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How to Improve a Successful Product? The Case of "Asproudi" of the Monemvasia Winery Vineyard

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Abstract: An interesting way to maintain genetic diversity in the vineyard could be based on selecting the desirable characters of each clone or variety in order to produce a high-quality poly-clonal or poly-varietal wine, according to the consumer's desire. The current study describes a holistic approach in viticulture towards wine production, applying a multidisciplinary methodology. Firstly, "Asproudi", a rare Greek variety, was analyzed molecularly. The initial hypothesis that "Asproudi" is a distinct variety was questioned; microsatellite analysis showed that "Asproudi" is a population of different genotypes, at least in the Monemvasia Winery vineyard. A targeted harvest of each genotype was performed during the same day and was followed by micro-vinifications. All standard analyses of must and wine were performed in the laboratory, while a sensory analysis by a professional team evaluated each of the produced wines, showing distinctive differences. The genetic relationship of some of the Monemvasia Winery "Asproudi" genotypes to the varieties maintained in the reference collection was revealed whereas some other genotypes remained unknown.

Keywords: *Vitis vinifera*; rare grapevine varieties; microsatellites; SSRs; micro-vinification; must analyses; wine analyses; sensory analysis

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1. Introduction

Viticulture and wine-making represent long-standing activities in Greece. The area around Mt Pangaion (Figure 1) in the north-east part of the country provides an example where myth and scientific evidence meet: according to myth, the god of wine-making, Dionysos, lived with his followers, the Maenands on the slopes of Mt Pangaion. Thousands of charred grapevine seeds, together with grapevine pressings and clay cups used for drinking the grape juice or wine, have been recently unearthed from the Neolithic settlement of Dikili Tash located in the valley on the north of Mt Pangaion, making this area the oldest known wine-making site in Europe [1].

The term "Asproudi" comes from the word "Aspo" ("Άσπρο" in Greek means white) and etymologically represents a case of diminution, a phenomenon quite common in the Greek language, through which new words are produced that enrich the original meaning with a delightful and cheerful mood while indicating new properties.

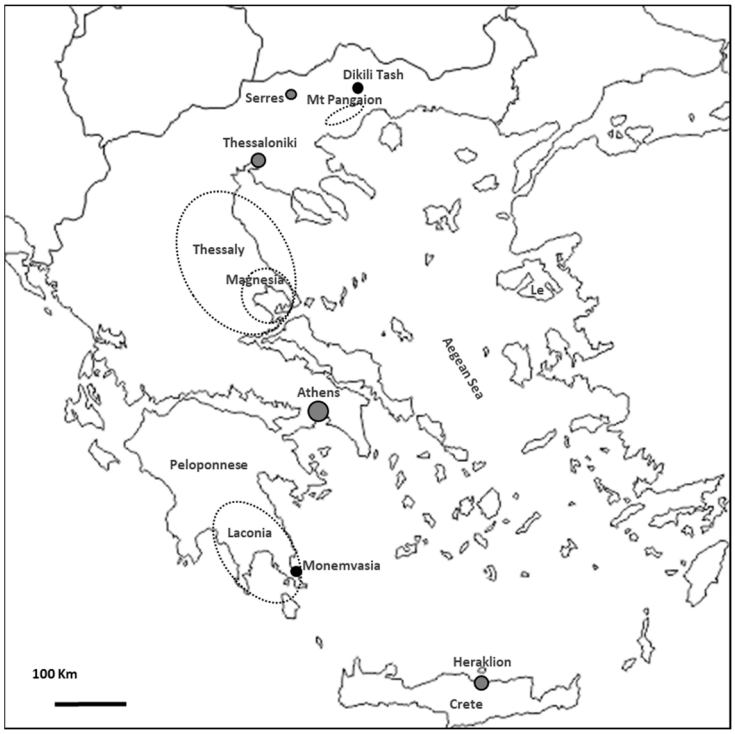

Figure 1. Map of Greece: the toponyms mentioned in the text are shown.

In the wine-related Greek literature, the term "Asproudi" was found as early as 1888 referring to grapevines cultivated in various areas of the country, such as in the central parts of Peloponnese, in Thessaly, and in the north Aegean islands (Figure 1) [2]; a few years later, however, the term was used to define the most dominant grapevine variety in the country [3] pointing out the color of the fruit. In the study "Greek Ampelography", a total of 12 varieties were described under the term "Asproudi" [4,5]. Nearly all of them were identified and discriminated either by the corresponding toponym of the cultivation area or by an adjective referring to a particular and well-observed feature. In recent years, some "heretic" statements, judging on the phenotypic characteristics, referred that "Asproudes" represent groups of white varieties that need to be separated [6], regardless of how difficult the task might be [7]. Therefore, a working hypothesis was emerged, according to which the term "Asproudi", together with its variants (e.g., "Asprouda", "Asproudes"), is a general term that has served as a verbal repository for grouping in the same heterogeneous group different varieties, exclusively on the basis of their light berry color and their time of maturation.

"Asproudi" vines are large with high vividness; it is considered as an early ripening local variety. Ripening occurs from early to mid-August, producing about four to five kilograms of grapes per vine. The wine that is produced by this variety has received many international awards pointing out the high value of this autochthonous grapevine material.

The Hellenic Agricultural Organization DIMITRA (ELGO-DIMITRA) officially maintains the reference collection of the country—more than five hundred autochthonous accessions—in three vineyards: (i) In Lykovrysi (Athens). This is the oldest and largest vineyard established in 1929. During the 1950s and 1960s, clonal experiments were performed for some of the most common autochthonous and international varieties, while in the 1980s numerous expeditions throughout the country ended up in the collection of nearly all of the autochthonous varieties. (ii) In Thermi (Thessaloniki). This is a copy of the Lykovrysi collection enriched with native varieties collected from the northern parts of the country. (iii) In Heraklion (Crete). This is a collection of the Cretan varieties as well as of varieties cultivated in the islands of the southern Aegean Sea.

From an economical/consumer point of view, the world market has started turning to distinct high-quality wines produced from the less-utilized autochthonous grapevine varieties imposing an absolute necessity to revalorize and preserve these varieties. This trend is not limited to specific regions and is observed in countries such as Italy [8], Germany [9], Argentina, Chile, and Bolivia [10]. The preservation and revitalization of these varieties aim to provide distinctive identities to wines and promote biodiversity in vineyards. Additionally, there is a need to adapt to changing environmental conditions, such as climate change, which may require the cultivation of new varieties for sustainable viticulture [11]. However, the market introduction of new resistant selections can be impeded by preconceptions about their wine quality. Overall, the cultivation and promotion of autochthonous grapevine varieties contribute to the diversity and sustainability of the wine market, investing in a novel high-quality wine product of unique organoleptic characteristics.

The pathway towards the valorization and utilization of such cultivars commences with the crucial phase of accurate varietal identification. This, however, could turn out to be a difficult task due to the occurrence of synonyms, homonyms, and misidentifications [12]. Ampelographic identification is based on phenotypic differences. The primary drawback of this approach is that identification solely relies on visual data, necessitating an expert with extensive training to ensure effectiveness. Nevertheless, misidentifications may still occur due to the sheer number of existing cultivars and their resemblance, exacerbated by the impact of external factors on grapevine morphology [13]. At present, grapevine identification is typically augmented through the use of molecular markers. Molecular analysis offers a more precise identification and characterization, as its outcomes are not influenced by environmental factors [14]. Among the various types of DNA markers, microsatellite markers (SSRs) have been widely employed in grapevine identification. These markers are highly informative and their application can yield distinctive profiles that provide unambiguous identification of grapevine cultivars, independent of environmental factors, diseases, or vineyard practices. SSR markers are locus-specific and co-dominant, enabling the inference of familial relationships between different grapevine cultivars [15].

