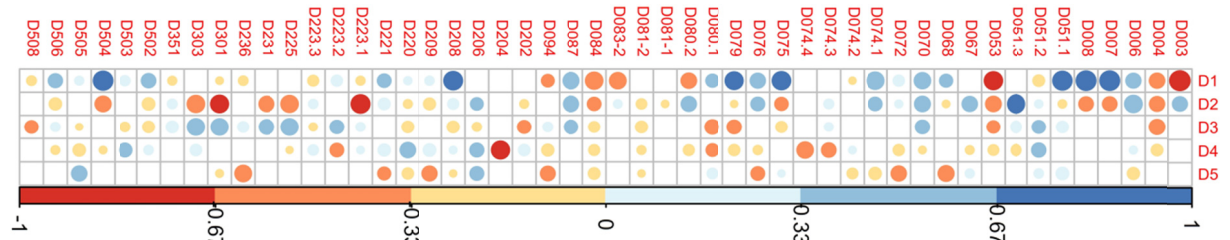


Figure 3. Correlations of the individual OIV descriptors (x axis) with each of the first five principal components (D1-5) with eigenvalues > 2.5 according to the PCA. The size of dot increases with the degree of correlation and the color of dot implies positive (blue) or negative (red) correlation

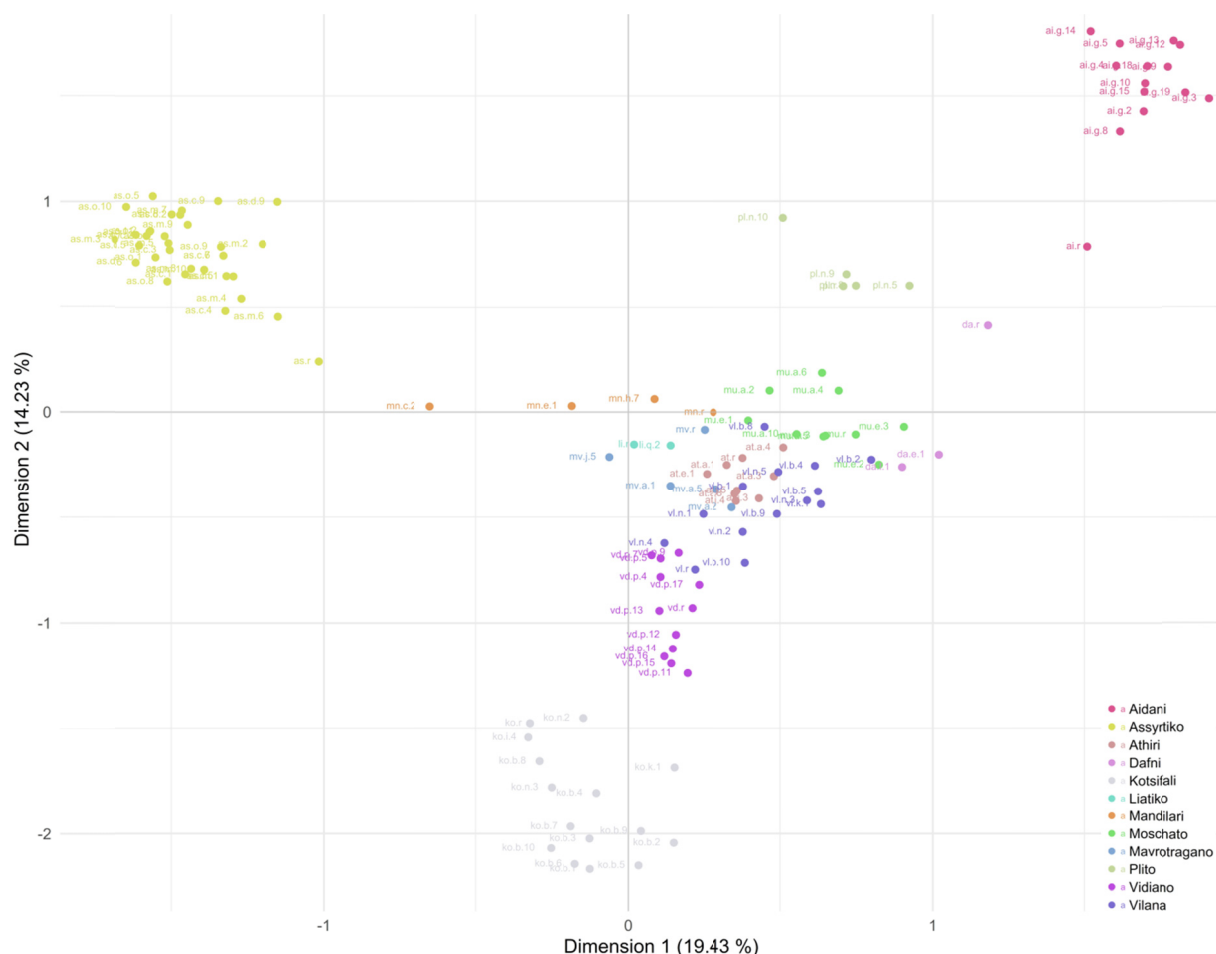


Figure 5. Ordination of 126 accessions of the 12 grapevine cultivars according to their PCA scores on the first (Dimension 1) and second (Dimension 2) principal components. PCA analysis performed on 42 OIV ampelographic descriptors

The ordination of the accessions on the PC 1 and 2 space revealed also significant intra-varietal variance of the studied phenotypic traits. This phenotypic variance is further evidenced in the cases of reference vines for ‘as.r’ (‘Assyrtiko’), ‘ai.r’ (‘Aidani lefko’), ‘da.r’ (‘Dafni’) which were placed relatively far by the rest of the accessions of their corresponding cultivar (Figure 5). Significant variation was observed on the PC 1 and 2 space, for ‘Vilana’, ‘Mandilaria’, and ‘Moschato Spinas’. The observed phenotypic diversity may be attributed to multiple factors, including variability in plant material, heterogeneous genetic origins of vine populations, fluctuations in viral load, epigenetic modifications, somatic mutations, or a combination of these elements (Garcia-Muñoz, Muñoz-Organero, de Andrés, & Cabello, 2011). Given the substantial variability in environmental conditions and vineyard management practices from which the selected accessions originated, it is not possible to definitively conclude that the observed phenotypic variation is attributable to genetic variation. Furthermore, a recent study (Krokida et al., 2024) has reported that the reference vines utilized in our investigation were likely virus-infected. To address these research questions, a controlled experiment should be conducted to investigate the phenotypic profiles of the selected accessions

Ampelography-based cluster analysis grouped all the samples studied in this work into distinct, cultivar specific majority clusters (Figure 6).

