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Varietal Authentication of Brunello di Montalcino Wine Using a Minimal Panel of DNA Markers

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Abstract: Wine DNA fingerprinting (WDF), retrieved from the amplification of a wider panel of Simple Sequence Repeat (SSR) marker mappings in the *Vitis vinifera* L. genome, was used to assess the monovarietal nature of Brunello di Montalcino wine. The reliability of the varietal assessment was carried out by estimating the PI values associated with resolutive unrooted dendrograms depicting the correct varietal nature of different wines. As few as five SSR DNA markers associated with a PI value of one over a million or less, PI $\leq 10^{-6}$, can identify the purity of Sangiovese against Merlot, Pinot Noir, Cabernet Sauvignon, Primitivo (Zinfandel), and genetic variants of the Sangiovese as plant references. WDF was used on other monovarietal wines obtained from Cabernet Sauvignon, Merlot, Chardonnay, and Pinot Noir to test the feasibility of the method. In blended wines, the test was able to trace the main varietal component in a three-variety blend, keeping the varietal fingerprint detectable when the main variety was at least 75% (*v*/*v*). The data confirm how local genetic variants of Sangiovese can be tracked in commercial wines, becoming, at wine makers' demand, part of an evidence synthesis of geographical origin.

Keywords: DNA markers; wine DNA fingerprinting; simple sequence repeats; Sangiovese; geographical origin

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1. Introduction

The quality, safety, and authenticity of food and beverages are currently an essential added cultural value in the agrifood industry globally. This is specifically linked to human health in contemporary society, which is increasingly aware of food consumption [1]. Additionally, due to the globalization of dietary markets and the increase in movements of various food products from one country to another, consumers are becoming more concerned with the geographical origin of their food and beverages [2]. Several countries have adopted quality, safety, and authenticity standards to safeguard their agrifood industries. Italy is currently the leading country in food and beverage authentication globally, followed by Spain, the United States of America, the United Kingdom, Germany, France, China, Portugal, Switzerland, and Greece [3]. There is an increasing interest in research to develop innovative analytical tools to control fraud and adulteration of food and beverages, such as wine [4].

Wine is an added-value beverage made using the fermented grape juice from grapevines (*Vitis vinifera* L.). It is made up of a complex mixture of compounds. The complex compounds contribute to the quality of wines because they are responsible for color, taste, and aroma [5,6]. The quality, composition, value, and functional properties of wine are influenced by the variety, geographical origin, and farming systems [7].

Consumers demand more informative labels on product origin, production process, and plant-derived components. Since the 1960s, wine quality has been granted by DOC and DOCG labels, from the moment that the geographical origin and varietal components were major targets of fraudulent practices in the wine-making industry. In addition to fraudulent practices, the wine and grape sector faces challenges of climate change.

Conservation of natural variability in the grapevine germplasm is essential for adaptation to the environment, and it is equally important for developing food and beverage authentication strategies. Food and wine authenticity is a crucial key factor that is rooted in the natural variability in vineyards because it provides important elements concerning the geographical origin of wine grape varieties. The availability of analytical tools for wine authentication protects consumers against fraud and preserves wine identity in the interest of producers. The European Union put in place regulations and laws, including Council Regulation NO. 1234/2007, Regulation EU NO. 1151/2012, NO. 664/2014, NO. 668/2014, which aimed to govern the entire European food sector [8]. Despite these laws and regulations, the wine sector continues to face major challenges due to competition for market demand and a trend of producing wines exhibiting good and high-quality flavors.

International wines, such as Brunello di Montalcino, are an example of wines that are expected to face fraud and adulteration in the local and international market [9]. Classical wine fraud and adulteration include change of labels, dilution with water, addition of alcohol, color, and flavor substances, blending with lesser quality wines, and misrepresentation of variety and geographical origin [10]. Brunello di Montalcino wine is an Italian wine produced from the Sangiovese grapevine variety in Tuscany, Italy [11]. The wine has fostered the economy of the Tuscany region and Italy as a whole. The wine fetches a high market price and is among the wines that have received recognition as quality and prestigious wines both at the national and international levels [12].

