Grapevine (*Vitis vinifera* L.) varietal assortment and evolution in the Marche region (central Italy)

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**ABSTRACT**

Italy is one of the richest countries in terms of grapevine varietal assortment, and the Marche region in central Italy has an ancient winegrowing tradition. Increasing interest in autochthonous grapevine varieties prompted efforts to recover and identify local minor germplasm also in this region, and to search for pedigree relationships and determine the evolution of varietal assortment. In the present study this was done using nuclear and chloroplast microsatellite markers and SNP markers from the *Vitis* 18kSNP chip. Eighteen new genotypes were found, of which seventeen belonged to well-known, less known and even unknown vines found to be specific to the Marche region. The rearranged pedigree highlighted the complex intertwining relationships between Marche varieties. Second-degree relationships were also derived. Some minor Marche varieties have increased the number of parent-offspring related members of Garganega, Sangiovese, Crepolino/Visparola and Sciaccarello, which are varieties already recognised as founders or recurrent parents of many Italian cultivars. Crepolino/Visparola was shown to be a key variety in the evolution of the Marche varietal assortment, having played the role of parent and grandparent, as explained by the presence of this genotype in the region in ancient times. Surprisingly, Semidano, a well-known Sardinian variety, played a clear role as parent or grandparent of three minor Marche varieties, testifying to its presence in central Italy in ancient times. Incrocio Bruni 54 was confirmed as an offspring of Sauvignon and Verdicchio, as declared by the breeder. The parentage of Lacrima was completed: this variety was found to be a spontaneous cross between Nera Rada and Aleatico.

A dendrogram of genetic similarity clearly resembled the groups defined with the pedigree reconstruction and it gave an indication of the genetic similarity of the varieties excluded from the strictest parentage links.

**KEYWORDS**

Italian grapevine germplasm, variety identification, old autochthonous varieties, pedigree relationships, microsatellite markers (SSR markers), Single Nucleotide Polymorphism (SNP markers)
INTRODUCTION

Italy is one of the richest countries in terms of grapevine varietal assortment, with almost 600 cultivars used in wine production registered in the Italian National Catalogue of Grapevine Varieties (http://catalogoviti.politicheagricole.it). The origin of the cultivated varieties can be determined by reconstructing their kinship; indeed, in the wine supply chain, describing a wine through the synergy between cultivation, quality and cultural issues is becoming increasingly important. The Marche region in central Italy and overlooking the Adriatic Sea has an ancient winegrowing tradition. In light of increasing consumer interest in local products, research on local grapevine germplasm and its conservation and exploitation play an important role in supporting the economic sustainability of farms. Viticulture is an important sector in the Marche region, with approximately 17,300 ha of vineyards and wine production of around one million hectolitres (https://www.assoenologi.it/wp-content/uploads/2020/09/report_previsioni_vendemmiali.pdf). Even if fifty-six grapevine varieties are suitable for cultivation in the Marche, only six are grown in over 70 % of the region’s vineyards, namely Sangiovese, Montepulciano, Verdicchio, Trebbiano Toscano/Biancame, Passerina and Pecorino. The first two varieties are used to produce the renowned Rosso Piceno wines and the third the Verdicchio dei Castelli di Jesi wines; together, these three varieties account for more than 50 % of the Marche appellation wines.

Significant efforts have been made to recover and identify local minor grapevine germplasm in many Italian regions, like Apulia, Campania, Emilia Romagna, Friuli Venezia Giulia, Liguria, Sardinia, Sicily, Tuscany (Costantini et al., 2005; De Mattia et al., 2007; Torello Marinoni et al., 2009; Carimi et al., 2010; Crespan et al., 2011; De Lorenzis et al., 2014; Schneider et al., 2014; Mercati et al., 2016; D’Onofrio et al., 2016; Gristina et al., 2017; Pastore et al., 2020), and variety pedigrees have been reconstructed for the most widespread and renowned ones and for those having local importance, helping to unravel the intricate skein of the structure of Italian grapevine populations (Di Vecchi-Staraz et al., 2007; Ruffa et al., 2016; Crespan et al., 2020; Raimondi et al., 2020; D’Onofrio et al., 2021). Similar efforts have been made in the Marche region, because the resulting knowledge is very useful in science and has cultural and marketing appeal. Since 1990, ASSAM has recovered local grapevine genetic resources for their conservation, characterisation and potential future exploitation. Over the period 1990-2012, some local, national and international grapevine varieties were evaluated and the most interesting ones were registered in the Italian National Catalogue of Grapevine Varieties, like Vernaccia nera grossa in 2008 and Garofanata in 2013 (http://catalogoviti.politicheagricole.it). In 2014, a larger recovery programme was set up: more than one hundred grapevine samples were collected in the Marche region and genotyped with microsatellite (SSR) markers to speed up their identification. Indeed, classical ampelographic identification is often difficult due to high varietal variability, often combined with poor vine health. Later, rare and unexplored genetic resources were preserved in a dedicated germplasm repository; a search for verbal and written information on these poorly known grapevines was also made. Pedigree studies were then implemented on varieties cultivated in the Marche region, in which 12 nuclear and 8 chloroplast SSRs were combined with SNPs from the Vitis18kSNP array. The very large number of point mutations in the grapevine genome make SNP (Single Nucleotide Polymorphism) markers a powerful tool with many applications. Next generation sequencing provided hundreds of thousands of SNP markers in the whole Vitis spp. genome. A first Vitis chip array, the Vitis9kSNP, was developed in 2010 (Myles et al., 2010), with 9,000 SNP detected in 11 Vitis vinifera L. varieties and six wild Vitis species. The GrapeReSeq Consortium developed a new one, the Vitis18kSNP, obtaining around 18,000 SNPs from 47 V. vinifera varieties, 12 wild Vitis species and 5 Muscadinia rotundifolia varieties (Le Paslier et al., 2013). This tool is used in pedigree studies as it contributes to finding new relationships, and it confirms or questions those found using only SSRs (Myles et al., 2011; Ruffa et al., 2016; Laucou et al., 2018; De Lorenzis et al., 2019; Crespan et al., 2020; Raimondi et al., 2020; D’Onofrio et al., 2021). Indeed, the Vitis18kSNP surpasses SSRs, because it can provide information on thousands of points in the genome in just one analysis, making up for their very low polymorphism.

MATERIALS AND METHODS

1. Plant material obtained during a sampling campaign in the Marche region, nSSR genotyping and varietal identification

One hundred and twenty-two vines were sampled in the Marche region and preserved in the ASSAM repository (Table 1); the sampling sites are
indicated when available and reported in Figure 1
for the accessions not listed or lacking an SSR
profile in the Vitis International Variety Catalogue (VIVC), or for those not enrolled in the Italian
catalogue.

Often these vines did not have a varietal name and
thus generic names were used for the samples,
referring, for example, to a vine trait, like grape
colour or shape, or to the plant owner’s name.

Genomic DNA was roughly extracted and
genotyped with 12 nuclear SSR (nSSR) markers,
including the nine used internationally for
grapevine identification (VVS2, VVMD5,
VVMD7, VVMD25, VVMD27, VVMD28,
VVMD32, VrZAG62, VrZAG79) (Maul et al.,
2012), plus ISV2 (VMC6e1), ISV4 (VMC6g1)
and VMCNG4b9 (Migliaro et al., 2013).
Fluorescent primers and an ABI3130xl genetic
analyser (Applied Biosystems, Foster City, CA)
were used to produce the nSSR profiles. Allele
calling was performed with GeneMapper software
version 5.0, with a homemade bin set obtained
from reference varieties. The SSR profiles of all
genotyped samples were identified using the CREA
Viticulture and Enology molecular database, the
available literature and the Vitis International
Variety Catalogue (VIVC, http://www.vivc.de).

2. Plant material selected for pedigree study

A selection of sixty-seven grapevine varieties
from two Italian repositories (CREA Viticulture
and Enology and ASSAM) were analysed for
pedigree relationship reconstruction (Table 2).
From the results of the sampling campaign carried
out on Marche Vitis germplasm, 36 Vitis vinifera
varieties used in wine production were selected
and analysed for the reconstruction of pedigree
relationships, along with other known grapevine
varieties of interest to the Marche region (Aleatico,
Garnacha tinta, Lacrima, Incrocio Bruni 54,
Malvasia bianca lunga and Vernaccia nera); a
total of 42 varieties were thus selected (in bold in
Table 2). This list also includes 25 other varieties,
which were selected for possible PO relationships
by screening around 4,000 unique SSR profiles
present in the CREA-Viticulture and Enology
molecular database (partially published).
### TABLE 1. Samples obtained during a sampling campaign in the Marche region. VIVC: *Vitis* International Variety Catalogue; IC: Italian Catalogue.

