


## Moscato Cerletti, a rediscovered aromatic cultivar with oenological potential in warm and dry areas

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### ABSTRACT

Baron Antonio Mendola was devoted to the study of grapevine, applying ampelography and dabbling in crosses between cultivars in order to select new ones, of which Moscato Cerletti, obtained in 1869, was the most interesting. Grillo, one of the most important white cultivars in Sicily, was ascertained to be an offspring of Catarratto Comune and Zibibbo, the same parents which Mendola claimed he used to obtain Moscato Cerletti. Thus the hypothesis of synonymy between Moscato Cerletti and Grillo or the same parentage for both sets of parents needs to be verified.

In the present study, historical documents were consulted and genetic analyses and ampelographic, agronomic and qualitative characterisation carried out to determine the distinctiveness of each cultivars. These were also compared with Catarratto Comune and Zibibbo in order to establish the Moscato Cerletti pedigree. Due to their different SSR profiles, Grillo and Moscato Cerletti were confirmed as two distinct cultivars; they also differed in ripening times and sugar storage ability, as well as in the aromatic grape produced by Moscato Cerletti only. The trio genotype genetic analysis confirmed that Zibibbo is a parent of Moscato Cerletti (justifying the aromatic grape), whilst the SSR profiles did not show Catarratto Comune to be a second parent.

Moscato Cerletti was found to have oenological potential in the production of sparkling muscat wines due to its ability to adapt to a changing climate in warm and dry environments and in different winegrowing regions.

### KEYWORDS

grapevine genetic resources, SSR (Simple Sequence Repeat), ampelographic description, agronomic traits, qualitative composition.

Supplementary data can be downloaded through: <https://oenone.eu/article/view/4605>

## INTRODUCTION

Sicily, the largest island in the Mediterranean Sea, is certainly one of the most important regions in terms of viticulture in the Mediterranean region. Today, this Italian region has the largest wine growing area (14.6 % of total Italian vineyard area) (AAVV, 2020a). In Sicily, viticulture has a long history and many autochthonous cultivars that have been re-discovered and exploited in recent decades (Ansaldi *et al.*, 2014). There are many ancient records testifying to the historical importance that Sicilian grape and wine has had since the XVII century (Cupani, 1696; Cupani, 1697), after which, Sicilian grape varieties have been described by Sestini (1812), Geremia (1834) and Minà Palumbo (1853), the latter being a “modern” description (according to the botanical method of Kolenati). Recently, a key role of *Magna Graecia* (namely Sicily and Calabria) in the grapevine dissemination from south to north Europe based on a wide genetic characterization of Italian germplasm was described (Mercati *et al.*, 2016; Sunseri *et al.*, 2018; De Lorenzis *et al.*, 2019; D’Onofrio *et al.*, 2021; Mercati *et al.*, 2021).

The Baron Antonio Mendola was born in Favara (province of Agrigento, Sicily) in 1828. He devoted his life to the study of grapevine, from ampelography, viticulture and oenology to the breeding of new cultivars (Cigo, 1904). A large repository of about four thousand varieties from Italy and all over the world was established in Favara for his studies. At that time, the collection was the richest and most celebrated in Italy and probably in Europe. Mendola was very active in national and international collaboration and the exchange of genetic material. Alexandre-Pierre Odart, who explained modern ampelography in his book *Ampélographie Universelle* (Odart, 1849), was his friend and mentor, and Victor Pulliat testified to Mendola’s interchange activities and scientific collaborations in the three editions of his book *Mille variétés de vignes* (Pulliat, 1888). Pulliat also published a list of the varieties sent to him by Mendola which were held in his collection in Chiroubles (Rhône), many of which were from Sicily and other Italian Regions. Pulliat shared these varieties and related information with famous Italian ampelographers from the regions of Piedmont, Lombardy and Veneto. In his work *Saggio di una Ampelografia Universale* (di Rovasenda, 1877), Giuseppe di Rovasenda cited the varieties selected by Mendola, which he claimed to have collected in his own repository in Bicocca (Piedmont, Italy).

In *Ampelografia* (Molon, 1906), Girolamo Molon described all the cultivars bred by Mendola which were in his grape collection.

When carrying out breeding, Mendola selected new varieties among which was Moscato Cerletti (or Catarratto Moscato Cerletti), which he dedicated to his friend Prof. Cerletti, director of the oenological Gattinara station (province of Vercelli, Italy). Mendola (1874) explained: “[...] in 1869, white Catarratto flowers were artificially fertilised with Zibibbo pollen in a vineyard located near Favara; the seeds were harvested on August 27 of the same year, sown in pots on March 3, 1870, and seedlings germinated around May 20 [...]”. In the autumn of the same year, Mendola tasted his very first Moscato Cerletti grapes and declared that they were among the best table grapes that he had ever obtained from seed in terms of size, beauty, taste and firmness of the berries.

The characteristic qualities of Moscato Cerletti have been confirmed by other authors. Molon gave a similar description of Moscato Cerletti to Mendola: “[...] *Vine with vigorous and healthy vegetation. Bunch of conical shape, medium size, with wings, the peduncle of primary bunch is short and lignified up to about the middle. Large berry just a little not uniform, from globose to broad ellipsoid shape, presence of bloom, skin with rare and light dotting, firmness of flesh, good and muscat flavour. Ripening in the third period [...]*”. Molon also wrote: “[...] *this variety, in our poor land, is very productive; if the bunch was less dense and earlier in ripening, we could designate it among the best [...]*”. In the last century, Moscato Cerletti was praised by Pirovano (Pirovano, 1925; Pirovano, 1933), Sannino (1920), Longo (1948), although Moscato Cerletti was considered extinct in Italy until now.

Different studies based on SSR profiles have demonstrated that Grillo, an autochthonous Sicilian cultivar famous for Marsala wine, is an offspring of Catarratto Comune and Zibibbo (or Muscat of Alexandria) (Di Vecchi Staraz *et al.*, 2007; Cipriani *et al.*, 2010; Lacombe *et al.*, 2013), who is intriguingly the same parents claimed by Mendola for Moscato Cerletti. This odd coincidence led to the hypothesis that Grillo is one of the new varieties obtained by Mendola or even a synonym of the disappeared Moscato Cerletti. As the present paper will show, the latter hypothesis is negated by our SSR analysis.

Nowadays, little information is available about the origins of Grillo, in particular whether it is an offspring from crossings carried out by Mendola himself. The first historical reference to Grillo in the Marsala area dates back to the time of Alagna Spanò (1873), as reported by Pastena (1971). Grillo has neither been mentioned by Cupani (1696) nor by Minà Palumbo (1853). Mendola provided Damiani (1885) with a list of the cultivars grown in Marsala and Trapani that included Grillo or *Griddu biancu*. In 1881, the French translation of the *Saggio di Ampelografia Universale* by di Rovasenda (1881) replaced the original name “Grello” with “Grillo”, that was present in Baron Mendola’s grapevine collection in Favara and attributing him this cultivar (textually: “Grillo, Sicile MEND”). This report contributed to the assumption that Grillo was one of the crosses produced by Mendola. Indeed, in contrast to the French translation, there is no explicit attribution of Grillo to Mendola in the original Italian text edition by di Rovasenda. Moreover, the cv. Grillo was not present in an earlier list of grapes in his large collection in Favara, which was drafted by Mendola in 1868, and after which there is no mention of the cultivar in any of his writings.

