

Article

New Insight into the Identity of Italian Grapevine Varieties: The Case Study of Calabrian Germplasm

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Citation: Fanelli, V.; Roseti, V.; Savoia, M.A.; Miazzi, M.M.; Venerito, P.; Savino, V.N.; Pirolo, C.; La Notte, P.; Falbo, M.; Petrillo, F.; et al. New Insight into the Identity of Italian Grapevine Varieties: The Case Study of Calabrian Germplasm. *Agronomy* **2021**, *11*, 1538. <https://doi.org/10.3390/agronomy11081538>

Academic Editors: Carlos Miranda and Jorge Urrestarazu

Received: 14 June 2021

Accepted: 28 July 2021

Published: 31 July 2021

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Abstract: Calabria is a region located in Southern Italy and it is characterized by a long tradition of viticulture practices and favorable pedoclimatic conditions for grapevine cultivation. Nevertheless, less than 2% of cultivated land is dedicated to grapevine growing in Calabria. The characterization of local grapevine accessions is crucial to valorize the local and peculiar Italian products and boost the Calabrian winemaking sector. With this purpose, we performed a deep characterization of two widespread Calabrian grapevine varieties—Magliocco Dolce and Brettio Nero, of which very little is known. In particular, a genetic and morphological analysis, a berry physico-chemical and polyphenolic compositions assessment, and oenological evaluation of monovarietal wines were carried out. Our results allowed us to demonstrate that Magliocco Dolce and Brettio Nero are unique and distinct varieties with peculiar morphological and chemical characteristics and show the suitability of these two varieties in high-quality wine production. Moreover, the obtained molecular profiles will be useful for authentication and traceability purposes.

Keywords: varietal identification; microsatellite; ampelography; polyphenols; chemical composition; wine

1. Introduction

Calabria is a region located at the extreme south of Italy between the Ionian and the Tyrrhenian seas. It is one of the most ancient regions of Southern Italy for grape cultivation [1], being supposed as a secondary center of grapevine domestication along with Sicily [2]. Indeed, the Greek colonization strongly influenced agriculture in Southern Italy, giving Calabria and Sicily an important role for the establishment of new grapevine varieties coming from the Eastern Mediterranean Sea and for their spreading through Northern Italy and France [3]. Moreover, the Calabria region is prevalently hilly or mountainous (90% of the territory) with a wide range of altitudes making Calabria a favorable region for growing grapes as the altitude is directly associated with environmental factors, such as temperature and humidity, that can strongly influence the wine terroir [4].

Although the long tradition of viticulture practices and the favorable pedoclimatic conditions for cultivating grapevine, as at March 2021 only 9160 ha in Calabria are cultivated with grapevine, representing less than 2% of the Calabrian cultivated area [5]. This percentage is very low compared to other Italian regions; however, an increase in Calabrian

PDO (Protected Designated of Origin) wine production was observed in the last years, implying the intention to boost the Calabrian winemaking sector. Nine and ten Calabrian wines are designated as PDO and PGI (Protected Geographical Indication), respectively. Among the Calabrian PDO wines, Terre di Cosenza is gaining in popularity following the success achieved in some popular wine exposition such as Vinitaly. Terre di Cosenza is a relatively new entry in PDOs since it was introduced in 2011, and it includes red, white, and rose variants. According to the disciplinary of production, Terre di Cosenza red wine is produced with the variety Magliocco in a minimal percentage of 60% [6]. The name Magliocco is used to indicate two different grapevine varieties: Magliocco Dolce and Magliocco Canino. These are two of the most widespread grapevine varieties in Calabria, cultivated mainly in the provinces of Crotone, Catanzaro, and Cosenza [7].

The name Mantonico Nero was used for a long time to indicate a black variety genetically different from Mantonico Bianco [2]. The use of the same name followed by a different adjective related to the color of berry skin is used to indicate varieties sharing the same genotype with a somatic mutation affecting fruit color, as observed for some grapevine varieties [8,9]. Therefore, the name Mantonico cannot be used to indicate both, Mantonico “Bianco” and Mantonico “Nero” (standing for white and black in Italian, respectively). Consequently, the name Brettio Nero, instead of Mantonico Nero, was indicated for the recent registration of this variety into the Italian National Registry of Grape Varieties. Mantonico Nero is used as synonymous of Magliocco Dolce in the disciplinary of production of Terre di Cosenza; although a distinct genetic profile was observed for this cultivar [2,10]. Mantonico and Magliocco are used also in the production of another Calabrian PDO wine, Lamezia, whose production has increased slightly over the last two years. According to the disciplinary of production, Lamezia red wine could be produced using Magliocco variety along with other Calabrian black varieties. Lamezia Mantonico-type wine is produced with Mantonico variety in a minimal percentage of 85%.

In grapevine, it is not unusual to come across varietal confusion with the occurrence of many synonymies and homonymies. To overcome this problem, over the last 10 years the research on grapevine genotyping through DNA-based analysis has increased [11–13]. Due to the great economic value of grapevine cultivation, the first draft of the grapevine genome was published in 2007 by Jaillon et al. [14] and by Velasco et al. [15] of the highly homozygous clone Pinot Noir PN40024 and highly heterozygous Pinot Noir ENTAV115 respectively. The availability of these resources and the development of next-generation sequencing techniques have also facilitated the large-scale genotyping of grape collections [16,17], the functional genomics studies [18], the marker-assisted selection plans [19,20], the transcriptomic analyses [21], and the development of genome walking methods [22].

Grapevine genetic diversity can be assessed through ampelographic and molecular characterization; these methods are useful to identify and study local cultivars and reveal cases of synonymy and homonymy. For a long time, ampelography has been the only technique used for grapevine characterization [23]; however, it is strongly affected by the operator’s experience. The “Organisation Internationale de la Vigne et du Vin” (OIV) set a list of descriptors in order to standardize the ampelographic evaluation among different operators [24]. Nevertheless, the morphological traits are influenced by several factors including environmental conditions, thus ampelography has been gradually integrated by molecular techniques that are highly reliable and reproducible since they are not influenced by environmental factors [25]. The most used molecular markers are Single Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) because they have a codominant nature, a high degree of polymorphism, and are distributed across the whole genome [26].

The molecular characterization of local and peculiar accessions is fundamental also for traceability and authentication purposes. Being a high-value product, wine is subjected to different kinds of fraud, such as the addition of coloring and flavoring substances, the use of undeclared varieties and mislabeling. Among the different analytical approaches used for food traceability and authentication, the isotopic ratio-based methods, the mass spectrometry, and the nuclear magnetic resonance (NMR) are the most used in wine surveil-

lance [27]. All these methods have been proven to be highly efficient in the identification of the geographical origin and detection of adulterants added to a product; however, they present remarkable limitations in the detection of contaminant species or variety. Conversely, molecular marker-based traceability allows the univocal identification of a specific variety making possible the authentication and traceability of wine products [28].

