

# First characterisation of minor and neglected *Vitis vinifera* L. cultivars from Mount Etna

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## Abstract

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Eight minor and neglected cultivars of *Vitis vinifera* L. were characterised according to their ampelographic and agronomic traits and discriminated by molecular analysis with SSR markers. These results are the first complete ampelographic description of these minor and neglected cultivars from the Mount Etna region. The results of this study reveal high morphological diversity of ancient grapevines growing in this region. SSR markers enabled us to discriminate the cultivars and revealed the genetic divergence between them and several autochthonous cultivars. Our efforts could contribute to a better knowledge of grape biodiversity based on morphological and molecular data and could be useful for the development of a reliable germplasm conservation strategy.

**Keywords:** Sicily; grapevine; biometric; SSR; identification

Grape cultivation in the Mount Etna region has extremely ancient origins. Archaeological findings (5<sup>th</sup> century BC) and the writings of Teocrito (3<sup>rd</sup> century BC) suggest that vineyards have been cultivated there for centuries (PÀSTENA 1989). As evidenced by GEREMIA (1834) and MOLON (1906), the grapevine germplasm of the Etna volcano was in the past very rich. Those authors estimated that the number of autochthonous cultivars was 50. Many of these cultivars are likely extinct, while others are still scattered throughout old vineyards. Moreover, the use of local names increases the uncertainty regarding the exact identification since homonyms or synonyms may occur. In this area, some minor and neglected cultivars still survive, even if subjected to genetic erosion.

Historical evidence and information, combined with morphological data (ampelography and am-

pelometry), are frequently used to characterise cultivars and to define relationships between them. Moreover, morphological data is an important tool that can be useful for the identification of genetic resources (THOMAS et al. 1993). Ampelography was also used to clarify the use of synonyms and homonyms (CRESPAN, MILANI 2001; SANTIAGO et al. 2007; SABIR et al. 2009). However, this technique has practical limitations, including the age and state of the plant, the presence of plant diseases, researcher subjectivity and, above all, environmental effects. In the last decades, molecular techniques were also employed to unequivocally discriminate varieties and clones by means of different molecular markers (BOURQUIN et al. 1993; BOWERS et al. 1996; YE et al. 1998; SEFC et al. 1999; ERGUL et al. 2002). Among these, microsatellites (SSR) are reliable and informative markers.

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In grape fingerprinting and genetic analyses, SSRs facilitate cultivar identification, pedigree analysis, population studies and genome mapping (SEFC et al. 2000). Since these markers are inherited in a co-dominant Mendelian manner, their analysis allows the reconstruction of pedigree. Furthermore, the high heterozygosity of the grapevine genome (69–88 %) according to THOMAS and SCOTT (1993) contributes to an increase in the number of possible allelic combinations at any SSR locus, thus increasing their discriminating power.

The aim of this research was to characterise by ampelographic, agronomical and molecular traits, eight minor and neglected autochthonous cultivars found in the Mount Etna region. These cultivars are described for the first time and are evaluated here-in for their potential use in oenological production in the Mediterranean basin.

## MATERIAL AND METHODS

**Plant material and field description.** Eight cultivars identified by growers as Terribile, Bianchetta, Madama Nera, Madama Bianca, Virdisi, Barbarossa Etna, Zzinèuro and Vispara Etna, were considered for characterisation. The cultivars were propagated and grafted onto 140 Ruggeri rootstocks and were cultivated from 2005 onwards in the experimental field of Catania University, Catania, Italy.

**Ampelographic description.** Ampelographic data were collected during 2012, 2013 and 2014 according to the protocols of the Organisation Internationale de la Vigne et du Vin (OIV 1983), as modified by EU project Genres 081 (1997). Thirty-two morphological descriptors, relative to shoots, young shoots, young leaves, mature leaves, inflorescence, bunches, berries and seeds were used (Table 1). Ten readings per each descriptor were taken on 20 plants (200 readings).

**Crop yield and analysis of berry quality.** The yield of each cultivar was harvested at maturity. For yield assessment, the number of clusters per vine was counted and then individually weighed to determine total yield per vine (kg/vine). In the laboratory, a sample of 20 clusters per genotype was used to determine the length, the peduncle length, the weight, width and length of the berry and the weight of seeds and skins. Data on the main phenological phases were also recorded in the field (Biologische Bundesanstalt, Bundessortenamt and Chemical in-

dustry – BBCH: inflorescence 57) (MEIER 2001). The must was analysed on a 100-berry subsample per cultivar. Total soluble solids (TSS) were measured using a digital refractometer (RX-5000 Atago Co., Ltd., USA) with temperature correction; titratable acidity (TA) and pH were measured using an automatic titrator (Titrimo model 798, Metrohm, Riverview, USA); TA was measured using a 5.0-ml aliquot of juice, titrating against 0.1 N NaOH up to pH 8.2 and was expressed as g/100 ml of tartaric acid equivalents.