At the beginning of the 20th century, in the post-phylloxera era, Grillo was considered a very precious cultivar in the area of Marsala (Paulsen, 1908), and it was noted for its valuable qualities among the varieties used to produce Marsala wine (Ray, 1919). Grillo rapidly became the most widespread white grape in the Marsala area (Paulsen, 1932) and in the second half of the century it became the third white cultivar in Sicily (5.2 % of vineyards surface area) after Catarratto and Inzolia (Pastena, 1971). Nowadays, Grillo is cultivated in an area of 8444 ha (AAVV, 2020b), representing 8.62 % of the Sicilian wine-growing area. Its widespread cultivation in Sicily is due to its tolerance to fungal diseases and its vegetative vigour, as well as the high alcohol content and scents of its wines. Grillo is still a key cultivar for Marsala wines, both as a single-variety and blended (Cammarerì Scurti and Alessandrini, 1891; Dell’Orto and Vajarello, 1926; Pastena, 1972). More recently, Pastena (1991) argued that Grillo could be used to obtain wines other than Marsala and dessert wines; nowadays it is also appreciated for sparkling and table wines (Moretti *et al.*, 2009).

For years Moscato Cerletti was thought to be extinct, but it was recently rediscovered by the Istituto Regionale Vino e Olio of Palermo at the INRAE Vassal-Montpellier Grapevine Biological Resources Center (France) ([https://www6.montpellier.inrae.fr/vassal\\_eng/](https://www6.montpellier.inrae.fr/vassal_eng/)). Indeed, Marés and Bouschet, friends and colleagues/collaborators of Mendola, kept their important ampelographic collections in Montpellier, including the Moscato Cerletti vine.

This study aims to shed light on the history and pedigree of Moscato Cerletti and to evaluate its agronomic and oenological potential for exploitation. Historical documents were examined and the phenotypic and genetic characterisation of Moscato Cerletti was carried out in order to demonstrate its distinctiveness from Grillo, as well as compare both varieties with Catarratto Comune and Zibibbo, the potential parents of both cultivars.

## MATERIALS AND METHODS

### 1. The experimental vineyard

Four cultivars, Moscato Cerletti, Grillo, Catarratto Comune and Zibibbo, were compared in a vineyard located in Marsala, western Sicily (80 m a.s.l., 37°47’41.01” - 12°33’22.15” E). The vineyard was not irrigated and the soil was 54 % clay, 21 % silt, 26 % sand, 13.5 % free lime and 0.6 % organic matter. The vines were planted in 2011, grafted onto 140 Ruggeri rootstock, trained on a VPS trellis and pruned, leaving one cane of eight buds and one spur of two buds. The inter- and intra-row vines were spaced at 2.20 m and 0.9 m respectively (5,050 vines/ha) and the row orientation was NE-SW. The vines were placed in two contiguous rows formed by fifty-five vines each. Fifteen vines with similar vigour were chosen for each cultivar for the agronomic and ampelographic evaluations.

### 2. Ampelographic description

The ampelographic traits of shoots, young and mature leaves, bunches and berries were recorded for two years, following the procedure reported in the 2nd Edition of the OIV Descriptor List for Grape Varieties and *Vitis* Species (OIV, 2009).

The morphological traits were recorded twice during the spring and summer seasons (2015 and 2016), using 51 OIV descriptors as suggested by the European GrapeGen06 project (Maul *et al.*, 2012).

The occurrence of budburst, flowering, veraison and harvest was recorded. The budburst (stage C), flowering (stages I) and veraison (stage M) dates were recorded when 50 % of buds, flowers and berries respectively showed the same phenological stage (Baggiolini, 1952).

Starting 20 days after veraison, total soluble solids (TSS) and titratable acidity (TA) (Jackson and Lombard, 1992) were measured on about 200 randomly picked berries. The sampling was weekly or more frequent (every 4 days) in order to be able to accurately define the date of harvest (stage N). Grapes were harvested when TSS did not increase for two subsequent measurements and TA was not lower than 5 g/L.

### 3. Agronomic traits and grape chemical composition

Shoot fruitfulness was assessed in 2015 and 2016. The yield and number of clusters - useful for determining cluster weight - of 15 selected vines were recorded at harvest. For each cultivar, one hundred berries (replicated three times) from twenty clusters, which had been harvested at technological maturity from several plants, were randomly sampled. The berries were weighed and their diameters (longitudinal-LD and transversal-TD) measured using a digital caliper (Digital Caliper 300 mm; Insize Co. Ltd.; China). One hundred berry seeds per replication were counted and weighed. The pruning mass and number of canes were recorded during the vegetative rest period.

The TSS (Brix) of 1 kg grape samples from each cultivar (replicated three times) was measured using an Atago® PR-32 digital refractometer (Atago®, Tokyo, Japan). TA was measured using a Crison Compact Titrator (Crison Instruments, Barcelona, Spain) by titration (0.1N NaOH) to pH 7 (expressed in gL<sup>-1</sup> of tartaric acid).

The free and glycosylated varietal aroma compounds were extracted from three replicates of 50 fresh grape berries, which were processed following the procedure described by Fracassetti *et al.* (2017) and Corona *et al.* (2020). The berries were de-seeded and the pulp was separated from the skin by adding Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> (100 mg). The skins were treated with 20 mL of methanol for 1 h to release the aroma compounds and to inactivate the glycosidase enzymes (which otherwise would have created artifacts; i.e., the formation of free varietal aroma compounds from glycosylated varietal aroma compounds),

and they were then crushed with a laboratory blender using a high-speed Ultra-Turrax T25 (IKA® Labortechnik, Staufen, Germany). The pulps were crushed separately with a laboratory blender using a high-speed Ultra-Turrax T25 (IKA Labortechnik, Staufen, Germany) and then mixed with the skin mixture. The skin and pulp mixture were centrifuged twice (7000 × g, 15 min, 4 °C) and the solid residue was washed with tartaric acid buffer (pH 3.2). The final extract (250 mL) was then clarified with a pectolytic enzyme (0.1 g) without secondary glycosidase activity (Rapidase® X-Press, DSM, The Netherlands) at room temperature for 2 h. 1-Heptanol was added as an internal standard (0.2 mL of 30 mg/L solution in 10 % ethanol) to the samples. Afterwards, all extract obtained was loaded onto a 5 g C18 reversed-phase solid-phase extraction (SPE) cartridge (Isolute®, SPE Columns, Uppsala, Sweden), which had been previously activated with 20 mL of methanol followed by 50 mL of deionized water at a flow-rate of approximately 3 mL/min, and then rinsed with 100 mL of deionized water to eliminate the sugars, acids, and other low molecular weight polar compounds. The free aromatic fraction was then eluted with 25 mL of dichloromethane. The eluate was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated to about 0.2 mL under a stream of nitrogen. This extract, which contained free volatile compounds, was immediately analysed by gas chromatography/mass spectrometry (GC/MS). Afterwards, the glycoconjugates aromas were eluted from the cartridge with 20 mL of methanol and the eluate was concentrated to dryness using a vacuum rotary evaporator set at 30 °C (Buchi R-210, Switzerland). This dried glycoside extract was dissolved in 5 mL of citrate-phosphate buffer (0.2 M, pH 5) and, using 50 mg of an AR-2000 commercial preparation with glycosidase side activities (DSM Oenology, The Netherlands), it was subjected to enzymatic hydrolysis at 40 °C for 24 h. After 24 h, 0.2 mL of 1-heptanol (30 mg/L solution in 10 % ethanol) was added as an internal standard, and the volatiles which had been generated by the enzymatic hydrolysis of glycosylated precursors were then extracted following the previously described SPE method. The obtained dichloromethane extract was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated to 0.2 mL and kept at -20 °C until analysis. A GC/MS analysis was performed with a Agilent 6890 Series GC system and Agilent 5973 Net Work Mass Selective Detector (Agilent Technologies) equipped with a DB-WAX column (30 m, 0.250 mm i.d., film thickness 0.25 µm; Agilent Technologies).