The revival of autochthonous grape varieties emphasizes the necessity of evaluating the produced wine, particularly due to the lack of previous research data. Molecular identification of the plant material that is used for the production of wine or related products together with their chemical analysis are needed to ensure authenticity [16]. A novel and more extensive approach involving molecular identification of the plant material used, together with a chemical analysis of the produced must and the wine, followed by sensory analysis of the final product, have been introduced recently [17] on the study of two minor Greek grapevine varieties, Karnachalades and Bogialamades. Previously, a multidisciplinary approach was used for the accurate ampelographic description of four Albanian varieties ultimately aiming to improve local economies [18].

Herein, we describe our involvement with the "Asproudi" grapevines of a commercial vineyard in Peloponnese. Initially, we were interested in revealing the molecular profile of this autochthonous Greek variety. Interestingly, however, we found out that it is a population of distinct genotypes. To evaluate their oenological potential, we performed targeted micro-vinifications for each of these genotypes; must and wine chemical analyses were carried out, followed by the sensory analysis on the final products. At later stages, when the molecular profile of the grapevine varieties maintained in the Greek reference collection became available, the molecular profiles of the genotypes found in the "Asproudi" population were compared to them. Some genotypes of the "Asproudi" population identified as varieties maintained and conserved in the reference collection while some others still remain unidentified.

2. Materials and Methods

2.1. Monemvasia Winery Management

The Monemvasia Winery vineyard, owned by the Tsimbidis family, is located in Monemvasia (36°40′59.1″ N 22°54′53.6″ E), the eastern part of Laconia facing the Aegean

Sea (Figure 1). It is planted in a homogeneous sandy-clay field in a sloppy hill. Vines are planted in R110 rootstocks, in 2.5 m × 1.1 m blocks, and trained on double cordon. All vine training, irrigation, and spraying operations were regularly performed each year. The pruning system that followed was three spurs in each cordon.

2.2. Molecular Studies

Young leaves had been collected from various plants of the Monemvasia Winery vineyard and were kept on ice until stored at −80 °C for further use. Genomic DNA was extracted from about 100 mg of the frozen tissue using the commercially available NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The integrity of the extracted genomic DNAs was evaluated by agarose gel electrophoresis, and the concentration was estimated by using a Quawell (Q3000 UV-Vis Spectrophotometer, Quawell Technology Inc., San Jose, CA, USA) spectrophotometer.

Polymerase chain reactions (PCRs) were performed as before [17,19], in a volume of 20 μL using 25 to 30 ng genomic DNA as a template, 200 mM of each dNTP, 10 pmol primers, 2 μL 10× KAPATaq DNA Polymerase buffer, and 1 U KAPATaq DNA Polymerase (KapaBiosystems, Cape Town, South Africa). Ten pairs of primers were used: VVS2 [20], VVMD5 and VVMD7 [21], VVMD25, VVMD27, VVMD28, and VVMD32 [22], and VrZAG62, VrZAG67, and VrZAG79 [23]. Forward primers were 5′–end fluorescently labeled with different fluorophores: FAM, HEX, ROX, and TAMRA. Primers were custom labeled according to (i) each dye's absorption and emission wavelength and (ii) the length of the amplified product to avoid overlapping during gel electrophoresis. PCR amplifications were performed in a 96-well MiniAmp Thermal Cycler (Applied Biosystems, Foster City, 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 3730xl DNA Analyzer (Applied Biosystems, CA, USA). Data analysis, sizing, and genotyping were performed using the GeneMapper (version 4.0) software. The GenAlEx 6.5 program [24] was used for statistical analysis (Genetic Distance—Codom Genotypic). Data were then exported to the MEGA4.1(Beta) program [25] as a Tri-Matrix using the default options. Dendrograms were constructed using the UPGMA method in the MEGA4.1(Beta) program.

2.3. Harvest and Standard Grape Juice Analysis

"Asproudi" vines from the Monemvasia Winery were grown under the same viticultural management until their manual harvest; they were all harvested during the same day, early in the morning in order to avoid oxidations [26].

The exact harvest date (last week of August 2019) was decided considering the same criteria as every year: desirable physiochemical parameters (°Brix, titratable acidity, and pH value) and technological maturity. The grapes were harvested by hand with care to ensure consistency and were pooled in one (or more) basket per group. Groups were defined according to the outcome of the microsatellite analysis. All baskets were transferred to the Laboratory of Enology and Alcoholic Drinks at the premises of the Agricultural University of Athens, and stored overnight in a cooling chamber (at 4 °C), before being moved to the experimental winery for vinification. As a reference, harvest of the "Asprouda" variety, maintained by ELGO-DIMITRA at Lykovrysi (38°04′09.1″ N 23°46′32.9″ E) was also performed.

Prior to fermentation, a set of physiochemical parameters related to maturity were conducted in the must. The analytical methods recommended by the International Organization of Vine and Wine (OIV) were used to determine the sugar concentration and titratable acidity of the grape juices. Thereafter, each group was divided into three small-scale vinifications. Due to lower acidity levels in white musts often causing the polymerization of phenolic compounds and resulting in brown deposits and therefore causing darkening of white wine [27], 1 kg/tn of tartaric acid was added in each vinification.

2.4. Small-Scale Vinification Protocol, and Must Analysis

Grapes were destemmed, crushed, and softly pressed—the grape juice was sulfated with 15 mg/L of sulfur dioxide (SO_2) during the crushing. The pressed juice was placed in 10 L plastic bottles, and 3 mg/L Safizym pectinase enzyme (Safizym Clean, Fermentis, Marquette-lez-Lille, France; endo-polygalacturonase) was added in order to facilitate sedimentation. The bottle headspace was purged with N_2 before the bottles were sealed and left overnight at 4 °C for sedimentation. Clear juice, coming from each group, was racked off the sediment. Conventional analysis (pH, °Brix, and titratable acidity) was conducted in the clear must of each group. Two liters (2 L) of clear must was transferred into clean 3 L tanks, in triplicates for each group. The clear grape must was inoculated with Safoeno GV 107 (Fermentis, Lille, France) yeast, prepared in accordance with the manufacturer's instructions. As for the yeast nutrient (20 g/100 kg), SpringFerm™ (Fermentis, Lille, France) was used—this includes inactivated yeast (rich in growth factors). Fermentations in triplicates of each group were conducted in a temperature-controlled environment (16 to 18 °C).

Alcoholic fermentation showed a regular trend, and was considered finished when the reducing sugar concentration was lower than 2 g/L. At the end of fermentation (approximately after 10 days), wines were racked and they were stored for the stabilization process at a controlled temperature (4 °C). Finally, before sealing the wines, 30 mg/L SO_2 was added for protection.

2.5. Analysis of Conventional Oenological Parameters and Total Phenolic Index

After sealing the wines in the plastic bottles, they were analyzed using the OIV methods [28] on alcohol percentage, reducing sugars, pH value, and total acidity.

The total polyphenol index (TPI) was determined by measuring the 280 nm absorbance of a 1:100 dilution of wine with a spectrophotometer, using a 10 mm quartz cuvette and multiplying the absorbance value by 100 [29]. The total polyphenol concentration was determined by the Folin–Ciocalteu assay, with the micro-scale protocol [30]. The results were expressed as mg/L of gallic acid equivalents (GAE).

A modification of the model described by Singleton and Kramling [31] was used to assess browning development. Wine lots of 30 mL were filtered and placed in a 30 mL, screw-cap glass vial (7.5 cm length, 2.1 cm internal diameter). Samples were subjected to heating at a constant temperature of 55.0 ± 0.2 °C in a heating chamber. Aliquots were withdrawn at 24 h intervals over a period of 13 days, and browning was measured at A420. The samples were then immediately returned to the vials to maintain the initial headspace volume.