| Sample name                                      | Recovery or Sampling site | Variety name              | VIVC variety number | IC code |
|-------------------------------------------------|---------------------------|---------------------------|---------------------|---------|
| Bianca (D’Onofrio)                              | Pedaso                    | Afus Ali (Regina)         | 122                 | 527     |
| Uva bianca (Zaghi)                              | Sirolo                    | Alfredo Marchetti         |                     |         |
| Negri o Neretto di Piobbico (Ulivello)          | Sarnano                   | Barbera                   | 974                 | 19      |
| Rossa acino ovale (Rossi)                       | Falerone                  |                           |                     |         |
| Bersigana                                       | ASSAM repository          | Bersigana                 |                     |         |
| Cacciuò nero                                    | ASSAM repository          | Besgano nero              | 1284                |         |
| Bianchetta                                      | ASSAM repository          | Bianchetta marchigiana    |                     |         |
| Pagadebito (Spreca)                             | Lapedona                  |                           |                     |         |
| Passerina F9                                    | ASSAM repository          | Bombino bianco/Passerina  | 1533                | 32/181  |
| Uva bianca 1907 (Amadio)                        | Campofilone               |                           |                     |         |
| Uva D'oro                                       | ASSAM repository          |                           |                     |         |
| Moscato nero (Ubaldi)                           | Montefalcone App.         | Cardinal                  | 2091                | 507     |
| Bianca fienile (Silvestri)                      | Cossignano                | Chasselas bianco (Chasselas dorato) | 2473                | 509     |
| Morettone (Remia)                               | Montegiorgio              |                           |                     |         |
| Cimiciola F38                                   | ASSAM repository          |                           |                     |         |
| Gaglioppa (Capecci)                             | Ripatransone              | Ciliegio                  | 2660                | 62      |
| Morettone (Capecci)                             | ASSAM repository          |                           |                     |         |
| Centenaria (Sgariglia)                          | Acquaviva                 | Cornichon bianco (Pizzutello bianco) | 16448                | 524     |
| Cocacciara                                      | ASSAM repository          |                           |                     |         |
| Fondazione fico (o pozzo?)                      | Montefiore dell’Aso       | Cocacciara                |                     |         |
| Pianta 2 porcile (Beato)                        | Montelparo                |                           |                     |         |
| Famoso                                          | Urbino (PU)               | Famoso marchigiano        |                     |         |
| Fava                                            | ASSAM repository          | Fava                      |                     |         |
| Forcese (Capecci)                               | Ripatransone              | Forcese                   |                     |         |
| Forcese (Ubaldi)                                | Montefalcone App.         |                           |                     |         |
| Forconese                                       | ASSAM repository          |                           |                     |         |
| Cotrognone                                      | ASSAM repository          |                           |                     |         |
| Moscatellone (Duri)                             | Serrapetrona              | Garofanata                | 24957               | 463     |
| Uva Bianca (Serboni)                            | Serrapetrona              |                           |                     |         |
| S. Maria/Luglia (Ulivello)                      | Monte Urano               | Italia                    | 5582                | 514     |
| Tintorino (Montalbini)                          | Arcevia                   | Jacquez                   | 5627                |         |
| Uva Luglia (Ulivello)                           | Montelparo                | Koenigin der weingarden (Regina dei vigneti) | 6350                | 528     |
| Luglia/Lugliola/S. Maria (Milanesi)             | Montefalcone App.         | Luglienga bianca (Sant’Anna di Lipsia) | 6982                | 544     |
| Bianca strada (Orlandi)                         | Villa Tara                |                           |                     |         |
| Fondazione strada grande                        | Montefiore dell’Aso       | Maceratino                | 7023                | 124     |
| Trebbiano (Spreca)                              | Lapedona                  |                           |                     |         |
| Variety (Synonym) | Location | Repository | Description | Code | Place |
|------------------|----------|------------|-------------|------|-------|
| Gallioppo (Silvestri) | Cossignano | ASSAM repository | Maiolica | 7136 | 126 |
| Gallioppo 75 | | | | | |
| Gallioppo GE | | | | | |
| Gallioppo GE F12 I9 P2 | | | | | |
| Malvasia bianca lunga (La Pila) | Montegiorgio | | | | |
| Malvasia? (Montalbini) | Arcevia | | | | |
| P1 (Scarabotti) | Recanati | | Malvasia bianca di Candia | 23555 | 131 |
| P2 (Scarabotti) | Recanati | | | | |
| San Niccolò TOR 1 (ITAS Vivarelli) | Fabriano | | | | |
| San Niccolò TOR 2 (ITAS Vivarelli) | Fabriano | | | | |
| San Niccolò TOR 4 (ITAS Vivarelli) | Fabriano | | | | |
| Sangiovese (ITAS Macerata) | Macerata | | Malvasia di Casorzo | 7264 | 134 |
| Bianca resistente (Anselmi) | Monte San Martino | | Malvasia istriana | 7269 | 138 |
| Premotico (Milanesi) | Montefalcone App. | | | | |
| Ulpetta nuova (Sgariglia) | Acquaviva | | Maturano bianco | 23347 | 424 |
| Ulpetta vecchia (Sgariglia) | Acquaviva | | | | |
| Uva cane | ASSAM repository | | | | |
| Melata Savini 2 (Silvestri) | Cossignano | | Melata | | |
| (Uva) Melata | | ASSAM repository | Merlot | 7657 | 146 |
| Granarello | | ASSAM repository | | | |
| Rossa foglia (Mattei Verde) | Arcevia | | Montepulciano | 7949 | 150 |
| Chiapparù | | ASSAM repository | Montonico bianco | 7960 | 151 |
| Uva Regno | ASSAM repository | | | | |
| (Uva) Moie | ASSAM Petritoli | | Morgentino | | |
| Uva Moscata p 2 Rossi | Falerone | | Moscato Cerletti | | |
| Bianca (Zanoni) | Pedaso | | | | |
| Moscatello Francese (Ulivello) | Monte Urano | | Muscat of Alexandria (Zibibbo) | 8241 | 343 |
| Pianta secolare (Maranesi) | Petritoli | | | | |
| Bianca Chiesa S. Domenico | Ancona | | Moscato di Terracina | 8053 | 281 |
| Malvasia Candia (La Pila) | Montegiorgio | | Moscato giallo | 8056 | 154 |
| Moscatello N. | Montefalcone loc. Faveto | | Muscat rouge de Madere | 8249 | |
| Moscianino | ASSAM repository | | Moscianello | 26683 | 889 |
| Bianca | | ASSAM repository | | | |
| Cacciù bianco 78/6 | | ASSAM repository | | | |
| Cacciù bianco (Botticelli) | Montottone | | Mostosa | 8075 | 157 |
| Cacciù bianco (Sprega) | Lapedona | | | | |
| Fondazione casa | Montefiore dell'Aso | | | | |
| Mostosa 49 | | ASSAM repository | | | |
| (Uva) nera rada | ASSAM repository | | Nera rada | | |
| Uva rossa (Iena) | Porto S. Elpidio | | Nerello mascalese | 8480 | 165 |
| Occhio nero | ASSAM repository | | Occhio nero | | |
| Vissanello (Orlandi) | Villa Tara | | Pecorino | 9072 | 184 |
| Pergolo (Vitali) | Montalparo | | Pergolo | | |
The recovery site is indicated, when available. The prime names of the varieties in the IVIC are shown in the “Variety name” column, and, when different, the name as registered in the IC is in brackets. The names of the 18 varieties lacking the SSR profile in the IVIC and not yet enrolled in the IC are in bold.
The search for compatible trios (parents and offspring) and duos (parent-offspring) was done based on 9 to 12 nSSRs in the CREA Viticulture and Enology database with Cervus 3.0 (Kalinowski et al., 2007) and GenAlEx 6.5 software (Peakall and Smouse, 2012), and in the IVTC using the “Relationships based on nine microsatellites” tool. The varieties which proved to be possible members of trios or duos were included in the sample set. Termarina and Verano accessions belonging to the Sciaccarello variety were both included to evaluate possible SNP polymorphisms, given their big phenotypic differences in grape morphology; Termarina is a parthenocarpic somatic variant of Sciaccarello and Verano shows the seeded, wild-type form (Crespan et al., 2016). Three parent-parent-child trios with well-established parent-offspring relationships were added for the evaluation of Mendelian incompatibilities and statistical comparisons: Manzoni bianco = Pinot x Riesling weiss (Grando and Frisinghelli, 1998; Cipriani et al., 2010.), Raboso Veronese = Raboso Piave x Marzemina bianca (Crespan et al., 2006), and Vitouska = Malvasia bianca lunga x Glera (Crespan et al., 2007).

3. Genomic DNA extraction and genotyping with chloroplast SSR and SNP markers for the pedigree study

Genomic DNA was extracted from young freeze-dried leaves from the samples listed in Table 2 using the QIAGEN DNeasy 96 Plant Kit (QIAGEN GmbH, Hilden, Germany) and according to the manufacturer’s protocols with the following modifications: AP1 buffer was added with 1.6 % PVP40 (Sigma Aldrich) and the samples were incubated at 65 °C for 5 min; the DNA was eluted in milliQ water at 65 °C. The DNA was quantified with Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific) by Synergy2 Fluorometer (Biotek). DNA quality was checked on an Agilent 2200 Tapestation (Agilent Technologies, CA) using the DNA genomic ScreenTape (Agilent Technologies) for DNA integrity detection, and the NanoDrop 8000 Spectrophotometer (Thermo Scientific, MA) for 260/230 and 260/280 ratios evaluation.

A check for correct sampling was performed on these DNAs using four nSSR (VVS2, VVMD5, VrZAG79 and VVMD28). Chlorotypes were assessed with eight chloroplast SSR markers (Arroyo-Garcia et al., 2006). Two multiplex PCR were organised using fluorescent primers and SSR allele calling was performed as described for the nSSRs.