**SSR analysis.** The molecular analysis included the eight studied minor and neglected cultivars. In order to exclude cases of synonymy and homonymy, 20 supplementary cultivars commonly found in Sicily ('Grillo', 'Zibibbo', 'Carricante', 'Nocera', 'Nerello Cappuccio', 'Alicante', 'Nerello Mascalese', 'Nero d'Avola', 'Frappato', 'Perricone', 'Grecanico', 'Catarratto', 'Inzolia', 'Damaschino', 'Malvasia di Lipari', 'Moscato di Noto', 'Vispara A', 'Vispara B', 'Visparola' and 'Barbarossa'), were also included in the analysis. DNA was extracted from 100 mg of fresh young leaf tissue with the Isolate Plant DNA Mini Kit (Bioline, London, UK).

The following 11 SSR loci labelled with Ned, Pet, Vic and 6-Fam dyes (Applied Biosystems, Foster City, USA) were selected for the analysis: VVMD5 (BOWERS et al. 1996), VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, (BOWERS et al. 1999), VVS2 (THOMAS, SCOTT 1993), VrZAG62 and VrZAG79 (SEFC et al. 1999) (Table 2). PCR amplifications were carried out as reported by DE LORENZIS et al. (2013).

The amplification products were separated in an automatic capillary sequencer ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA). A Liz-500 size standard (Applied Biosystems, USA) was used to estimate the approximate molecular weight of each fragment. The Genescan 3.1.2 software program (Applied Biosystems, USA) was used to analyse the samples. All of the samples were analysed with the same methodology and under the same conditions to facilitate comparison of the results.

**Statistical analysis.** The morphological values obtained over 3 years were reported as modal values. The genetic distance was calculated from SSR data using the simple matching dissimilarity index. From the dissimilarity matrix obtained, a weighted Neighbour-Joining tree (SAITOU, NEI 1987) was computed using the dissimilarity analysis and representation for Windows (DARwin5) software

Table 1. List of the morphological descriptors investigated in the study during 2012, 2013 and 2014 according to the protocols of the *Organisation Internationale de la Vigne et du Vin* (OIV 1983), as modified by EU project GENRES 081 (1997), relative to shoots, young shoots, young leaves, mature leaves, inflorescence, bunches, berries and seeds, according to their discriminating ability

| OIV code | Vine part     | Description of the character                |
|----------|---------------|---|
| OIV 001  | young shoot   | form of tip                                 |
| OIV 003  | young shoot   | intensity of anthocyanin colouration of tip |
| OIV 004  | young shoot   | anthocyanin colouration of tip              |
| OIV 005  | young shoot   | density of erect hairs on tip               |
| OIV 006  | shoot         | attitude (habit)                            |
| OIV 007  | shoot         | colour of dorsal side of internode          |
| OIV 008  | shoot         | colour of ventral side of internode         |
| OIV 011  | shoot         | density of erect hairs on node              |
| OIV 016  | shoot         | number of consecutive tendrils              |
| OIV 017  | shoot         | length of tendril                           |
| OIV 051  | young leaf    | colour of upper surface                     |
| OIV 053  | young leaf    | density of prostrate hairs between veins    |
| OIV 054  | young leaf    | density of erect hairs between veins        |
| OIV 065  | mature leaf   | size of blade                               |
| OIV 067  | mature leaf   | shape of blade                              |
| OIV 068  | mature leaf   | number of lobes                             |
| OIV 076  | mature leaf   | shape of teeth                              |
| OIV 079  | mature leaf   | general shape of petiole sinus              |
| OIV 081  | mature leaf   | tooth at petiole sinus                      |
| OIV 084  | mature leaf   | density of prostrate hairs between veins    |
| OIV 085  | mature leaf   | density of erect hairs between veins        |
| OIV 151  | inflorescence | sex of flower                               |
| OIV 204  | bunch         | density                                     |
| OIV 206  | bunch         | length of peduncle                          |
| OIV 502  | bunch         | single bunch weight                         |
| OIV 223  | berry         | shape                                       |
| OIV 225  | berry         | skin colour (without bloom)                 |
| OIV 238  | berry         | pedicel length                              |
| OIV 241  | berry         | presence of seeds                           |
| OIV 503  | berry         | single berry weight                         |
| OIV 243  | seed          | 100-seed weight                             |
| OIV 244  | seed          | transversal ridges on side                  |

version 5.0 (PERRIERAND, JACQUEMOUD-COLLET 2006), and the robustness of branches was tested using 1,000 bootstraps.