The GC-MS conditions which were used are reported by Corona *et al.* (2020). The detection was carried out by electron impact mass spectrometry in total ion current (TIC) mode using an ionisation energy of 70 eV. The mass acquisition range was  $m/z$  30–330. Volatile organic compounds were identified by comparing their mass spectra and GC retention times with those of the pure commercial standard compounds or others prepared in our laboratory, as well as by comparing their mass spectra with those in the NIST/EPA/NIH Mass Spectral Library database (Version 2.0d, build 2005). The concentration ( $\mu\text{g}/\text{kg}$  berries) of volatile compounds was determined as 1-heptanol equivalents.

#### 4. Statistical analysis

The variation in the forty-three OIV descriptors of the four cultivars was visualised via a heatmap using the ggplot2 R package (<https://cran.r-project.org/web/packages/ggplot2/index.html>). Different colours and gradients were associated with the scale and combination for each category.

A two-way analysis of variance (ANOVA) was carried out to examine year and cultivar effects, and their interaction, followed by within-cultivar Tukey's HSD multiple-comparison test for yield components, pruning mass and grape quality traits. The cultivar effect was tested by one-way ANOVA for free and glycosylated volatile compounds, followed by a multiple-comparison Tukey's HSD test.

A Principal Component Analysis (PCA) was run using yield, pruning mass, quality traits and glycosylated volatile grapes compounds through the R package FactoMiner (Le *et al.*, 2008). Finally, the Pearson correlation coefficient ( $p < 0.05$ ) was calculated for the profiles of all pairs of selected traits using Hmisc R/package (<https://cran.r-project.org/web/packages/Hmisc/index.html>).

#### 5. Genotyping with nuclear and chloroplast SSR markers

Genomic DNA was extracted from 100 mg young leaf tissue (1–2 cm diameter) from the four varieties using the QiagenDNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The DNA quality (260/230 and 260/280 ratios) and concentration were checked with a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA, USA). Pinot noir and Sangiovese were included in the analysis as reference cultivars for allele calling. The samples were analysed with thirteen nuclear SSRs (nSSR), VrZag62, VrZag79, VVMD5, VVMD7, VVMD25,

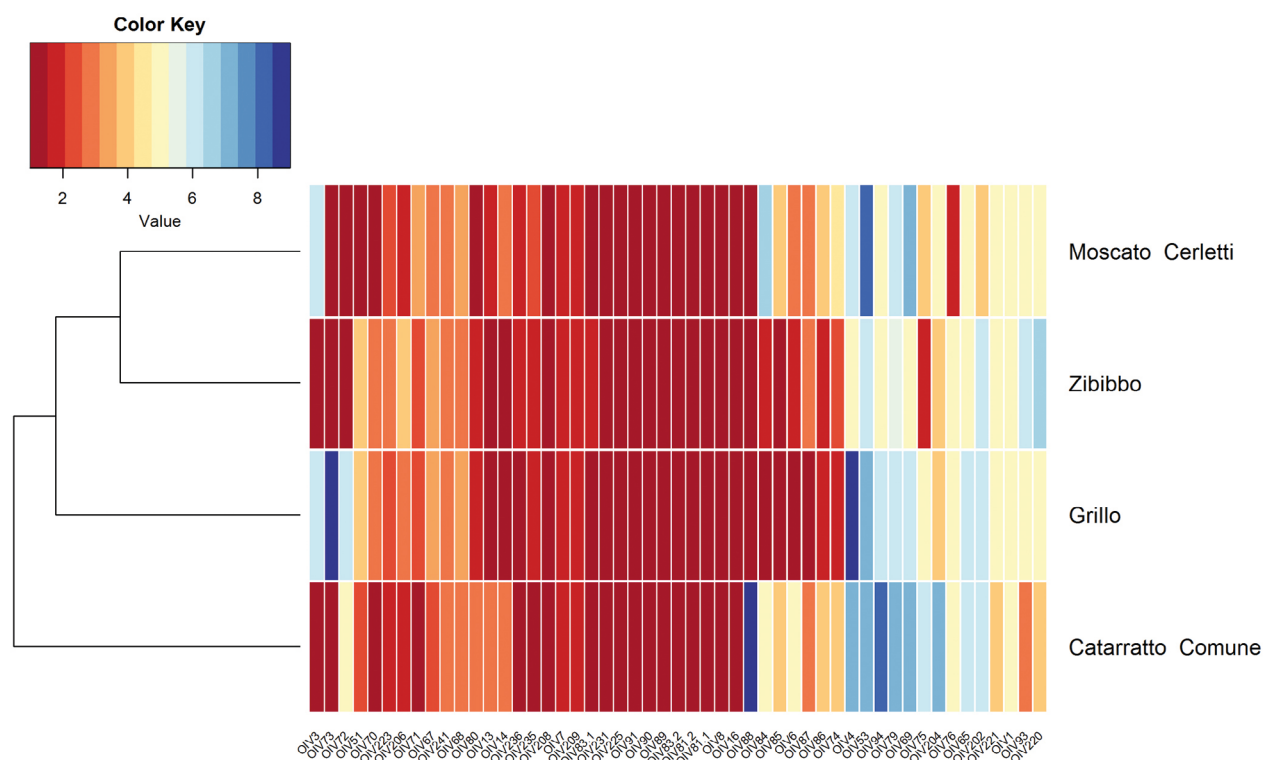
VVMD27, VVMD28, VVMD32, VVS2, ISV2, ISV3, ISV4 and VMCNG4B9 (Thomas and Scott, 1993; Bowers *et al.*, 1996; Bowers *et al.*, 1999; Sefc *et al.*, 1999; Crespan, 2003), nine of which are proposed as a standard set for grapevine genotyping in the framework of the GrapeGen06 European project (<https://www1.montpellier.inra.fr/grapegen06/>). In addition, six chloroplast microsatellite loci (cpSSR) were analysed using the consensus primer pairs designed by Weising and Gardner (1999) for ccmp3, ccmp4, ccmp5, ccmp6 and ccmp10, and by Bryan *et al.* (1999) for ccSSR23. Amplification reactions were performed in 20  $\mu\text{L}$  final volume following the procedure described in Mercati *et al.* (2013). The chlorotypes and cpSSR markers were those used in Arroyo-García *et al.* (2006).