All the samples were genotyped using the Infinium® II Vitis18k SNP array, which comprises 18,071 SNPs (GrapeReSeq Consortium, Illumina), following the Infinium® HD Assay Ultra protocol (Illumina Inc., San Diego, CA). The samples were scanned using an Illumina HiScan.

4. Data processing for parentage relationships

For the SNP data analysis, a no-call threshold of 0.15 was applied as a GenCall cutoff using GenomeStudio Genotyping Module v2.0 of ILLUMINA. The SNP selection was performed with ASSiST (Automatic SNP ScorIng Tool) software (Di Guardo et al., 2015) v. 1.02, applying the default parameters for germplasm material. No pedigree or map information was given. A larger set of 192 unique genotypes, mainly comprising Italian varieties, was used to obtain a more consistent SNP classification into the groups obtained with the software; only Robust, OneHomozygRare_HWE and OneHomozygRare_notHWE SNPs were retained, as reported in Crespan et al. (2021).

4.1. IBS/IBD estimation

The filtered SNPs were used to prepare ‘map’ and ‘ped’ files for analysis with PLINK v1.09 software (Purcell et al., 2007) (http://pngu.mgh.harvard.edu/purcell/plink). Identical by state (IBS) and Identical by Descent (IBD) indexes were estimated for pairs of samples, using the parameters for PO relationships, namely Z0, Z1, Z2 and PI-HAT with reference indexes of 0, 1, 0 and 0.5 respectively.

4.2. Mendelian inconsistencies

Mendelian inconsistencies between pairs of samples were computed on the larger set of 192 unique genotypes by searching for PO related varieties. First degree related genotypes were then combined in all parent-parent-offspring trios and related Mendelian inconsistencies were computed. Home-made algorithms were written in Excel Visual Basic for Application (VBA) to perform these computations.

4.3. Full-sib relationships

Possible full-sib relationships were evaluated using Colony software version 2.0.6.5 (July 30, 2018), which is freely available at https://www.zsl.org/science/research-projects/software. Both nSSR and SNP markers were used as data input, and parentage relationships were inferred by combining PLINK information, 12 SSRs and Mendelian inconsistencies. The following main settings were applied: markers error rate 0.00001,
no sibship prior indicator, one medium run, FL (full likelihood) analysis method and medium precision when calculating FL.

5. Genetic similarity

MEGA X software version 10.1.8 (Kumar et al., 2018) was used to obtain an rooterved dendrogram of genetic similarity using the 8,770 ASSIsT-selected SNP markers. Pairwise genetic distances were computed using the Kimura 2-parameter method. Missing data were removed for each sequence pair, using the ‘pairwise deletion’ option. A dendrogram of genetic similarity was obtained using the Unweighted Pair-Group Arithmetic Average Method (UPGMA). A bootstrap test of 2,000 replicates was used to define the percentage of replicate trees in which the associated genotypes clustered together; these values were shown next to the branches. Only branches with bootstrap values higher than 75 were taken into consideration.

RESULTS

In the first part of this study, a sampling campaign on the minor local grapevine germplasm grown in the Marche region was performed for vine cultivar identification supported by SSR profiles. In the second part, the pedigree relationships of 42 varieties of interest to the Marche region (17 of them being new entries identified in the first part of this study) were evaluated also in comparison with an additional 25 potentially PO-related varieties based on 9-12 SSR markers, thus totaling 67 varieties.

1. Identification of the vines recovered during surveys 1990-2020

The 30-year sampling campaign in the Marche region made it possible to find and preserve in the ASSAM repository 122 accessions of Vitis showing 61 different SSR profiles. Forty-three profiles were shared with the Vitis International Variety Catalogue (ITVC), allowing the name of the variety and the corresponding code number to be confirmed or assigned. The remaining 18 genotypes, except for Moscato Cerletti, are new and possibly local Marche grapevine germplasm. The names assigned to 12 of them are those indicated during the survey phase, including the names ‘Bianchetta marchigiana’ and ‘Famoso marchigiano’, which were assigned to distinguish these varieties from other Bianchetas (like Bianchetta genovese and Bianchetta trevigiana) and from the muscat flavoured Famoso from the Emilia-Romagna region respectively.

The name ‘Alfredo Marchetti’ was assigned to Uva bianca Zaghi after the noble, ancient owner of the villa in Sirolo where this vine was found. Scrochiona is the dialectal translation of the generic name ‘Uva croccante’ (meaning crispy grape). Ripanea comes from the nickname of Mister Serboni, Ripanè, meaning the one who works on ‘steep slopes’ (‘ripe’ in Italian). Morgentino, Moscato Cerletti and Tenerone were assigned according to the CREA Viticulture and Enology SSR molecular database. The identification results are summarised in Table 1.

Among the 61 genotypes recovered in the sampling campaign, 8 table grape varieties were found (Afus Ali, Cardinal, Chasselas blanc, Cornichon blanc, Italia, Koenigin der weingarten, Luglienga bianca, and Pizzutello nero), as well as two hybrids (Jacquez and Villard blanc). The remaining 51 genotypes included not only wine varieties from other Italian regions (Barbera, Nerello mascalese, Terrano, Uva tosca), but also from countries on the other side of the Adriatic Sea, like the Croatian Plavina crna and the Slovenian Zunek. Five Muscats were recognised: the reknown and worldwide Muscat of Alexandria and the Italian Moscato di Terracina, Moscato giallo and Muscat rouge de Maderie. It was a big surprise to also find Moscato Cerletti, concealed as an unknown vine with a muscat flavour. Moscato Cerletti was obtained by Baron Antonio Mendola of Favara (Sicily) in 1870 during his breeding activities and was selected and praised as a very nice table grape; however, it was thought to have disappeared in Italy (Antonio Sparacio, personal communication).

The most frequently found wine varieties were Malvasia bianca di Candia (7 samples), Mostosa (6), Sgranarella (6), Ciliegiolo (5), followed by Bombino bianco/Passerina, Maiolica, Maturano bianco and Sangiovese with 4 samples. All these are Italian varieties, most of them already well known, except for Sgranarella, which was enrolled in the Italian Catalogue in 2019 (http://catalogoviti. politicheagricole.it). Three Cacciù bianco samples were collected in the survey, and all corresponded to Mostosa. Cacciu nero, however, was shown to be a homonym of at least two different varieties, Besgano nero and Ripanea. The correspondence between the Marche Gallioppo (not to be confused with Gallioppo of Calabria) and Maiolica was confirmed.

Additional information was retrieved by comparison with the CREA Viticulture and Enology SSR molecular database, showing a
**TABLE 2.** List of the 67 varieties used for the pedigree study.