Several genetic parameters were determined by PowerMarker (LI, MUSE 2005): major allele frequency, number of genotypes detected, number of alleles per locus, observed heterozygosity ( $H_o$ ), cal-

culated as the number of heterozygotes at a given locus divided by number of individuals typed) and polymorphism information content ( $PIC = 1 - \sum gi^2$ , where  $gi$  is the frequency of the  $i^{\text{th}}$  allele carried by the population) were calculated for each locus in order to estimate the level of genetic diversity (BOTSTEIN et al. 1980).

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Table 2. Sequences and references for SSR primers chosen according to their ability to distinguish closely related cultivars

| Name    | Forward                            | Reverse                       | References            |
|---------|------------------------------------|-------------------------------|-----------------------|
| VVMD5   | NED - CTAGAGCTACGCCAATCCAA         | TATACCAAAAATCATATTCCTAAA      | BOWERS et al. 1996    |
| VVMD7   | VIC - AGAGTTGCGGAGAACAGGAT         | CGAACCTTCACACGCTTGAT          | BOWERS et al. 1999    |
| VVMD21  | NED - GGTTGTCTATGGAGTTGATGTTGC     | GCTTCAGTAAAAAGGGATTGCG        | BOWERS et al. 1999    |
| VVMD24  | VIC - GTGGATGATGGAGTAGTCACGC       | GATTTTAGGTTTCATGTTGGTGAAGG    | BOWERS et al. 1999    |
| VVMD25  | VIC - TTCCGTTAAAGCAAAAAGAAAAAGG    | TTGGATTTGAAATTTATTGAGGGG      | BOWERS et al. 1999    |
| VVMD27  | VIC - GTACCAGATCTGAATACATCCGTAAGT  | ACGGGTATAGAGCAAACGGTGT        | BOWERS et al. 1999    |
| VVMD28  | PET- AACAAATTCATGAAAAGAGAGAGA-GAGA | TCATCAATTTTCGTATCTCTATTT-GCTG | BOWERS et al. 1999    |
| VVMD32  | PET - TATGATTTTTTAGGGGGGTGAGG      | GGAAAGATGGGATGACTCGC          | BOWERS et al. 1999    |
| VVS2    | 6-FAM - CAGCCCGTAAATGTATCCATC      | AAATTCAAAAATTCTAATTCAACTGG    | THOMAS and SCOTT 1993 |
| VrZAG62 | 6-FAM - GGTGAAATGGGCACCGAACACACGC  | CCATGTCTCTCCTCAGCTTCTCAGC     | SEFC et al. 1999      |
| VrZAG79 | 6-FAM - AGATTGTGGAGGAGGGAACAAACCG  | TGCCCCCATTTTCAAACCTCCCTTCC    | SEFC et al. 2000      |

## RESULTS AND DISCUSSION

### Morphological and agronomical characterisation

Sicily has one of the oldest traditions of viticulture from all of the Italian regions is a centre of genetic diversity. In this work, the biodiversity of some ancient grapevines cultivated in the Mount Etna region was investigated and confirmed by SSR molecular markers. The eight cultivars showed several differences for most of the morphological descriptors studied (Table 3). Only six of the 32 studied descriptors did not show differences in the analysed cultivars with no significant variations observed over the 3 years (data not shown). The characters ‘form of tip in young shoots’ and ‘number of consecutive tendrils on the shoot’ confirmed that the studied cultivars belonged to *Vitis vinifera* L. and excluded the possibility of any American origin. Other previously studied cultivars within the Mount Etna region were reported as hybrids with American species from the post-phylloxera age (ALLEWELDT 1997). Some ampelographic characters, such as general shape of petiole sinus, are under strong genetic control, and are not influenced

by the environment; therefore, differences in these characters indicate differences in genotype (ZULINI et al. 2005). Therefore, ampelographic description has an important role in supporting the genetic analysis.