2.6. Sensory Evaluation of the Wine

Sensory trials were carried out by twelve trained assessors (recruited by the Laboratory of Enology and Alcoholic Drinks; equal sex distribution) with professional experience in the wine industry, and at least one year experience in tasting white wines. Wine samples used for the sensory evaluation were unfiltered, and the evaluation was performed two months after sealing the tanks.

Samples (25 mL) of wines were presented in a randomized order for each participant. Samples were served in ISO standard glasses 3591 [32] and numbered by a random three-digit number; each glass was covered with a glass cup in order to avoid diffusion of odorants [33,34]. The evaluation consisted of describing the appearance, aroma, taste, and harmony of each wine sample; the taster participants were first asked to describe each wine by a list of seven descriptors ("Color Intensity", "Aroma Intensity", "White Fruits/Flowers", "Vegetal Aroma", "Taste Balance", "Acidity", "Aftertaste"), and then to proceed to a quantitative assessment, using a scale of 0 to 10 (from lowest to highest intensity).

2.7. Statistical Analysis

Small scale vinifications were performed in triplicates. All values are presented as the mean ± standard error. All values are presented as the mean and standard deviation. Statistical analyses were performed using the Statgraphics Centurion application (version 1.0.1.C). The significance of the results was determined with an unpaired *t*-test or one-way ANOVA with Tukey's test. Multivariate statistical data analysis (MVA) of the samples was performed with XLstat (XLSTAT 2017: Data Analysis and Statistical Solution for Microsoft Excel; AddinSoft, Paris, France, 2017).

The sensory evaluation data were analyzed by a non-parametric Kruskal–Wallis one-way analysis of variance using Statgraphics Centrurion. The Kruskal–Wallis Non-Parametric Hypothesis Test is used when a variable does not meet the normality assumptions of a one-way ANOVA. When the *p*-values were <0.05, a post-hoc Mann–Whitney–Wilcoxon test was applied to compare, one by one, the wines for each variable.

3. Results

3.1. Molecular Studies Showed That "Asproudi" of the Monemvasia Winery Is a Population of Different Genotypes

Searching for autochthonous grapevine material in Peloponnese, an initial sampling mission was performed at the early stages of the vegetative period (1 June) of 2018 in the Monemvasia Winery vineyard. A total of sixteen samples were collected from the vineyard; eight of the samples were collected randomly from the vines of the white variety that was known as "Asproudi". Molecular identification on microsatellite loci was performed on these samples and on the autochthonous varieties "Monemvassia" (spelling according to the updated national Catalogue: ya530_57378_020322-2), "Kydonitsa", "Assyrtiko, "Glykerithra", and "Gaidouria" which are commonly cultivated in the wider geographical area of Monemvasia, and in other parts of the Monemvasia Winery vineyard. Samples of these five reference varieties were also collected from the grapevines that are maintained in the reference collection at Lykovrysi. This analysis ended up in the distinction of the eight Monemvasia Winery "Asproudi" samples in four different and discreet groups not related to the five reference varieties analyzed (Figure 2; Groups i, ii, iii, iv); since the empirical names of the Monemvasia Winery working team were considered, the molecular analysis detected misnamings (indicated by asterisks in Figure 2): "Glykerithra-MW-2018" and "Gaidouria-MW-2018" are indeed "Assyrtiko". As no studies, neither ampelographic nor molecular, were performed prior to that analysis, this distinction was the first evidence that the Monemvasia Winery "Asproudi" material is actually a heterogeneous population of different genotypes. The emerging question was whether all genotypes that make up "Asproudi" have been represented in this primary analysis. To answer this question, sampling was repeated the following year, when bunches were mature (the last days of July 2019). Thirty-one samples were collected: phenotypic differences were considered when choosing the vines to sample. In addition, twenty-one samples of white grapevines cultivated in the surrounding vineyards ("Unknown" samples from Estate Loulouda and Estate Sarra) were also collected. The analysis on the same microsatellite loci brought up seven groups (Table 1 and Figure 3; hereafter, the constituting groups are called "Groups": A, B, C, D, E, F, G).

Table 1. Microsatellite analysis at nine loci; the microsatellite loci are designated in the top line. Allele size (in base pairs) are shown; asterisks (*) indicate the varieties that are maintained by ELGO-DIMITRA at the Lykovrysi's grapevine vineyard (Athens); "MW": "Monemvasia_Winery"; "Est_Loulouda" and "Est_Sarra" are estates located in the wider Monemvasia area; "2018" or "2019" indicate the year the samples were collected. Xinomavro, Koundoura lefki, and Cabernet Sauvignon were included as reference material. Data for the VVMD5 are not shown because the data produced were not available for all samples.

	VVS2		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VrZAG62		VvZAG67		VvZAG79	
Asproudi-MW-2018-1	141	155	238	246	243	253	183	187	259	279	250	254	195	203	138	160	241	241
Asproudi-MW-2018-2	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249

Table 1. *Cont.*

	VVS2		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VrZAG62		VvZAG67		VvZAG79	
Asproudi-MW-2018-3	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	249
Asproudi-MW-2018-4	133	143	246	248	237	239	187	191	249	259	256	268	201	203	138	150	241	249
Asproudi-MW-2018-5	143	151	238	252	253	261	179	183	249	261	248	256	187	187	124	130	249	257
Asproudi-MW-2018-6	143	151	238	252	253	261	179	183	249	261	248	256	187	187	124	130	249	257
Asproudi-MW-2018-7	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	249
Asproudi-MW-2018-8	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249
Asproudi-MW-2019-1	141	155	238	246	243	253	183	187	259	279	250	254	195	203	138	160	241	241
Asproudi-MW-2019-2	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	247
Asproudi-MW-2019-3	143	143	240	252	237	239	177	187	255	259	248	254	187	199	130	148	241	249
Asproudi-MW-2019-4	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	247
Asproudi-MW-2019-5	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Asproudi-MW-2019-6	131	143	238	242	239	239	179	191	245	261	248	254	187	187	130	138	239	253
Asproudi-MW-2019-7	143	151	240	252	253	261	179	183	251	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-8	143	149	238	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-9	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Asproudi-MW-2019-11	143	143	240	252	237	239	177	187	255	259	248	254	187	199	130	148	241	249
Asproudi-MW-2019-13	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	138	241	247
Asproudi-MW-2019-14	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	138	241	247
Asproudi-MW-2019-15	143	151	240	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-16	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Asproudi-MW-2019-17	143	151	240	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-18	133	135	238	246	237	239	177	177	255	259	250	270	187	187	148	148	241	247
Asproudi-MW-2019-19	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	247
Asproudi-MW-2019-20	143	149	238	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-21	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	247
Asproudi-MW-2019-22	143	149	238	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-23	143	151	238	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-24	143	143	240	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Asproudi-MW-2019-25	143	149	238	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asproudi-MW-2019-26	143	143	240	252	237	239	177	187	255	259	248	254	187	199	130	148	241	249
Asproudi-MW-2019-27	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Asproudi-MW-2019-28	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	249
Asproudi-MW-2019-29	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	247
Asproudi-MW-2019-30	133	143	246	248	237	239	187	191	259	259	256	270	201	203	138	150	241	249
Unknown-Est_Loulouda-46	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Unknown-Est_Loulouda-47	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249
Unknown-Est_Sarra-48	133	143	246	248	237	239	187	191	249	259	256	268	201	203	138	150	239	239
Unknown-Est_Sarra-49	143	151	240	252	253	261	179	183	251	261	248	256	187	187	124	130	247	255
Unknown-Est_Sarra-50	143	143	238	252	237	239	177	187	255	259	248	254	187	199	130	148	241	247
Unknown-Est_Sarra-51	143	151	240	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Unknown-Est_Sarra-52	143	149	238	252	253	261	179	183	249	261	248	256	187	187	124	130	247	255
Asprouda_Aitoloakarnanias-1 *	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249
Asprouda_Aitoloakarnanias-2 *	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249
Asprouda-1 *	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249
Asprouda-2 *	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	249
Asprouda or Dimitreiko-1 *	133	143	246	248	237	239	187	191	249	259	256	270	201	203	138	150	241	247
Asprouda or Dimitreiko-2 *	133	143	246	248	237	239	187	191	249	259	256	268	201	203	138	150	241	247
Xinomavro *	131	131	248	248	237	239	177	179	229	245	248	250	193	203	122	136	235	247
Koundoura lefki *	139	143	244	248	239	247	183	191	237	259	250	256	195	201	144	154	235	247
Cabernet Sauvignon *	137	151	238	248	237	247	173	187	235	237	238	238	187	193	122	136	243	243