The amplification fragments were detected on an ABI PRISM 3500 Genetic Analyser (Applied Biosystems® by Life Technologies, Foster City, CA, USA), and the alleles were measured for their size by GeneMapper™ Software v4.0 (Applied Biosystems by Life Technologies). The obtained SSR profiles were compared with the Italian *Vitis* database (<http://www.vitisdb.it>), the European *Vitis* database (<http://www.eu-vitis.de/index.php>), the Vitis International Variety Catalogue database (<http://www.vivc.de/>) and the CREA - Viticulture and Enology database (partially published in <http://catalogoviti.politicheagricole.it>).

## RESULTS

### 1. Ampelographic description

The ampelographic results are represented in a heatmap which shows the expression level of each OIV descriptor (Figure 1), and main trait descriptions are summarised in Table S1. Moscato Cerletti and Grillo showed clear differences, the most important being reported hereafter. The anthocyanin level of the shoot tip prostrate hairs was medium in Grillo and high for Moscato Cerletti; the density of the shoot tip prostrate hairs was high for Grillo and from high to very high for Moscato Cerletti; the colour of the 4th leaf upper side blade was a reddish copper for Grillo and a green-yellow for Moscato Cerletti; the two sides of the teeth of a mature leaf were either both straight or both convex for Grillo, while they were just both straight for Moscato Cerletti; the density of the prostrate hairs between the main veins on the lower side of the blade was from medium to high for Moscato Cerletti, and very low for Grillo; the density of the erect hairs between the main veins on the lower side of the blade was from low



**FIGURE 1.** Fifty-one OIV descriptors recorded for each cultivar.

The OIVs were represented by a heatmap. Different colours and gradients represent the categories for each descriptor (see Table S1).

**TABLE 1.** Summary of phenological stages of the investigated cultivars.

Phenological stages	Bud break	Flowering	Véraison	Harvest
Cultivar				
Catarratto Comune	Early April	Mid to late May	Late July	Early to mid-September
Zibibbo	Late March	Late May	Late July	Late August
Grillo	Early April	Mid to late May	Late July	Late August to early September
Moscato Cerletti	Early April	Late May	Early August	Mid-September

to medium for Moscato Cerletti, and very low for Grillo. Bunch density was medium-loose for Grillo and medium for Moscato Cerletti (Figure S1); the peduncle length of the primary bunch was from very short to short for Moscato Cerletti, and short for Grillo; the muscat flavour of the berry was absent for Grillo, but present for Moscato Cerletti. The berry flesh was from slightly firm to very firm for Moscato Cerletti, and slightly firm for Grillo.

The phenological stages are shown in Table 1. Zibibbo had the earliest bud break (early April) and ripening (late August). Moscato Cerletti and Catarratto Comune ripened the latest (mid-September), while Grillo ripened after Zibibbo and before Moscato Cerletti.

## 2. Agronomic traits and grape chemical composition

The yield parameters, pruning mass and grape macrostructure of the four cultivars are given in Table 2. Catarratto Comune and Zibibbo were characterised by average values of fruitfulness, and Grillo and Moscato Cerletti by low values. Moreover, the latter were not suitable for spur pruning, because of the low basal fruitfulness (data not shown). All cultivars showed a medium-high cluster weight. Moscato Cerletti, Grillo and Zibibbo were similar in berry size. Consistent with the ampelographic traits (Table S1), the berry length/width ratio was higher than 1.10 for Zibibbo, but lower than 1.10 for the other three cultivars, whose berry shape was obloid to globose.

**TABLE 2.** P-values of two-way analysis of variance to evaluate year and cultivar effects, and their interaction, followed by within-cultivar Tukey's HSD multiple-comparison test for yield components, pruning mass and grape quality traits.

Source	Fruitfulness	Cluster weight (g)	Berry weight (g)	Berry length (mm)	Berry width (mm)	Berry length/width ratio	Seed number (n)/berry	Seed weight (g)/berry	Weight of a single seed (g)	Cane (g)	TTS (Brix)	pH	Titrateable acidity (g/L)
	p value	p value	p value	p value	p value	p value	p value	p value	p value	p value	p value	p value	p value
Year (Y)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cultivar (C)	0.044	0.034	0.000	0.000	0.000	0.000	0.002	0.042	0.001	ns	0.002	0.024	0.010
Cataratto Comune	1.5 a*	288 a	1.8 C	14.5 C	13.9 C	1.04 B	1.10 B	0.05 b	0.04 B	81.2 B	19.6 B	3.2 b	7.8 A
Zibibbo	1.5 a	263 a	4.5 A	21.2 A	18.5 A	1.15 A	2.30 A	0.10 a	0.04 B	75.0 B	23.1 AB	3.6 a	5.0 B
Grillo	1.3 ab	234 b	3.2 B	16.8 B	15.9 B	1.06 B	1.45 B	0.07 b	0.05 A	72.9 A	25.8 A	3.3 ab	6.5 AB
Moscato Cerletti	1.15 b	271 a	3.3 B	16.8 B	16.1 B	1.05 B	1.60 AB	0.08 b	0.05 A	84.0 A	21.7 B	3.4 ab	4.9 B
Interaction													
Y x C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

\*Upper, lowercase and different letters within the same column indicate statistically significant differences at  $p < 0.01$  and  $p < 0.05$  respectively, among cultivars according to Tukey's HSD test. ns = not significant.

The number of seeds per berry of Grillo and Moscato Cerletti was intermediate - between Catarratto Comune and Zibibbo. The low number of seeds per berry suggests a reduced rate of auto-fertilisation, which frequently caused chicken berries to form on Catarratto Comune and/or a loose/medium bunch density for Grillo and Moscato Cerletti. Zibibbo had the highest seed weight per berry due to its higher number of seeds, while Grillo and Moscato Cerletti were intermediate – between Catarratto and Zibibbo. Grillo and Moscato Cerletti had the highest single seed weight. All four cultivars were characterised by medium cane vigour.

Grillo had the highest TSS content, while Moscato Cerletti came between Catarratto Comune and Zibibbo with a moderate TSS content. A higher variation between cultivars can be observed in terms of titratable acidity; the coefficient of variation was 22.4 % for this trait compared to

11.6 % for TSS. Catarratto Comune had the highest acidity and the lowest pH, followed by Grillo.

Varietal free volatile compounds (Table 3) were detected in Zibibbo and Moscato Cerletti only, which is to be expected as they are classified as aromatic varieties. Twenty free volatile compounds were identified, among them 17 monoterpenes, the most abundant class, and 3 benzenoids. Monoterpenes were almost four times more abundant in Zibibbo than in Moscato Cerletti, also showing a different aromatic profile: in Zibibbo the main monoterpenes were linalool, 2,6-dimethyl-3,7-octadiene-2,6-diol, geranic acid, geraniol e trans-piran linalool ox.; in Moscato Cerletti they were geranic acid, linalool, 2,6-dimethyl-3,7-octadiene-2,6-diol and geraniol. Benzenoids were five times higher in Zibibbo than in Moscato Cerletti, 2-phenylethanol and benzyl alcohol being the most abundant in both cultivars.