The yield per vine was always < 3.1 kg/vine except for ‘Barbarossa Etna’. This cultivar, together with ‘Vispara Etna’, showed longer clusters, whereas ‘Bianchetta’ and ‘Virdisi’ had the highest cluster weight. Barbarossa also had the highest berry weight (Table 4).

Concerning the phenological phases, the cultivars ripened from the third ten days of August (‘Vispara Etna’) to the third ten days of September (‘Barbarossa Etna’, ‘Madama Nera’, ‘Zzineuro’) and the first ten days of October (‘Terribile’) (Table 5).

The must composition of all the cultivars exhibited medium-high TSS (°Brix) values that ranged from 18.4 to 22.5. ‘Terribile’ and ‘Zzineuro’ reached the highest values. ‘Titratable’ acidity ranged from 3.6 to 10.0 g/l, and pH ranged from 3.15 to 3.65 (Table 6). Therefore, the cultivars have a high potential for wine production. In particular, ‘Terribile’ and ‘Zzineuro’, common in old vineyards, showed a good-quality cluster and a strong skin colour that could contribute to improve the quality and red colour of wines.

Table 3. Values of ampelographic characters relative to young shoots, shoots, young leaves, mature leaves, inflorescence, clusters, berries and seeds, according to the OIV descriptor list for grape varieties (OIV 1983; GENRES-81 1997); values represent the average of modal data recorded in 2012, 2013 and 2014

| OIV code | Barbarossa Etna | Bianchetta | Madama bianca | Madama nera | Terribile | Virdisi | Vispara Etna | Zzinèuro |
|----------|-----------------|------------|---------------|-------------|-----------|---------|--------------|----------|
| OIV 001  | 7               | 7          | 7             | 7           | 7         | 7       | 7            | 7        |
| OIV 003  | 7               | 3          | 5             | 3           | 3         | 7       | 3            | 5        |
| OIV 004  | 3               | 5          | 5             | 7           | 7         | 5       | 7            | 5        |
| OIV 005  | 1               | 1          | 3             | 1           | 1         | 3       | 1            | 3        |
| OIV 006  | 3               | 1          | 1             | 3           | 1         | 1       | 1            | 3        |
| OIV 007  | 2               | 1          | 1             | 2           | 1         | 1       | 1            | 1        |
| OIV 008  | 3               | 1          | 1             | 3           | 1         | 3       | 1            | 1        |
| OIV 011  | 1               | 1          | 1             | 1           | 1         | 1       | 1            | 1        |
| OIV 016  | 1               | 1          | 1             | 1           | 1         | 1       | 1            | 1        |
| OIV 017  | 1               | 1          | 3             | 3           | 3         | 3       | 5            | 7        |
| OIV 051  | 4               | 3          | 2             | 3           | 2         | 4       | 3            | 3        |
| OIV 053  | 1               | 3          | 3             | 3           | 3         | 3       | 5            | 7        |
| OIV 054  | 1               | 3          | 3             | 3           | 5         | 3       | 5            | 5        |
| OIV 065  | 3               | 5          | 5             | 5           | 9         | 7       | 5            | 5        |
| OIV 067  | 3               | 4          | 3             | 3           | 3         | 3       | 3            | 4        |
| OIV 068  | 3               | 2          | 3             | 2           | 4         | 2       | 3            | 2        |
| OIV 076  | 3               | 2          | 3             | 3           | 2         | 2       | 2            | 3        |
| OIV 079  | 3               | 2          | 2             | 4           | 3         | 2       | 2            | 4        |
| OIV 081  | 1               | 1          | 1             | 1           | 1         | 1       | 1            | 1        |
| OIV 084  | 1               | 5          | 7             | 7           | 3         | 5       | 7            | 3        |
| OIV 085  | 1               | 3          | 5             | 5           | 3         | 5       | 5            | 3        |
| OIV 151  | 3               | 3          | 3             | 3           | 3         | 3       | 3            | 3        |
| OIV 204  | 7               | 7          | 5             | 5           | 5         | 7       | 5            | 5        |
| OIV 206  | 1               | 3          | 5             | 3           | 3         | 1       | 5            | 3        |
| OIV 502  | 3               | 3          | 3             | 3           | 3         | 3       | 3            | 3        |
| OIV 223  | 3               | 4          | 2             | 3           | 2         | 4       | 3            | 3        |
| OIV 225  | 5               | 1          | 1             | 5           | 6         | 1       | 1            | 6        |
| OIV 238  | 3               | 3          | 7             | 7           | 5         | 3       | 7            | 5        |
| OIV 241  | 3               | 3          | 3             | 3           | 3         | 3       | 3            | 3        |
| OIV 243  | 3               | 1          | 3             | 3           | 3         | 1       | 1            | 3        |
| OIV 244  | 0               | 0          | 0             | 0           | 0         | 0       | 0            | 0        |
| OIV 503  | 7               | 5          | 3             | 3           | 3         | 5       | 1            | 5        |

