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Genetic relationships among local *Vitis vinifera* cultivars from Campania (Italy)

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Summary

A total of 114 accessions putatively corresponding to 69 local grape cultivars from Campania (Southern Italy) were analysed with 8 microsatellite markers (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD31, VrZAG62 and VrZAG79) in order to evaluate their genetic diversity and relationships. According to their unique genotype at SSR loci finally 56 varieties were found. Interesting cases of synonymy, *i.e.* Greco di Tufo and Asprinio, Palummina and Piedirosso, and homonymy were disclosed. Pairwise genetic distances were calculated between all cultivars. Clustering of cultivars did not reflect their current distribution and this suggests that grape cultivars of Campania might have been introduced from various and distinct geographic areas.

K e y w o r d s : Genotyping, microsatellites, grapevine.

Introduction

The spread of viticulture from Greece to Western Europe crossed the southern part of Italy and herewith the region of Campania (Unwin 1991). The factors that favoured the maintenance of a high viticultural biodiversity in this area are of historical, cultural and geographical nature. Various foreign dominations and the presence of many ports along the coast promoted intensive exchanges of plant material with several countries of the Mediterranean area (Del Treppo 1967). During the past centuries, the propagation of grape by layers was a widespread practice in Southern Italy, which contributed to pressure existing genotypes and clones (Arcuri 1887). Moreover, we noticed fragmentation of plots which may have contributed to the preservation of numerous ancient varieties by reducing the spread of modern viticulture with a small number of common cultivars. Finally, we hypothesize that the volcanic origin of several soils might have averted the spread of phylloxera, thus preventing the extinction of ancient vineyards and the following substitution of local cultivars by international ones. As a consequence, Campania nowadays provides very high numbers of different *Vitis vinifera* varieties, some of which have been growing here since the first half of the 19th century (Arcuri 1887). Among the numerous putatively autochthonous cultivars of this region, only 10 are actually registered in the National Catalogue of Italian Grapevines for wine production: Aglianico, Asprinio, Biancolella, Coda di volpe, Falanghina, Fiano, Forastera, Greco di Tufo, Piedirosso and Sciascinoso. Several other varieties are neither identified nor described.

In the present study DNA microsatellite markers (or simple sequence repeats, SSRs) were used to compare and to identify varieties. Their abundant and random distribution in the genome, high polymorphism, co-dominant Mendelian inheritance, reproducibility and ease of scoring made them ideal markers for several applications, including scion and rootstock genotype identification (Thomas and Scott 1993, Grando and Frisinghelli 1998, Sefc *et al.* 1998), as well as evaluation of genetic relationships among individuals and parentage studies (Sefc *et al.* 1997, Bowers *et al*. 1999 a, Vouillamoz *et al*. 2003). We used microsatellite analysis to evaluate the current grape germplasm in Campania in order (a) to characterise the local grape varieties, (b) to study their genetic diversity and relationships and (c) to preserve vines for breeding purposes.

Material and Methods

G r a p e v i n e m a t e r i a l : Leaves or woody tissues of 114 samples putatively corresponding to 69 cultivars were collected from local collections and private farms all over Campania (Tab. 1). If possible, the oldest vines of each variety were compared with recently planted vines. In several cases, cultivars with the same name were sampled at different sites in order to identify possible homonyms. Finally, different clones of Aglianico and Greco di Tufo were analysed, including commercial plant material kindly supplied by VCR (Vivai Cooperativi di Rauscedo, Italy). The ampelographic description according to the minimum list of O. I. V. characters has been presented for 34 cultivars (Monaco and Manzo 2001).

D N A e x t r a c t i o n : DNA was extracted according to Doyle and Doyle (1990) as modified by Steenkamp *et al*. (1994) for the CTAB solution [3 % (w/v) CTAB, 1.4 M NaCl, 0.2 % (v/v) 2-mercaptoethanol, 20 mM EDTA, 1 M Tris-HCl (pH 8.0) and 1 % (w/v) polyvinylpyrrolidone (PVP-40)].