Many studies about the grapevine genetic diversity were performed about the authentication of Calabrian varieties [2,29–31], allowing to unravel several cases of misnaming. As for Magliocco Dolce and Brettio Nero varieties, the identification remains unsolved. Pellerone et al. [29] obtained SSR profiles of variety Magliocco Canino, highlighting a genetic similarity to variety Perricone. Afterward, several synonymies for both Magliocco Dolce and Magliocco Canino were identified [11,30]; among these, a synonymy between Magliocco and Notardomenico, a variety commonly used in the production of several Apulian PGI wines, was identified. More recently, Sunseri et al. [2] affirmed that Magliocco Dolce is synonymous of Greco Nero, a variety widely cultivated in Southern Italy and used in many Calabrian PDO and PGI wines production. About the variety Mantonico, which indicates two color variants, white (Bianco) and black (Nero), only a few studies were performed to clarify the identity of this cultivar [2,10].

In our study, we intended to carry out a deep study through a multidisciplinary approach about the varieties Magliocco Dolce and Brettio Nero to define their main characteristics, as these two varieties were recently registered into the Italian National Registry of Grape Varieties (INRGV) [32] and the available information about them was limited. Genetic analysis, ampelographic evaluation, berry chemical, and polyphenolic assessment were used in the past with the purpose to characterize and valorize local and peculiar Italian grapevine varieties [11,33–35]; however, this is the first time that a combined approach based on a multidisciplinary analysis is used to deeply characterize and valorize two local varieties. In detail, we verified if Magliocco Dolce and Brettio Nero were unique and distinct varieties and if they were synonymous of any other Italian variety. We evaluated several ampelographic characters related to shoot, leaf, bunch, and berry. Moreover, we measured some chemical and polyphenolic compounds in berries at maturity assessing the effect of their levels on chemical and polyphenolic composition and sensory characteristics of monovarietal red wine. In addition, a genetic analysis of different Calabrian and Italian accessions was performed, comparing the obtained molecular profiles with those available in the Italian Vitis Database (IVD) [36], INRGV [32], and the European Vitis Database (EVD) [37] with the purpose to clarify the correct denomination of different neglected Calabrian varieties. The ultimate goal was the valorization of these two local grapevine varieties through a deep definition of their main characteristics and their oenological properties and the production of a molecular profile useful for their traceability and the protection of their identity.

2. Materials and Methods

2.1. Plant Material

Fresh leaves of Magliocco Dolce, Brettio Nero, Greco Nero, and Guarnaccia Bianca provided by Agenzia Regionale per lo Sviluppo dell'Agricoltura in Calabria (ARSAC) were used for genetic analysis. In addition, 60 Italian grape genotypes sampled in different areas of Italy were also included in the analysis (Table 1). Among these, 24 samples were collected in Calabria region, and 36 were sampled in other Italian regions. In addition, morphological evaluation was performed on 50 plants for each accession, Magliocco Dolce and Brettio Nero, in four different localities of Cosenza province: Saracena, Bisignano, San Marco Argentano and Cosenza (district Donnici). Finally, chemical characterization and polyphenolic content evaluation of berries were conducted on 24 plants for each accession.

Table 1. List of accessions used for genetic diversity analysis. Sixty-four accessions were sampled in different Italian regions, while the profile of 33 varieties was retrieved from 3 molecular grapevine databases (INRGV = Italian National Registry of Grape Varieties; IVD = Italian Vitis Database; EVD = European Vitis Database).

Accession	Sampling Region	Accession	Database Used to Retrieve the Molecular Profile
Altamura 1	Calabria	Aglianico del Vulture	INRGV
Altamura 2	Calabria	Carricante	INRGV
Altamura 3	Calabria	Frappato	INRGV
Arvino	Calabria	Glera	INRGV
Brettio Nero ARSAC	Calabria	Guarnaccia b.	INRGV
Canino	Calabria	Lambrusco di Sorbara	INRGV
Doraca	Calabria	Lambrusco Grasparossa	INRGV
Greco Nero ARSAC	Calabria	Magliocco Canino	INRGV
Guarnaccia	Calabria	Mantonico Bianco	INRGV
Guarnaccia Bianca	Calabria	Montonico Bianco	INRGV
Guarnaccia Bianca ARSAC	Calabria	Moscato di Scanzo	INRGV
Guarnaccia Saracena	Calabria	Notardomenico	INRGV
Guarnaccia Bianca-like	Calabria	Tintilia	INRGV
Guarnaccia Nera	Calabria	Trebbiano di Soave	INRGV
Lagrima Nera	Calabria	Trebbiano Romagnolo	INRGV
Magliocco Dolce ARSAC	Calabria	Zibibbo	INRGV
Magliocco Canino	Calabria	Guarnaccia Nera	IVD
Magliocco Dolce	Calabria	Guarnaccia Bianca	IVD
Magliocco Falvo	Calabria	Nero d'Avola	IVD
Magliocco Sanmarco	Calabria	Aleatico	EVD
Mantonico Nero	Calabria	Barbera	EVD
Mantonico Bianco	Calabria	Catarratto Bianco comune	EVD
Mantonico d'Alessandria	Calabria	Chardonnay	EVD
Mantonico di Roggiano	Calabria	Greco	EVD
Mantonico-like	Calabria	Falanghina	EVD
Unknown 1	Calabria	Fiano	EVD
Unknown 2	Calabria	Malvasia delle Lipari	EVD
Unknown 3	Calabria	Malvasia Nera	EVD
Caldarese	Puglia	Montonico Bianco	EVD
Bianco d'Alessano	Puglia	Nebbiolo	EVD
Bombino Bianco	Puglia	Nerello Mascalese	EVD
Bombino Nero	Puglia	Syrah	EVD
Maresco	Puglia	Tempranillo	EVD
Minutolo	Puglia		
Moscattello selvatico	Puglia		
Moscattidone	Puglia		
Moscato Reale	Puglia		
Negroamaro	Puglia		
Ottavianello	Puglia		
Primitivo	Puglia		
Prunesta	Puglia		
San Martino	Puglia		
Somarello Rosso	Puglia		
Susumaniello	Puglia		
Trebbiano Toscano	Puglia		
Uva di Troia	Puglia		
Ciliegiolo	Abruzzo		
Gaglioppo	Abruzzo		
Montepulciano	Abruzzo		
Moscato Bianco	Abruzzo		
Moscato d'Alessandria	Abruzzo		
Moscato di Terracina	Abruzzo		
Mostosa	Abruzzo		
Malvasia	Toscana		

Table 1. Cont.

Accession	Sampling Region	Accession	Database Used to Retrieve the Molecular Profile
Merlot	Toscana		
Moscato d'Amburgo	Toscana		
Pinot Nero	Toscana		
Sangiovese	Toscana		
Cabernet	Veneto		
Moscato Giallo	Veneto		
Alicante	Sicilia		
Perricone	Sicilia		
Greco Bianco	Lazio		
Aglianico	Basilicata		

2.2. DNA Extraction and Microsatellite Analysis

DNA was extracted from young leaves collected in the field and stored at $-80\text{ }^{\circ}\text{C}$ until extraction. The extraction was carried out following the protocol of Spadoni et al. [38] Quantity and quality of extracted DNA were checked through Nano-Drop™ 2000C Spectrophotometer (Thermo Scientific, Waltham, MA, USA) and 0.8% agarose gel electrophoresis.

Samples were genotyped using a set of 12 simple sequence repeat (SSR) markers: VVS2, VVMD5, VVMD7, VVMD21, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG21, VrZAG62, VrZAG64, and VrZAG79 [39–42]. Each PCR reaction was performed in a final volume of 25 μL containing 50 ng of template DNA, 1X of PCR buffer, 0.25 μM of forward and reverse primer mix, 0.1 mM of each dNTP and 1 U of DreamTaq polymerase (Thermo Fisher, Waltham, MA, USA). The forward primer was labeled at 5' end with one of the FAM, VIC, PET, and NED fluorescent dyes (Sigma-Aldrich, St. Louis, MO, USA).

