


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.

KEY WORDS

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 *Vitis*18kSNP 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 *Vitis*9kSNP, 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 *Vitis*18kSNP, 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 *Vitis*18kSNP 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

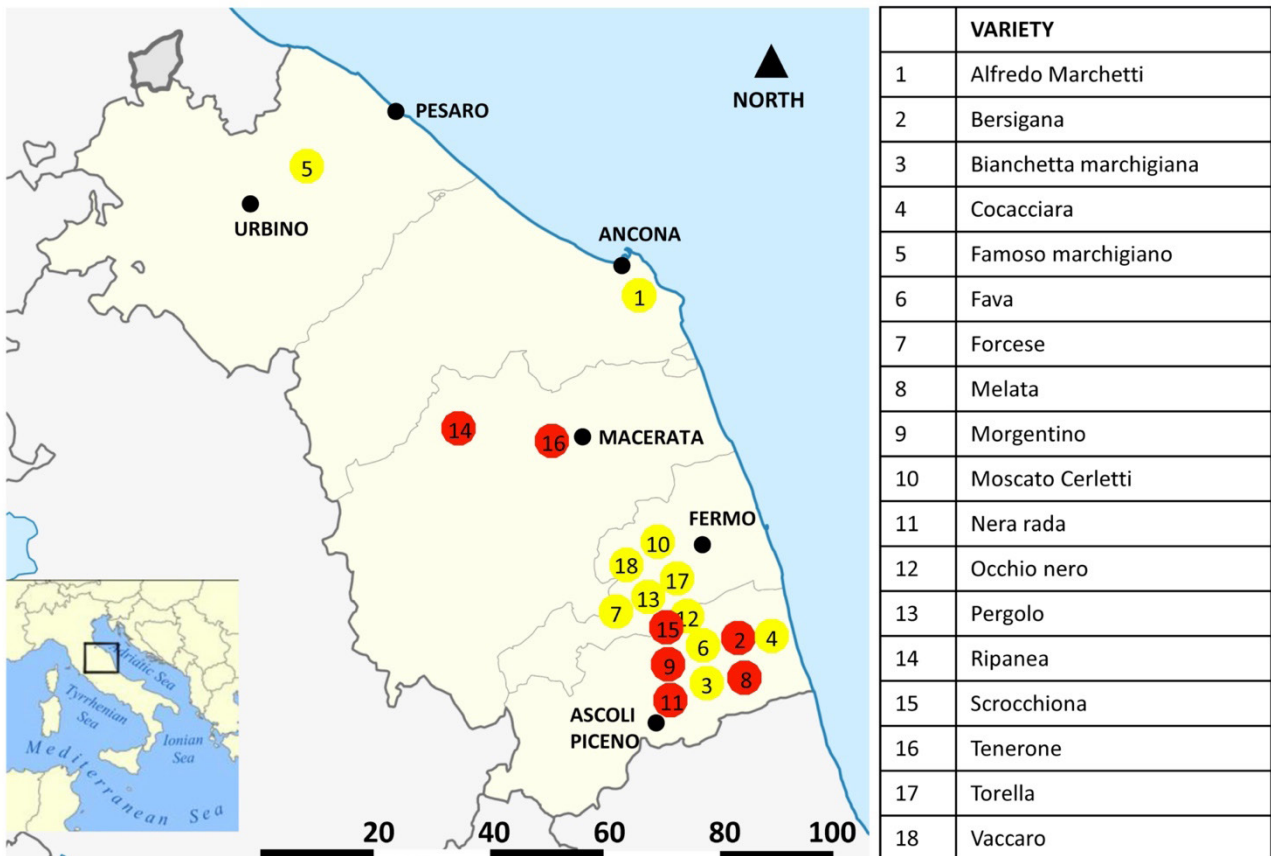


FIGURE 1. Sites of recovery in Marche region of the 18 grapevine varieties lacking the SSR profile in the *VIVC*, which are not yet enrolled in the Italian catalogue and in bold in Table 1.