| ID | Variety name | Accession name          | Berry colour | Repository | Country of origin |
|----|--------------|-------------------------|--------------|------------|-------------------|
| 1  | Aleatico     | Vernaccia di Pergola    | B            | CREA       | Italy             |
| 2  | Alfredo Marchetti | Bianca Zaghi         | W            | ASSAM      | Italy             |
| 3  | Bersigana    | Bersigana               | B            | ASSAM      | Italy             |
| 4  | Bianchetta marchigiana | Bianchetta       | W            | ASSAM      | Italy             |
| 5  | Bombino bianco/Passerina | Bombino bianco      | W            | CREA       | Italy             |
| 6  | Bombino nero | Bombino nero           | B            | CREA       | Italy             |
| 7  | Caloria      | Caloria                 | B            | CREA       | Italy             |
| 8  | Capibianchi  | Capibianchi            | B            | CREA       | Italy             |
| 9  | Ciliegiolo   | Ciliegiolo              | B            | CREA       | Italy             |
| 10 | Cocacciara   | Cocacciara             | W            | ASSAM      | Italy             |
| 11 | Crepolino/Visparola | Seacco            | W            | CREA       | Italy             |
| 12 | Drupeggio    | Drupeggio Deruta       | W            | CREA       | Italy             |
| 13 | Empibotte    | Borbottone              | W            | CREA       | Italy             |
| 14 | Famoso       | Famoso                  | W            | CREA       | Italy             |
| 15 | Famoso marchigiano | Famoso marchigiano    | W            | ASSAM      | Italy             |
| 16 | Fava         | Uva Fava                | W            | ASSAM      | Italy             |
| 17 | Fogarina     | Fogarina                | B            | CREA       | Italy             |
| 18 | Foglia tonda | Foglia tonda            | B            | CREA       | Italy             |
| 19 | Forcese      | Forcese                 | W            | ASSAM      | Italy             |
| 20 | Gabbavolpe   | Gabbavolpe              | W            | CREA       | Italy             |
| 21 | Garganega    | Garganega               | W            | CREA       | Italy             |
| 22 | Garnacha tinta | Alicante            | B            | CREA       | Spain             |
| 23 | Garofanata   | Garofanata              | W            | CREA       | Italy             |
| 24 | Grero        | Grero                   | B            | CREA       | Italy             |
| 25 | Incrocio Bruni 54 | Incrocio Bruni 54     | W            | CREA       | Italy             |
| 26 | Lacrima      | Lacrima di Morro d'Alba| B            | CREA       | Italy             |
| 27 | Livornese/Rollo | Livornese           | W            | CREA       | Italy             |
| 28 | Maceratino   | Maceratino             | W            | CREA       | Italy             |
| 29 | Maiolica     | Gallioppo marchigiano   | B            | CREA       | Italy             |
| 30 | Malvasia bianca di Candia | Malvasia bianca di Candia | W       | CREA       | Italy             |
| 31 | Malvasia bianca lunga | Malvasia bianca lunga | W            | CREA       | Italy             |
| 32 | Maturano bianco | Maturano bianco       | W            | CREA       | Italy             |
| 33 | Melata       | Uva melata              | R            | ASSAM      | Italy             |
| 34 | Minutolo     | Minutolo                | W            | CREA       | Italy             |
| 35 | Montepulciano | Montepulciano          | B            | CREA       | Italy             |
| 36 | Montonico bianco | Montonico bianco     | W            | CREA       | Italy             |
| 37 | Morgentinio  | (Uva) Moie              | P            | ASSAM      | Italy             |
| 38 | Muscat rouge de Madere | Moscato violetto      | R            | CREA       | Italy             |
| 39 | Moscianello  | Moscianino              | W            | ASSAM      | Italy             |
| 40 | Mostosa      | Mostosa                 | W            | CREA       | Italy             |
| ID | Variety                   | Color | Institution | Country   |
|----|---------------------------|-------|-------------|-----------|
| 41 | Negroamaro                | B     | CREA        | Italy     |
| 42 | Nera rada (Uva) nera rada | B     | ASSAM       | Italy     |
| 43 | Nuragus                   | W     | CREA        | Italy     |
| 44 | Occhio nero               | W     | ASSAM       | Italy     |
| 45 | Pecorino                  | W     | CREA        | Italy     |
| 46 | Pergolo                   | W     | ASSAM       | Italy     |
| 47 | Quaglione                 | B     | CREA        | Italy     |
| 48 | Ripanea Uva rossa (Serboni)| B    | ASSAM       | Italy     |
| 49 | Sangiovese                | B     | CREA        | Italy     |
| 50 | Sauvignon                 | W     | CREA        | France    |
| 51 | Sciaccarello Verano       | B     | CREA        | Italy     |
| 52 | Sciaccarello Termarina    | B     | CREA        | Italy     |
| 53 | Sercocchina Rossa crocante| B    | ASSAM       | Italy     |
| 54 | Semidano                  | W     | CREA        | Italy     |
| 55 | Sgranarella Vesperino     | W     | CREA        | Italy     |
| 56 | Somarello rosso           | R     | CREA        | Italy     |
| 57 | Tenerone Grugnini         | R     | ASSAM       | Italy     |
| 58 | Torella (Uva) Torell Piermarini| W | ASSAM  | Italy     |
| 59 | Trebbiano abruzze Trebbiano abruzze | W | CREA   | Italy     |
| 60 | Trebbiano perugino        | W     | CREA        | Italy     |
| 61 | Trebbiano toscano         | W     | CREA        | Italy     |
| 62 | Vaccaro                   | W     | ASSAM       | Italy     |
| 63 | Verdicchio                | W     | CREA        | Italy     |
| 64 | Vernaccia nera  Vernaccia nera grossa (di Cerreto) | B     | CREA       | Italy     |
| 65 | Vernaccia nera grossa     | B     | CREA        | Italy     |
| 66 | Vulpea Quaiara            | B     | CREA        | Austria   |
| 67 | Zunek Zivi                | W     | ASSAM       | Slovenia   |

**Additional varieties**

| ID | Variety               | Color | Institution | Country   |
|----|-----------------------|-------|-------------|-----------|
| 68 | Glera                 | W     | CREA        | Italy     |
| 69 | Manzoni bianco        | W     | CREA        | Italy     |
| 70 | Marzemina bianca      | W     | CREA        | Italy     |
| 71 | Pinot                 |       | CREA        | France    |
| 72 | Raboso Piave          | B     | CREA        | Italy     |
| 73 | Raboso veronese       | B     | CREA        | Italy     |
| 74 | Riesling weiss        | W     | CREA        | Germany   |
| 75 | Vitouska              | W     | CREA        | Italy     |

Berry colour: B = black, W = white, R = red, P = pink. CREA: CREA Viticulture and Enology, Susegana (TV), Italy; ASSAM: ASSAM, Petrioli (FM), Italy. The 42 varieties of interest to the Marche region are highlighted in bold. Additional 8 varieties with known pedigree relationships were used as references and are listed in italics at the end of the table (ID 68-75).
greater diffusion than previously known for some minor genotypes: i) additional synonyms of Sgranarella are Vesprino (still used in Marche Region), and Rosciolo (used in the nearby Lazio region), and ii) Grugnintì is a synonym for the Tuscan Boggione rosso and Tenerone; Tenerone was chosen as the prime name for this genotype due to the ampelographic description given by Bandinelli et al. (2005).

No differences were found between the SNP profiles of Verano, the seeded form of Sciaccarello, and Termarina, the partenocarpic somatic variant.

2. Parentage relationships

In the pedigree studies on the sixty-seven grapevine cultivars, 66 different SSR and SNP profiles were found, thus further supporting the molecular synonymy between Sciaccarello and Termarina. The nSSR profiles and related chlorotypes of these 67 varieties and of the eight additional ones are reported in Table S1. All the varieties were univocally identifiable with the 14 SNP set selected by Laucou et al., 2018, except for Sciaccarello and Termarina, whose profiles are provided in Table S2.

The Scacco accession of CREA-Viticulture and Enology shared the same SSR profile as the Tuscan Crepolino described by Armani et al. (2008) and the Sicilian Visparola (Carimi et al., 2010; De Lorenzis et al., 2014). Scacco was also analysed by Pastore et al. (2020) as a cultivar grown in Emilia Romagna where it is known under the synonym Rossola (Tebano). Therefore, this genotype was shown to have a range spreading from southern to northern Italy. Given that the only available ampelographic description for this variety is Crepolino, and that previous pedigree relationships are related to Visparola (D’Onofrio et al., 2021), the combination of these two names, Crepolino/Visparola, is used hereafter for this genotype.

Excluding the eight additional varieties listed in Table S1 from the computation, three chlorotypes, A, C and D, were found with large differences in frequency: type D was prevalent (75.8 %; 50/66), followed by type A (21.2 %; 14/66) and type C (only 3.0 % 2/66).

SNP pruning performed with ASSIsT software retained 8,770 SNP out of 18,071 (48.53 %): 3,407 were classified as Robust (18.9 %), 2,784 as OneHomozygRare_HWE (15.4 %) and 2,579 as OneHomozygRare_NotHWE (14.3 %).

The 8,770 SNP profiles related to the 74 unique varieties are reported in Table S3.

2.1. Duos and trios

The same pairs of first-degree related varieties were recognised using PLINK parameters and Mendelian inconsistencies computation (Table 3). The Z1 PLINK parameter was between 0.8334 and 1.

The distribution of Mendelian inconsistencies for all pairs of genotype combinations (computed on 192 unique genotypes) is shown in Figure 2: a clear Gaussian curve is represented, referring to non-PO related varieties. However, a well separated, small group of pairs outside the Gaussian curve and located on the left side of the figure can be seen; this small group is in strong agreement with the presence of PO relationships and shows the inconsistencies found for PO related varieties, with a maximum of 22 mismatching loci, while for the pairs inside the Gaussian distribution the Mendelian inconsistencies were from 53 onwards.

A complex network of first- and second-degree relationships was found.

Table 3 shows that some varieties were found to be PO related to more than one other variety: Garganega shows the highest number of PO relationships (10), then Crepolino/Visparola (6), Sciaccarello (6), Semidano (4), Sangiovese (3) and Mostosa (2); seven pairs of PO related varieties were also found.

First degree related varieties, selected according to the MI on duos, were then compared in all possible parent-parent-offspring combinations and the MI were computed accordingly. The distribution of the MI on trios is reported in Figure 3.

A group of trios with a maximum of 37 MI was shown to be well separated from the others at 268 MI onwards; the three parent-parent-child combinations used as reference also fall into this little group (Table 4).

One selfing and six trios were established based on PLINK parameters for PO relationships combined with 12 SSR data and MI in comparison with the three well-established parent-parent-child relationships used as references (Table 4 and Figure 4).