The cycling program consisted of a touchdown protocol: 5 min of initial denaturation at $94\text{ }^{\circ}\text{C}$, followed by 10 cycles composed by (i) denaturation at $94\text{ }^{\circ}\text{C}$ for 45 s; (ii) annealing at $60\text{ }^{\circ}\text{C}$ for 45 s, with a decrement of $0.5\text{ }^{\circ}\text{C}$ per cycle to reach $55\text{ }^{\circ}\text{C}$; and (iii) extension at $72\text{ }^{\circ}\text{C}$ for 30 s. Then, the annealing temperature was maintained at $54\text{ }^{\circ}\text{C}$ for the 25 successive cycles.

Amplification products (2 μL) were mixed with 14 μL of formamide and 0.5 μL of the GeneScan 600 LIZ size standard (Life Technologies, Carlsbad, CA, USA), and then used for capillary electrophoresis. This was performed by the ABI PRISM 3100 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA) and allele sizes were assigned through the GeneMapper Software version 3.7 (Life Technologies, Carlsbad, CA, USA).

2.3. Genetic Diversity Assessment

The molecular profiles of Magliocco Dolce and Brettio Nero were compared with the genetic profile obtained by the other Calabrian and Italian accessions and with the molecular profiles of 33 varieties available in three different databases (Italian Vitis database, Italian National Registry of Grape Varieties and European Vitis Database) (Table 1). For each marker, the following parameters were calculated by the GENALEX software version 6.51b2 [43]: number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), observed (Ho) and expected (He) heterozygosity, and fixation index (F). Moreover, the polymorphic information content (PIC) and the frequency of null alleles (Nu) were calculated using Cervus version 3.0 [44]. Frequency-based genetic distances were calculated in order to construct an unweighted neighbor-joining dendrogram for all the grapevine accessions, using DARWIN version 6.0.010 [45].

2.4. Morphological Evaluation and Chemical Characterization of Berries

A set of 45 OIV descriptors was used for the observations of shoot (OIV 001, 003, 004, 007, 008, 016, 351, 352, 353), young and mature leaves (OIV 051, 053, 065, 067, 068, 070, 072, 074, 075, 076, 078, 079, 080, 081-1, 081-2, 083-2, 084, 087), flower sex, bunch and berry

characteristics (OIV 151, 152, 153, 202, 204, 206, 208, 209, 220, 223, 225, 228, 235, 236, 241, 502, 503) and grape yield (OIV 504) [24].

Ripening curves referred to sugar concentration, pH, and total acidity were built for both Magliocco Dolce and Brettio Nero varieties. Analysis was performed on berries collected at 4 distinct stages of ripening: beginning of veraison (E-L 35), middle veraison (E-L 36), end of veraison (E-L 37), and maturity (E-L 38). Samples were collected for three seasons (2016–2018). Moreover, the following parameters were measured in berries at maturity: total anthocyanins were measured in the skin, total flavonoids, total polyphenols, proanthocyanidins, and flavans were estimated in skin and seed, and hydroxycinnamic acids were measured in the pulp. These compounds were determined by spectrophotometric methods [46]. Analysis was performed for two seasons (2016–2017).

2.5. Chemical and Sensorial Analysis of Wines

Magliocco Dolce and Brettio Nero were used to produce two monovarietal red wines at the experimental wine cellar of Center for Research, Experimentation and Training in Agriculture (CRSFA) Basile Caramia (Locorotondo, Italy), through the following winemaking process. Whole grapes were refrigerated for 24 h at 4–6 °C, then destemmed with the addition of 8 g/hl of potassium metabisulphite. Cryomaceration was performed for 6 h at 4 °C followed by natural heating of the crushed grapes up to 20 °C. Afterward, ammonia salts with thiamine (10 g/hl) were added and yeasts were inoculated. After reaching 4–5° and 9° alcohol, oxygenation of the whole mass and addition of ammonia salts with thiamine (15 g/hl) was performed. Afterward, a pressing at the complete exhaustion of sugars was carried out. The wine was subjected to static clarification at 0 °C for one week and, then, naturally heated up to 20 °C. Then, lactic acid bacteria were inoculated and malolactic fermentation was monitored. Finally, the wine was transferred with oxygenation and integrated with free SO₂ at 25 mg/L. Refinement and stabilization of the wine with controlled oxygenation were performed every 15 days.

For each of the two wines, the following physico-chemical parameters were determined: alcoholic degree, total acidity, pH, density, dry extract, volatile acidity, ash, malic acid, tartaric acid, citric acid, glycerol, potassium and sulfates, and polyphenols (total anthocyanins, total flavonoids, total polyphenols, proanthocyanidins, and flavans). Methods of analysis used for measuring each parameter are listed in the Official Journal of the European Union C 43/01 [47]. Determination of polyphenolic compounds was performed using spectrophotometric determination as described by Di Stefano et al. [48]. Six replicates of each wine were used for analysis.

A sensorial analysis was also carried out approximately six months after bottling. Hedonic sensory evaluation of wines was performed with a panel of consumers from 18 to 65 years old who have been asked to evaluate the two wines based on 24 parameters related to their color, odor, flavor, hit in mouth, persistence and overall liking on a 10-point hedonic scale. The scale of values ranged from “extremely high” to “extremely low” corresponding to the highest and lowest scores of “10” and “1”, respectively. Consumers tasted the two samples of wines served in a glass cup adequately coded and served in a randomized order. Water was available for rinsing.

2.6. Statistical Analysis

The statistical significance of the data was analyzed using standard unpaired Student's *t*-test. $p < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Genetic Diversity Analysis

A high overall genetic diversity was observed among the 97 Italian grape accessions, supporting the effectiveness of the selected SSR markers set in discriminating among the accessions (Table 2).

Table 2. Diversity indices of 12 SSR markers detected in a set of 97 grapevine accessions.

SSR Marker	Size Range	Na	Ne	I	Ho	He	F	PIC	F (Null)
VVS2	131–155	10	4.82	1.83	0.87	0.79	−0.09	0.77	−0.050
VVMD5	225–251	11	5.12	1.88	0.81	0.80	−0.01	0.78	−0.007
VVMD7	233–263	8	4.24	1.68	0.76	0.76	0.00	0.73	0.005
VVMD21	228–266	9	3.88	1.64	0.77	0.74	−0.03	0.71	−0.011
VVMD25	239–269	11	4.73	1.82	0.79	0.79	−0.01	0.76	−0.010
VVMD27	174–193	8	4.79	1.75	0.82	0.79	−0.04	0.77	−0.023
VVMD28	218–270	14	7.58	2.20	0.93	0.87	−0.07	0.85	−0.035
VVMD32	241–273	9	6.07	1.97	0.77	0.84	0.07	0.82	0.038
VRZAG21	179–215	7	4.07	1.54	0.82	0.75	−0.09	0.72	−0.049
VRZAG62	178–204	10	7.03	2.04	0.91	0.86	−0.06	0.84	−0.030
VRZAG64	135–189	11	5.98	1.94	0.89	0.83	−0.07	0.81	−0.034
VRZAG79	236–260	12	6.44	2.05	0.82	0.84	0.02	0.83	0.010
Total		120							
Mean		10	5.40		0.83	0.81	−0.03	0.78	
Min.		7	3.88	1.54	0.76	0.74	−0.09	0.71	−0.050
Max.		14	7.58	2.20	0.93	0.87	0.07	0.85	0.038