The eight genotypes analyzed in 2018 were identified in these seven groups, together with the samples from the wider area (Estate Loulouda and Estate Sarra). These results demonstrated that the "Asproudi" of the Monemvasia Winery vineyard represents indeed a population of at least seven genotypes. Some of these genotypes are represented in high numbers (Groups A and D) in the "Asproudi" population of the Monemvasia Winery vineyard while some others are represented minimally (Groups B, C, and G consisted only of one vine). At that point, the emerging question was whether the seven genotypes that make up the "Asproudi" population in the Monemvasia Winery vineyard represent unknown autochthonous material or they are registered in the National Catalogue and in the Greek bibliography. To answer this question, the molecular profile of the seven Monemvasia Winery Groups were compared to the molecular profile of many of the varieties maintained in the reference collection by ELGO-DIMITRA; this comparison was performed in summer 2022 (when such molecular data became available for the genetic material maintained in the reference collection). The analysis showed that: Group A possesses a high degree of similarity to the variety "Asprouda" (synonym: Dimitreiko), originally collected from Arkadia (the central part of Peloponnese), and "Asprouda Aitoloakarnanias"; both maintained in the reference collection. Group D is related to "Arkadino", also a variety of Arkadia, while Groups E and F are both highly close to the variety "Proimo aspro", a white variety

collected from the area of Serres—a region in the very north of the country. For the single member Groups B, C, and G, only the latter was found to be closely related to the variety "Svarna" collected from Magnesia (central Greece), while the former two remained unidentified. Some of the Monemvasia Winery "Asproudi" genotypes have also been detected in neighboring vineyards (Estate Loulouda and Estate Sarra; "Unknown" samples 46 to 52; Figure 3 and Table 1) supporting the concept that these genotypes represent common local grapevine genetic material.

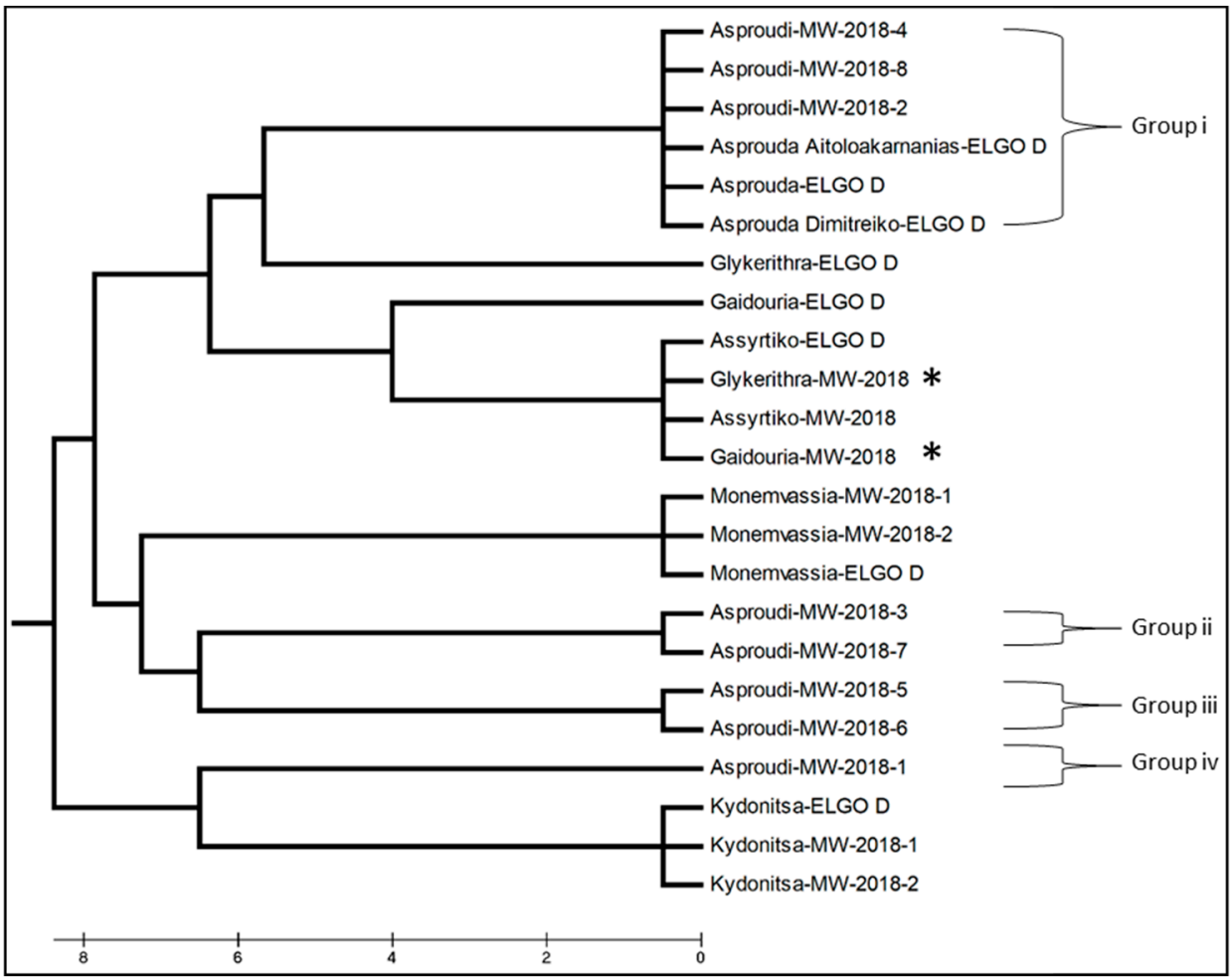

Figure 2. Dendrogram showing the relationship among the "Asproudi" genotypes of the Monemvasia Winery (MW) and the reference samples collected from the reference collection maintained by ELGO-DIMITRA (ELGO D). Since the empirical names of the MW were used, misnamings have been detected (indicated by asterisks): "Glykerithra-MW-2018" and "Gaidouria-MW-2018" are indeed "Assyrtiko".

3.2. Oenological Potential of the Monemvasia Winery "Asproudi" Constituting Genotypes

As long as it was clear that the Monemvasia Winery "Asproudi" was indeed a population of discreet genotypes, the aim of this work was to evaluate the oenological potential of the constituting genotypes.