Due to the importance of quality and authenticity requirements, several biotechnology methodologies for the determination of quantitative and qualitative compounds in wine have been established to confirm wine products. They include mineral analysis (spectrometry), organic profiling (chromatography), isotopic ratios, and DNA analysis [13]. Other analytical methods include capillary electrophoresis and sensory analysis [14]. DNA analysis is the most powerful methodology for determining the risk of adulteration of wines. In comparison to other types of markers, like proteins, which are affected by environmental factors, DNA is thought to be more consistent, even given external variations. Additionally, DNA markers are stable and numerous in number and, therefore, can form a wide basis to provide reliable information [15]. The idea and history of wine biological origin authentication through DNA testing dates back to the DNA fingerprinting and identification of grapevine cultivars, with emphasis on the selection process, which, from alien accessions of wild grapes, brought the differentiation of actual *V. vinifera* varieties [16]. WDF and other multi-analytical approaches have proved to be very promising tools for wine varietal authentication [13,15,17–22]. WDF, a method for detecting genotype rarity in grapevine varieties, uses nuclear genomic DNA amplification to reconstruct individual genotypes. The validation of WDF relies on the statistical validation of allelic detection from wine that is able to match the existing grapevine datasets [23]. Genotyping-based methodologies in wine and forensic medicine applications are closely related, as they depict DNA profiles of contributors in difficult conditions [17]. DNA from wine is often degraded and present in small quantities, requiring the definition of low template DNA (LT-DNA). The high value and reliability of molecular testing in forensics, even in extreme conditions, reinforce the idea of using DNA as a varietal authentication marker for wine quality. SSR-based genotyping is self-regulated, but a predictive key for correctly addressing wine DNA analysis could save time and money [24]. DNA fingerprinting technology is widely accepted for

grapevines by the International Organization of Vine and Wine (OIV). The technique is PCR-based and utilizes SSR markers and CE detection systems for the characterization of grapevine germplasm diversity [25]. The utilization of SSR markers has been effective in identification, percentage assignment, and genetic mapping of *Vitis vinifera* genomes [26]. SSRs are universally distributed throughout eukaryotic genomes, and there are many studies on agronomically important plant species using SSRs [27]. DNA fingerprinting has been applied successfully for wine traceability. The term WDF was first introduced by Vignani and Scali (2016) [28], and several prior papers used DNA-based methods for wine varietal authentication [29]. According to the WDF definition, the SSR-based approach, in conjunction with statistical elaboration, is used for wine varietal authentication. WDF provides information on the probability of the presence of a particular variety of grapes in a wine bottle.

The aim of this study was to propose a basis for the development of a molecular single-test kit for Brunello di Montalcino varietal authentication and for developing wine databanks by using bioinformatics. These can be achieved through integrated wet lab procedures with statistical validation of genetic tests. In addition, the molecular genetic variability set of data provided from the wet lab molecular analysis of wines, which includes experimental (IN7, CB17 Sangiovese wines) and commercial monovarietal wines, enables the tracking of wine origin. Evidence of geographical origin is among the information that wine makers demand and can be fulfilled by tracking grapevine genetic variants in different wines. Small labs may consider gaining the ability to carry out wine quality assessment through the integrated molecular biology and bioinformatics approach to determine the varietal composition of wines with increasing complexity. This reduces operational costs by reducing unnecessary analysis. Furthermore, a low level of expertise and technical requirements are needed. This work builds upon the data and findings from a previously published work [17]. In comparison to the previous study, this study demonstrates the efficiency and accuracy of using a minimal panel of 5 SSR markers compared to the 10 SSR markers used in the authentication of Brunello di Montalcino wine.

2. Materials and Methods

2.1. Wine and Grapevine References

The wine list and relative description are reported in Table 1. The wines included experimental and commercial wines. The experimental Sangiovese wines (CB17 and IN7), obtained by the small-scale fermentation of grapevine berries given at harvest (2014) by Case Basse di Gianfranco Soldera (Montalcino, Italy), were kindly provided by the University of Florence. The commercial Brunello and Rosso di Montalcino wines were kindly provided by local wine makers (Tenuta Le Potazzine, Tenuta Caprili, and Azienda Agricola Palazzo, Montalcino, Siena, Italy). A Brunello di Montalcino blended with 5% Pinot Noir was included as an experimental wine.