The total number of alleles (Na) was 120, ranging from 7 at the VRZAG21 locus to 14 at the VVMD28 locus. The number of effective alleles (Ne) ranged from 3.88 to 7.58 for VVMD21 and VVMD28 markers, respectively. Shannon’s information index (I) ranged from 1.54 (VRZAG21) to 2.20 (VVMD28). The null allele frequencies were estimated lower than 0.05 for all the loci, ranging between −0.050 and 0.038, for VVS2 and VVMD32 markers, respectively. The mean observed heterozygosity (Ho) was 0.83 and expected heterozygosity (He) was estimated as 0.81, determining a mean value for the fixation index (F) of −0.03. The efficiency of the SSR markers in distinguishing the genotypes was estimated by calculating the PIC index. This value indicated a powerful discrimination ability of markers. In fact, it ranged from 0.71 (VVMD21) and 0.85 (VVMD28) with a mean value of 0.78.

The dendrogram constructed through the unweighted neighbor-joining method clustered the accessions in three main groups (I, II, III) (Figure 1).

Group I split into two sub-clusters (I-a and I-b) containing, respectively, 50 and 14 accessions. The cluster I-a may be in turn divided into two sub-cluster (I-a1 and I-a2). The sub-cluster I-a1 contained most of the Calabrian accessions, highlighting several cases of homonymies and synonymies (Table S1). The samples Guarnaccia and Guarnaccia Nera showed a different profile compared with Guarnaccia Nera present in the Italian Vitis database, indicating a possible case of misnaming. Mantonico Nero and Brettio Nero shared identity, showing also a high similarity with Mantonico d’Alessandria and Mantonico di Roggiano. The samples named Guarnaccia Bianca and Guarnaccia Bianca-like clustered with the Guarnaccia Bianca registered into the Italian Vitis database but differed from Guarnaccia Bianca present in the Italian National Registry of Grape Varieties. A similar discrepancy was observed for the variety Montonico Bianco in the Italian National Registry of Grape Varieties and the European Vitis database, located in cluster I-b and sub-cluster I-a2, respectively. The accession Greco Nero provided by ARSAC showed a different profile compared with Greco Bianco indicating misuse of the same name followed by “Bianco” and “Nero” designation to indicate different varieties.

The sub-cluster I-a2 consisted mostly of profiles retrieved from the molecular databases corresponding to accessions cultivated in different Italian regions and included the Calabrian accessions Canino and Altamura 2.

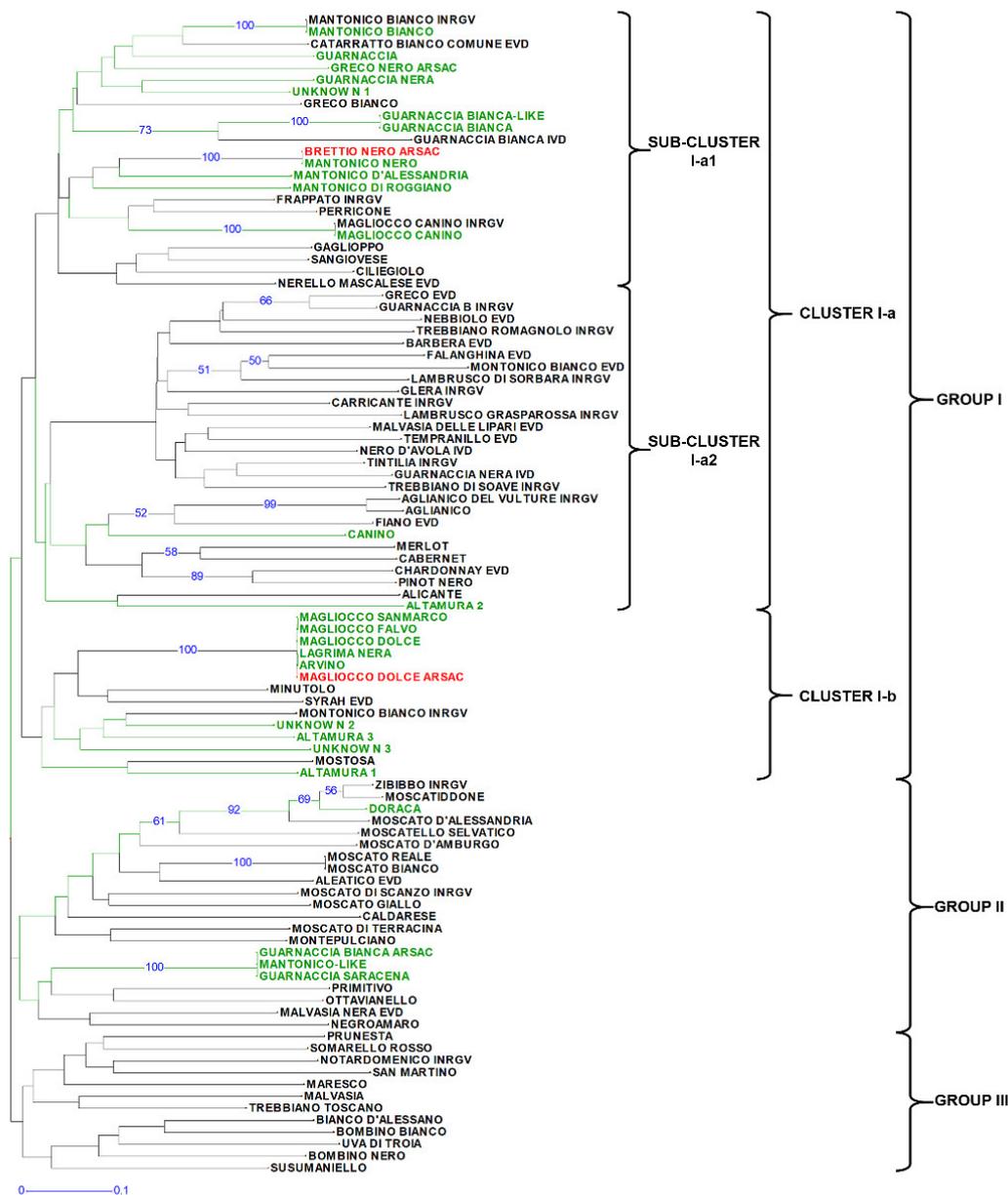


Figure 1. Unweighted neighbor-joining dendrogram obtained with SSR data from 64 grape accessions and 33 molecular profiles taken from three different grapevine databases (Italian National Registry of Grape Varieties, Italian Vitis Database and European Vitis Database). Samples collected in the Calabria region are green. Brettio Nero and Magliocco Dolce provided by ARSAC are in red.

The cluster I-b contained several Calabrian accessions. Arvino, Lagrima Nera, and Magliocco Dolce shared the same molecular profile indicating a case of synonymy. Their profile is completely different compared to that of Greco Nero provided by ARSAC.