Harvest time was decided by the experts of the Monemvasia Winery taking into consideration the technological maturity of the grapes; harvest was performed in the same day (27 August 2019; Table 2) for all groups. Group G was heavily infected by fungal infections; therefore, it was excluded from further experimentation on vinification. An additional sample was harvested on 4 September from "Asprouda" (synonym: "Dimitreiko") maintained in the reference ELGO-DIMITRA grapevine collection (hereafter: Group H). Table 2 shows the sugar concentration and titratable acidity of the grape juices of the different

groups, confirming that they were at different ripen stages: °Brix values in musts varied from 17.2 (Group C) to 26.3 (Group B). In the oenological field, the different groups provide grape berries with different levels of maturity (Table 2): the pH values varied between 3.47 (Group C) and 3.93 (Group H). Group H showed by far the highest content of total acidity (4.95 g/L) compared to the lowest 3.1 value (Groups B and D).

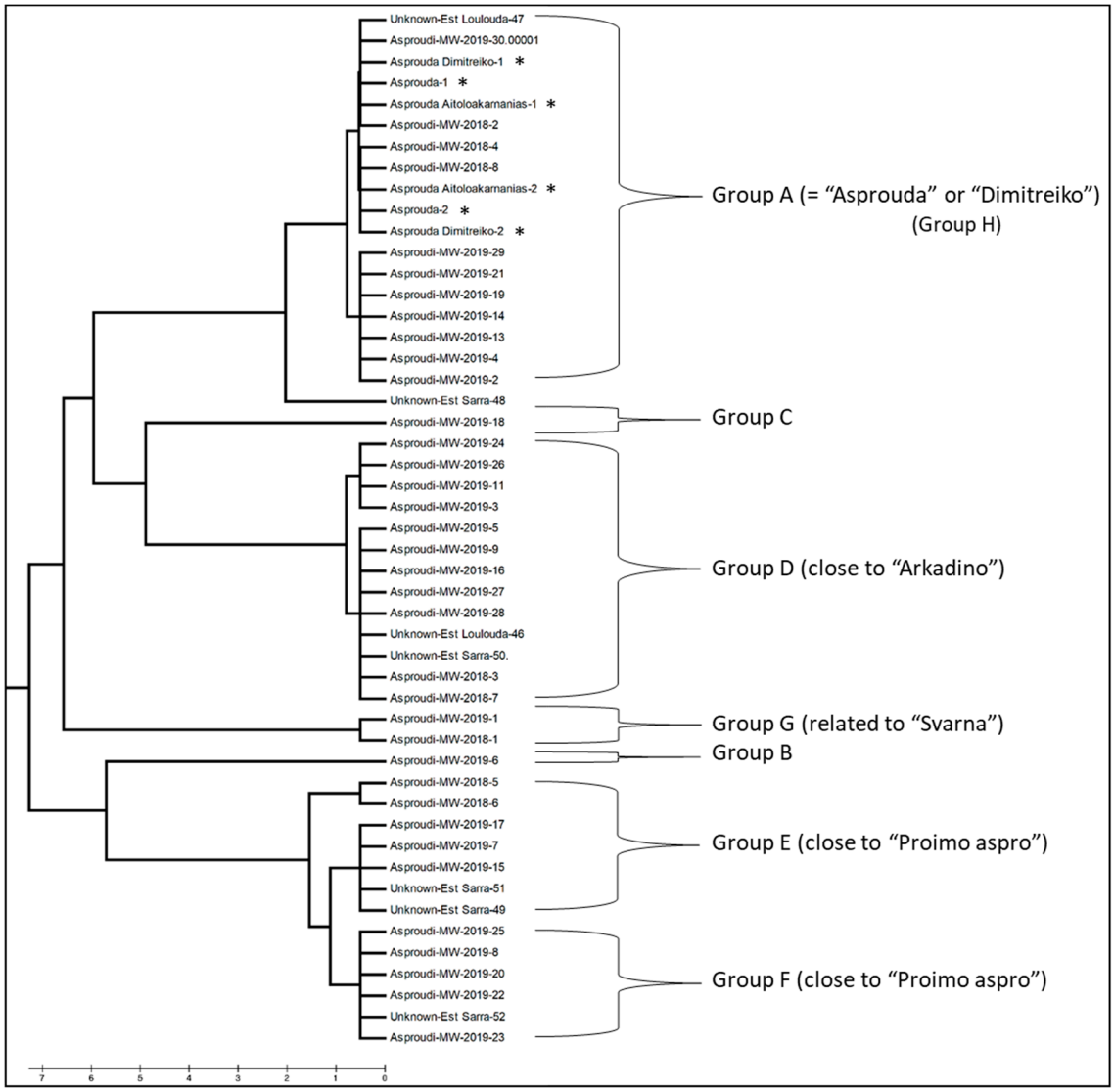

Figure 3. Dendrogram showing the distinction of the "Asproudi" Monemvasia Winery (MW) genotypes to seven groups (A to G). Samples from the area that surrounds the Monemvasia Winery vineyards were also included in this analysis (Unknown-Est_Loulouda and Unknown-Est_Sarra) together with reference material from the reference collection (indicated by an asterisk). "2018" and "2019" indicate the year that sampling occurred.

A key factor to produce wines with high-quality characteristics is the distinctive aroma. The warm and dry summer of the respective vintage of Monemvasia during the ripening period provided must with low levels of total acidity, recorded in all Monemvasia Winery "Asproudi" groups. Therefore, tartaric acid was added to the musts to adjust the pH of the must and ensure a smooth alcoholic fermentation.

Wines were analyzed approximately two months after the end of fermentation to record the evolution of ethanol content, pH, and total acidity in the micro-vinifications (Table 3). The ethanol content of the wines produced by the Groups B, D, A, and G was high; this is in agreement with the corresponding sugar concentration observed in grapes.

An exception is recorded: the wine produced from Group D. Total acidity indicates the freshness of the wines, and the stability of a wine over time; in this respect, the wines from Groups A and B attained the highest values of acidity (7.8 and 7.42 tartaric acid g/L, respectively; Table 3), whereas the lowest values were recorded by wines from Group H and F (5.24 and 5.77 tartaric acid g/L, respectively). Group H had the highest wine pH value (3.6), whereas Group C had the lowest (2.88). All wines can be considered as dry wines because the reducing sugar content was less than 2 g/L (Table 3).

Table 2. Details of harvest and vinification (cultivation area, harvest time, and vinification time), and conventional must analysis (°Brix, pH, and total acidity) of all groups analyzed. Groups A to F refer to the Monemvasia Winery vineyard "Asproudi" plant material, while Group H refers to the "Asprouda" variety that is maintained in the ELGO-DIMITRA vineyard at Lykovrysi. Means ± Standard errors followed by a different letter, in the column, do differ by Tukey's HSD test at 5% probability. Values represent means of triplicate determinations ± standard error.

Grape Group	Cultivation Area	Harvest Date	Vinification Date	Total Soluble Solids (°Brix)	pH	Total Acidity (Tart. Ac. g/L)
A	Monemvasia	27 August 2019	28 August 2019	24.1 ± 0.3 c	3.62 ± 0.04 b	4.30 ± 0.1 b
B	Monemvasia	27 August 2019	28 August 2019	26.3 ± 0.2 a	3.87 ± 0.07 a	3.10 ± 0.16 d
C	Monemvasia	27 August 2019	28 August 2019	17.2 ± 0.3 f	3.47 ± 0.04 c	3.75 ± 0.10 c
D	Monemvasia	27 August 2019	28 August 2019	24.8 ± 0.4 b	3.8 ± 0.09 a	3.10 ± 0.21 d
E	Monemvasia	27 August 2019	28 August 2019	20.9 ± 0.5 d	3.68 ± 0.07 ab	3.75 ± 0.19 c
F	Monemvasia	27 August 2019	28 August 2019	21.4 ± 0.6 de	3.69 ± 0.07 ab	3.70 ± 0.07 c
G	Monemvasia	27 August 2019	---	---	---	---
H	Lykovrysi	4 September 2019	5 September 2019	22.5 ± 0.4 e	3.93 ± 0.06 a	4.95 ± 0.04 a

Table 3. Conventional wine analysis (alcohol volume, pH, and total acidity) of the wines from all seven groups; Groups A to F refer to the Monemvasia Winery "Asproudi" Groups, while Group H refers to the "Asproudi" variety that is maintained in the ELGO-DIMITRA vineyard at Lykovrysi. Means ± Standard errors followed by a different letter, in the column, do differ by Tukey's HSD test at 5% probability. Values represent means of triplicate determinations ± standard error.