### SSR analysis

SSR markers have already been used to solve cases of homonyms and synonyms (FOSSATI et al. 2001) and to fingerprint varieties (ROSSONI et al. 2003). In our research, the SSR analysis identified a high degree of genetic variability among the cultivars. On the whole, the 11 tested SSRs generated

multiple fragments in the studied cultivars. In total, 82 alleles were scored with an average of 7.5 alleles per locus; the number of alleles ranged from a minimum of five alleles (for VVMD25) to a maximum of 11 alleles (for VVMD28) (Table 7).

The observed heterozygosity ranged from 0.643 for primers VVMD7 and VVMD24 to 0.963 for VVMD32 (mean 0.800), while the poly-

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Table 4. Average values of the quantitative parameters measured for vine, bunch, berry and seed (years 2012, 2013 and 2014 ± standard deviation)

| Cultivar        | Yield/vine (kg)         | Cluster length (cm)      | Cluster weight (g)         | Peduncle length (cm)   | Berry weight (g)        | Berry width (mm)        | Pedicle length (mm)    | Seed weight (mg)      |
|-----------------|-------------------------|--------------------------|----------------------------|------------------------|-------------------------|-------------------------|------------------------|-----------------------|
| Barbarossa Etna | 4.6 <sup>a</sup> ± 0.4  | 17.0 <sup>a</sup> ± 1.4  | 121.4 <sup>b</sup> ± 36.2  | 2.3 <sup>c</sup> ± 0.9 | 7.1 <sup>a</sup> ± 0.6  | 21.2 <sup>a</sup> ± 1.0 | 4.1 <sup>b</sup> ± 0.7 | 35.2 <sup>b</sup> ± 3 |
| Bianchetta      | 2.6 <sup>b</sup> ± 0.7  | 14.3 <sup>b</sup> ± 1.9  | 190.5 <sup>a</sup> ± 44.1  | 4.2 <sup>b</sup> ± 0.7 | 4.2 <sup>b</sup> ± 0.4  | 18.2 <sup>b</sup> ± 0.8 | 3.8 <sup>b</sup> ± 0.6 | 43.3 <sup>a</sup> ± 5 |
| Madama bianca   | 2.1 <sup>b</sup> ± 0.6  | 14.1 <sup>b</sup> ± 1.5  | 114.7 <sup>c</sup> ± 42.8  | 6.1 <sup>a</sup> ± 1.3 | 2.3 <sup>bc</sup> ± 0.1 | 15.0 <sup>c</sup> ± 0.4 | 5.3 <sup>a</sup> ± 0.6 | 38.9 <sup>b</sup> ± 3 |
| Madama nera     | 2.9 <sup>ab</sup> ± 0.3 | 15.9 <sup>b</sup> ± 1.5  | 161.5 <sup>ab</sup> ± 63.0 | 5.2 <sup>b</sup> ± 0.8 | 2.5 <sup>bc</sup> ± 0.2 | 14.4 <sup>c</sup> ± 0.7 | 5.6 <sup>a</sup> ± 0.6 | 36.6 <sup>b</sup> ± 3 |
| Terribile       | 2.3 <sup>b</sup> ± 0.5  | 12.7 <sup>b</sup> ± 0.9  | 141.2 <sup>ab</sup> ± 60.6 | 4.5 <sup>b</sup> ± 1.0 | 1.8 <sup>bc</sup> ± 0.3 | 13.1 <sup>c</sup> ± 0.8 | 3.1 <sup>b</sup> ± 0.9 | 27.2 <sup>c</sup> ± 3 |
| Virdisi         | 2.5 <sup>b</sup> ± 0.3  | 16.4 <sup>ab</sup> ± 2.7 | 176.1 <sup>a</sup> ± 86.6  | 2.0 <sup>c</sup> ± 0.6 | 4.0 <sup>b</sup> ± 0.9  | 17.0 <sup>b</sup> ± 0.4 | 3.5 <sup>b</sup> ± 0.7 | 44.4 <sup>a</sup> ± 4 |
| Vispara Etna    | 3.1 <sup>ab</sup> ± 1.1 | 17.5 <sup>a</sup> ± 2.7  | 108.1 <sup>c</sup> ± 43.1  | 6.3 <sup>a</sup> ± 1.2 | 1.0 <sup>c</sup> ± 0.2  | 12.0 <sup>c</sup> ± 0.5 | 5.6 <sup>a</sup> ± 0.8 | 23.6 <sup>c</sup> ± 3 |
| Zzinèuro        | 2.2 <sup>b</sup> ± 0.3  | 16.0 <sup>ab</sup> ± 2.8 | 129.3 <sup>b</sup> ± 39.8  | 4.2 <sup>b</sup> ± 0.9 | 3.1 <sup>bc</sup> ± 0.4 | 16.8 <sup>b</sup> ± 0.9 | 5.1 <sup>a</sup> ± 0.7 | 38.1 <sup>b</sup> ± 5 |