Group II consisted of 21 samples collected in different Italian regions, including several members of the “Moscato family”. Among these, Moscato Reale and Moscato Bianco represent a case of synonymy (Table S1). The samples named Guarnaccia Saracena and Mantonico-like shared the same molecular profile of Guarnaccia Bianca provided by ARSAC and they are different from both Guarnaccia Bianca retrieved in the Italian National Registry of Grape Varieties and Italian Vitis database. Finally, group III contained 12 samples and included most of the Apulian accessions.

To verify the profile of Greco Nero, Guarnaccia Bianca, Magliocco Dolce, and Brettio Nero provided by ARSAC, we checked it into the Vitis International Variety Catalogue

(VIVC) [49]. For Magliocco Dolce and Brettio Nero, the molecular profile was identical to the one obtained in our study. The VIVC profile of Guarnaccia Bianca was different from the other profiles of Guarnaccia Bianca and the VIVC profile of Greco Nero was different from both Greco Nero ARSAC and Greco Bianco indicating a widespread confusion on the identity of these two varieties (Table S2).

3.2. Ampelographic Characterization and Chemical Analysis of Berries

The phenotypic profile of Magliocco Dolce and Brettio Nero based on 45 OIV descriptors was developed (Table 3). The two accessions exhibited very similar phenotypes, with some differences in mature leaf characters (OIV code 070, 072, and 079) and berry length and shape (OIV code 220 and 223).

Table 3. Phenotypic profile of Magliocco Dolce and Brettio Nero accessions based on 45 OIV descriptors.

OIV Code	Characteristic	Levels	Magliocco Dolce	Brettio Nero
001	young shoot: aperture of tip	1 closed, 3 half open, 5 fully open	5	5
003	young shoot: intensity of anthocyanin coloration on prostrate hairs of tip	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1
004	young shoot: density of prostrate hairs on tip	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5	5
007	shoot: color of dorsal side of internodes	1 green, 2 green and red, 3 red	1	1
008	shoot: color of ventral side of internodes	1 green, 2 green and red, 3 red	1	1–2
016	Shoot: number of consecutive tendrils	1 green, 2 green and red, 3 red	1	1
051	Young leaf: color of the upper side of blade (4th leaf)	1 green, 2 yellow, 3 bronze, 4 copper-reddish	1	1
053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	5–7	5–7
065	Mature leaf: size of blade	1 very small, 3, small, 5 medium, 7 large, 9 very large	5–7	5–7
067	Mature leaf: shape of blade	1 cordate, 2 wedge-shaped, 3 pentagonal, 4 circular, 5 kidney-shaped	3	3
068	Mature leaf: number of lobes	1 one, 2 three, 3 five, 4 seven, 5 more than seven	2–3	2–3
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	1 absent, 2 only at the petiolar point, 3 upto the 1st bifurcation, 4 up to the 2nd bifurcation, 5 beyond the 2nd bifurcation	1	2
072	Mature leaf: goffering of blade	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong	3	1
074	Mature leaf: profile of blade in cross section	1 flat, 2 V-shaped, 3 involute, 4 revolute, 5 twisted	2–5	5
075	Mature leaf: blistering of upper side of blade	1 absent or very weak, 2 weak, 3 medium, 4 strong, 9 very strong	5–7	5
076	Mature leaf: shape of teeth	1 both sides concave, 2 both sides straight, 3 both sides convex, 4 one side concave on side convex, 5 mixture between both sides straight and both sides convex	5	5
078	Mature leaf: length of teeth compared with their width	1 very short, 3 short, 5 medium, 7 long, 9 very long	5	3–5
079	Mature leaf: degree of opening/overlapping of petiole sinus	1 very wide open, 3 open, 5 closed, 7 overlapped, 9 strongly overlapped	5	7
080	Mature leaf: shape of base of petiole sinus	1 U-shaped, 2 brace-shaped, 3 V-shaped	3	3
081-1	Mature leaf: teeth in the petiole sinus	1 none, 9 present	1	1
081-2	Mature leaf: petiole sinus base limited by veins	1 not limited, 3 on one side, 3 on both sides	1	1
083-2	Mature leaf: teeth in the upper lateral sinuses	1 none, 9 present	1	1
084	Mature leaf: density of prostrate hairs between the main veins on lower side of blade	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	3–5	5

Table 3. Cont.

OIV Code	Characteristic	Levels	Magliocco Dolce	Brettio Nero
087	Mature leaf: density of erect hairs on main veins on lower side of blade	1 none or very low, 3 low, 5 medium, 7 high, 9 very high	1	1
151	Flower: sexual organs	1 fully developed stamens and no gynoecium, 2 fully developed stamens and reduced gynoecium, 3 fully developed stamens and fully developed gynoecium, 4 reflexed stamens and fully developed gynoecium	3	3
152	Inflorescence: insertion of 1st inflorescence	1 up to the 2nd node, 2 3rd and 4th node, 3 from the 5th node on	1–2	1–2
153	Inflorescence: number of inflorescences per shoot	1 up to 1 inflorescence, 2 1,1 to 2 inflorescences, 3 2,1 to 3 inflorescences, 4 more than 3 inflorescences	2	2
202	Bunch: length (peduncle excluded)	1 very short, 3 short, 5 medium, 7 long, 9 very long.	3–5	5–7
204	Bunch: density	1 very loose, 3 loose, 5 medium, 7 dense, 9 very dense	5–7	5–7
206	Bunch: length of peduncle of primary bunch	1 very short, 3 short, 5 medium, 7 long, 9 very long	3–5	3–5
208	Bunch: shape	1 cylindrical, 2 conical, 3 funnel shaped	2	2
209	Bunch: number of wings of the primary bunch	1 absent, 2 1–2 wings, 3 3–4 wings, 4 5–6 wings, 5 more than 6 wings	1–2	1–2
220	Berry: length	1 very short, 3 short, 5 medium, 7 long, 9 very long	3	5–7
223	Berry: shape	1 obloid, 2 globose, 3 broad ellipsoid, 4 narrow ellipsoid, 5 cylindrical, 6 obtuse ovoid, 7 ovoid, 8 obovoid, 9 horn shaped, 10 finger shaped	2	3
225	Berry: color of skin	1 green yellow, 2 rose, 3 red, 4 grey, 5 dark red violet, 6 blue black	6	6
228	Berry: thickness of skin	1 very thin, 3 thin, 5 medium, 7 thick, 9 very thick	7	7
235	Berry: firmness of flesh	1 soft, 2 slightly firm, 3 very firm	2	2
236	Berry: particularity of flavor	1 none, 2 muscat, 3 foxy, 4 herbaceous, 5 other flavor	1	1
241	Berry: formation of seeds	1 none, 2 rudimentary, 3 complete	3	3
351	Vigor of shoot growth	1 very weak, 3 weak, 5 medium, 7 strong, 9 very strong	5	5
352	Growth of axillary shoots	1 absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong	5	5
353	Length of internodes	1 very short, 3 short, 5 medium, 7 long, 9 very long	3–5	3
502	Bunch: weight of a single bunch	1 very low, 3 low, 5 medium, 7 high, 9 very high	3	3
503	Berry: single berry weight	1 very low, 3 low, 5 medium, 7 high, 9 very high	1–3	1–3
504	Yield per m ²	1 very low, 3 low, 5 medium, 7 high, 9 very high	5	5

Ripening curves referred to sugar concentration, pH, and total acidity were built for the two varieties, considering 4 distinct stages of ripening (from E-L 35 to E-L 38). In both varieties, during veraison sugar concentration increases rapidly, also pH increases as total acidity decreases. Both, Brettio Nero and Magliocco Dolce exhibited similar profiles reaching approximately the same value of sugar concentration and pH at maturity (Figure 2). The principal compounds responsible for berry phenolic profile and representing some of the principal factors affecting red wine quality were also measured in the two varieties (Figure 3).