**TABLE 3.** Aromas of grapes in mg/kg: free volatile compounds in Zibibbo and Moscato Cerletti.

Cultivar	Zibibbo	Moscato Cerletti		
Traits	average	average	sign p	p value
Benzenoids				
Benzyl Alcohol	20.4	6.3	**	0.0079
2-Phenylethanol	144.8	25.5	**	0.0003
Eugenol	5.1	2.8	**	0.0100
Monoterpenes				
Trans furanlinalool OX	31.1	8.7	*	0.0136
Cis furanlinalool OX	70.2	112.1	**	0.0077
Linalool	940.7	174.3	**	0.0012
HoTrienol	54.2	14.7	**	0.0002
$\alpha$ -Terpineol	48.0	6.0	**	0.0000
Trans piran Linalool ox	388.3	40.4	**	0.0001
Cis piran Linalool ox	172.9	17.3	**	0.0000
Citronellol	5.9	25.1	*	0.0137
Nerol	72.7	18.1	**	0.0000
Geraniol	541.1	129.5	**	0.0002
2,6-Dimethyl-3,7-octadiene-2,6-diol	818.1	144.2	**	0.0001
2,6-Dimethyl-7-octene-2,6-diol	10.2	2.2	*	0.0192
OH-Citronellol	6.8	5.3	ns	
Trans 8-OH Linalool	64.4	23.5	*	0.0338
OH Geraniol	41.4	23.5	ns	
Cis 8-OH Linalool	38.8	9.8	**	0.0000
Geranic acid	466.1	223.9	**	0.0035

\*, \*\* indicate statistically significant differences at  $p < 0.01$  and  $p < 0.05$  respectively between cultivars according to Tukey's HSD test. ns = not significant



**TABLE 4.** Aromas of grape: glycosylated volatile compounds in the four investigated cultivars.

Cultivar	Catarratto Comune		Zibibbo		Grillo		Moscato Cerletti	
Traits	average		average		average		average	
Benzenoids								
Benzaldehyde	22.3	ns	2.1		1.0		2.0	
Methyl Salicylate	43.6	ns	24.3		4.2		5.7	
Benzyl Alcohol	792.0	A	84.1	B	67.1	B	76.6	B
2-Phenylethanol	267.5	A	121.6	B	51.4	B	197.3	A
Eugenol	69.1	A	9.1	B	10.3	B	12.6	B
Isoeugenol	8.1	a	1.0	c	1.3	c	3.2	b
Vanillin	7.3	a	7.0	a	4.5	ab	3.1	b
Zingerone	10.5	A	0.7	B	2.2	B	0.8	B
Homovanillic Alcohol	116.3	A	82.5	A	2.8	B	14.8	B
Dihydroconiferyl Alcohol	37.8	b	111.8	a	84.1	a	100.3	a
Norisoprenoids								
3,4-Dihydro-3-oxo- $\alpha$ -ionol (I)	9.3	B	19.8	A	5.1	B	33.2	A
3,4-dihydro-3-oxo- $\alpha$ -ionol (II)	13.4	B	40.9	A	6.5	B	42.4	A
3-OH-b-Damascone	26.8	b	18.3	c	48.6	A	24.3	BC
3-oxo- $\alpha$ -ionol	213.1	a	134.5	b	54.7	C	112.5	bc
Blumenol C	3.1	B	15.2	A	1.7	B	20.5	A
3,9-Dihydroxy-Megastigma-5-Ene	2.1	C	13.9	B	10.1	B	23.7	A
3-OH- $\beta$ -ionone	35.8	A	14.6	B	19.3	B	13.3	C
Vomifoliol	141.4	b	173.9	b	51.8	c	223.5	a
Monoterpenes								
Trans Furan Linalool Ox	4.2	C	319.1	A	0.7	C	74.5	B
Cis Furan Linalool Ox	5.4	C	75.6	B	1.5	C	114.7	A
Linalool	2.3	C	1785.7	A	1.4	C	649.4	B
HoTrienol	n.d.	B	81.4	A	n.d.	B	2.6	B
Neral	n.d.	B	18.3	A	0.6	B	19.0	A
$\alpha$ -Terpineol	12.4	B	91.8	A	6.1	B	104.6	A
Geranial	1.0	C	48.9	A	2.4	C	27.8	B
Trans Pyran Linalool Oxide	1.6	C	105.9	B	0.5	C	155.1	A
Cis Pyran Linalool Oxide	6.6	C	38.7	A	4.8	C	17.3	B
Citronellol	n.d.	C	27.8	B	0.7	C	105.5	A
Nerol	9.4	B	647.6	A	1.3	B	553.3	A
Geraniol	50.5	c	1154.1	a	15.7	c	833.8	b
2,6-Dimethyl-3,7-octadiene-2,6-diol	1.5	c	743.8	a	0.6	c	149.3	b
Endiol	2.4	C	82.7	A	1.9	C	58.0	B
2,6-Dimethyl-7-octene-2,6-diol	n.d.	C	54.4	A	3.7	C	35.3	B
OH-Citronellol	7.3	B	26.3	A	0.2	C	11.0	B
8-OH-Dihydrolinalool	8.8	C	140.4	B	0.7	C	264.3	A
OH-Nerol	n.d.	C	23.6	A	n.d.	C	12.3	B
Trans-8-OH-Linalool	13.4	B	176.0	A	5.2	B	160.2	A
Cis-8-OH-Linalool	8.8	B	120.9	A	16.7	B	114.4	A
OH-Geraniol	11.3	B	168.1	A	12.0	B	17.9	B
Geranic acid	19.4	B	905.7	A	9.3	B	804.5	A
p-Menth-1-ene-7,8-Diol	71.5	A	38.7	B	32.0	B	32.2	B
8-OH-Nerol	n.d.	B	29.2	A	2.5	B	29.0	A
8-OH-Geraniol	n.d.	C	128.4	A	3.2	C	24.8	B

Upper, lowercase and different letters within row indicate statistically significant differences at  $p < 0.01$  and  $p < 0.05$  respectively, among cultivars according to Tukey's HSD test. ns = not significant

In all four cultivars, forty-three varietal glycosylated volatile compounds were identified and quantified (10 benzenoids, 8 norisoprenoids and 25 monoterpenes; Table 4). Catarratto Comune and Grillo were classified as non-aromatic due to the absence of aromatic compounds, as shown by the very low amounts of glycosylated monoterpenes. Grillo also showed the lowest amounts of norisoprenoids. Benzenoids were significantly high in Catarratto Comune and intermediate in Zibibbo and Moscato Cerletti, but at trace levels in Grillo (Tables 4 and 5).

Catarratto Comune showed the highest concentrations of benzenoid compounds (benzaldehyde, methyl salicylate, benzyl alcohol, 2-phenylethanol and eugenol), monoterpenes

(p-menth-1-ene-7,8-diol), and norisoprenoids (3-oxo-a-ionol and 3-OH-b-ionone). Grillo displayed the highest quantities of 3-OH-b-damascone, which were considerably higher than Zibibbo and Moscato Cerletti in particular. In terms of monoterpene compounds, Zibibbo was high in linalool, geraniol, geranic acid, 2,6-dimethyl-3,7-octadiene-2,6-diol and 8-OH geraniol, while Moscato Cerletti was high in geraniol, geranic acid, linalool and nerol (Table 4).