means marked by different letters within a column are significantly different ( $p \leq 0.05$ ) based on Fisher's least significant difference (LSD)

morphic information content (PIC) values ranged from 0.619 for VVMD24 to 0.838 for VVMD28 (mean 0.705). The highest discriminating power was observed in VVMD28, which permitted the discrimination of 18 singular cultivars and displayed the lowest major allele frequency; conversely, VVMD21 and VVMD25 revealed much lower allele numbers, facilitating the detection of only nine different genotypes (Table 6).

The selected markers allowed the discrimination of all of the analysed cultivars. The UPGMA dendrogram (Fig. 1) confirmed the genetic divergence between the studied genotypes and confirmed that the eight cultivars examined in this study are different from the most widespread grape varieties in Sicily. No cases of synonyms were detected in the analysed cultivars. The cultivars that are known in Sicily by the same or similar names (Vispara/Visparola' and Barbarossa Etna/Barbarossa) were shown to be unique in their genetic profiles; all the genotypes of Vispara group were very closely related

among each other, with the exception of 'Vispara A', which showed high similarity with 'Carricante'. Interestingly, the genotypes belonging to the same group of 'Vispara Etna' are characterised by higher levels of soluble solids, a thin skin and early ripening period. SSR analysis of this group showed that the four genotypes, despite their phenotypic similarity, were genetically different, suggesting that their common name is likely due to their remarkable phenotypic similarities. According to SSR analysis, 'Barbarossa' from Etna differs from 'Barbarossa' found in the west of Sicily; several varieties with this name are described in the Italian National Register and it would be interesting to compare the local variety with all the others and with the 'French Barbarouz Provençal' in order to ascertain any common origin.

As for 'Bianchetta', this name refers to the light skin colour of the berries. The Italian National Register reports a 'Bianchetta Trevigiana' from the Veneto region and it could be interesting to deter-

Table 5. Main phenological phases of the eight minor and neglected cultivars

|                | Barbarossa Etna | Bianchetta      | Madama bianca   | Madama nera     | Terribile    | Virdisi         | Vispara Etna | Zzinèuro        |
|----------------|-----------------|-----------------|-----------------|-----------------|--------------|-----------------|--------------|-----------------|
| Budbreak       | 21–30 March     | 10–20 March     | 21–30 March     | 21–30 March     | 11–20 April  | 21–30 March     | 11–20 March  | 1–10 April      |
| Flowering      | 11–20 May       | 11–20 May       | 11–20 May       | 11–20 May       | 21–30 May    | 11–20 May       | 1–10 May     | 11–20 May       |
| Veraison       | 21–30 July      | 21–30 July      | 21–30 July      | 21–30 July      | 1–10 August  | 21–30 July      | 11–20 July   | 21–30 July      |
| Berry ripening | 21–30 September | 11–20 September | 11–20 September | 21–30 September | 1–10 October | 11–20 September | 21–30 August | 21–30 September |