Grape Group	Alcohol Volume (V.V.%)	pH	Total Acidity (Tartaric Acid g/L)	Residual Sugars (g/L)
A	13.5 ± 0.01 c	3.093 ± 0.014 bc	7.80 ± 0.07 f	0.76 ± 0.02 c
B	15.4 ± 0.04 e	2.975 ± 0.021 ab	7.42 ± 0.10 e	0.61 ± 0.02 f
C	9.2 ± 0.07 a	2.888 ± 0.016 a	6.82 ± 0.035 d	0.67 ± 0.02 df
D	14.6 ± 0.11 d	2.948 ± 0.044 ab	6.86 ± 0.051 d	1.40 ± 0.02 a
E	12.3 ± 0.11 b	3.150 ± 0.096 c	6.11 ± 0.018	0.93 ± 0.02 b
F	12.2 ± 0.09 b	3.026 ± 0.040 abc	5.77 ± 0.03 b	0.72 ± 0.023 cd
H	13.3 ± 0.07 c	3.601 ± 0.026 f	5.24 ± 0.03 a	1.44 ± 0.023 a

Groups B and D were at a higher degree of maturity (Table 2), thus provided wines with higher values of absorption and phenolics, in contrast to Groups C, E, and F which had not reached comparable maturity levels. Similar results have been reported [35]: wines from early harvests showed a pale yellow-soft color and lower color intensity. The wines produced from grapes with a high degree of phenolic maturation (Groups B and D) had a higher absorption value at 420 nm (Table 4), so they appeared darker in color and with higher values of phenolics (Figure 4). Therefore, the degree of the grapes ripening during harvest is a critical factor for the color of the produced wine as well as for its aromatic potential. Wines from Groups B, D, and A have shown maximum ethanol content, the highest values of absorbance at 420 nm, and degrees in TPI and K factor (Table 4). The results imply that the identified factors are unique to each distinct group, underscoring the significance of scrutinizing these groups more extensively with regards to viticultural and oenological utilization and exploitation. It can be stated that the wines from the genotypes

of the Monemvasia Winery "Asproudi" plant material can be distinguished according to their classic parameters.

Table 4. Compositional factors and browning characteristics determined in the wines from all groups analyzed: Groups A to F refer to the Monemvasia Winery "Asproudi" Groups, while Group H refers to the "Asproudi" variety that is maintained in the ELGO-DIMITRA vineyard at Lykovrysi. Means ± Standard errors followed by a different letter, in the column, do differ by Tukey's HSD test at 5% probability. Values represent means of triplicate determinations ± standard error.

Grape Group	420 nm	Folin Ciocalteu (Gallic Acid mg/L)	TPI	k Factor
A	0.0965 ± 0.0016 d	2.8511 ± 0.0303 b	9.5266 ± 0.3407 c	0.0026 ± 0.00026 c
B	0.3485 ± 0.0025 f	3.9944 ± 0.0032 cd	14.1266 ± 0.0878 d	0.0082 ± 0.00016 e
C	0.0525 ± 0.0007 a	2.5947 ± 0.1097 a	7.21330 ± 0.0838 a	0.0013 ± 0.00017 ab
D	0.1780 ± 0.0016 e	3.8696 ± 0.0488 c	13.9866 ± 0.1215 d	0.0068 ± 0.00048 d
E	0.0610 ± 0.0009 b	2.8063 ± 0.0092 b	8.2200 ± 0.07540 b	0.0006 ± 0.00040 a
F	0.0515 ± 0.0002 a	2.5097 ± 0.0013 a	7.8400 ± 0.08210 ab	0.0022 ± 0.00031 bc
H	0.0880 ± 0.0009 c	4.0590 ± 0.0382 f	7.5266 ± 0.2087 a	0.0008 ± 0.000005 a

Figure 4. Visual wine color evaluation—from left to right, the wines of Groups A to H (no wine from Group G).

In the analysis of compositional factors and browning characteristics, a repeated pattern was observed: wines of Groups B, D, and A received the highest values in the absorption at 420 nm, Folin index, TPI factor, and K factor (Table 4) with one exception: wine from Group H in the Folin index. These results might be explained by the high oxidation of those wines and the tendency to be oxidized in a shorter period than the rest of the wines.

Absorbance at 420 nm varied from 0.0515 to 0.3485 (Groups F and B, respectively). Total phenolics showed great variance: the values of equivalent of gallic acid mg/L in the Folin Ciocalteu assay ranged between 2.5097 (Group F) and 4.059 (Group H), whereas the values of the TPI ranged between 7.2133 (Group C) and 14.1266 (Group B). Finally, the accelerated browning test provided significant differences among the k factor (from 0.0008 to 0.0082) (Table 4). The wines that showed significantly lower values regarding the k factor were those made from Groups E and H: practically, these two groups produce wines which would develop a brown color later than the others.

3.3. Sensory Analysis Confirmed That Different Wines Are Produced by Different Genotypes

Sensory analysis was conducted two months after the end of alcoholic fermentation—during this period wines were kept at 4 °C for protein and tartaric stabilization. Quantitative descriptive sensory analysis was assessed for average wine odor and taste intensity scores (Table 5 for statistical data elaboration; Figure 5).

The sensory descriptor "Color Intensity" showed a diverse performance, with the wines of Groups B and D possessing the highest values. In "Aroma Intensity", wine from Group H showed by far the highest score, whereas all the other groups possessed comparable scores. As for the odoriferous "White Fruits/Flowers" character, wine produced by Group B presented the lowest value—all other wines presented a comparable performance. Wine from Group A received the highest score in "Vegetal Aroma". In respect to the

descriptor of "Taste Balance", the lower scores were recorded by the Groups B and C. The panelists judged wines B and D with the highest value for the "Acidity" descriptor while wine H received the lowest value. Finally, no significant differences were observed among the wines for the descriptor "Aftertaste".

Table 5. The Kruskal–Wallis test and, when significant, ($p < 0.05$) the Mann–Whitney–Wilcoxon test were applied for multiple comparisons to the results of the sensory scores for the wines produced by the different fertilization treatments. Test statistics: the Kruskal–Wallis test was statistically significant when $p < 0.05$. Different letters in each row indicate significant differences ($p < 0.05$) among different samples.