To test the method on wines other than Brunello di Montalcino, we used the following monovarietal control experimental wines: 100% Sauvignon Blanc, 100% Merlot, 100% Cabernet Sauvignon, and 100% Chardonnay.

To test the limit of detection of the main variety in a plurivarietal wine, we used several blended wines (Wine Institute, San Francisco, CA, USA). In detail, the blended wines, containing up to three varieties each, were the following: 75% Cabernet Sauvignon + Tempranillo, 76% Merlot + Rubired + Rubi Cabernet, 76% Chardonnay + Viognier, Symphony, and 76% Sauvignon Blanc + French Colombard + Chardonnay.

Plant references were obtained from the National Italian Grapevine Register held at CREA (Conegliano Veneto, TV, Italy). Plant references and relative genotypes are conserved and updated as ex situ data DNA collection by the SERge-GEnomics S.r.L. spin-off company

(Siena, Tuscany, Italy). The grapevines used as references were Sangiovese, Pinot Noir, Cabernet Sauvignon, Merlot, CB17-Sangiovese genetic variant, IN7-Sangiovese genetic variant, Sauvignon Blanc, Chardonnay, Tempranillo, Rubired, Rubi Cabernet, Viognier, French Colombard, and Symphony. SSR genotypes were either obtained in our laboratory or retrieved from the open data available in databanks.

Table 1. List of wines.

Wine Name	Vintage	Year of Analysis	Origin	Type (E = Experimental) (C = Commercial)
1. IN7	2014	2019	University of Florence	E
2. CB17	2014	2019	University of Florence	E
3. 95% Sangiovese + 5% Pinot Noir	2014	2019	Siena, Italy	E
4. Brunello di Montalcino	2014	2019	Montalcino, Siena, Italy	C
5. Rosso di Montalcino	2014	2019	Montalcino, Siena, Italy	C
6. 100% Sauvignon Blanc	2018	2019	Siena, Italy	E
7. 100% Merlot	2018	2019	Siena, Italy	E
8. 100% Cabernet Sauvignon	2018	2019	Siena, Italy	E
9. 100% Chardonnay	2018	2019	Siena, Italy	E
10. 75% Cabernet Sauvignon + Tempranillo	2016	2019	USA	C
11. 76% Merlot + Rubired + Rubi Cabernet	2016	2019	USA	C
12. 76% Chardonnay + Viognier, Symphony	2016	2019	USA	C
13. 76% Sauvignon Blanc + French Colombard + Chardonnay	2016	2019	USA	C

2.2. DNA Extraction from Grapevine Leaves

DNA from grapevine leaves was extracted using a DNAeasy Plant mini kit (Qiagen, Milano, Italy), following the manufacturer's instructions. Homogenization was performed by grinding 100 mg of fresh leaves in a 1.5 mL Eppendorf tube with a plastic pestle at room temperature. The DNA was then diluted in 100 µL elution buffer (10 mM Tris-HCl pH 8.5).

2.3. DNA Purification from Wines

DNA was purified from the wines following the protocol by Bigliazzi et collaborators [30], starting from 400 mL of wine. Quantification of *Vitis vinifera* DNA was performed following a validated protocol [31].

2.4. SSR-Genotyping and Allele Sizing

Wine DNA fingerprinting (WDF) was obtained by genotyping the *V. vinifera* genomic DNA from the wine, as described in [17].

2.5. DNA Amplification

The DNA extracted from the wines and plants was genotyped by using SSR markers VVS2 [32], VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD31, VVMD32, VVMD34, and VVMD36 [33], and Vrzag21, Vrzag47, Vrzag64, Vrzag79, and Vrzag83 [34]. Polymerase chain reaction was carried out in double replicas in a PCR plate of 96 wells at a total volume of 12.5 µL, containing 2.5 µL DNA, 0.25 mM of dNTPs, 0.25 µM of each primer (one of them fluorescently labeled), 1× Green GoTaq reaction buffer containing 1.5 mM $MgCl_2$, and 0.1 U GoTaq Flexi DNA polymerase (Promega, Milano, Italy). The PCR conditions included denaturation steps for 5 min at 95 °C, 39 cycles of 30 s at 95 °C, 30 s at 48–56 °C, depending on the specific marker, 1 min at 72 °C, and an extension stage at 72 °C for 10 min. Grape DNA can be genotyped using the same protocol.