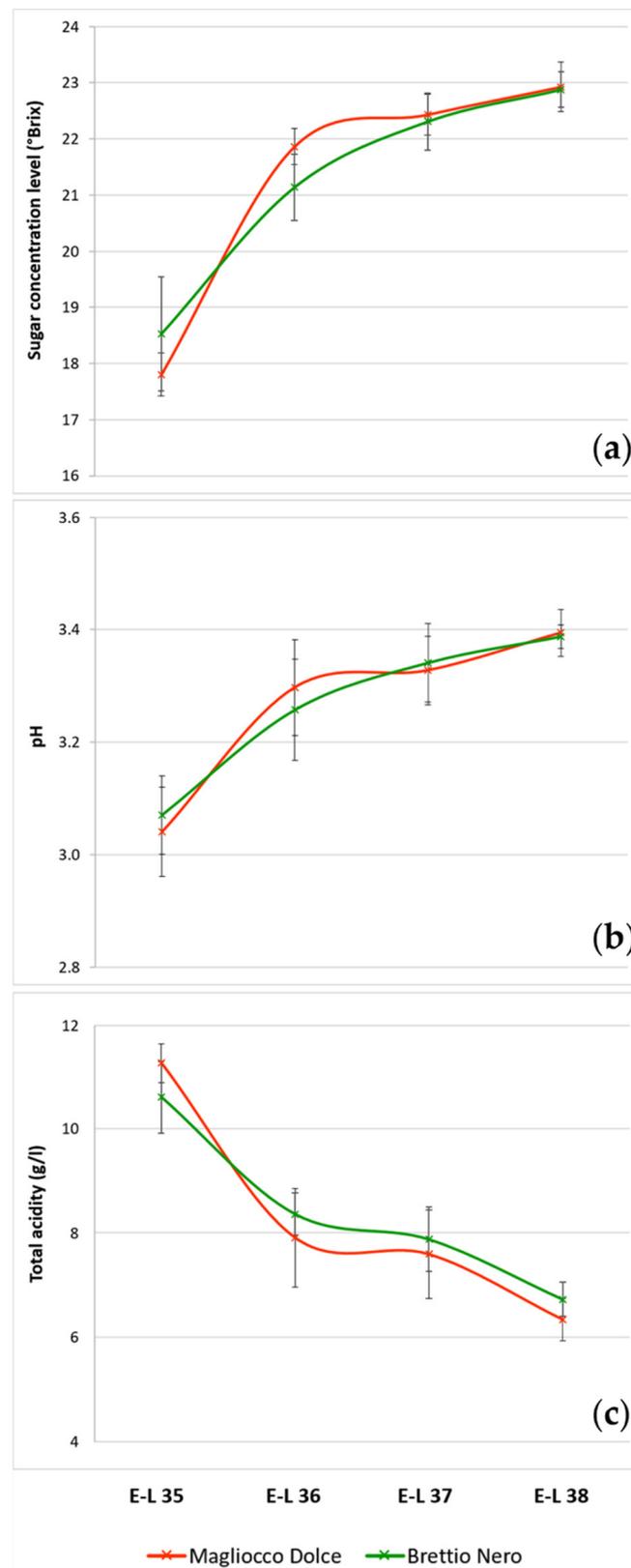


Figure 2. Changes in sugar concentration (a), pH (b), and total acidity (c) during veraison in Magliocco Dolce and Brettio Nero varieties. Samples were collected for three seasons (2016–2018) at 4 distinct time points: beginning of veraison (E-L 35), middle veraison (E-L 36), end of veraison (E-L 37), and maturity (E-L 38). Standard unpaired Student's *t*-test was used for comparison of two varieties.

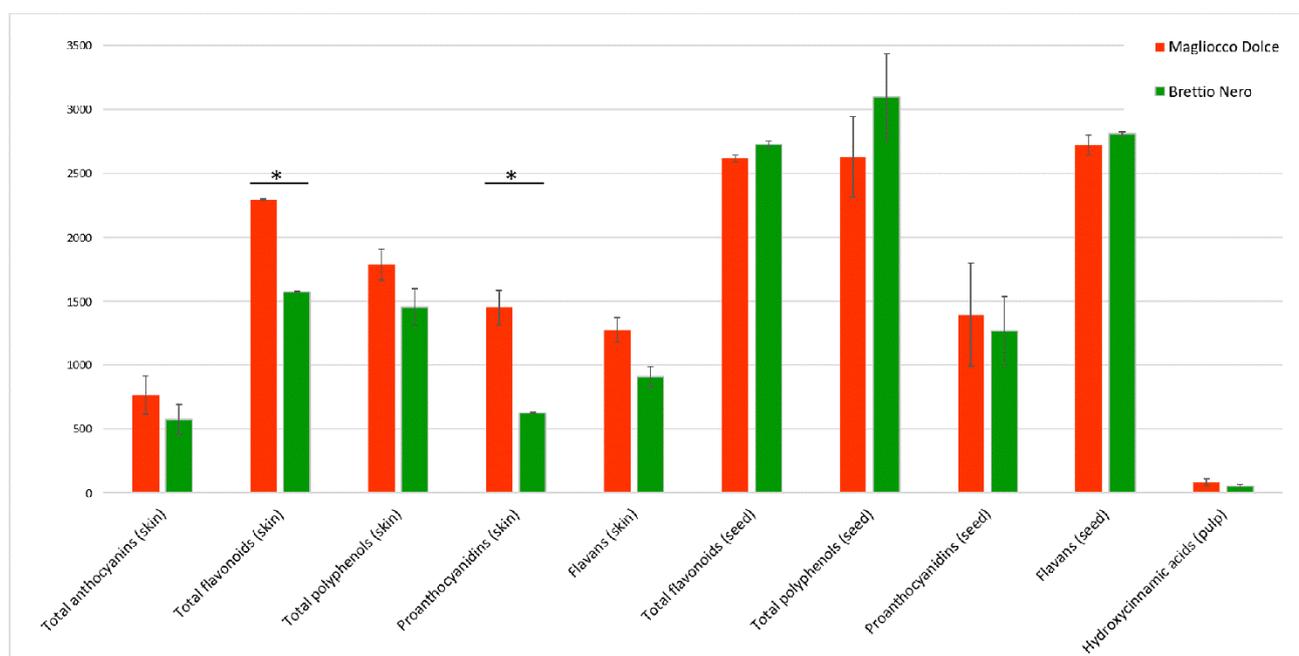


Figure 3. Polyphenols content measured in berry of Magliocco Dolce and Brettio Nero at maturity. Data were collected for two seasons (2016–2017). Standard unpaired Student's *t*-test was used for comparison of two varieties, * $p < 0.05$.