Chlorotypes helped in some cases to establish the sexual role played by the parents in generating their offspring. Alfredo Marchetti was found...
### TABLE 3. Duos: parent-offspring (PO) relationships inferred with PLINK parameters and Mendelian inconsistencies (MI).

| First variety          | Second variety             | PLINK parameters | MI | Reference literature |
|------------------------|-----------------------------|------------------|----|----------------------|
|                        |                             | Z0 | Z1    | Z2    | PI_HAT |                |
| Famoso marchigiano     | Garofanata                  | 0.0091 0.942 0.0489 0.5199 | 5   | present paper        |
| Forcese                | Moscianello                 | 0.0218 0.9612 0.017 0.4976 | 12  | present paper        |
| Empibotte              | Forcese                     | 0.0255 0.8927 0.0818 0.5282 | 14  | Crespan et al., 2008 |
| Malvasia bianca di Candia |                             | 0.0109 0.925 0.0641 0.5266 | 6   | Di Vecchi-Staraz et al., 2007 |
| Marzemina bianca      |                             | 0.0309 0.9613 0.0077 0.4884 | 17  | Crespan et al., 2008 |
| Montonico bianco      |                             | 0.0109 0.9279 0.0611 0.5251 | 6   | Crespan et al., 2008 |
| Pergolo               |                             | 0.0091 0.9202 0.0707 0.5308 | 5   | present paper        |
| Somarello rosso       |                             | 0.0019 0.8704 0.1187 0.5539 | 6   | Di Vecchi-Staraz et al., 2007 |
| Trebbiano perugino    |                             | 0.0291 0.9517 0.0192 0.495 | 16  | present paper        |
| Trebbiano toscano     |                             | 0.0182 0.9815 0.0003 0.4911 | 10  | Di Vecchi-Staraz et al., 2007 |
| Vernaccia nera grossa |                             | 0 1 0 0.5 | 9   | present paper        |
| Maiolica               | Negroamaro                  | 0.0146 0.9577 0.0277 0.5066 | 8   | D’Onofrio et al., 2021 |
| Montepulciano          | Bombino bianco              | 0.0218 0.9393 0.0389 0.5085 | 12  | Lacombe et al., 2013 |
| Bersigiana             |                             | 0.0146 0.9633 0.0222 0.5038 | 8   | present paper        |
| Caloria                |                             | 0.0237 0.9526 0.0263 0.5013 | 13  | Di Vecchi-Staraz et al., 2007 |
| Famoso                 |                             | 0 1 0 0.5 | 7   | D’Onofrio et al., 2021 |
| Tenerone               |                             | 0 1 0 0.5 | 13  | present paper        |
| Livornese/Rollo       |                             | 0.0127 0.9348 0.0524 0.5198 | 7   | Di Vecchi-Staraz et al., 2007 |
| Muscat rouge de Madere |                             | 0.0109 0.9891 0 0.4946 | 6   | Di Vecchi-Staraz et al., 2007 |
| Sangiovese             | Capibianchi                 | 0.0218 0.9513 0.0268 0.5025 | 12  | Di Vecchi-Staraz et al., 2007 |
| Foglia tonda           |                             | 0.02 0.9207 0.0593 0.5197 | 11  | Crespan et al., 2008 |
| Scroccichiana          |                             | 0.0197 0.9803 0 0.4901 | 11  | present paper        |
| Semidano               | Bianchetta marchigiana      | 0.0164 0.9016 0.082 0.5328 | 9   | present paper        |
| Drupeggio              |                             | 0.0146 0.9646 0.0208 0.5031 | 8   | present paper        |
| Nuragus                |                             | 0.0146 0.9349 0.0505 0.518 | 8   | present paper        |
| Occhio nero            |                             | 0.0091 0.9485 0.0424 0.5167 | 5   | present paper        |
| Torella                |                             | 0.0255 0.9252 0.0493 0.5119 | 14  | present paper        |
| Mostosa                | Trebbiano abruzese           | 0.0182 0.8962 0.0856 0.5337 | 10  | present paper        |
| Vaccaro                |                             | 0.0273 0.8334 0.1393 0.556 | 15  | present paper        |
| Verdichio              | Maceratino                  | 0.02 0.976 0.0039 0.492 | 11  | Lacombe et al., 2013 |

Expected values for PO relationships: Z0 = 0, Z1 = 1, Z2 = 0, PI_HAT = 0.5.
to be derived from selfing of Maturano bianco. Bombino nero was confirmed to be the progeny of Bombino bianco × Quagliano (Bergamini et al., 2016); Ciliegiolo was a spontaneous cross between Sangiovese and Muscat rouge de Madere; Forcese derived from Garganega × Crepolino/Visparola; Incrocio Bruni 54 was confirmed as a cross between Verdicchio and Sauvignon; Lacrima derived from Aleatico × Nera rada, and finally Morgentino was a cross between Forcese and Sciaccarello.

No reliable full-sibs were found using Colony software.

3. Dendrogram of genetic similarity

A dendrogram of genetic similarity was produced by applying the UPGMA method on all the genotypes selected for parentage studies, including additional reference varieties for known trios, totalling 74 unique SNP genotypes; nine clusters were found with bootstrap values higher than 75 (Figure 5).
### TABLE 4. Trios selected by combining PLINK parameters data for PO relationships using 8770 SNP ASSISt-selected, 12 SSRs and Mendelian inconsistencies (MI).

| Offspring            | First candidate       | Second candidate       | PLINK parameters | MI  | Reference literature               |
|----------------------|-----------------------|------------------------|------------------|-----|-----------------------------------|
|                      |                       |                        | Z0   | Z1   | Z2   | PI_HAT   |                  |
| Alfredo Marchetti    | Maturano bianco       | Maturano bianco        | 0.0036 | 0.2891 | 0.7073 | 0.8518   | 2                 | present paper   |
| Bombino nero         | Bombino bianco        | Quagliano              | 0.0091 | 0.9459 | 0.045  | 0.518    |                  | Bergamini et al., 2016 |
|                      | Bombino bianco        | Quagliano              | 0.0237 | 0.9461 | 0.0302 | 0.5033   | 18               |
| Ciliegiolo           | Sangiovese            | Muscat rouge de Madere | 0.0218 | 0.978  | 0.0002 | 0.4962   |                  | Di Vecchi-Staraz et al., 2007 |
|                      | Sangiovese            | Muscat rouge de Madere | 0.0237 | 0.9596 | 0.0168 | 0.4965   |                  |
| Forcese              | Garganega             | Crepolino              | 0.0164 | 0.91    | 0.0736 | 0.5286   |                  | present paper   |
|                      | Garganega             | Crepolino              | 0.0237 | 0.8722 | 0.1041 | 0.5402   |                  |
| Incroci Bruni 54     | Verdicchio            | Sauvignon              | 0.0382 | 0.9361 | 0.0257 | 0.4937   | 22               | present paper   |
|                      | Verdicchio            | Sauvignon              | 0.0382 | 0.9361 | 0.0257 | 0.4937   |                  |
| Lacrima              | Aleatico              | Nera rada              | 0.0182 | 0.8999 | 0.0819 | 0.5319   |                  | D’Onofrio et al., 2021 |
|                      | Aleatico              | Nera rada              | 0.0219 | 0.9064 | 0.0718 | 0.5249   |                  |
| Morgentino           | Forcese               | Sciaccarello           | 0.0255 | 0.9275 | 0.047  | 0.5108   | 26               | present paper   |
|                      | Forcese               | Sciaccarello           | 0.0255 | 0.9275 | 0.047  | 0.5108   |
| Reference trios      | Pinot                 | Riesling weiss         | 0.0109 | 0.9871 | 0.002  | 0.4955   |                  | Grando and Frisighelli, 1998 |
|                      | Pinot                 | Riesling weiss         | 0.0401 | 0.8672 | 0.0927 | 0.5263   |                  |
| Raboso veronese      | Raboso Piave          | Marzemina bianca       | 0.0252 | 0.9748 | 0    | 0.4874   | 31               | Crespas et al., 2006 |
|                      | Raboso Piave          | Marzemina bianca       | 0.0252 | 0.9748 | 0    | 0.4874   |
| Vitouska             | Malvasia bianca lunga | Glera                  | 0.0273 | 0.8543 | 0.1184 | 0.5456   |                  | Crespas et al., 2007 |
|                      | Malvasia bianca lunga | Glera                  | 0.0164 | 0.9155 | 0.0682 | 0.5259   |                  |
|                      | Malvasia bianca lunga | Glera                  | 0.0164 | 0.9155 | 0.0682 | 0.5259   | 24               |

**Note:** Z0, Z1, Z2, and PI_HAT are parameters from the PLINK analysis. MI indicates the Mendelian inheritance consistency. The reference literature provides the source of the trios used in the study.
Group A is the largest group (16 members), nine of the members being first or second degree related and the reference variety being Crepolino/Visparola. Group G is the second largest group (14 members), with two reference varieties, Sangiovese and Sciaccarello. The group of Garganega, F, has 11 members; the group of Semidano, D, has 8 members and the smallest groups, B, C, E, H, I, have from 5 to 2 members.

**DISCUSSION**

Sixteen genotypes were shown to be local Marche varieties/vines which were previously either poorly known or completely unknown: Alfredo Marchetti, Bersigiana, Bianchetta marchigiana, Cocacciara, Famoso marchigiano, Fava, Forcese, Melata, Morgentino, Nera rada, Occhio nero, Pergolo, Ripanea, Scrochiona, Torella and Vaccaro. The rearranged pedigree highlighted the complex intertwining of relationships between the Marche varieties. No putative full sibs were found, indicating not only that there is a large number of missing vines necessary to complete the puzzle, but also the liveliness of local wine growers in selecting new varieties.

Some varieties already recognised as founders or recurrent parents of many Italian cultivars, like Garganega, Sangiovese and Sciaccarello (Di Vecchi-Staraz *et al*., 2007; Crespan *et al*., 2008; Lacombe *et al*., 2013), increased their PO related members. The long list of varieties which are PO related with Garganega testifies and confirms the role of founder that is played by this very ancient cultivar from northern to southern Italy. The molecular data supports previous findings related to Empibotte, Malvasia bianca di Candia, Montonico bianco, Somarello rosso, Trebbiano peruginu, Trebbiano toscano and adds two local Marche varieties to the list, Pergolo and Vernaccia nera grossa. Very little information is available for Pergolo. Costanzo Felici da Piobbico (1525-1585, in Felici, 1986) cites a grapevine named “la pergola”; the Ampelographic Bulletin number XVI (1883) mentions the Pergolo as being one of the varieties spread over the Appignano, Venarotta and Ascoli Piceno area; and oral sources cite Pergolo as a variety found in Montelparo and Santa Vittoria in Matenan (Fermo province).