2.6. Capillary Electrophoresis

The polymorphism of the SSRs was examined by allele sizing on a capillary electrophoresis. First, 1 µL of PCR product and 10 µL loading mix, containing highly deionized formamide and 0.2 µL internal standard (GS-400 Rox Standard, Thermofisher, Monza, Italy), were denatured at 95 °C for 5 min and kept on ice for 2 min before loading into an AB3130 genetic analyzer (Applied Biosystems, Waltham, MA, USA) machine. Electrophoresis was performed with the following parameter settings: separation temperature of 60 °C, injection voltage of 1.6 kV, injection time of 8 s, run voltage of 15 kV, and run time. At least two technical replicates were performed for each sample of DNA extracted.

2.7. Genotyping

The allele size was obtained using Gene Marker Version 2.2.0 software (SoftGenetics, State College, PA, USA). The PI was calculated to estimate the discrimination power of each panel of markers in discriminating varieties. The PI values were calculated by use of Identity software, Identity 4.0, Centre for Applied Genetics, University of Agricultural Sciences, Vienna [34]. Each sample was processed in triplicate.

2.8. Consistency of WDF After Data Merging

WDF analysis was performed according to [30]. Each experiment was repeated at least three times, and the data from each experiment were gathered in one major dataset. An estimate of the discrimination power of the test in resolving varieties was determined by calculating the PI using the minimal number of markers able to depict the varietal nature and propaedeutically to produce graphic outputs.

3. Results

3.1. Estimating Informativeness of WDF Testing: Setting a Minimal Panel of Markers

The extractable DNA out of a five-year aged wine is estimated by use of RT-PCR in the range of 30–40 ng/mL, and it is usually used for downstream genotyping applications with standard panels consisting of 10–16 SSR loci in order to reconstruct source vine identity [30]. The discrimination power of the SSR panel of markers was calculated with Identity software by setting the WDF marker panel consisting of seven SSR markers using Sangiovese, Sangiovese-IN7, Sangiovese-CB17, Merlot, Pinot Noir, Cabernet Sauvignon, and Zinfandel as grapevine references. The estimated PI value for the correct genetic profiling of monovarietal wines using the seven SSR markers was calculated to be in the order of about one over ten billion (PI = 1.04×10^{-10}) [17].

To reduce the number of tests, the panel was decreased to five SSRs. The WDF performed on both the experimental wines (IN7 and CB17) and the Brunello di Montalcino, Rosso di Montalcino, and SG-C wines using the five-SSR panel suitable for the Sangiovese-based monovarietal is referred to as the minimal panel, and it is associated with a discriminatory power in the order of 1 over a million or less (PI $\leq 10^{-6}$) (Table 2).

To simulate a two-variety blend or an experimental mixing of Pinot Noir in a Sangiovese almost pure wine, a mock Brunello di Montalcino wine, added with 5% Pinot Noir, was tested by WDF. The WDF carried out using five SSR markers allowed for the detection of Pinot Noir-specific alleles at four SSR markers out of five (Table 3).

The data demonstrate a significant correspondence between the genotype that can be obtained from wine DNA and the standard genotype profile of the respective vines, confirming the reliability of the varietal nature of wine prediction, even using the minimal panel of markers (Table 2). The monovarietal nature of the Brunello and Rosso di Montalcino wines is confirmed by the consistency in observing, in all markers, a systematic lack of multi-allelic profiles, indicative of multiple biological sources. More in detail, this panel

seems to effectively differentiate monovarietal Sangiovese wines from other experimental monovarietal wines, such as CB17, IN7, and SG-C, all deriving from varietal variants of Sangiovese in historical vineyards in Montalcino.

Table 2. The data shown represents the first molecular assay using a minimal panel of markers, potentially adjustable to a one-tube PCR test that is designed for varietal authentication of high-quality Brunello di Montalcino and Rosso di Montalcino wines. The IN7, CB17, and SG-C wines show the allelic variants of the respective Sangiovese vineyards linked to the presence of a few historical forms of local plant strains. Alleles matching between wines and plant references are in bold.