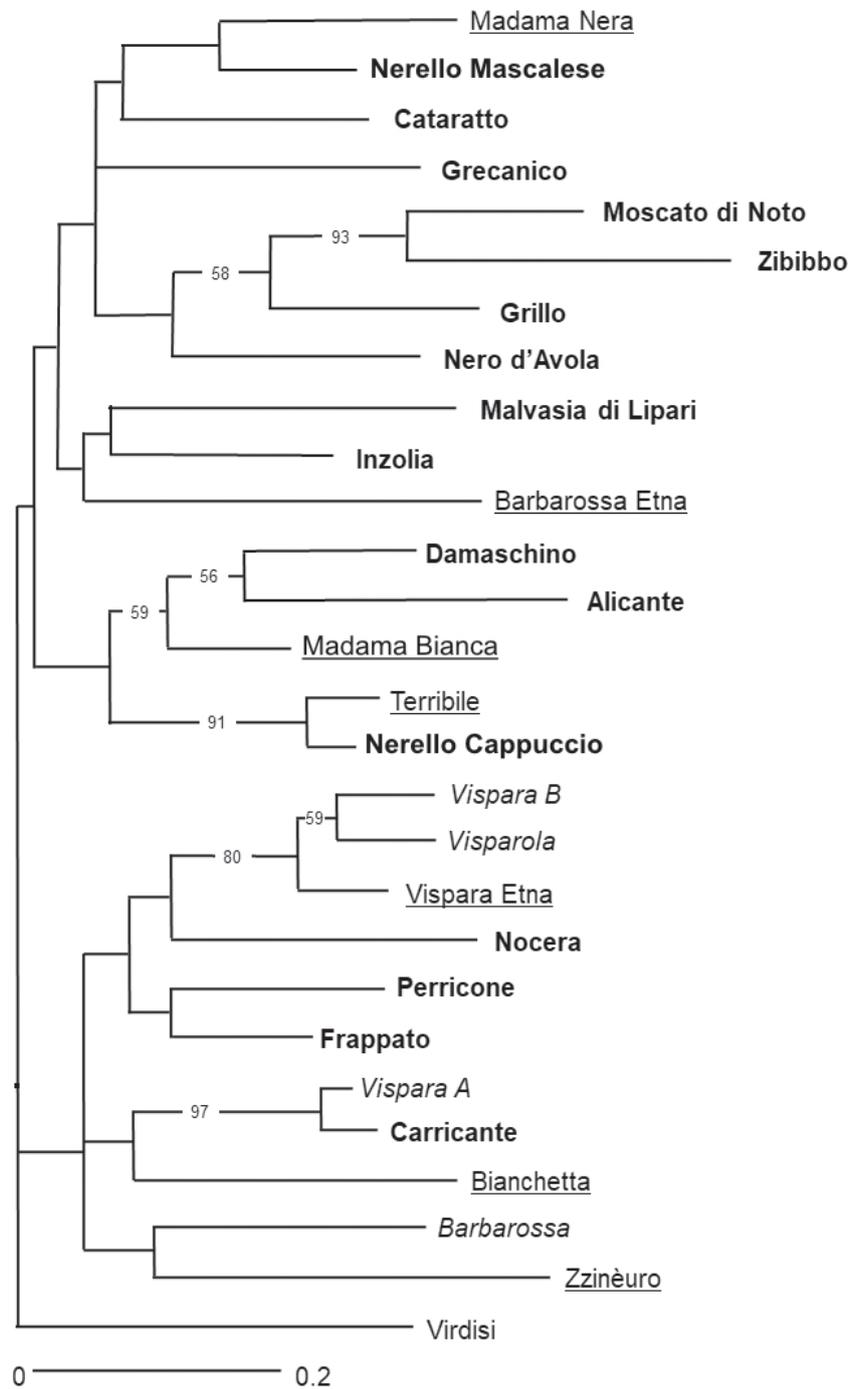


Fig. 1. Dendrogram obtained using the UPGMA clustering method. The analysis revealed the genetic distinctiveness of the eight neglected cultivars from the Mount Etna region (underlined characters) and their genetic relationship with the main (in bold) and some minor (in italics) cultivated vines in Sicily

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Table 6. Total soluble solids (TSS), pH and titratable acidity (years 2012, 2013 and 2014  $\pm$  standard deviation); means marked by different letters within a column are significantly different ( $p \leq 0.05$ ) based on Fisher's least significant difference (LSD) between cultivars and parameters