‘Moschato Spinas’, ‘Vilana’, ‘Athiri’, ‘Dafni’, ‘Plyto’ and ‘Aidani lefko’, while the second one consisted of the red grapevine varieties ‘Kotsifali’, ‘Liatiko’ and ‘Mavrotragano’ and the white-berry varieties ‘Assyrtiko’ and ‘Vidiano’. Among the varieties of the first sub-cluster, ‘Moschato Spinas’ genotypes forms two separate sub-groups (one group included six accessions from the Archanes cultivation area (Heraklion, Crete) and one accession from Katsampas area (Heraklion, Crete) and the second group comprised the remaining two accessions from Katsampas and the cultivar preserved in the ampelographic collection of ELGO-DIMITRA) exhibiting a relatively high degree of phenotypic distance between them. Interestingly, within the ‘Athiri’ and ‘Aidani lefko’ clusters, the reference samples from the national collection of ELGO-DIMITRA at Lykovryssi (Athens) stand fairly apart from the samples of the two cultivars collected from Cretan and Thira vineyards. In addition, the ampelographic description revealed that the accessions of ‘Mandilaria’ from Thira (‘mn.c.2’) are phenotypically differentiated from the Cretan accessions and the reference ones (‘mn.e.1’; ‘mn.h.7’ and ‘mn.r’). It, also, should be noted that the red cultivar ‘Kotsifali’ shows a relative ampelographic similarity (shares similar ampelographic features) with the white cultivar ‘Vidiano’, rather than the red ones ‘Liatiko’ and ‘Mavrotragano’.

Ampelographic and molecular characterization of native grape individuals growing in vineyards across the broader south Aegean area enhances our understanding of the overall phenotypic and genetic diversity of the species, reveals valid relationships among grapevine genotypes and offers insights into a historically significant germplasm, since viticulture in the south Aegean has a history of over 6 thousand years. Our findings, based on OIV descriptors, highlight the high level of diversity existing among the 12 grapevine cultivars studied. Furthermore, to analyze the genetic diversity among the studied grapevine germplasm, a similarity dendrogram was obtained based on the SSR profiles (Figure 7).

In the SSR dendrogram (Figure 7), in all cases but the ‘Athiri’ group, the reference sample from the national germplasm collection at Lykovryssi is grouped with the corresponding samples from the cultivation centers. In the ‘Athiri’ group the reference sample, although highly close to the ‘Athiri’ samples from the cultivation centers, is not similar with them. This is a case that needs to be resolved with further studies.