Sensory Descriptors	Kruskal–Wallis Test *p*-Value	Post-Hoc Mann–Whitney–Wilcoxon Test						
		A	B	C	D	E	F	H
Color Intensity	2.004×10^{-8}	b	d	ab	c	d	a	ab
Aroma Intensity	0.007	a	a	a	a	a	a	b
White Flowers/Fruits	0.004	a	b	a	a	a	a	a
Vegetal Aroma	0.03	a	ab	b	ab	ab	b	b
Taste Balance	0.0124	a	b	b	ab	a	ab	a
Acidity	0.02	a	a	a	a	ab	ab	b
Aftertaste	0.04	ab	a	b	a	ab	ab	a

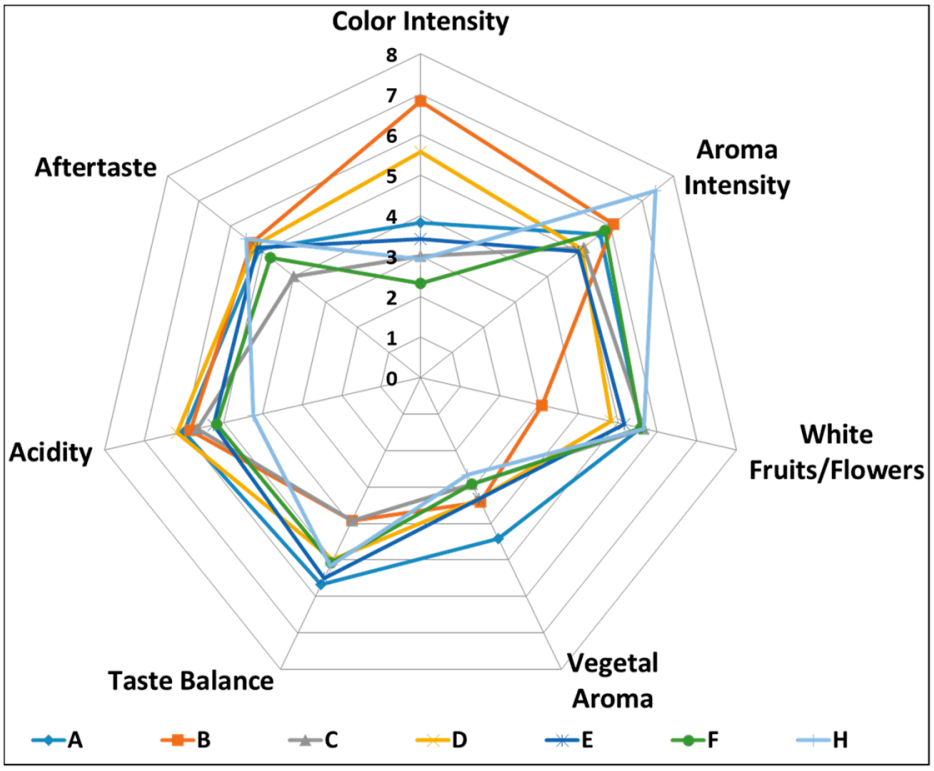

Figure 5. Spider plot of the sensory profile of the experimental wines produced by the various Monemvasia Winery "Asproudi" Groups, as outlined by a group of trained panelists from the Laboratory of Enology and Alcoholic Beverages of the Agricultural University of Athens. Wines were judged using predefined quality attributes on a scale from 1 (absent) to 10 (high).

The Kruskal–Wallis test was applied due to the fact that it is a rank-based test that is similar to the Mann–Whitney U test, but can be applied to one-way data with more than two groups. The Kruskal–Wallis test does not address hypotheses about the medians of the groups. Instead, the test addresses if it is likely that an observation in one group is greater than an observation in the other (Table 5). The outcome of the Kruskal–Wallis test unravels

differences among the groups, but does not unravel which groups are different from other groups; this was achieved by performing the post-hoc Mann–Whitney–Wilcoxon testing (Table 5).

Principal component analysis (PCA) showed that the wines produced by the various Monemvasia Winery "Asproudi" genotypes differ in their classic parameters, confirming the assumption that different varieties produce different wines. Projection on the plot clearly separated the samples into two main groups: one on the left and one on the right of the Y axis (Figure 6a). Each group is subdivided into two subgroups: one above and one below the X axis, so as, in the end, an even distribution of the "Asproudi" Groups in the plot is observed providing additional support to the concept that different and distinctive characteristics of each variety lead in the production of wines with a particular sensory profile while applying the same winemaking technique. It is noted that the same genotype cultivated in different terroirs (Group A in Monemvasia and Group H in Lykovrysi) may produce wines with different chemical and sensorial characteristics.

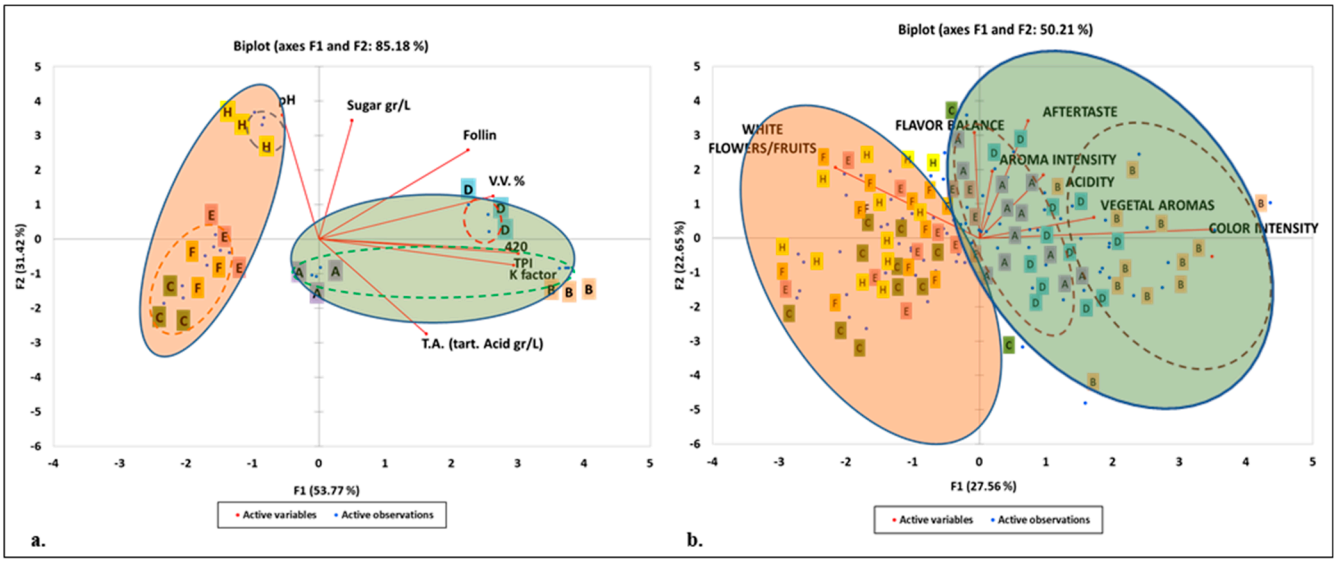

Figure 6. Unsupervised classification using Principal component analysis (PCA). (**a**) PCA applied to chemical parameters of wines of the different groups identified in the Monemvasia Winery "Asproudi" population. (**b**) PCA applied to the seven sensory descriptors from the experimental wines of the different "Asproudi" Groups. Samples in the score plots was colored according to the groups.

To analyze further the relationships of the attributes to the wine samples, PCA was conducted on the covariance matrix using seven additional terms ("White Flowers/Fruits", "Flavor Balance", "Aroma Intensity", "Aftertaste", "Acidity", "Vegetal Aromas", "Color Intensity") (Figure 6b). PC I explained 27.56% of the variance, whereas PC II explained 22.65% of the variance, totaling 50.21% of the variation of the data represented in the biplot. PCA showed that "Color Intensity" and "Vegetal Aroma" were associated mostly with the A, B, and D Groups, whereas "White Flower/Fruits" was associated with the G, C, and F Groups. As a consequence, the wines produced by different groups could be determined after building two PCAs: the wines of the H, F, C, and E groups are localized in the left side and the wines produced by the A, B, and D groups are localized on the right part of the two PCAs (Figure 6a,b).