While Grillo was mostly poor in glycosylated volatile compounds, Zibibbo was particularly rich, mainly in total monoterpenes (Table 5). Moscato Cerletti also showed high monoterpene values, even if they were lower than Zibibbo's.

**TABLE 5.** Aromas of grape in mg/kg: total glycosylated volatile compounds (benzenoids, norisoprenoids and monoterpenes) in the four investigated cultivars.

Cultivar	Catarratto Comune		Zibibbo		Grillo		Moscato Cerletti		
Traits	average		average		average		average		p value
Benzenoids	1374.4	A	444.4	B	228.9	B	416.4	B	0.0000
Norisoprenoids	445.0	A	431.1	A	197.9	B	493.4	A	0.0030
Monoterpenes	237.9	C	7033.0	A	123.8	C	4370.9	B	0.0000

Uppercase and different letters within row indicate statistically significant differences at  $p < 0.01$  among cultivars according to Tukey's HSD test

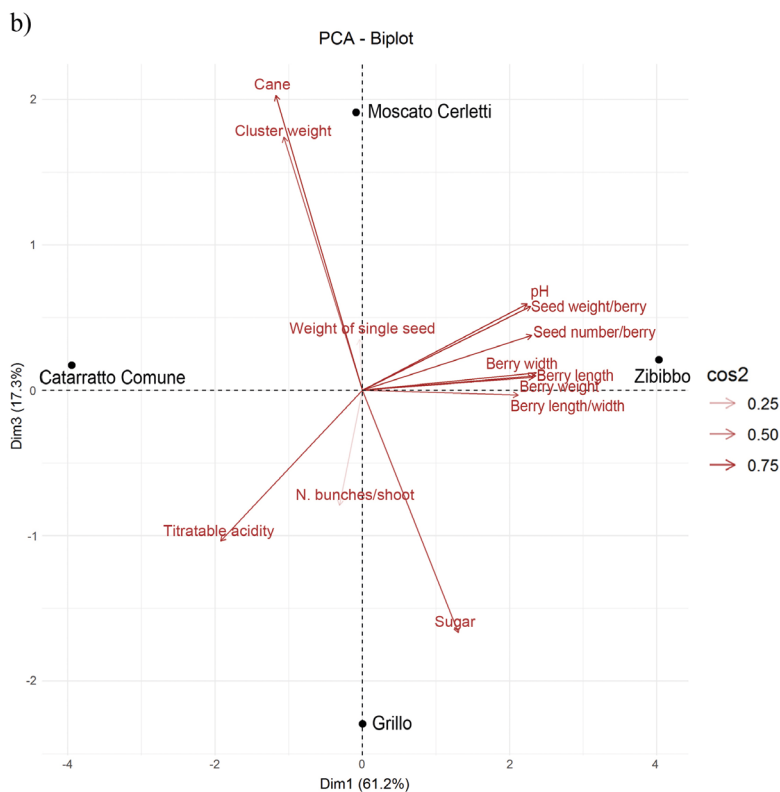
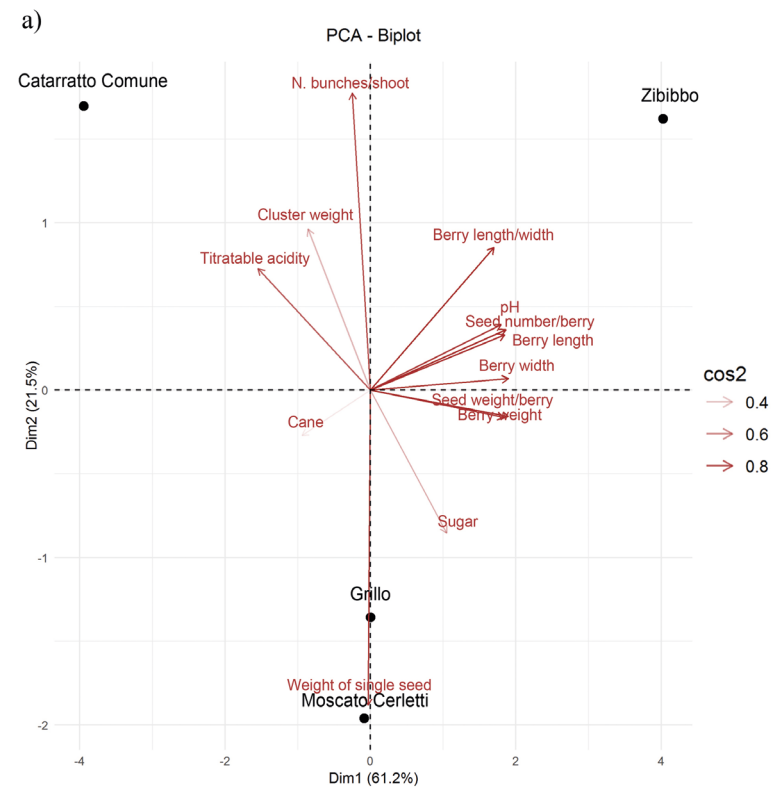
### 3. Principal Component Analysis (PCA)

A PCA was carried out to assess the traits useful for cultivar discrimination by extracting the main orthogonal variables which explain the divergence among samples (Figures 2a, 2b). The first PCA distinguished Catarratto Comune and Zibibbo, while the second differentiated Catarratto Comune and Zibibbo from Grillo and Moscato Cerletti, the latter two being between Catarratto Comune and Zibibbo. The single seed weight and number of bunches/shoots weighed the most on Dim1, while the seed weight/berry, and berry width and weight mostly contributed to the variability explained by Dim2 (Figure 2a). Moscato Cerletti and Grillo were distinguished by the third component (Figure 2b), where sugar and cluster weight weighed the most were the most weighing and represented 17.29 % of total variance.

Nearly 83 % of total variance was explained by the first two PCAs, with berry length/width, pH, seed number/berry, berry length, berry width, seed weight/berry and berry weight being the main variables ( $\cos^2 \geq 0.9$ ). This evidence is in

agreement with the contributing values for each variable at the three dimensions of principal components (Figure S2), as well as with the correlation analysis (Figure S3). The Pearson correlations between variables were highly significant ( $p < 0.05$ ): cluster weight and cane were highly and negatively correlated with sugar; berry weight was highly and positively correlated with number of seeds/berries and pH, and highly and negatively correlated with titratable acidity; seed weight and number were highly and negatively correlated with titratable acidity; berry weight was highly and positively correlated with berry length and width (Figure S3).