| Cultivar        | TSS<br>(°Brix)                | pH                           | Titratable acidity<br>(g/l) |
|-----------------|-------------------------------|------------------------------|-----------------------------|
| Barbarossa Etna | 20.1 <sup>ab</sup> $\pm$ 0.21 | 3.61 <sup>a</sup> $\pm$ 0.05 | 5.1 <sup>b</sup> $\pm$ 0.4  |
| Bianchetta      | 20.7 <sup>ab</sup> $\pm$ 0.49 | 3.21 <sup>a</sup> $\pm$ 0.02 | 6.3 <sup>b</sup> $\pm$ 0.2  |
| Madama bianca   | 18.4 <sup>b</sup> $\pm$ 0.71  | 3.58 <sup>a</sup> $\pm$ 0.03 | 3.8 <sup>c</sup> $\pm$ 0.3  |
| Madama nera     | 18.9 <sup>b</sup> $\pm$ 0.25  | 3.65 <sup>a</sup> $\pm$ 0.02 | 3.6 <sup>c</sup> $\pm$ 0.5  |
| Terribile       | 22.5 <sup>a</sup> $\pm$ 0.32  | 3.45 <sup>a</sup> $\pm$ 0.04 | 6.0 <sup>b</sup> $\pm$ 1.0  |
| Virdisi         | 19.0 <sup>b</sup> $\pm$ 0.15  | 3.15 <sup>a</sup> $\pm$ 0.03 | 10.0 <sup>a</sup> $\pm$ 1.9 |
| Vispara Etna    | 19.0 <sup>b</sup> $\pm$ 0.47  | 3.48 <sup>a</sup> $\pm$ 0.02 | 5.9 <sup>b</sup> $\pm$ 0.4  |
| Zzinèuro        | 21.9 <sup>a</sup> $\pm$ 0.20  | 3.37 <sup>a</sup> $\pm$ 0.06 | 5.5 <sup>b</sup> $\pm$ 0.3  |

mine if the Sicilian Bianchetta grapevine, never described before, could share a common origin with the cultivar from Veneto.

The names 'Madama Bianca' and 'Madama Nera' are probably derived from the French "Madame", suggesting that the grapevines might have originated in France.

'Terribile' and 'Zzinèuro' are genotypes quite common in old vineyards in the Mount Etna region. Although never described before, they show high-quality bunches and could be successfully coupled with 'Nerello Mascalese' (the main genotype of this area) in order to improve colour and structure during wine production.

A similar study about the ampelographic make-up of Sardinia was performed by MATTIA et al. (2007). Also, CRESPIAN et al. (2006) investigated several local varieties of the Malvasia family, describing syn-

onyms and identifying several local cultivars. All these authors reported the potential of both ampelographic and molecular tools for solving problems of cultivar identification; the suitability of these tools is probably due to different factors including the absence of a comprehensive germplasm database and problems in naming the different varieties.

## CONCLUSION

In this work, we identified, recovered and characterised eight minor, neglected and never before described cultivars still found in old vineyards in the region of Mount Etna. The results of this work can boost the protection of local grape biodiversity, and the use of the autochthonous genetic resources described here may contribute to the promotion of

Table 7. Genetic parameters of 11 microsatellite markers in 20 main and eight minor grapevine cultivars

| Marker  | Major allele frequency | Genotype No. | Allele No. | Heterozygosity | PIC   |
|---------|------------------------|--------------|------------|----------------|-------|
| VVMD5   | 0.402                  | 11           | 7          | 0.798          | 0.702 |
| VVMD7   | 0.500                  | 12           | 8          | 0.643          | 0.632 |
| VVMD21  | 0.464                  | 9            | 7          | 0.821          | 0.634 |
| VVMD24  | 0.536                  | 10           | 6          | 0.643          | 0.619 |
| VVMD25  | 0.464                  | 9            | 5          | 0.786          | 0.622 |
| VVMD27  | 0.393                  | 12           | 7          | 0.821          | 0.707 |
| VVMD28  | 0.214                  | 18           | 11         | 0.929          | 0.838 |
| VVMD32  | 0.296                  | 12           | 9          | 0.963          | 0.786 |
| VVS2    | 0.446                  | 11           | 7          | 0.714          | 0.665 |
| VRZAG62 | 0.268                  | 15           | 7          | 0.857          | 0.797 |
| VRZAG79 | 0.411                  | 14           | 8          | 0.821          | 0.750 |
| Mean    | 0.400                  | 12.09        | 7.5        | 0.800          | 0.705 |

viticulture in the Mount Etna region. The selected cultivars are also potentially of oenological interest, since they were found in a region with a high degree of diversification among different vineyards; in such a context, rare and endangered cultivars may be identified and re-evaluated to obtain standard products with high potential for the wine market.

The results of the molecular analysis confirm the high variability of the *Vitis* germplasm in the Mount Etna region and represent a highly significant contribution to better evaluating aspects related to the movement of grapevine varieties in Southern Italy. Finally, they also represent an important contribution to our understanding of grape genetic resources in danger of extinction.

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