Vernaccia nera grossa, locally named Vernaccia Cerretana, was recovered in old tree lines in the municipality of Cerreto d’Esi (Macerata province) and neighbouring areas; it was enrolled in the Italian Catalogue in 2008. The oldest available citation of this variety is as a local cultivar in the

**FIGURE 4.** Reconstruction of the pedigree of the Marche varieties.

The chlorotypes (in brackets) are codified in letters according to Arroyo-García *et al*. (2006). Solid lines indicate the links inferred with present molecular data or the confirmation of previous findings. Arrows show the cross direction when possible. Well-known information from the literature not provided in this paper is represented as dotted lines.

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Ampelographic Bulletin number X (1877) by Professor Carlo Morbelli. It spread moderately throughout the Fabriano area towards the end of the 19th century, then its importance waned, and it was neglected in the viticulture reconstitution phase after the Second World War.

Our data confirm the following varieties to be PO related with Sangiovese: Capibianchi and Foglia tonda, and add a new one, Scrocchiona.

Six varieties were PO related with Sciaccarello, of which the new entries were Bersigana and Famoso. The only citation of Bersigana grapes is very recent and comes from the poet Umberto Piersanti (2008). Famoso marchigiano was at risk of extinction, but was recovered from an old vineyard in Pesaro area by the winemaker Giancarlo Soverchia in the 1980s. Famoso marchigiano, described as a synonym of Uva della Madonna in 1872 (De Bosis, 1873), was briefly described in the Ampelographic Bulletin number VI (1876) as being one of the main, white-berried varieties of the Pesaro Urbino province.

Crepolino/Visparola was shown to be a key variety in the evolution of the Marche varietal assortment, having played the role of parent and grandparent, which can be explained by the ancient presence of this genotype in the region. Nowadays, it is highly threatened (only one vine was found during sampling not included in this study) in Marche, but some plants are still grown in Tuscany, Sicily and Emilia Romagna. Crepolino/Visparola is also PO related with Vulpea. Vulpea was already recognised as a parent of many other varieties, especially in the Friuli Venezia Giulia region (Crespan et al., 2020), as well as in Croatia (Žulj Mihaljević et al., 2020), and its country of origin is thought to be Austria. The presumed origin of Crepolino/Visparola is ascribed to Greece, because of its full-sib relationship with the Greek cultivar Augustiatitis (D’Onofrio et al., 2021). Crepolino/Visparola could then have spread along two different routes from the south of the Balkans to Austria and from Sicily to the north of the Italian peninsula. The presence of its progeny in the Marche region supports the second route.

Semidano and Nuragus are two well-known Sardinian varieties which are, to our knowledge, not grown outside of the island. The first historical report on Semidano dates back to 1870, while Nuragus was cited for the first time in 1837 (Nieddu, 2011). The first-degree relationship between them is not surprising, also given their morphological resemblance. Even if inexplicable at present, molecular data give clear evidence of the role played by Semidano in the birth of Bianchetta marchigiana and Occhio nero, which are two Marche varieties, and of Druppeggio, a variety shared by the nearby regions of Tuscany, Lazio and Umbria, and which in turn is PO related to Torella. The place of origin of some Sardinian varieties is still unclear and debated; for example, Spergola, a variety grown in the northern part of the Italian Apennine area, was discovered to be synonymous with the more renowned Sardinian Vernaccia di Oristano, and recent pedigree studies confirm that this genotype was imported into Sardinia from the Italian mainland (Raimondi et al., 2020; D’Onofrio et al., 2021).

No information is available on Bianchetta marchigiana. Occhio nero may correspond to an old Marche variety called Occhietto bianco, which is briefly described in the VII Ampelographic Bulletin (1877). The cultivar Torella (meaning ‘little bull’) was grown for a long time in the countryside of Ortezzano (Fermo province) by the Piermarini family. Its name refers to a Piermarini ancestor called "bull" ('toro’ in the Italian language) due to his strength when carrying bags of wheat and also to the large and compact clusters of the grape, which comprises big, sweet and aromatic berries suitable for being dried.

The Alfredo Marchetti vine is a rare case of selfing. Its parent is Maturano bianco, a well-known variety autochthonous of the Latium region, and also grown in the Marche region under different names, like Uva d’oro, Premotico and Ulpetta. The trunk of this vigorous, ungrafted vine grows throughout the Fabriano area towards the end of the Fabriano area towards the end of the 19th century, then its importance waned, and it was neglected in the viticulture reconstitution phase after the Second World War.

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The Alfredo Marchetti vine is a rare case of selfing. Its parent is Maturano bianco, a well-known variety autochthonous of the Latium region, and also grown in the Marche region under different names, like Uva d’oro, Premotico and Ulpetta. The trunk of this vigorous, ungrafted vine grows throughout the walls of a noble house in Sirolo (Ancona) up to the second floor, and the roots reach below the underground tanks. It can be assumed that a grape seed brought by some animal, probably a bird, was dropped and generated this special vine.

Incrocio Bruni 54 was confirmed as an offspring of Sauvignon and Verdicchio, which was obtained from a grape seed brought by some animal, probably a bird, was dropped and generated this special vine.

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The Alfredo Marchetti vine is a rare case of selfing. Its parent is Maturano bianco, a well-known variety autochthonous of the Latium region, and also grown in the Marche region under different names, like Uva d’oro, Premotico and Ulpetta. The trunk of this vigorous, ungrafted vine grows throughout the walls of a noble house in Sirolo (Ancona) up to the second floor, and the roots reach below the underground tanks. It can be assumed that a grape seed brought by some animal, probably a bird, was dropped and generated this special vine.

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namely ‘Selezione Bruni 54’ from the CREA-VE repository, did not correspond to the true Incrocio Bruni 54. In 2010, only 12 ha were cultivated with Incrocio Bruni 54 throughout the Marche region (ISTAT 2010), but interest in growing this cultivar is increasing due to the excellent structure and peculiar sensorial profile (spicy hints of aromatic herbs) of its wine, which is different to that of both its parents.

Maceratino was shown to be another progeny of Verdicchio and to probably be a spontaneous cross. First cited by Rastelli (1808) and Brignoli (1809) as Maceratese, Maceratino was described by Santini (1875) as having a long list of synonyms, like Montecchiese, Greco Maceratese, Greco Montechiese, Matelicano, Ribona and Verdicchio sirolese among others. This long list testifies to its ancient and diffuse presence in the Marche region. Its resemblance to Verdicchio had already been noted both for its ampelographic and oenological traits; therefore, the molecular data confirm previous observations. Maceratino is currently quite successful as a result of new interest in the “Ribona” appellation.

Mostosa and Trebbiano abruzzese are morphologically very similar and shown to be PO related; Mostosa is also first degree related to Vaccaro. Little is known about Vaccaro: it was cited in the Ampelographic Bulletin number XVI (1883) as being one of the varieties of the Ascoli Piceno province, and oral testimonies have referred to this variety as being grown in the Campofilone and Ortezzano areas.

The pedigree of Lacrima was completed: Aleatico had already been identified as one parent (D’Onofrio et al., 2021) and as a result of the chlorotype polymorphism in the present study, it was possible to determine its role as father; our data also identified the mother, Nera Rada, a previously unknown vine. It is worth noting that Aleatico is locally known as Vernaccia di Pergola. The muscat flavour of Lacrima was clearly inherited from Aleatico, which, in turn, is one of the numerous offspring of Moscato bianco. The survey conducted by the ampelographic commission in the late 1800s in the Marche region showed a widespread presence of Aleatico in all the provinces of this region. No information was found for Nera Rada. Nowadays Aleatico is mainly cultivated in the Cesano Valley and more precisely in the Pergola area (Pesaro and Urbino province); it is the main grape variety used in the “Pergola” appellation wines, and is characterised by a rose and cherry flavour.

Combined clusters and second-degree relationships are the result of Garganega, Crepolino/Visparola and Sciaccarello crossed in different combinations - often with still unknown vines - that gave rise to new varieties; for example, Forcense is the offspring of Garganega and Crepolino/Visparola; in turn Forcense was crossed with Sciaccarello to produce Morgentino. Morgentino is listed in the Ampelographic Bulletin number XVI (1883) as Brugnentino, Moglia or Uva Moglia in Montelparo area, Servigliano, Santa Vittoria in Matenano and other synonyms in Ascoli Piceno province.

Garofanata, described during the ampelographic exhibition held in Ancona in 1872 (De Bosis, 1873), was shown to be second degree-related to Crepolino/Visparola through Famoso marchigiano.

Crepolino/Visparola is a variety of central and southern Italy. It is PO related to the Marche varieties Famoso marchigiano, Forcense and Sgranarella, as well as to Maiolica (central Italy), and the Apulian Minutolo. These findings are evidence that Crepolino/Visparola was more common in the past than it is today. Maiolica is widespread in Tuscany, where it was rediscovered as Sanforte and enrolled a second time with this name in the Italian Catalogue.