In general, Magliocco Dolce showed higher levels of polyphenolic compounds in the skin compared to Brettio Nero, in particular, a significant difference was observed for total flavonoids and proanthocyanidins ($p < 0.05$), whereas the levels of polyphenolic compounds were comparable between the two varieties in the seed. Finally, hydroxycinnamic acids in berry pulp were slightly lower in Brettio Nero compared to Magliocco Dolce.

3.3. Oenological Parameters and Wine Sensory Analysis

A total of 20 oenological parameters were analyzed in the two monovarietal wines obtained from Magliocco Dolce and Brettio Nero. Both wines showed similar values for all parameters with only two exceptions of ashes, lower in Brettio Nero compared to Magliocco Dolce, and malic acid, higher in Brettio Nero compared to Magliocco Dolce (Table 4).

In general, the polyphenols content (except flavans) showed to be higher in Magliocco Dolce wine than Brettio Nero wine, according to polyphenols content observed in berry skin of the two varieties. Although, it should also be noted that the standard deviation of data (especially the one related to total proanthocyanidins and flavans) showed very high values, indicating a high variation of data in analyzed replicates (Table 4).

The wine sensory evaluation of the two monovarietal wines, performed on 24 sensorial parameters, indicated a higher limpidity and color intensity of Brettio Nero wine compared to Magliocco Dolce one. In general, Brettio Nero wine showed to be more aromatic than Magliocco Dolce, while no differences were observed in smell intensity, persistency, and quality. The 50% of tasters indicated a much higher acidity and tasteness of Brettio Nero wine, while no significant differences were observed in other flavor features of the two wines (Figure 4).

Table 4. Chemical parameters and polyphenols content measured in Magliocco Dolce and Brettio Nero monovarietal wines. Analysis was performed for two seasons (2016–2017). Standard unpaired Student's *t*-test was used for comparison of two varieties, * $p < 0.05$.

Parameter	Magliocco Dolce	Brettio Nero
Alcoholic degree (% $\pm v/v$)	14.02 \pm 0.59	13.37 \pm 0.64
Total acidity (g/L)	6.55 \pm 0.32	6.51 \pm 0.52
pH	3.48 \pm 0.17	3.52 \pm 0.16
Density (20°/20°)	1.04 \pm 0.07	1.05 \pm 0.07
Total dry extract (g/L)	32.24 \pm 1.63	28.67 \pm 0.27
Volatile acidity (g/L)	0.5 \pm 0.03	0.39 \pm 0.13
Ashes (g/L)	3.15 \pm 0.01	2.95 \pm 0.06 *
Malic acid (g/L)	3.11 \pm 0.06	3.73 \pm 0.16 *
Tartaric acid (g/L)	3.28 \pm 1.17	2.93 \pm 1.16
Citric acid (g/L)	0.1 \pm 0.04	0.16 \pm 0.01
Glycerol (g/L)	9.68 \pm 0.48	8.95 \pm 0.82
Potassium (g/L)	1.39 \pm 0.35	1.44 \pm 0.27
Sulfates (g/L)	0.42 \pm 0.13	0.51 \pm 0.07
Color intensity	8.82 \pm 0.69	7.23 \pm 1.74
Color tonality	0.85 \pm 0.32	0.92 \pm 0.42
Total anthocyanins (mg/L)	171.81 \pm 21.15	82.87 \pm 31.44
Total flavonoids (mg/L)	973.12 \pm 314.93	715.44 \pm 75.37
Total polyphenols (mg/L)	2207.23 \pm 309.91	1759.63 \pm 505.08
Proanthocyanidins (mg/L)	2370.49 \pm 1280.70	1709.40 \pm 915.09
Flavans (mg/L)	471.13 \pm 227.00	756.52 \pm 402.89

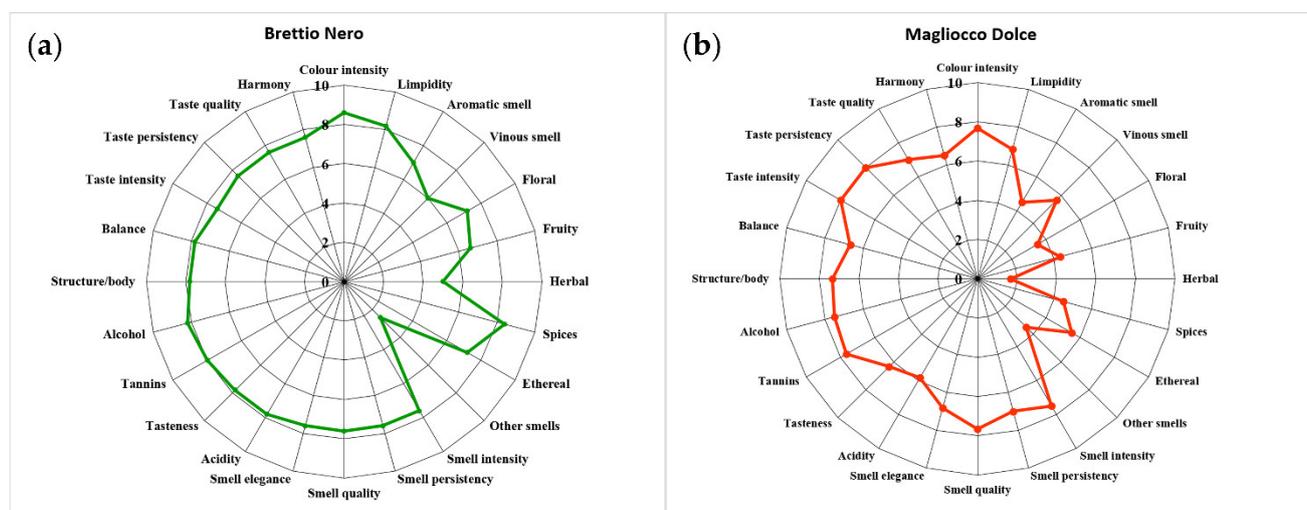


Figure 4. Brettio Nero (a) and Magliocco Dolce (b) monovarietal wine sensorial profiles based on 24 parameters evaluated by consumer volunteers.

4. Discussion

The increase in Calabrian wine production along with the growing interest shown by consumers towards local and peculiar Italian products make crucially important the characterization of local grapevine varieties along with the clarification of naming of these cultivars since a general variety name confusion is rather widespread. Our work has covered a series of genetic, viticultural, and oenological analyses in order to unequivocally characterize two widespread Calabrian grapevine varieties: Magliocco Dolce and Brettio Nero.

ARSAC-authenticated plants of the varieties Magliocco Dolce and Brettio Nero were genotyped with 12 SSR markers. The obtained molecular profiles were compared with that of 26 popular Calabrian accessions and 36 Italian varieties. Moreover, a comparison with

some of the most spread Italian cultivars available in three different molecular databases was also made. The dendrogram built through the unweighted neighbor-joining method highlighted the presence of three principal groups (Figure 1), with most of the Calabrian accessions clustered together in the sub-cluster I-a1 and the cluster I-b. Mantonico Nero accession sampled in Calabria resulted to be genetically identical to the ARSAC-authenticated Brettio Nero confirming to be a specific genotype different from any other accession included Mantonico Bianco. Despite the general practice to use the same varietal name for berry colors variant, derived from a somatic mutation affecting fruit color [8,9], in the case of the name Mantonico this could not be used to indicate the white “Bianco” and black “Nero” variants. Thus, the new name Brettio Nero was proposed instead of Mantonico Nero for the registration of this variety into the Italian National Registry of Grape Varieties. The misuse of the same name followed by “Bianco” and “Nero” designation to indicate different varieties is not uncommon. Indeed, Greco Bianco showed a different molecular profile compared to Greco Nero and the same situation is observed for Bombino Bianco and Bombino Nero (Figure 1).