Besides demonstrating the associations among the descriptors, PCA can also be used to display the relative "locations" of the samples with respect to each other and their characterizing attribute. Furthermore, a significant finding is that the discrimination regarding the chemical and sensorial analysis of the Monemvasia Winery "Asproudi" plant material is closely related with the grouping according to the molecular classification.

4. Discussion

For years, the identification of grapevine varieties was based solely on ampelographic descriptions. This method, however, is time consuming, requires experienced personnel, and depends on the terroir, the cultivation techniques, and the sanitary stage of the vines. During the last two decades, microsatellite analysis, a method based on DNA technology, has been used instead [36]. A complementary combination of the former, traditional ampelography, and the latter, modern ampelography, should be used, to achieve scientific progress.

"Asproudi" has long been cultivated in the central and south-east parts of Peloponnese. It is considered as a discreet grapevine variety, regardless of the recent statements that support it is a population of different genotypes [6,7]; it is noted, however, that in the recently updated National Catalogue of cultivated grapevine varieties in Greece (ya530_57378_020322-2), the term "Asproudi" has been replaced by the term "Asproudes" (plural of "Asproudi"). Our current work demonstrates that the Monemvasia Winery "Asproudi" is comprised of at least seven genotypes. One of these seven genotypes was identified as "Asprouda" (synonym: "Dimitreiko"), a registered variety maintained in the reference grapevine collection at the ELGO-DIMITRA premises. It was also found that the microsatellite profile of "Asprouda" is similar to the profile of "Asprouda_Aitoloakarnanias", another accession in the ELGO-DIMITRA reference collection. Another four of the seven genotypes were found to be genetically related to three registered accessions, whereas two genotypes remained unidentified. Future work could aim towards a comprehensive ampelographic description in combination with the molecular analysis of the four genotypes and also of the two unidentified (and undescribed) ones.

The chemical as well as the polyphenolic profile of a given grapevine cultivar serves as a significant indicator of its inherent genetic and commercial potential, and can be used as a valuable tool for discerning between distinct cultivars. The wines underwent analysis approximately two months subsequent to the cessation of the fermentation process. It is necessary to focus on the differences of total acidity among the "Asproudi" genotypes. This feature has added value due to climate change, which is considered as the main factor for the decreased acidity of wines, as well as the production of unbalanced wines [37].

Moreover, noteworthy variations were detected among the genotypes with regards to the maturity of grape berries. Therefore, it is important to ensure that cultivars do not ripen too early because ripening during the hottest period of the summer results in unbalanced wines that can be high in alcohol, lacking acidity, freshness, and aroma expression features [37–39].

During the grape ripening period, the sugar concentration increases whilst the acidity level declines. Grapes from cooler areas have higher levels of acidity, which is linked to slower grape ripening, compared to grapes from warmer climate areas [40]. It has also been reported that lower acidity levels in white wines are often the cause of the polymerization of phenolic compounds, resulting in brown deposits and therefore causing darkening of white wine [27].

The absorbance at 420 nm exhibits comparability with the previously reported values pertaining to the phenomenon of the browning test observed in Greek white wines over time [41]. According to the determined k factor, the genotypes of the Monemvasia Winery "Asproudi" provide wines with a decreased color intensity, low phenolic compounds, and the tension to brown later than the other Greek autochthonous white grapevine varieties, such as Assyrtiko, Moschofilero, Roditis, Petroulianos, and Malagousia [41,42].

"Color Intensity" is a key descriptor in the sensory evaluation; the wines of Groups B and D displayed the most elevated levels. Consistent with prior investigations on white wines, it is plausible that amplified mouthfeel qualities may be linked to a heightened phenolic compound presence [43]. Previous studies differentiated clones of the white grapevine variety Albarino based on their physiochemical parameters [44].

Our efforts extensively documented the chemical and sensory variability of wines obtained from different "Asproudi" groups that were grafted onto the identical rootstock and

cultivated under similar mesoclimatic conditions. It is important to note that altered chemical and sensory characteristics of wine were observed across different groups even when the same vinification protocol was applied. Six groups of the Monemvasia Winery "Asproudi" underwent evaluation, revealing significant differences in the classic parameters among musts and wines, as well as in sensory profiles. The outcome of this examination led to the conclusion that chemical compounds can serve as a valuable tool for the classification and differentiation of various clones, and those wines may possess distinct characteristics not only in relation to varieties but also to groups within a given variety. The application of PCA demonstrated that wines originating from different groups differ in their classic parameters. For instance, Groups B, D, and A exhibited the highest levels of ethanol content. Classic parameters analysis confirmed that distinct groups produce different wines. These findings suggest that the parameters are characteristic to individual groups, highlighting the importance of characterizing these groups for industrial use and consumer preference.

Characterizing autochthonous grapevine germplasm is crucial for preventing the erosion of genetic resources and the loss of local oenological products that are deeply rooted in the traditions of the region. However, in addition to conventional molecular and ampelographic analyses, it is also necessary to consider information about the natural environment in order to assess the optimal growth conditions for the genotype–environment relationship. Moreover, understanding the spatial distribution of local biodiversity within the indigenous territory can provide insights into the degree of erosion risk, which is inversely related to the extent of diffusion, as well as the importance of on-farm conservation. Abandoning vineyards would not only result in landscape loss and land degradation, but also undermine the preservation of the local biodiversity. Therefore, in the current research it is proposed that integrated criteria for evaluating and promoting local varieties are adopted as the means to promote the sustainability of grapevine production in the face of climate-related obstacles.

The multidisciplinary approach—molecular identification, must and wine evaluation, and sensory analysis—indicates that the genotypes found under the name "Asproudi" in the Monemvasia Winery vineyard could satisfactorily be used separately. This practice can satisfy the various grapevine growing and oenological requirements in different environmental conditions, taking advantage of their individualities, such as earliness of ripening, productivity, and ability to influence wine sensory characteristics in terms of body and complexity of aroma. Shaping the final quality features of the Monemvasia Winery, "Asproudi" grapes at harvest can offer valuable support to orient viticultural practices aimed at enhancing the quality of grape production in light of growing site and clone/variety preference. Alternatively, depending on the range of oenological objectives, it may appear advantageous for the simultaneous cultivation of several clones, chosen according to their level of production and aromatic complexity that can be used in mixes.

The objective of a sustainable agriculture/viticulture, as advocated by the new Rural Development Program (2014–2020) in alignment with the European 2020 Horizon objectives, will also entail the preservation of agro-biodiversity and landscape, as well as the mitigation of habitat fragmentation or simplification. In the Monemvasia region, numerous autochthonous grapevine cultivars are currently at risk alongside their respective landscapes. This investigation focuses on a local grapevine genotype, namely "Asproudi," and furnishes an analysis of its molecular characteristics, as well as the chemical and sensory descriptors, with the ultimate aim of utilizing distinct grapevine genotypes for the sustainable exploitation of genetic resources.

5. Conclusions

The multidisciplinary approach incorporated in the current study managed to distinguish a population of varieties that are known as "Asproudi". Each of these genotypes was evaluated in terms of vinification by chemical and sensory analysis.

Therefore, to answer the initial question, "How to improve a successful product", our suggestion would be to use scientific research as an essential tool in everyday practice. This,

although it may be considered as a self-evidenced assumption, is still not the main issue in many cases. The application of modern scientific research could be an important advantage to the primary sector representing two-way communication and cooperation between the scientists and the people of the primary sector (viticulturists, farmers, nurseries, etc.): the common goal would be to improve agricultural production, and ultimately the national economy and the private income. Our current work aims to serve as a first step towards this perspective.

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Informed Consent Statement: Panelists gave informed consent before participating in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author (pending privacy and ethical considerations).

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Conflicts of Interest: The authors declare no conflict of interest.

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