The Marche Gallioppo is one of the recognised synonyms of Maiolica, and it was widespread in the Marche in the 19th century. Maiolica was shown to be PO related to Negroamaro, an autochthonous Apulian variety not cultivated outside that region; this link and others already reported by D’Onofrio et al. (2021) highlight that Maiolica was more widespread in southern Italy in the past. The link between Marche and Apulia is also clear from the synonymy between Bombino bianco (Apulia) and Passerina (Marche).

The nine groups in the dendrogram clearly resemble those suggested by pedigree reconstruction and indicate a genetic similarity in the varieties excluded from the strictest parentage links. Group A refers to the varieties found to be first or second degree linked to Crepolino/Visparola, except for Morgentino, which is clustered with its second parent, Sciaccarello. The inclusion of Glera, Malvasia bianca lunga and Vitouska is easily explained, because Glera is one of the numerous offspring of Vulpea (Crespan et al., 2020). Group A also comprises varieties that, at a first glance, seem completely unrelated, such as Lacrima and its parents; their association with the group suggests a missing, still unknown link.
Group G clearly assembles the same varieties already linked by strict parentage relationships to Sangiovese or Sciaccarello; most of them are commonly assigned to central Italy grapevine germplasm. Pecorino is the only one that escaped pedigree reconstruction; its place in the dendrogram suggests this variety also belongs to central Italy, even if there is a missing link.

Cluster D, with Semidano as the most representative variety, surprisingly groups two additional varieties beyond the expected ones: Melata and Zunek. Zunek has been recovered in the Marche as Zivi, a denomination present in the Ampelographic Bulletin number XI (1879) of a variety grown in the province of Macerata, which was was not held in high esteem.

Group C is another solid group which is 100% supported by bootstrap values. Group C links Maturano bianco and its selfing progeny Alfredo Marchetti to Cocaccia, Fava and Gabbavolpe. In the Ampelographic Bulletin number XVI (1883) Cocaccia and Uva Fava are listed as varieties of the Ascoli Piceno and Fermo provinces.

Grero is the only stand alone variety.

CONCLUSIONS

One third of the ampelographic assortment of Marche is characteristic of this region. It originated partly from already well-known founders or main parents, like Sangiovese, Garganega and Sciaccarello, and partly from a recently discovered founder for this region, Crepolino/Visparola. A surprising link with the Sardinian Semidano was revealed by the molecular analyses, but no information is available at this time to explain this finding. Incrocio Bruni 54 was confirmed to be the progeny of Verdicchio and Sauvignon, as declared by the breeder.

Given previous experiences, like for Pecorino and more recently for Garofanata, we are confident that local grapevine biodiversity, with its territorial exclusivity and qualitative characteristics, can be a valuable resource for farms that need to create new marketing spaces within an increasingly demanding and competitive market. Shortly ASSAM and CREA will proceed with the ampelographic, agronomic and oenological characterisation of the still undescribed varieties to identify the best performing ones that may eventually be enrolled in the Italian Catalogue.

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REFERENCES

Armanini, A.B., Stanzione, G., & Storchi, P. (2008). Crepolino. In: Il germoplasma viticolo della Toscana. 2. Vitigni ad uva bianca. ARSIA Regione Toscana, 74–78.

Arroyo-Garcia, R., Ruiz-Garcia, L., Bolling, L., Ocete, R., López, M.A., Arnold, C., Ergul, A., Söylemezoğlu, G., Uzn, H.I., Cabello, F., Ibáñez, J., Aradhya, M.K., Atanassov, A., Atanassov, I., Balint, S., Cenis, J.L., Costantini, L., Goris-Lavets, S., Grando, M.S., Klein, B.Y., McGovern, P.E., Merdinoğlu, D., Pejić, I., Pelsy, F., Primikirios, N., Risovannaya, V., Roubelakis-Angelakis, K.A., Snoussi, H., Sotiri, P., Tamhankar, S., This, P., Troshin, L., Malpica, J.M., Lefort, F., & Martinez-Zapater, J.M. (2006). Multiple origins of cultivated grapevine (Vitis vinifera L. ssp. sativa) based on chloroplast DNA polymorphisms. Mol. Ecol. 15(12), 3707–14. https://doi.org/10.1111/j.1365-294X.2006.03049.x

Bandinelli, R., Boselli, M., & Pisani, P.L. (2005). Tenerone. In: Il germoplasma viticolo della Toscana. 1. Vitigni ad uva nera. ARSIA Regione Toscana, 242–245.

Bergamini, C., Perniola, R., Cardone, M.F., Gasparro, M., Pepe, R., Caputo, A.R., & Antonacci, D. (2016). The molecular characterization by SSRs reveals a new South Italian kinship and the origin of the cultivar Uva di Troia. Springerplus, 5, 1562. https://doi.org/10.1186/s40064-016-3228-8

Brignoli, B. (1809). Istruzione sul miglioramento de’ vini del dipartimento del Metauro. Ancona, Per Arcangelo e figlio Sartori.

Carimi, F., Mercati, F., Abbate, L., & Sunseri, F. (2010). Microsatellite analyses for evaluation of genetic diversity among Sicilian grapevine cultivars. Genet. Resour. Crop Evol., 57, 703–719. https://doi.org/10.1007/s10722-009-9506-3

Cipriani, G., Spadotto, A., Jurman, I., Di Gaspero, G., Crespan, M., Meneghetti, S., Frare, E., Vignani, R., Cresti, M., Morgante, M., Pezzotti, M, Pè, E., Policriti, A., Testolin, R. (2010). The SSR-based molecular profile of 1005 grapevine (Vitis vinifera L.) accessions uncovers new synonymy and parentages, and reveals a large admixture amongst varieties of different geographic origin. Theor. Appl. Genetics, 121(8): 1569–1585. https://doi.org/10.1007/s00122-010-1411-9

Costantino, L., Monaco, A., Vouillamoz, J.F., Forlani, M., & Grando, M.S. (2005). Genetic relationships among local Vitis vinifera cultivars from Campania (Italy). Vitis, 44(1), 25–34. https://doi.org/10.5073/vitis.2005.44.25-34

Crespan, M., Cancelleri, S., Chies, R., Giannetto, S., Meneghetti S. (2006). Individuati i genitori del Raboso veronese: una nuova ipotesi sulla sua origine.
(The parents of Raboso veronese were discovered: a new hypothesis on its origin). *Riv. Vit. Enol.*, 1, 3–12.

Crespin, M., Crespin, G., Giannetto, S., Meneghetti, S., & Costacurta, A. (2007). “Vitouska” is the progeny of “Prosecco tondo” and “Malvasia bianca lunga”. *Vitis*, 46(4), 192–194. https://doi.org/10.5073/vitis.2007.46.192-194

Crespin, M., Calò, A., Giannetto, S., Sparacio, A., Storchi, P., & Costacurta, A. (2008). ‘Sangiovese’ and ‘Garganega’ are two key varieties of the Italian grapevine assortment evolution. *Vitis*, 47(2), 97–104. https://doi.org/10.5073/vitis.2008.47.97-104

Crespin, M., Fabbro, A., Giannetto, S., Meneghetti, S., Petrucci, C., Del Zan, F., & Sivilotti, P. (2011). Recognition and genotyping of minor germplasm of Friuli Venezia Giulia revealed high diversity. *Vitis*, 50(1), 21–28. https://doi.org/10.5073/vitis.2011.50.21-28

Crespin, M., Carraro, R., Giust, M., & Migliaro, D. (2016). The origin of Termarina cultivar, another grapevine (*Vitis vinifera* L.) parthenocarpic somatic variant. *Australian Journal of Grape and Wine Research*, 22(3), 489–493. https://doi.org/10.1111/ajgw.12236

Crespin, M., Migliaro, D., LARGER, S., Pindo, M., Petrucci, C., Stocco, M., Rusjan, D., Sivilotti, P., Velasco, R., & Maul, E. (2020). Unraveling the genetic origin of ‘Glera’, ‘Ribolla gialla’ and other autochthonous grapevine varieties from Friuli Venezia Giulia (northeastern Italy). *Scientific Reports*, 10, 7206. https://doi.org/10.1038/s41598-020-64061-w

De Bosis, F. (1873). La esposizione ampelografica marchigiana-abruzzese tenuta in Ancona il settembre 1872 e studi sulla vite e sul vino della provincia anconitana. Tipografia del Commercio, Ancona, Italy.