Circle colours correspond to accession berry colour, black (red circles) or white (yellow circles).

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 VMCG4b9 (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	<i>VIVC</i> variety number	<i>IC</i> code
Bianca (D'Onofrio)	Pedaso	Afus Ali (Regina)	122	527
Uva bianca (Zaghi)	Sirolo	Alfredo Marchetti		
Negrì o Neretto di Piobbico (Ulivello)	Sarnano	Barbera	974	19
Rossa acino ovale (Rossi)	Falerone			
Bersigiana	ASSAM repository	Bersigiana		
Cacciù 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 blanc (Chasselas dorato)	2473	509
Morettone (Remia)	Montegiorgio			
Cimiciola F38	ASSAM repository			
Gaglioppa (Capecci)	Ripatransone	Ciliegiolo	2660	62
Morettone	ASSAM repository			
Morettone (Capecci)	Ripatransone			
Centenaria (Sgariglia)	Acquaviva	Cornichon blanc (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 (Ubaldi)	Montefalcone App.	Forcese		
Forconese	ASSAM repository			
Cotrognone	ASSAM repository			
Moscattellone (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			

Gallioppo (Silvestri)	Cossignano			
Gallioppo 75	ASSAM repository			
Gallioppo GE	ASSAM repository	Maiolica	7136	126
Gallioppo GE F12 I9 P2	ASSAM repository			
Malvasia bianca lunga (La Pila)	Montegiorgio			
Malvasia? (Montalbini)	Arcevia			
P1 (Scarabotti)	Recanati			
P2 (Scarabotti)	Recanati	Malvasia bianca di Candia	23555	131
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			
Ulpetta vecchia (Sgariglia)	Acquaviva	Maturano bianco	23347	424
Uva cane	ASSAM repository			
Melata Savini 2 (Silvestri)	Cossignano			
(Uva) Melata	ASSAM repository	Melata		
Granarello	ASSAM repository	Merlot	7657	146
Rossa foglia (Mattei Verde)	Arcevia	Montepulciano	7949	150
Chiapparù	ASSAM repository			
Uva Regno	ASSAM repository	Montonico bianco	7960	151
(Uva) Moie	ASSAM Petritoli	Morgentino		
Uva Moscata p 2 Rossi	Falerone	Moscato Cerletti		
Bianca (Zanoni)	Pedaso			
Moscanello 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
Moscanello 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			
Cacciù bianco (Spreca)	Lapedona	Mostosa	8075	157
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		

Pianta 4 (Giammarini)	Marina Palmense			
Rossa foglia (Mattei Rossa)	Arcevia	Petit Bouschet	1619	
Cornetta? (Ulivello)	Magliano di Tenna	Pizzutello nero	9524	
Pianta 1 (Giammarini)	Marina Palmese			
Pianta 2 (Giammarini)	Marina Palmese	Plavina crna	9557	893
Cacciù nero	ASSAM repository			
Uva Rossa (Serboni)	Serrapetrona	Ripanea		
Balsamina	ASSAM repository			
Pianta 1 entrata (Beato)	Montelparo			
Sangiovese	Ripatransone	Sangiovese	10680	218
Uva Rosata pianta 3 (Rossi)	Falerone			
Rossa croccante	ASSAM repository	Scrocchiona		
Ciciu	ASSAM repository			
Ciciu F42	ASSAM repository			
Cimicino	ASSAM repository			
Pianta 3 bosco 1 (Beato)	Montelparo	Sgranarella	26656	900
Pianta 4 bosco 2 (Beato)	Montelparo			
Pianta 5 bosco 3 (Beato)	Montelparo			
Grugninti (Fattobene) campo	Pollenza			
Grugninti (Fattobene) argine	Pollenza	Tenerone		
Tinturina (Ubaldi)	Montefalcone App.			
Uva nera (Virgili)	Monte San Martino	Terrano	12374	233
(Uva) torella (Piermarini)	Ortezzano	Torella		
San Niccolò DA 1 (ITAS Vivarelli)	Fabriano			
Uva Fico	ASSAM repository	Trebbiano toscano	12628	244
Vite Bastarda (Ulivello)	Monte Urano	Uva Tosca	12836	249
Cacciù (Capecci)	Ripatransone			
Vaccaro	ASSAM repository	Vaccaro		
Vaccù	ASSAM repository			
Bianca precoce (Colasanti)	ASSAM repository			
Vummi	ASSAM repository	Verdicchio	12963	254
Bordoletto (Duri)	Serrapetrona	Vernaccia di S. Gimignano	12998	261
Brugentile	ASSAM repository			
Brugentile N1	Cerreto d'Esì	Vernaccia nera grossa	23042	415
Brugentile Casanao 2	Coccure			
Uva francese (Ulivello)	Fermo			
Uva Bianca pianta 1 (Rossi)	Falerone			
Bianca resistente (Cossignani)	Montefiore dell'Aso	Villard blanc	13081	
Bianca (Potentini)	Porto Recanati			
Uva Bianca (Marchetti)	Cupra Marittima			
Zivi (Milanesi)	Montefalcone App.	Zunek	17739	
Zivi F41	ASSAM repository			