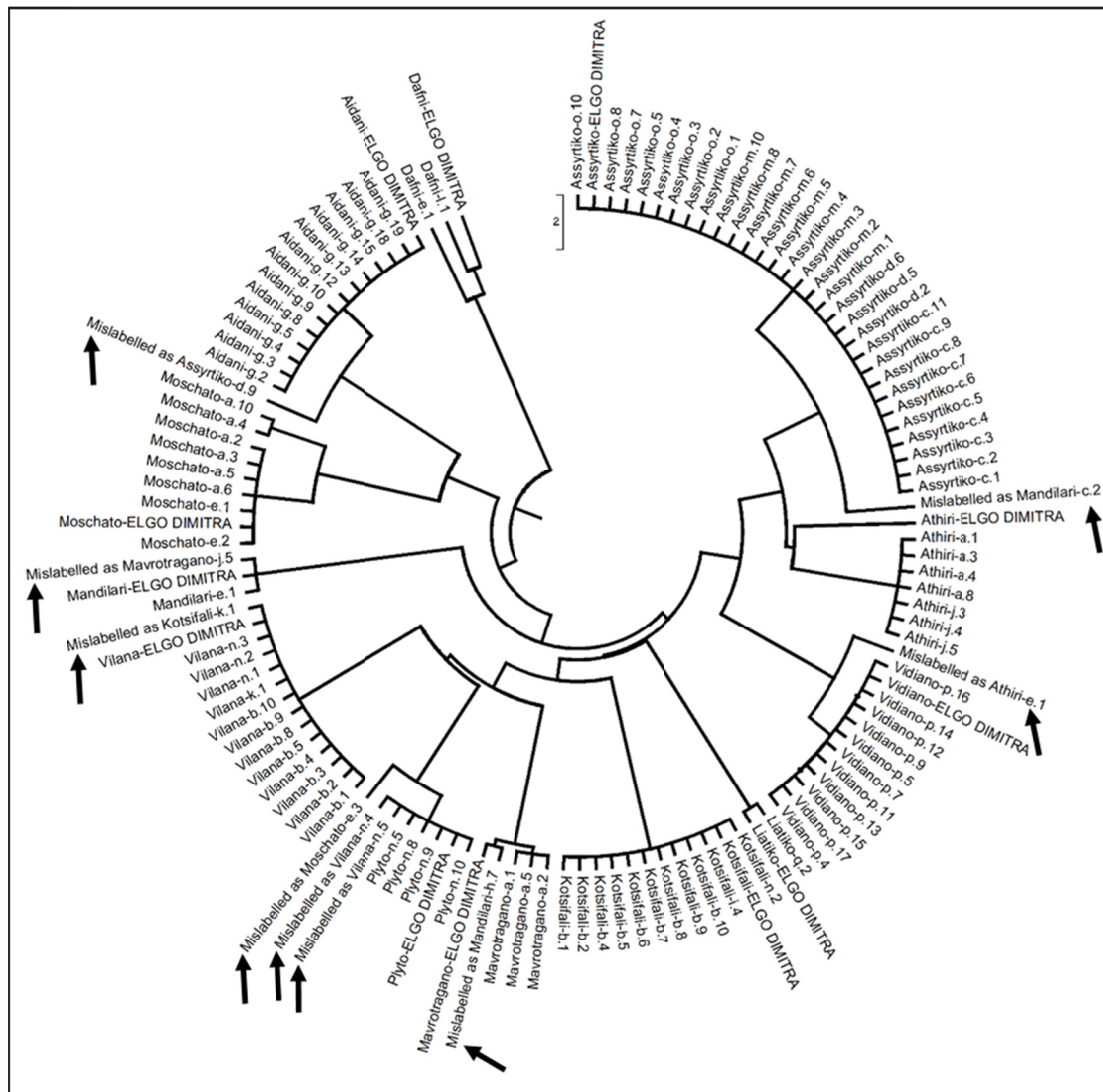


Figure 7. UPGMA dendrogram of the 12 autochthonous *Vitis* genotypes studied in the present analysis. Arrows point to mislabeling occasions. Reference samples are indicated by the suffix 'ELGO-DIMITRA'