	VVS2	**VVMD25**	**VVMD27**	**VrZag21**	**VrZag83**
Grapevine type					
Sangiovese	135–135	241–241	179–185	202–204	191–195
Wines					
IN7	–	**238**–241	179–185	204–204	191–**201**
CB17	135–**146**	241–**255**	185–**189**	–	191–**201**
SG-C	135–**145**	241–**254**	–	204–**206**	191–195
Rosso di Montalcino		241–241	179–185	202–204	191–195
Brunello di Montalcino	135–135	241–241	179–185		191–195

Table 3. Brunello di Montalcino mock wine added with 5% Pinot Noir. Alleles matching Pinot Noir are in bold.

	100% Sangiovese	**100% Pinot Noir**	**95% Sangiovese + 5%Pinot Noir**
VVMD25	241–241	241–249	241–**249**
VrZag21	202–204	201–207	**201**–204–**207**
VrZag83	191–195	190/202 or 189–201	191–**201**
VVS2	135–135	140–154	135–**140–154**
VVMD27	179–185	185–189	

3.2. Varietal Assessment in the Control Monovarietal and Blended Wines

The detection of minor varietal components in blended wines by use of SSR markers is linked to a low risk of losing genetic information (low allele drop risk). As already shown, to address the reliability of the genetic test for the Brunello di Montalcino wine, the system was tested on the experimental wine in which 5% of Pinot Noir was added. Six informative alleles matching Pinot Noir and not Sangiovese were detected (alleles in bold, Table 3). To assess the detection limits of minor varietal components in the other wine types, four additional wines (wines 10, 11, 12, and 13) were used. The composition of the varietal blends was only partially known, reflecting the real situation of most commercial wines. The scope of the characterization of the four blended wines was specifically to determine if minor quantities of several varieties added to the blend would impair the identification of the major variety. Table 4 shows the allele profiles that were detected by Random Match Probability on the blended wines.

Table 4. Allele profiles detected in the blended wines. The alleles in bold are those that do not match the main varietal component genotype. The wines include the initial CS = Cabernet Sauvignon, M = Merlot, C = Chardonnay, and SB = Sauvignon Blanc. The number of SSR full loci or single alleles (in brackets) detected by Random Match Probability matching the major variety are reported in bold at the bottom of each column.

	Wine 10			**Wine 11**			**Wine 12**			**Wine 13**		
	CS + Tempranillo	Control wine 100% CS	CS Plant	**M** + Rubired, Ruby Cabernet, etc.	Control wine 100% M	M Plant	**C** + Viognier, Symphony, etc.	Control wine 100% C	C Plant	**SB** + French Colombard + Chardonnay, etc.	Control wine 100% SB	SB Plant
SSR markers												
VVMD27				**194**		185 189				175	175–189	175–189
VVMD25	240–249	240	240–249	240	240–249	240–249	240–255	240–255	240–255	240–248	240–248	240–248
VVMD21			249–258	243–249		243–249	249	249	249	249	243–249	243–249
VVMD24	208–216	208–216	208–216	208–212–**214**		208–212	208–216	208–216	208–216	216–**208**	216	216
VVMD32			240	240		240		240	240–271		240–256	240–256
VVMD31			206–210	212	212	212–216			214–216	216	210–216	210–216
VVMD36			252–262			252			252–274		262	262
VVMD34			239–247	239		239			239		238–247	238–247
VVMD7			238			238						238–256
VVS2	141		141–153	139	139	139–153	139	139	139–145	136	136	136–153
ZAG47	152–166		152–166		166	166	166	158–166	158–166	153–166	153–166	153–166
ZAG79			245			245			243			245
ZAG21	201		201–207	201–**220**		201			201–207	205–207	205–207	205–207
ZAG83	201		201	195		195–201		189	189–201		187–201	187–191–201
ZAG64			139–160			139–160		240–255	160–164			
	4 loci+2(1/2)			**5 loci+3(1/2)**			**3 loci+3(1/2)**			**4 loci+4(1/2)**		