The recovery site is indicated, when available. The prime names of the varieties in the *VIVC* 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 *VIVC* 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 GenAIEx 6.5 software (Peakall and Smouse, 2012), and in the *VIVC* 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-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: API 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 unrooted 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 (IVC), 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 Bianchettas (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. Scrocchiona 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 weingarden, 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 toscana), 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 Madere. 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), Ciliegiole (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. Cacciù 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	Scacco	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	Morgentino	(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

41	Negroamaro	Negroamaro	B	CREA	Italy
42	Nera rada	(Uva) nera rada	B	ASSAM	Italy
43	Nuragus	Nuragus	W	CREA	Italy
44	Occhio nero	Occhio nero	W	ASSAM	Italy
45	Pecorino	Pecorino	W	CREA	Italy
46	Pergolo	Pergolo	W	ASSAM	Italy
47	Quagliano	Quagliano	B	CREA	Italy
48	Ripanea	Uva rossa (Serboni)	B	ASSAM	Italy
49	Sangiovese	Sangiovese	B	CREA	Italy
50	Sauvignon	Sauvignon	W	CREA	France
51	Sciaccarello	Verano	B	CREA	Italy
52	Sciaccarello	Termarina	B	CREA	Italy
53	Scrocchiona	Rossa croccante	B	ASSAM	Italy
54	Semidano	Semidano	W	CREA	Italy
55	Sgranarella	Vesprino	W	CREA	Italy
56	Somarello rosso	Somarello rosso	R	CREA	Italy
57	Tenerone	Grugninti	R	ASSAM	Italy
58	Torella	(Uva) Torella Piermarini	W	ASSAM	Italy
59	Trebbiano abruzzese	Trebbiano abruzzese	W	CREA	Italy
60	Trebbiano perugino	Trebbiano perugino	W	CREA	Italy
61	Trebbiano toscano	Trebbiano toscano	W	CREA	Italy
62	Vaccaro	Vaccaro	W	ASSAM	Italy
63	Verdicchio	Verdicchio	W	CREA	Italy
64	Vernaccia nera	Vernaccia nera	B	CREA	Italy
65	Vernaccia nera grossa	Vernaccia nera grossa (di Cerreto)	B	CREA	Italy
66	Vulpea	Quaiara	B	CREA	Austria
67	Zunek	Zivi	W	ASSAM	Slovenia
Additional varieties					
68	<i>Glera</i>		W	CREA	Italy
69	<i>Manzoni bianco</i>		W	CREA	Italy
70	<i>Marzemina bianca</i>		W	CREA	Italy
71	<i>Pinot</i>			CREA	France
72	<i>Raboso Piave</i>		B	CREA	Italy
73	<i>Raboso veronese</i>		B	CREA	Italy
74	<i>Riesling weiss</i>		W	CREA	Germany
75	<i>Vitouska</i>		W	CREA	Italy