ARSAC-authenticated Magliocco Dolce (cluster I-b) showed to be completely different from ARSAC-authenticated Greco Nero (sub-cluster I-a1) which, thus, cannot be considered synonymous of Magliocco Dolce [2]. In the same way, it showed a different molecular profile from that of Notardomenico retrieved from INRGV, excluding also, in this case, the hypothesis that Magliocco Dolce and Notardomenico are synonymous [11]. By contrast, the names Lagrima Nera, Arvino, and Magliocco Dolce were confirmed as synonymies [31]. The genetic analysis showed different molecular profiles for Magliocco Dolce and Magliocco Canino and genetic similarity of the latter with the variety Perricone, in accord with Pellerone et al. [29]. Based on our results, we can sustain that Magliocco Dolce and Brettio Nero represent two unique varieties not matching with any other registered Calabrian variety. Moreover, we highlighted the necessity to define the new name Brettio Nero instead of Mantonico Nero in order to clearly distinguish this cultivar from the Mantonico Bianco variety.

For a long time, ampelography was the only methodology used to identify grape varieties [50,51]. Today, despite the introduction of more reliable genetic methods, it is still fundamental for the identification and description of grape cultivars. We used 45 OIV descriptors to morphologically describe Magliocco Dolce and Brettio Nero varieties (Table 3), including all that required for registration of a new variety into the Italian National Registry of Grape Varieties. Magliocco Dolce and Brettio Nero varieties were investigated also for physico-chemical parameters of berry, including the content of some classes of polyphenols. Grape berries are a non-climacteric fruit, and the ripening process involves numerous physiological and metabolic activities occurring during the whole veraison phase till the berry reaches maturity making them suitable for vinification. These changes make the ripening process a determining factor in the quality of grapes and wines [52]. For both Magliocco Dolce and Brettio Nero varieties, several chemical parameters were measured in berries at different ripening stages. The ripening curves showed a sigmoidal trend with a rapid increase of sugar concentration reaching the highest value at maturity (about 23 °Brix for both varieties). Simultaneously, total acidity decreases inducing an increase of pH from about 3.1 at the beginning of veraison to about 3.4 at maturity (Figure 2).

The evaluation of the sugar content and acidity level alone does not fully express the real oenological potential of a grape accession. The knowledge of the polyphenolic profile of grapes allows careful planning of the winemaking process and the exploitation of the full potentiality of a variety [53]. In red wines, the polyphenolic content of berry at maturity (grape phenolic maturity) is one of the major factors affecting quality. Most of the wine’s sensory characteristics, such as color, astringency, and bitterness, are directly associated with the profile of anthocyanins and proanthocyanidins [54,55]. We evaluated the grape phenolic maturity of Magliocco Dolce and Brettio Nero through the measurement of principal phenolic compounds. Our results showed to be highly variable, being more affected by

climatic conditions and viticulture practices than the grape cultivar. Nevertheless, they are in line with findings obtained by other authors [33,56,57].

Wine quality and flavor are affected by several factors including the physico-chemical characteristics of berries at harvesting and the operating conditions adopted during grape maceration that can strongly influence the amount of extracted phenols. In order to evaluate the effects of berry composition on the content and flavor of wine during the winemaking process, 20 oenological parameters were checked in Magliocco Dolce and Brettio Nero monovarietal wines. Moreover, a sensorial analysis based on the detection of 24 parameters was performed by six consumer volunteers. The two wines reached the minimum percentage for the volume of alcohol established by law (>9% volume; Council Regulation (EC) No 1234/2007) [47] and a high alcoholic sensation in the sensory analysis was perceived. Acidity, which plays an important role in wine tasting, as it confers crispness and freshness to the wine, reached similar levels in the two varieties. Both wines showed normal values of total acidity (between 5 e 8 g/L) and pH (between 3 and 3.7) and low volatile acidity (<1 g/L) [58]. Although both wines showed a comparable level of acidity (Table 4), acidity was perceived more in Brettio Nero wine compared to Magliocco Dolce. Color intensity and tonality, which are mostly determined by anthocyanin content [59], showed, as expected, very high values; in particular, color intensity showed to be higher in Magliocco Dolce wine compared to Brettio Nero wine, according to the higher content of anthocyanins in berry skin; nevertheless, Brettio Nero wine was judged more intensely colored by consumers. Phenolic compounds play an important antioxidant activity in wines, especially in red ones [60]. The levels of polyphenols compounds found in the two monovarietal wines are comparable with that observed commonly in red wines [61]. In general, a higher polyphenols content was observed in Magliocco Dolce wine compared to Brettio Nero wine, as well as a higher polyphenols content was observed in Magliocco Dolce berries. Regarding the sensorial analysis, in general, both wines were judged as harmonious and tasty. The wine produced with Brettio Nero variety presented an intense floral and fruity smell while Magliocco Dolce wine was judged slightly perfumed.

5. Conclusions

In our study, we deeply characterized two Calabrian varieties—Magliocco Dolce and Brettio Nero—with the purpose to valorize them and boost their use in wine production. The genetic analysis allowed us to verify that they are unique and distinct accession and to define a genetic profile useful for molecular traceability purposes. The morphological evaluation highlighted the peculiar characteristics of Magliocco Dolce and Brettio Nero allowing their discrimination from any other Calabrian variety. Finally, the chemical analysis of berry and the evaluation of oenological properties of Magliocco Dolce and Brettio Nero demonstrated the suitability of these varieties in high-quality wine production.

In the future, the characterization of other Calabrian grapevine samples following the same multidisciplinary approach will allow the valorization of local and peculiar accessions and their registration in the Italian National Registry of Grape Varieties. This will contribute to promote the quality and value of the wine produced with these new varieties and highlight the potential of Calabria region in the wine sector.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11081538/s1>, Table S1: List of synonyms found in our study and comparison with synonyms recognized by the Italian National Registry of Grape Varieties, Table S2: Molecular profile obtained for the ARSAC-authenticated accessions and comparison with the profiles contained in the Vitis International Variety Catalogue (VIVC).

Author Contributions: Conceptualization, V.F., V.R., V.N.S. and C.M.; Data curation, V.R. and P.V.; Formal analysis, V.F.; Methodology, V.F., V.R., M.A.S., P.V. and C.P.; Project administration, V.N.S., P.L.N. and C.M.; Resources, M.F. and F.P.; Software, V.F., M.A.S. and M.M.M.; Supervision, C.M.; Writing—original draft, V.F.; Writing—review and editing, V.F., V.R., M.M.M. and C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by CAMERA DI COMMERCIO DI COSENZA, Deliberazione n. 28 del 19/04/2016. Valentina Fanelli was supported by MIUR-PON Ricerca e Innovazione 2014–2020 (project AIM1809249-attività 2, linea 1).

Data Availability Statement: All data are included in the manuscript.

Acknowledgments: The authors would like to thank Marcello Bruno and Claudio Fuoco from Agenzia Regionale per lo Sviluppo dell’Agricoltura in Calabria (ARSAC) for their help in sampling plant material.

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

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