De Lorenzis, G., Las Casas, G., Brancadoro, L., & Sciencia, A. (2014). Genotyping of Sicilian grapevine germplasm resources (*V. vinifera* L.) and their relationships with Sangiovese. *Scientia Horticulturae*, 169, 189–198. https://doi.org/10.1016/j.scienta.2014.02.028

De Lorenzis, G., Mercati, F., Bergamini, C., Cardone, M. F., Lupini, A., Mauceri, A., Caputo, A.R., Abbate, L., Barbagallo, M.G., Antonacci, D., Sunseri, F., & Brancadoro, L. (2019). SNP genotyping elucidates the genetic diversity of Magna Græcia grapevine germplasm and its historical origin and dissemination. *BMC Plant Biol.*, 19, 7. https://doi.org/10.1186/s12870-018-1576-y

De Mattia, F., Imazio, S., Grassi, F., Lovicu, G., Tardagiuila, J., Failla, O., Maitt, C., Sciencia, A., & Labra, M. (2007). Genetic characterization of Sardinia grapevine cultivars by SSR markers analysis. *Journal International des Sciences de la Vigne et du Vin*, 41(4), 175–184. https://doi.org/10.20870/oeno-one.2007.41.4.837

Di Guardo, M., Micheletti, D., Bianco, L., Koehorst-van Putten, H.J.J., Longhi, S., Costa, F., Aranzana, M.J., Velasco, R., Arus, P., Troggio, M., & van de Weg, E.W. (2015). ASSiST: An Automatic SNP ScorIng tool for in- and out-breeding species. *Bioinformatics*, 31(23), 3873–4. https://doi.org/10.1093/bioinformatics/btv446

Di Vecchi-Staraz, M., Bandinelli, R., Boselli, M., This, P., Boursiquot, J.M., Laucou, V., Laccombe, T., & Varèd, D. (2007). Genetic structuring and parentage analysis for evolutionary studies in grapevine: Kin group and origin of the cultivar Sangiovese revealed. *J. Amer. Soc. Hort. Sci.* 132, 514–524. https://doi.org/10.21273/JASHS.132.4.514

D’Onofrio, C., Fausto, C., Matarase, F., Materazzi, A., Scalabrelli, G., Fiorani, F., Poli, I. (2016). Genotyping of grapevine varieties from Garfagnana (Northern Tuscany): evidence of a historical center of diversity. *American Journal of Enology and Viticulture* 67, 120–126. https://doi.org/10.5344/ajev.2015.15046

D’Onofrio, C., Tumino, G., Gardiman, M., Crespin, M., Bignami, C., De Palma, L., Barbagallo, M.G., Muganu, M., Morcia, C., Novello, V., Schneider, A., & Terzi, V. (2021). Parentage atlas of Italian grapevine varieties as inferred from SNP genotyping. *Front. Plant Sci.*, 11, 2265. https://doi.org/10.3389/fpls.2020.605934

Felici, C. (1986). Scritti Naturalistici I. “Dell’Insalata e piante che in qualunque modo vengono per cibo del’homo”, a cura di Guido Arbizzoni, Edizioni QuattroVenti, Urbino

Grando, M.S., & Frisinghelli, C. (1998). Grape microsatellite markers: sizing of DNA alleles and genotype analysis of some grapevine cultivars. *Vitis*, 37(2), 79–82. http://hdl.handle.net/10449/16291

Gristina, A.S., De Michele, R., Garfi, G., La Mantia, T., Fontana, I., Spinelli, P., Motisi, A., & Carimi, F. (2017). Urgent need for preservation of grapevine (*Vitis vinifera* L. subsp. *vinifera*) germplasm from small circumsicilian islands as revealed by SSR markers and traditional use investigations. *Genetic Resources and Crop Evolution*, 64(6), 1395–1415. https://doi.org/10.1007/s10722-016-0444-6

Kalinowski, S.T., Taper, M.L., & Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* 16, 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35, 1547–1549. https://doi.org/10.1093/molbev/msy096

Laccombe, T., Boursiquot, J. M., Laucou, V., Di Vecchi-Staraz, M., Peros, J. P., & This, P. (2013). Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.). *Theoretical and Applied Genetics*, 126, 401–414. https://doi.org/10.1007/s00122-012-1988-2
Laucou, V., Launay, A., Bacilleri, R., Lacombe, T., Adam-Blondon, A. F., Bérard, A., Chauveau, A., de Andres, M.T., Hausmann, L., Ibañez, J., Le Paslier, M.C., Maghradze, D., Martinez-Zapater, J.M., Maul, E., Ponnaiah, M., Toepfer, R., Peros, J.P., Boursiquot, J.M. (2018). Extended diversity analysis of cultivated grapevine Vitis vinifera with 10K genome-wide SNPs. *PLoS One* 13, e0192540. https://doi.org/10.1371/journal.pone.0192540

Le Paslier, M.C., Choisne, N., Bacilleri, R., Bounon, R., Boursiquot, J.M., Bras, M., Brunel, D., Di Gaspero, G., Hausmann, L., Lacombe, T., Laucou, V., Launay, A., Martinez-Zapater, J.M., Morgante, M., Raj, P.M., Ponnaiah, M., Queuneville, H., Scalabrin, S., Torres-Perez, R., Adam-Blondon, A.F. (2013). The GrapeReSeq 18 k Vitis genotyping chip. In 9th International Symposium Grapevine Physiology and Biotechnology: International Society for Horticultural Science, p. 123.

Maul, E., Sudharma, K.N., Kecke, S., Marx, G., Müller, C., Audeguin, L., Boselli, M., Boursiquot, J.M., Bucchetti, B., Cabello, F., Carraro, R., Crespan, M., de Andrés, M.T., Eiras Dias, J., Ekhvaia, J., Gaforio, L., Gardiman, M., Grando, S., Gyropoulos, D., Margadade, D., Marinoni, D., Maletic, E., Moreira, F., Muñoz-Organero, G., Nakhutsrishvili, G., Pepe, I., Peterlunger, E., Pitsoli, D., Pospisilova, D., Preiner, D., Raimondi, S., Regner, F., Savin, G., Savvides, S., Schneider, A., Sereno, C., & Simon, S. (2012). The European Vitis Database (www.eu-vitis.de) – a technical innovation through an online uploading and interactive modification system. *Vitis*, 51, 79–86. https://doi.org/10.5073/VITIS.2012.51.79-85

Mercati, F., De Lorenzis, G., Brancadoro, L., Lupini, A., Abenavoli, M.R., Barbagallo, M.G., Di Lorenzo, R., Scienza, A., & Sunseri, F. (2016). Genetic characterization of grape cultivars from Sicily. *Tree Genetics & Genomes*, 12, 59. https://doi.org/10.1007/s11295-016-1021-z

Migliaro, D., Morreale, G., Gardiman, M., Landolfo, S., Crespan, M. (2013). Direct multiplex PCR for grapevine genotyping and varietal identification. *Plant Genetic Resources: Characterization and Utilization* 11(2), 182–185. https://doi.org/10.1017/S1479262112000433

Myles, S., Chia, J.M., Hurwitz, B., Simon, C., Zhong, G.Y., Buckler, E., & Ware, D. (2010). Rapid genomic characterization of the genus Vitis. *PLoS One*, 5, e8219. https://doi.org/10.1371/journal.pone.0008219

Myles, S., Boyko, A. R., Owens, C. L., Brown, P. J., Grassi, F., Aradhya, M. K., Prings, B., Reynolds, A., Chiaj, J.M., Warech, D., Bustamante, C.D., & Buckler, E.S. (2011). Genetic structure and domestication history of the grape. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3530–3535. https://doi.org/10.1073/pnas.1009363108

Nieddu, G. (2011). Vitigni della Sardegna. Notizie storiche, distribuzione geografica, sinonimi. Ed. CONVISAR.

Pastore, C., Fontana, M., Raimondi, S., Ruffa, P., Filippetti, I., & Schneider, A. (2020). Genetic characterization of grapevine varieties from Emilia-Romagna (Northern Italy) discloses unexplored genetic resources. *American Journal of Enology and Viticulture*, 71, 334–344. https://doi.org/10.5344/ajev.2020.19076

Peakall, R., & Smouse, P.E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics*, 28, 2537–2539 (2012). https://doi.org/10.1093/bioinformatics/bts460

Piersanti, U. (2008). L'albero delle nebbie. Einaudi Ed., Torino

Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., & Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population based linkage analyses. *Am J Hum Genet*, 81, 559–575. https://doi.org/10.1086/519795

Raimondi, S., Tumino, G., Ruffa, P., Bocacci, P., Gambino, G., & Schneider, A. (2020). DNA-based genealogy reconstruction of Nebbiolo, Barbera and other ancient grapevine cultivars from northwestern Italy. *Sci Rep*, 10, 15782. https://doi.org/10.1038/s41598-020-72799-6

Rastelli, A.A. (1808). Il dottore della villa su tutti i principali oggetti dell’agricoltura. Tomo I. Stamperia Bonelli, Jesi.

Ruffa, P., Raimondi, S., Bocacci, P., Abbà, S., & Schneider, A. (2016). The key role of “Moscato bianco” and “Malvasia aromatica di Parma” in the parentage of traditional aromatic grape varieties. *Tree Genetics & Genomes* 12, 1–14. https://doi.org/10.1007/s11295-016-1006-y

Santini, S. (1875). Ampelografia del Circondario di Macerata (Estratto dall’Economista delle Marche anno V N. 3-4) Tipografia dei Fratelli Mancini, Macerata.

Schneider, A., Raimondi, S., Pirola, C.S., Marinoni, D.T., Ruffa, P., Venerito, P., & La Notte, P. (2014). Genetic characterization of grape cultivars from Apulia (Southern Italy) and synonyms in other Mediterranean regions. *American Journal of Enology and Viticulture*, 65(2), 244–249. https://doi.org/10.5344/ajev.2013.13082

Torello Marinoni, D., Raimondi, S., Ruffa, P., Lacombe, T., & Schneider, A. (2009). Identification of grape cultivars from Liguria (north-western Italy). *Vitis*, 48(4), 175–183. https://doi.org/10.5073/vitis.2009.48.175-183

Žulj Mihaljević, M., Maletić, E., Preiner, D., Zdunić, G., Bubola, M., Zyprian E., & Pejić, I. (2020). Genetic diversity, population structure, and parentage analysis of Croatian grapevine germplas.