Berry colour: B = black, W = white, R = red, P = pink. CREA: CREA Viticulture and Enology, Susegana (TV), Italy; ASSAM: ASSAM, Petritoli (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 Sciacarello, 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 Sciacarello 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 Sciacarello 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 Armanni *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), Sciacarello (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-offspring 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		
Crepolino/ Visparola	Famoso marchigiano	0.0109	0.9853	0.0038	0.4964	6	present paper
	Forcese	0.0237	0.8722	0.1041	0.5402	13	present paper
	Maiolica	0.018	0.982	0	0.491	10	D'Onofrio <i>et al.</i> , 2021
	Minutolo	0.0146	0.9551	0.0303	0.5079	8	Lacombe <i>et al.</i> , 2013
	Vesprino	0.0218	0.9594	0.0188	0.4985	12	present paper
	Vulpea	0.0218	0.8395	0.1387	0.5584	12	D'Onofrio <i>et al.</i> , 2021
Famoso marchigiano	Garofanata	0.0091	0.942	0.0489	0.5199	5	present paper
Fava	Gabbavolpe	0.0218	0.9506	0.0275	0.5028	12	present paper
Forcese	Moscianello	0.0218	0.9612	0.017	0.4976	12	present paper
Garganega	Empibotte	0.0255	0.8927	0.0818	0.5282	14	Crespan <i>et al.</i> , 2008
	Forcese	0.0164	0.91	0.0736	0.5286	9	present paper
	Malvasia bianca di Candia	0.0109	0.925	0.0641	0.5266	6	Di Vecchi-Staraz <i>et al.</i> , 2007
	Marzemina bianca	0.0309	0.9613	0.0077	0.4884	17	Crespan <i>et al.</i> , 2008
	Montonico bianco	0.0109	0.9279	0.0611	0.5251	6	Crespan <i>et al.</i> , 2008
	Pergolo	0.0091	0.9202	0.0707	0.5308	5	present paper
	Somarello rosso	0.0109	0.8704	0.1187	0.5539	6	Di Vecchi-Staraz <i>et al.</i> , 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 <i>et al.</i> , 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 <i>et al.</i> , 2021
Montepulciano	Bombino bianco	0.0218	0.9393	0.0389	0.5085	12	Lacombe <i>et al.</i> , 2013
Sciaccarello	Bersigana	0.0146	0.9633	0.0222	0.5038	8	present paper
	Caloria	0.0237	0.95	0.0263	0.5013	13	Di Vecchi-Staraz <i>et al.</i> , 2007
	Famoso	0	1	0	0.5	7	D'Onofrio <i>et al.</i> , 2021
	Tenerone	0	1	0	0.5	13	present paper
	Livornese/Rollo	0.0127	0.9348	0.0524	0.5198	7	Di Vecchi-Staraz <i>et al.</i> , 2007
	Muscat rouge de Madere	0.0109	0.9891	0	0.4946	6	Di Vecchi-Staraz <i>et al.</i> , 2007
Sangiovese	Capibianchi	0.0218	0.9513	0.0268	0.5025	12	Di Vecchi-Staraz <i>et al.</i> , 2007
	Foglia tonda	0.02	0.9207	0.0593	0.5197	11	Crespan <i>et al.</i> , 2008
	Scrocchiona	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	Drupeggio	0.0255	0.9252	0.0493	0.5119	14	present paper
Mostosa	Trebbiano abruzzese	0.0182	0.8962	0.0856	0.5337	10	present paper
	Vaccaro	0.0273	0.8334	0.1393	0.556	15	present paper
Verdicchio	Maceratino	0.02	0.976	0.0039	0.492	11	Lacombe <i>et al.</i> , 2013

Expected values for PO relationships: Z0 = 0, Z1 = 1, Z2 = 0, PI_HAT = 0.5.