Both in the blended and control wines, the data show a tendency of allele drop risk in the amplified SSR loci, which appears likely to be marker-dependent. Depending on the type of blend, the allele drop might be critical if the "lost" allele tracks the presence of the main component. For instance, the Cabernet Sauvignon wines tend to lose alleles 249, 153, and 207; the Merlot wines tend to lose alleles 249, 216,153, and 201; the Chardonnay wines tend to lose alleles 145, 158, 201, and 271; and the Sauvignon Blanc wines tend to lose alleles 189, 243, 210, and 153. Allele drop risk does not seem to increase in relation to the complexity of the wine blends, in agreement with the hypothesis of a marker-type-dependent effect rather than a wine-composition-related issue. Allele drop risk opens issues concerning allele call and constitutes an implicit invitation to adopt rigorous methodological guidelines, which include the use of technical replicas and a minimal threshold for DNA quality and quantity, to keep a high confidence level of the WDF test. The above components may help to assess the feasibility of undertaking WDF and support molecular evidence with specific statistical elaboration. In addition, this supports the main effort in WDF as a varietal diagnostic tool, which focuses on further improvement in the grapevine DNA extraction method from wine. This is achieved by scaling down the initial volume of wine and simplifying procedures. Notably, this would ease the production of technical replicas, which seem crucial for the validation of observed alleles in WDF profiles.

3.3. Allele Sizing Reflects Vineyard Diversity

Some local Sangiovese grapevine strains, despite the overall phenotypic asset recalling the grapevine cultivar, exhibit minor allelic differences, as shown in Table 5. Historical vineyards (>40 years) in Montalcino reveal natural diversity that may be used as an indication of the geographical origin of the wines. As a matter of fact, some alleles informative of vineyard origin may be tracked in the wines (Table 2).

Table 5. Allelic variants of Sangiovese_IN7 and CB17 potentially indicative of microgeographic origin.

	VVS2	**VVMD27**	**VVMD25**	**VVMD21**	**VVMD24**	**VVMD36**	**Vrzag21**	**Vrzag83**
Sangiovese	135	179–185	241	243–249	206–212	201–203	202–204	191–195
Sangiovese_CB17	135–**146**	185–**189**	241–**255**	249	206–212	203–**205**	202–**205**	191–**201**
Sangiovese_IN7	n.d.	179–185	**238**–241	**231**–243	212–**216**	**252–262**	204	191–**201**

n.d. stands for: "Not Detected".

4. Discussion

Previous work by a research team [17] demonstrated that WDF can effectively describe the varietal nature of wines after the calculation of different distance matrices by Neighbor-Joining (NJ), followed by Principal Component Analysis (PCA), in a wine-dedicated bioinformatics elaboration. The WDF of Sangiovese-based wines and experimental wines remains informative for assessing the varietal origin, even in the case of using a minimal panel of informative SSR markers. The data show how the WDF for the Sangiovese experimental and commercial wines is consistent with their varietal nature, even after merging data from multiple experiments, if the target of the test is to identify the Sangiovese profile against specific varieties, namely, Merlot, Pinot Noir, and Cabernet Sauvignon. Additionally, the PI value generated can serve as an essential tool to validate the methodology. As shown in Supplementary Table S1, the PI value associated with the use of five SSR markers ($PI \leq 1.0 \times 10^{-6}$), with respect to an extended panel of seven SSRs, appears to be stringent enough to guarantee the correct varietal assignment.

The Brunello di Montalcino wine was analyzed seven years from its plurivarietal production, and the Rosso di Montalcino five years from production. Both DOCG wines did not lose the capability of the molecular detection of the Sangiovese variety even after

wine aging. To resolve a blended varietal wine containing 75% of the main variety, the PI is sensibly decreased due to the need to increase the number of SSR markers to be used for carrying out the WDF test. Namely, a PI value in the order of 10^{-7} was observed for the blended whites taken from the US market, and the order of 10^{-13} for some red wines commercially available and also produced in the US [17]. Point-of-care technology demand along the wine-making chain focuses on rapid, low-cost, and reliable analytical methodologies [35]. In a mid-sized private laboratory setting, monitoring nonconformance is essential in determining areas where further improvements can be made. DNA-based technologies are rapidly moving towards the use of consolidated biotechnology applications, which allow quick and low-cost approaches for wine varietal characterization, even in small and basic molecular biology laboratories.