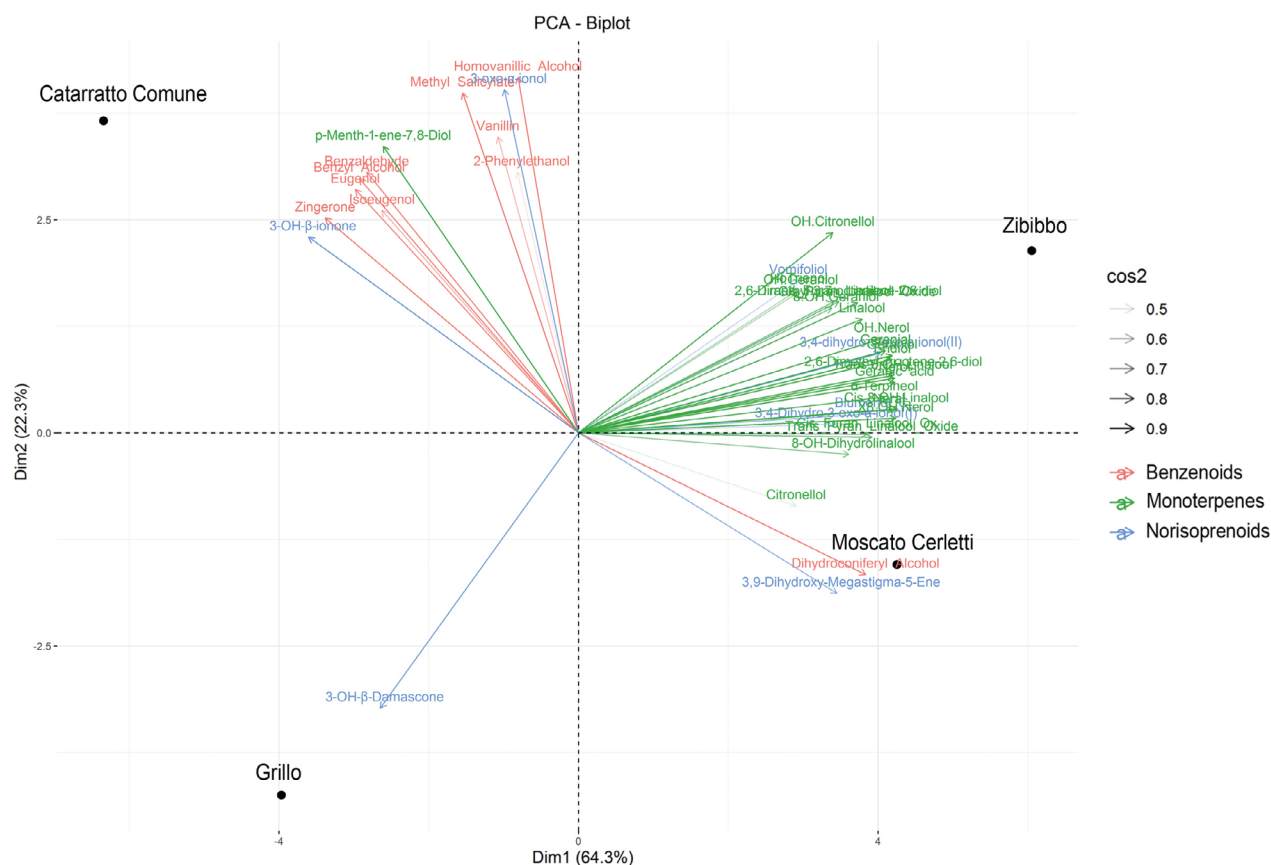
The PCA allowed the cultivars to be differentiated in terms of glycosylated volatile compounds (Figure 3). Sixty-four and 22 % of total variance was explained by the first and the second PCAs respectively. The first PCA differentiated Catarratto Comune and Grillo from Zibibbo and Moscato Cerletti, in agreement with the latter's aromatic profile, and with their typically high content in glycosylated monoterpenes and norisoprenoids.



**FIGURE 2.** a) First two principal components extracted by PCA developed using yield parameters, pruning mass and grape quality traits recorded for the studied cultivars. b) Third and first principal components extracted by PCA developed using yield parameters, pruning mass and grape quality traits recorded for the studied cultivars.

a) Traits associated with cultivar discrimination are indicated in the plot, along with their significance values ( $0.4 < \cos^2 < 0.8$ );

b) Traits associated with cultivar discrimination were indicated in the plot, along with their significance values ( $0.4 < \cos^2 < 0.8$ ).



**FIGURE 3.** First two principal components extracted by PCA developed using glycosylated volatile compounds of grapes recorded for cultivars studied.

Traits associated to cultivar discrimination were indicated in the plot, underlining their significance values ( $0.4 < \cos^2 < 0.8$ ).

In contrast, a lower content of these compounds was observed in Catarratto Comune and Grillo. In the second component, Catarratto Comune is well-differentiated, especially in terms of its high benzenoid content, which contrasts with the low values observed for Grillo (Figure 3).

#### 4. Genotyping with nuclear and chloroplast SSR markers

Thirteen nuclear SSR loci were adopted for grapevine cultivar identification and the parentage analysis. The four different profiles of each cultivar, which were confirmed in the comparison with public databases, are reported in Table 6.

The molecular profiles of Catarratto Comune, Zibibbo and Grillo are available in many public databases. The SSR profile of Moscato Cerletti was compared with its accession grown in the repository of CREA Viticulture and Enology in Susegana (Treviso, Italy). Our data confirmed previous results of studies on the Grillo pedigree, Grillo being one of the rare

cultivars with known parents and grandparents (Di Vecchi Staraz *et al.*, 2007; Cipriani *et al.*, 2010; Lacombe *et al.*, 2013).

Unfortunately, the results of the SSR analyses confirmed only one of the two cultivars claimed by Mendola to be the parents of Moscato Cerletti: Zibibbo. The high number (5) of mismatching SSR loci excluded the cultivar Catarratto as the second parent, and the research into Moscato Cerletti's other parent using VIVC and CREA molecular databases was also unsuccessful.

In addition, the cpSSR analysis showed that Catarratto, Grillo and Moscato Cerletti shared the same chlorotype (D), while Zibibbo had chlorotype B. Taking into account the results of both SSR analyses, Catarratto could be the female parent of Grillo, while Zibibbo may be the male parent of both cultivars (Grillo and Moscato Cerletti), as expected for the maternal inheritance of plastids in grapevine (Arroyo-García *et al.*, 2002). Further analysis is required to identify the female parent of Moscato Cerletti.

**TABLE 6.** Nuclear and chloroplast SSR profiles of the four cultivars studied. The fragment lengths are in bp.

Markers	Type	Catarratto Comune	Zibibbo	Grillo	Moscato Cerletti
VVS2	nSSR	143	133	143	133
		151	149	149	149
VVMD5	nSSR	225	227	225	225
		225	231	227	231
VVMD7	nSSR	239	249	249	239
		249	251	249	251
VVMD27	nSSR	179	179	179	179
		179	194	194	194
VrZAG62	nSSR	200	186	186	186
		202	204	202	202
VrZAG79	nSSR	251	247	247	249
		251	255	251	255
VVMD25	nSSR	259	250	250	240
		259	250	259	250
VVMD28	nSSR	231	247	239	239
		239	271	247	247
VVMD32	nSSR	251	265	253	265
		253	273	273	273
ISV2	nSSR	165	141	143	143
		169	143	169	165
ISV3	nSSR	139	133	133	133
		145	139	145	139
ISV4	nSSR	177	169	191	177
		191	195	195	195
VMCNG4B9	nSSR	150	158	150	158
		176	158	158	172
ccmp3	cpSSR	107	106	107	107
ccmp4	cpSSR	128	128	128	128
ccmp5	cpSSR	104	105	104	104
ccmp6	cpSSR	107	107	107	107
ccmp10	cpSSR	115	115	115	115
ccSSR23	cpSSR	281	281	281	281
Chlorotypes	-	D	B	D	D

## DISCUSSION

Moscato Cerletti, acquired from the INRAE Montpellier collection, showed ampelographic traits that are in agreement with those previously reported (Mendola, 1874; Molon, 1906; Sannino, 1920; Longo, 1948). The comparison with the SSR profile of another Moscato Cerletti accession, available from the CREA research Center for

Viticulture and Enology of Conegliano (Treviso, Italy), confirmed the identity of the genotype obtained from France. Therefore, it is possible to conclude that the Moscato Cerletti used in the study was true to type. Taking into consideration the historical documents, molecular analyses, ampelographic, phenological and grape quality traits assessment, it can be concluded that Grillo and Moscato Cerletti are clearly distinct cultivars.