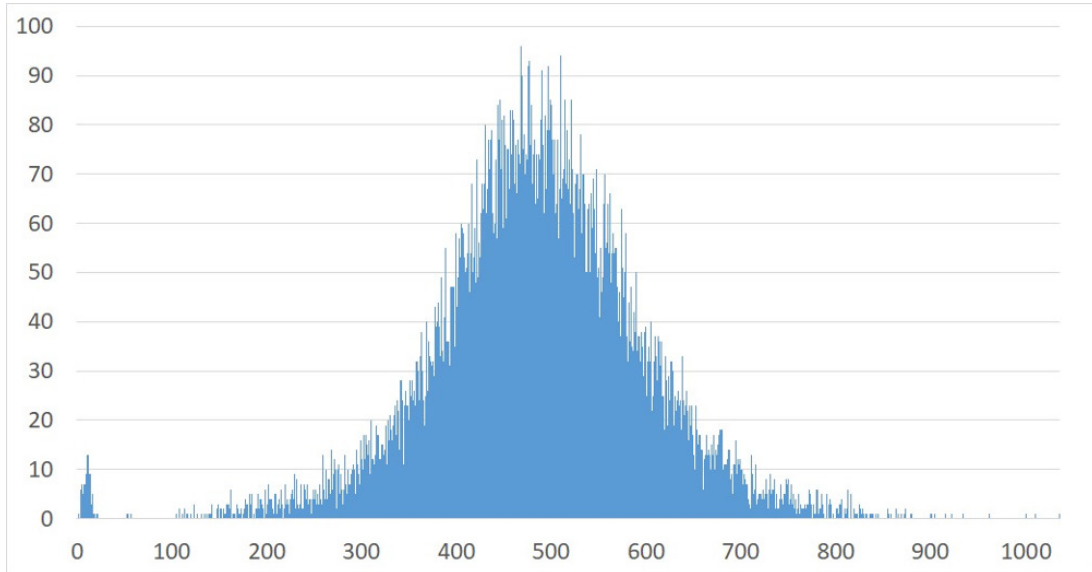


FIGURE 2. Distribution of Mendelian inconsistencies computed on pairs of samples in the larger set of 192 unique genotypes.