WDF is highly dependent on the DNA extraction procedure from wine. The state of DNA in wine has been a subject of debate. Some authors emphasize the need to consider the development of specific molecular markers for wine and the sensitivity of detection methods since wine contains a mixture of phenolic compounds, such as acids and phenols, that interfere with DNA extraction and the downstream performance of DNA [36]. Also, fermentation introduces DNA into wine from multiple biological sources, while WDF is interested in grape DNA only. Optimal protocols for DNA extraction from wine are necessary, as it is the bottleneck of DNA analysis. As a matter of fact, it was proven that DNA extracted with optimized protocols from several wine types could be genotyped to reconstruct the varietal composition of monovarietal wines [30]. After this achievement, the varietal authentication was also used on blended wines, where the system estimates the probability of the presence of specific grapevines by bioinformatics [17]. More recently, research on wine bio-molecular integrated compositional analysis was published [15,37,38]. This work took a different multidisciplinary approach to wine authentication by demonstrating that the bioinformatics tools that validate DNA evidence in wine are common to other more "classical" compositional analyses, such as chemical profiling, and open a new perspective on wine ontology and anti-counterfeiting measures. Genotype profiles of the original grapevine varieties of Sangiovese monovarietal wines, both experimental and commercial, were identified via SSR PCR-based amplification. In addition, the data form the basis for the definition of predictive key features associated with the analysis.

Given that the authentication of wines is of major interest to the wine industry, consumers, and governmental policymakers responsible for food and wine conformity checks, we believe that the findings presented here could stimulate the high-throughput processability of the analysis focusing on wine varietal authentication. This work expands on the prior research on the varietal authentication of wines [39]. Since most certifications promote technological advancements, the proposed methodology could influence the wine certification process by contributing to the upgrading of major global building certification schemes. Additionally, the technique could boost regulatory compliance through the development of a more robust wine traceability system that addresses geographical provenance and wine variety authentication [40]. With the growing demand for wine consumption globally, the methodology could also influence consumer trust. Consumers are able to access information on wine labels linked to geographical origin [41]. For most wine samples, a significant correspondence between the genotype obtained from wine DNA and the standard plant profile of the respective vines was calculated, through PI, to be lower than one over a million ($PI \leq 10^{-6}$). The data shown here represent the first molecular assay performed on Brunello di Montalcino and Rosso di Montalcino using a minimal set of informative SSRs in WDF experimental plots to reconstruct the varietal identity of the source vine with sufficient statistical reliability.

5. Conclusions and Perspectives

The sequencing of many grape genomes, the availability of techniques to quantify nucleic acids in wine, and the innovations in bioinformatics and chemoinformatics provide the grounds to explore a new direction in wine quality and product safety assessment. Despite the fact that the DNA fingerprinting approach is reliable for wine varietal authentication [28], the efficiency of the approach should still be tested on other wine types, and the validation of allele observation is essential for organizing genetic profiling databases for wine. There is also a need to integrate multi-omics analytical approaches with other quality evaluation parameters regarding wines; information on the history, cultural, and chemical features beyond the product is also required to complete a circumstantial picture of a high-quality wine. There is also a need to simplify the results for a wider use of the authentication technique and for its acceptance by the general public. Molecular integrated approaches have played a role in predicting the composition of wines [24]. The application of the statistical validation of genetic data has ensured high-quality control standards, given that a number of analyses, such as chemical, physical, cultural, historical, and statistical analyses, provide information on the characteristics of wine products that will be part of an innovative ontology databank for wine.

In the future, to create cutting-edge block-chain databanks, molecular, chemical, and metabolomics profiling should be combined to produce extensive wine ontology databanks provided with innovative and informative wine labels. To avoid needless analysis, even in volunteer certification, the test of varietal authentication on various wines must be proven to be reliable. In this regard, this study shows that high-commercial-value monovarietal Brunello di Montalcino may be subjected to a reliable WDF test.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages11030081/s1; Table S1: Among the 15 SSR markers, 7 (bold, first column) were efficiently used for WDF in Sangiovese wines in previous work [17]. A limited panel of five SSR markers (bold, third column from left) were successfully used to obtain confirmation of the Sangiovese monovarietal nature of the wines.

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