Mendola (1874) reported his first grape seed plantations in 1860; afterwards, regular and accurate observations were made of all seedlings produced from the selfing of different grapes and the artificial hybridisation of cultivars. Moscato Cerletti was derived from a cross performed in 1869; Grillo is reported as being extensively cultivated in the area of Marsala only five years later, thus it is clear that Grillo was obtained before the cross that produced Moscato Cerletti.

Intriguingly, the parents that are at the origin of Grillo are the same that Mendola claims he used to obtain Moscato Cerletti, which is also the case for the female role putatively played by Catarratto in both crosses. It is worth noting that the time gap between the appearance and successful spreading of Grillo and the breeding activities of Mendola is very short, thus it is unlikely that Grillo derives from Mendola's breeding activity.

The SSR profiles of the trio genotypes excluded the possibility of Catarratto being the female parent of Moscato Cerletti, but points to Zibibbo as the male one, which is further corroborated by the results of the cpSSR analysis. Mendola's writings suggest that the Moscato Cerletti seedling was noticed among many others produced by the putative cross of Catarratto x Zibibbo, because it stood out for its vigour and leaf colour and hairs. In contrast, our data indicates that Moscato Cerletti seedlings could have ended up there from another group of seeds by accident or mistake; the discovery of the complementary parent would help to understand what really happened.

Therefore, due to the valuable traits that it is praised for, a preliminary evaluation of the oenological potential of Moscato Cerletti was carried out. Moscato Cerletti grapes showed lower titratable acidity than Grillo, but both cultivars shared similar components of grape yield, except for the bunches, which were tighter and heavier from Moscato Cerletti. In the past, Zibibbo, Grillo and Moscato Cerletti were classified as table grapes; currently, Grillo is solely and extensively used as a wine grape, while Moscato Cerletti is not yet in cultivation. It is hoped that this first report will promote the exploitation of Moscato Cerletti for its valuable features.

Finally, Moscato Cerletti is an aromatic cultivar, as is its male parent, Zibibbo; indeed, it was characterised by twenty free volatile compounds (17 monoterpenes and 3 benzenoids), even if in lower concentrations than Zibibbo.

Furthermore, Moscato Cerletti was found to be rich in norisoprenoids, except for 3-OH-b-Damascone and 3-OH-b-ionone, as well as in monoterpenes, like geraniol, geranic acid, linalool and nerol. This aromatic profile further supports the possibility of a first-degree relationship with Zibibbo, which is also confirmed as being conceivable by historical documents and SSR data.

The characterisation of Moscato Cerletti in the present study is in agreement with descriptions published by different authors (Mendola, 1874; Sannino, 1920; Longo, 1948), except in terms of its ability to produce high levels of alcohol for Marsala's wines: Moscato Cerletti showed lower sugar levels, perhaps due to the vineyard management adopted during the first decade of the 20th century differing to the current ones. In the past, traditional management in dry conditions utilised the bush system, with spur pruning, low buds per vines and 10,000 or more vines/ha planting density (Pastena, 1989). In contrast, vineyards are nowadays trained to a "vertical shoot position trellis" system with Guyot pruning and a much lower planting density, as was adopted in this experimental trial.

The late harvest date recorded for Moscato Cerletti was probably related to its low ability to accumulate sugars, especially when compared to Grillo. Late ripening could be considered a favourable trait for adaptation to a changing climate, mainly in warm and dry environments, contributing to maintaining the freshness and aromatic complexity of the wines (van Leeuwen *et al.*, 2019).

In future scenarios, the quality of wine produced in warm and dry areas (Mediterranean countries, California and Australia) will be seriously endangered by climate change, as a result of reduced rainfall, increased temperatures and extreme events, such as heatwaves (IPCC, 2014).

It is believed that climate change (Santos *et al.*, 2020) is causing, and, to a larger extent, will cause in the future, the degradation of grape organic acids, an increase in sugar content and a loss of secondary metabolites, more precisely of grape phenolic and volatile compounds.

Many varieties from southern Europe will therefore no longer be adapted to warm and dry climates, and new strategies for vineyard management and oenological processes will need to be applied in the face of climate change (Scafidi *et al.*, 2013; Palliotti *et al.*, 2017; Alfonzo *et al.*, 2020; Frioni *et al.*, 2020b).

It is predicted that the varieties cultivated in the Mediterranean Basin will shift to the northern European viticultural areas, while in the warmer wine regions later ripening varieties will be planted (Van Leewen *et al.*, 2019). Hence, the reintroduction of autochthonous relict cultivars to their “terroir” is a potential strategy for preventing a reduction in wine quality (Frioni *et al.*, 2020a). Furthermore, in the future, such neglected varieties may also be able to thrive in non-traditional areas. Moscato Cerletti is one such variety that could be considered and added to the other cultivars grown in a warm climate area to produce sparkling muscat wines. Recently, warm and dry climate areas of grape cultivation, such as Portugal, Brazil and Australia, as well as some Southern Italian regions (Sicily, Campania and Lazio), have seen an increase in sparkling wine production in response to growing global consumer demand (OIV, 2020). In Sicily, sparkling wine production jumped from 2.414 hl in 2015 to 7.301 hl in 2019, representing a 202 % increase (Salvia, 2020). Nowadays, about 100 sparkling wine labels are produced, of which about 25 % and 75 % rosé and white respectively, obtained from either autochthonous or international cultivars widespread in Sicilian vineyards (Salvia, 2020).

However, there is also a need to introduce late-ripening varieties in other areas, as shown by the increase in global sparkling wine production: as much as +57 % since 2002, according to the International Organization of Vine and Wine (OIV) in 2018 and amounting to as much as 20 million hl.

## CONCLUSION

Moscato Cerletti, recovered in France, is true to type and distinct from Grillo. Grillo and Moscato Cerletti showed different ripening times and sugar storage capacities; furthermore, Moscato Cerletti is an aromatic cultivar, as is its male parent, Zibibbo. In contrast, Grillo is a non-aromatic cultivar, as is its female parent, Catarratto Comune (Di Vecchi Staraz *et al.*, 2007; Cipriani *et al.*, 2010; Lacombe *et al.*, 2013). The claim that the female parent of Moscato Cerletti is the cultivar Catarratto Comune, is not supported by the results of the molecular analysis and remains unknown.

Today, Moscato Cerletti should be enrolled in the Italian Catalogue and exploited for its favourable adaptation to a changing climate and its quality grape traits in order to produce wines, such as sparkling muscat wines. Hence, Moscato Cerletti should be considered and added to the other

cultivars grown in warm and dry environments, as well as in other winegrowing regions.

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