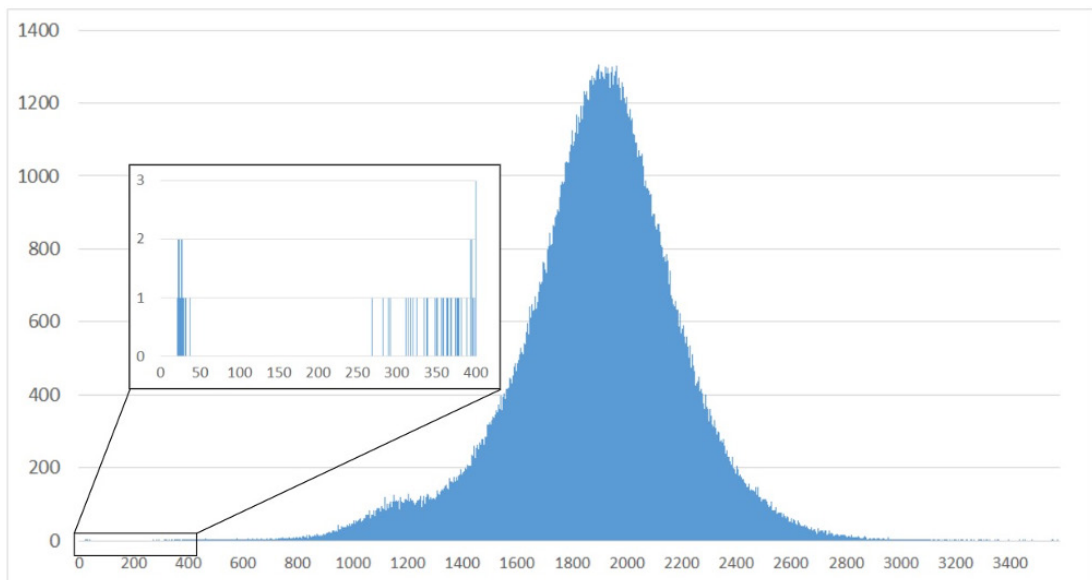


FIGURE 3. Distribution of Mendelian inconsistencies computed on first degree related genotypes combined in all possible parent-parent-offspring trios.

to be derived from selfing of Maturano bianco. Bombino nero was confirmed to be the progeny of Bombino bianco \times Quagliano (Bergamini *et al.*, 2016); Ciliegiolo was a spontaneous cross between Sangiovese and Muscat rouge de Madere; Forcese derived from Garganega \times Crepolino/Visparola; Incrocio Bruni 54 was confirmed as a cross between Verdicchio and Sauvignon; Lacrima derived from Aleatico \times 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		0.0091	0.9459	0.045	0.518	18	Bergamini <i>et al.</i> , 2016
		Quagliano	0.0237	0.9461	0.0302	0.5033		
Ciliegiolo	Bombino bianco	Quagliano					24	Di Vecchi-Staraz <i>et al.</i> , 2007
	Sangiovese	Muscat rouge de Madere	0.0218	0.978	0.0002	0.4892		
Forcese	Sangiovese	Muscat rouge de Madere	0.0237	0.9596	0.0168	0.4965	22	present paper
	Garganega	Crepolino	0.0164	0.91	0.0736	0.5286		
Incrocio Bruni 54	Garganega	Crepolino	0.0237	0.8722	0.1041	0.5402	35	present paper
	Verdicchio	Sauvignon	0.0382	0.9361	0.0257	0.4937		
Lacrima	Verdicchio	Sauvignon	0.0255	0.9672	0.0072	0.4909	22	D'Onofrio <i>et al.</i> , 2021
	Aleatico	Nera rada	0.0182	0.8999	0.0819	0.5319		
Morgentino	Aleatico	Nera rada	0.0219	0.9064	0.0718	0.5249	26	present paper
	Forcese	Sciaccarello	0	1	0	0.5		
Reference trios	Forcese	Sciaccarello	0.0255	0.9275	0.047	0.5108	22	present paper
	Manzoni bianco	Pinot	0.0109	0.9871	0.002	0.4955		
Raboso veronese	Pinot	Riesling weiss	0.0401	0.8672	0.0927	0.5263	31	Crespan <i>et al.</i> , 2006
	Raboso Piave	Marzemina bianca	0	1	0	0.5		
Vitouska	Raboso Piave	Marzemina bianca	0.0252	0.9748	0	0.4874	24	Crespan <i>et al.</i> , 2007
	Malvasia bianca lunga	Glera	0.0273	0.8543	0.1184	0.5456		
	Malvasia bianca lunga	Glera	0.0164	0.9155	0.0682	0.5259		

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, Bersigana, Bianchetta marchigiana, Cocacciara, Famoso marchigiano, Fava, Forcese, Melata, Morgentino, Nera rada, Occhio nero, Pergolo, Ripanea, Scrocchiona, 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 perugino, 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 Matenano (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