Moreover, mislabeling have been detected in nine samples from the cultivation centers (shown by arrows in Figure 7). Namely, the 'Mandilari.c.2' was found to be highly close to the 'Assyrtiko' group; the 'Athiri.e.1' was grouped close to the 'Vidiano' sub-cluster; the 'Mandilari.h.7' was grouped together with the 'Mavrotragano' individuals; both 'Vilana.n.4' and 'Vilana.n.5' and also the 'Moschato.e.3' were all grouped to the 'Plyto' group; the 'Kotsifali.k.1' was grouped to the 'Vilana' group; the 'Mavrotragano.j.5' was grouped to the 'Mandilaria' group and the 'Assyrtiko.d.9' was grouped together with the 'Aidani' ones. The cultivar 'Dafni' was significantly separated from the other grapevine cultivars included in the study (Figure 7). This individual grouping of 'Dafni' is also reported by Stavarakakis et al. (1997) employing 9 RAPD markers. Grapevine cultivars 'Moschato Spinias' and 'Aidani lefko' also exhibited unique genotypes (with the exception of the 'Moschato Spinias' 'mu.e.2' accession which is located in the 'Plyto' sub-cluster) and they were differentiated from each other, as well as, from the rest of the cultivars. The differentiation found among the 'Moschato Spinias' individuals employing the ampelographic description was also revealed from the analysis of the microsatellite profile and is in agreement with the report of Bibi et al. (2020). In accordance, this clear separation of 'Moschato Spinias' from the rest Cretan grapevine germplasm is also reported by Avramidou et al. (2023) who concluded that 'Moschato Spinias' is not an indigenous Cretan cultivar but rather transferred to Crete from Samos island. Furthermore, the low degree of genetic similarity between the reference and the rest of the 'Athiri' samples confirmed the results of the

ampelographic data (whereas the above finding was not supported for ‘Aidani lefko’ cultivar). We also have to mention that ‘Mandilaria’ genotypes did not exhibit a strong monophyletic signal, since ‘Mandilaria’ samples did not appear as a single cluster in the SSR-based analysis (in contrast with the cultivar-specific signal reported for the cultivar in Avramidou et al. (2023) report). This pattern of scattered distribution of ‘Mandilaria’ accessions revealed by the grouping based on genetic distances established via SSR markers was not confirmed by the OIV descriptors dendrogram (Figure 6). Moreover, we have to notice that the high degree of similarity between ‘Vilana’ and ‘Vidiano’ reported by Avramidou et al. (2023) and to a lesser extent by Lefort and Roubelakis-Aggelakis (2001) was not confirmed by our data since the hierarchical structure analysis, employing both ampelographic and microsatellite data (Figures 6 and 7), sets the two cultivars in different sub-clusters.

4. Conclusion

In light of the challenges faced by modern viticulture and winemaking sector in meeting the demands of the global market, as well as, considering the risk of genetic erosion of the indigenous grapevine populations, we evaluated the phenotypic and genetic diversity of grapevines from cultivation centers and the national ampelographic collection. Variety labeling is essential, especially in old, multi-varietal vineyards (as the Greek vineyard), since it provides a consensus description of the grapevine planting material and assists with the certification of the winemaking process. Based on the results presented in this paper and considering the relatively small extent of the viticultural area under study, we concluded that local cultivars grown in Cretan and Thira vineyards are characterized by ample inter- and intra-cultivar variability, reflecting both the phenotypic and genetic differences amongst them. Despite this, the vast majority of the studied accessions (with few exceptions already mentioned) were grouped along cultivar-specific clusters further supporting, not only, the validity of the ampelographic description with the combined use of SSR-based analysis, when it comes to studying polyclonal grapevine cultivars, but also the initial identity attributed to the cultivars since we detect no case of synonyms or homonyms. This study facilitates the establishment of the first multi-accession germplasm repository in Greece and enables further investigation of the intra-varietal diversity of the 12 cultivars under a common experimental environment. The overall outcome of this research will contribute to the accurate identification of the primary Cretan and Thira winemaking cultivars and can be directly applied to clonal selection programs to identify the most valuable clones of Cretan and Thira grapevine germplasm. Accordingly and following the National Regulations and the EU Directives for the production of basic and certified (along with pathogen-free) propagation material, the varieties examined in our study are grown in pots in nethouses. In order to maintain a relatively large intravarietal diversity and to produce clones that are conceivably of benefit to the grapevine growers, the next step involves the evaluation of the agronomical and oenological characteristics of the candidate clones in potential terroirs.

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Authors Contributions

G.D. was responsible for project administration, data collection and writing of the original manuscript. T.P. was responsible for ampelographic data collection and analysis. G.M. and L.R. performed the determination and analysis of the SSR markers. P.R. revised the manuscript. D.T. handled the correlation of OIV descriptors, the multivariate analysis of ampelographic traits and revised the manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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