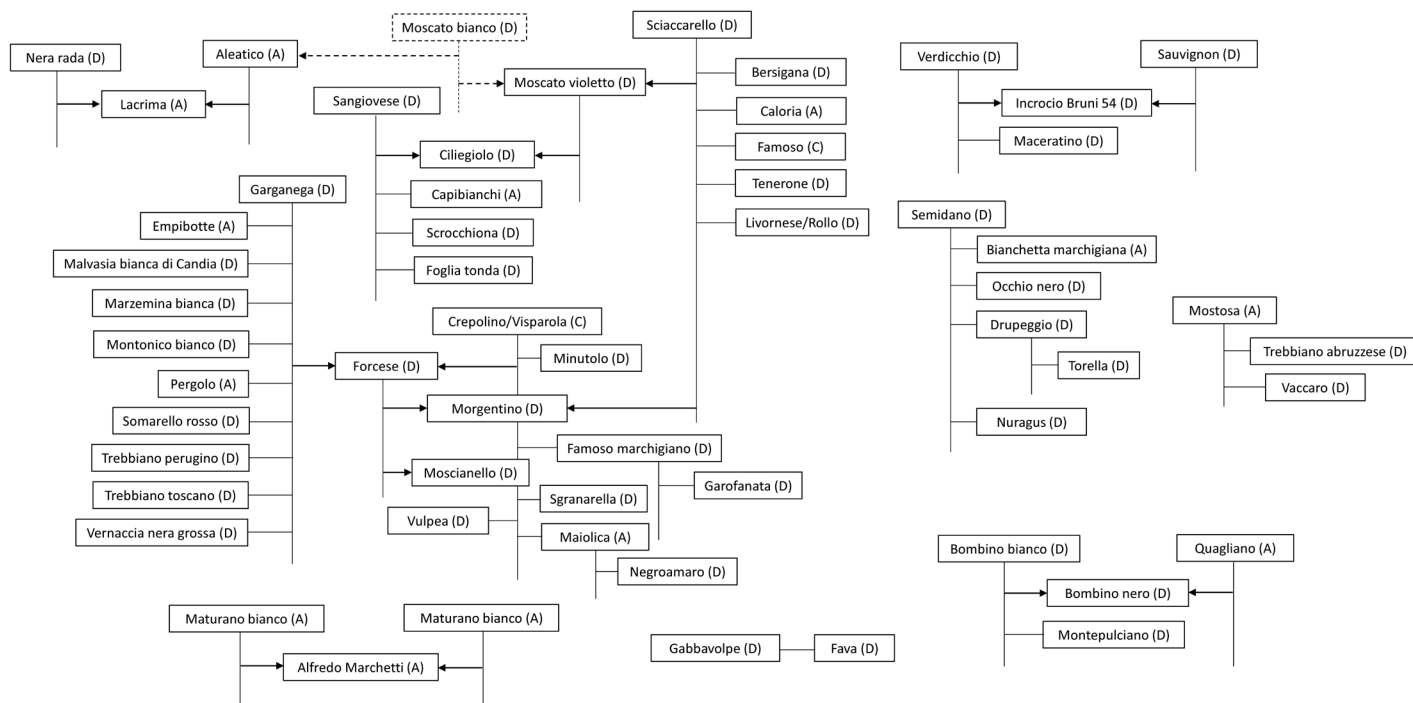
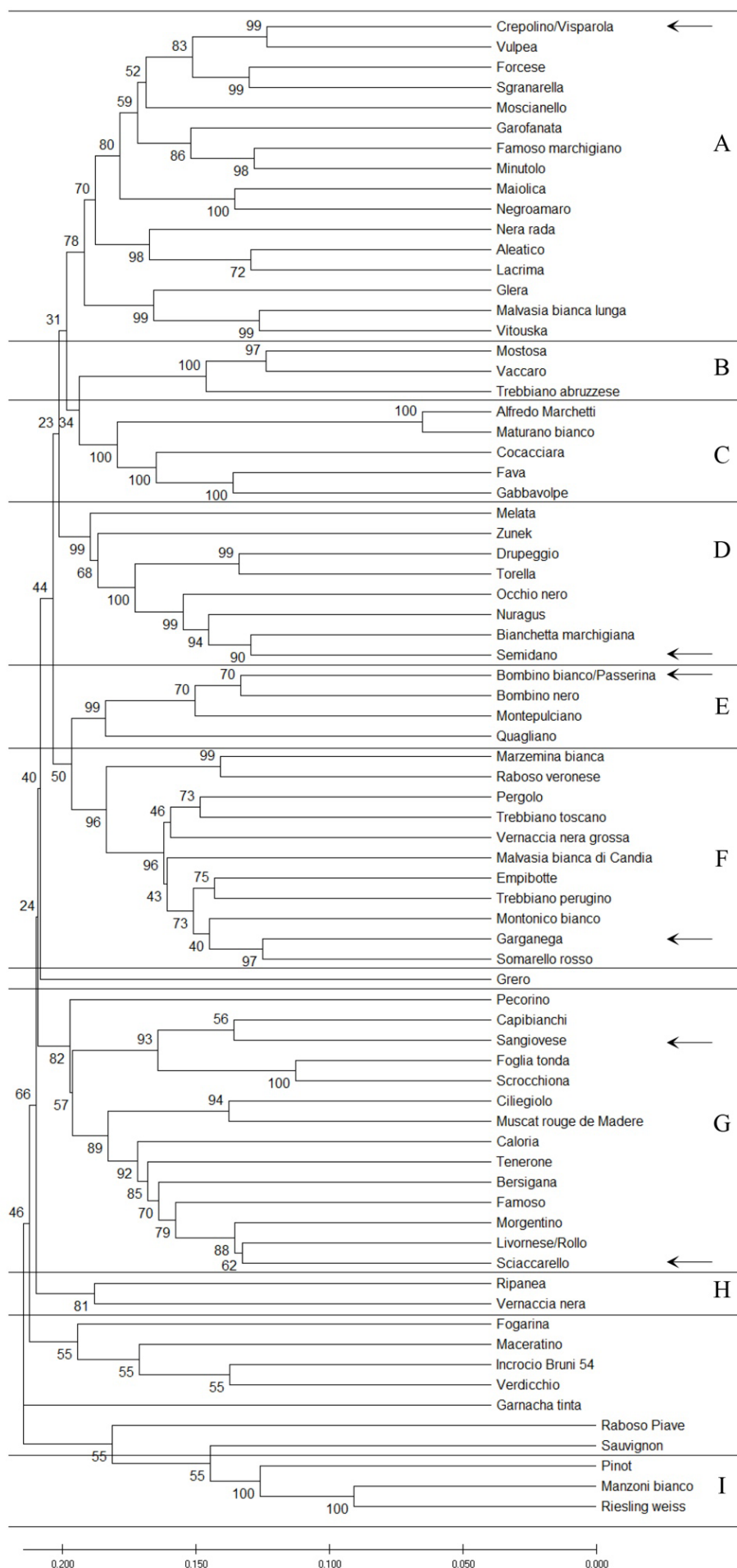


FIGURE 4. Reconstruction of the pedigree of the Marche varieties.

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.



◀ **FIGURE 5.** Unrooted optimal dendrogram for 74 varieties using the UPGMA method and a bootstrap test of 2,000 replicates (related values are shown next to the branches).

Pairwise distances were computed using the Kimura 2-parameter method. The analyses were conducted in MEGA X.

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 Augustiatis (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 Drupeggio, 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 inside 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 in 1936, as declared by the breeder, and enrolled in the Italian catalogue in 1971. This result invalidates the claim in Cipriani *et al.* (2010) that Incrocio Bruni 54 is the offspring of Aleatico × Lacrima, because the accession considered in that paper,

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 Montecchiese, 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, Forcese is the offspring of Garganega and Crepolino/Visparola; in turn Forcese 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, Forcese 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 Sciacarello; 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 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 Cocacciara, Fava and Gabbavolpe. In the Ampelographic Bulletin number XVI (1883) Cocacciara 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 Sciacarello, 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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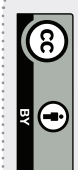
Sector, under the Regional Law of 3 June 2003, no. 12 “Protection of animal and plant genetic resources of the Marche region”.

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