M i c r o s a t e l l i t e a n a l y s i s : The 114 samples (see Tab. 1) were genotyped at 8 microsatellite loci: VVS2 (Thomas and Scott 1993), VVMD5, VVMD7 (Bowers *et al.* 1996), VVMD25, VVMD27, VVMD31 (Bowers *et al.* 1999 b),

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Table 1

Grape cultivars analysed in this study

Cultivars[a]	Place of sampling[b]	No. of samples	Cultivation area[c]
Aglianichello	AV	1	C, E
Aglianico	AV	4	A, B, C, D, E
Aglianico amaro	BN	1	B
Aglianico di Napoli	Regional collection (BN)	1	E
Aglianico Pannarano	BN	1	B
Aglianicone	SA	1	D
Aleatico	CE	1	A
Asprinio	CE	4	A
Barbarossa (Barbera piemontese)	SA	1	D
Barbera del Sannio	BN	1	B
Biancatenera	SA	2	D
Biancazita	SA	3	D
Biancolella	NA	3	E
Cacamosca	NA	1	D, E
Calabrese (Averno)	NA	1	E
Calabrese (Montenuovo)	NA	1	E
Calabrese or Nero d'Avola	Regional collection	1	not Campania
Caprettone	Regional collection (CS)	1	E
Casavecchia	CE	2	A
Castiglione	NA	1	E
Catalanesca	NA	1	E
Cavalla	NA	1	E
Coda di pecora	CE	2	A
Coda di volpe	Regional collection (CS)	5	A, B, C, E
Falanghina beneventana	BN	1	B
Falanghina Campi Flegrei	Regional collection (BN)	1	A, E
Falanghina Pigna Piccola	NA	1	E
Fenile	SA	2	D
Fiano	AV	2	C
Forastera	NA	1	E
Ginestra	SA	2	D
Greco di Tufo	AV	11	A, C
Ianese	Regional collection (SA)	1	D
Livella (Battipaglia)	Regional collection (SA)	1	C, E
Livella (Ischia)	NA	1	E
Livella (Mirabella)	NA	1	E
Malvasia del Chianti	BN	1	B
Mangiaguerra	BN	1	B, D, E
Moscatello	AV	1	C
Moscato di Baselice	BN	1	B
Nerella	NA	2	E
Pallagrello	AV	1	C
Pallagrello	CE	3	A
Palummina	NA	1	E
Palummina (Mirabella)	NA	1	E
Pellecchiona	Regional collection (SA)	1	D
Pepella	SA	2	D
Piedirosso avellinese	Regional collection (BN)	1	C
Piedirosso beneventano	Regional collection (BN)	1	B
Piedirosso napoletano	Regional collection (BN)	6	A, B, D, E
Ricciulella	NA	1	E
Ripolo	SA	2	D
Rosso Antico	CE	1	A

Tab. 1, continued

Cultivars[a]	Place of sampling[b]	No. of samples	Cultivation area[c]
Sanginella	SA	1	D
S. Antonio	CE	1	A
S. Nicola	NA	2	E
S. Pietro	CE	1	A
Santa Sofia	SA	1	D
Sciascinoso	AV	3	C, E
Summariello	BN	3	B
Suppezza	NA	1	E
Tintiglia	AV	1	C
Tintore di Tramonti	SA	1	D
Trebbiano toscano	Regional collection (BN)	1	A, B
Tronta	Regional collection (SA)	1	D, E
Uva anonima nera	AV	1	C
Uva rosa	NA	1	E
Uva strone	AV	1	C
Zagarese	NA	1	E

[a] Names in bold type refer to cultivars provided with the minimum OIV descriptive list.
[b] Abbreviations: AV = Avellino, BN = Benevento, CE = Caserta, NA = Napoli, SA = Salerno.
[c] See Fig. 3 for codes A-E.

VrZAG62 and VrZAG79 (Sefc et al. 1999). In order to facilitate comparison with other data from literature or databases, the core set of microsatellites, applied for the screening of grapevine collections in Europe within the frame of the GENRES#081 research project (Dettweiler 1997, This *et al.* 2004), was included in the analysis (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79). In the case of Asprinio and Greco di Tufo, 8 additional SSR loci were analysed: VVS1, VVS3, VVS4 (Thomas and Scott 1993), VVMD6, VVMD8 (Bowers *et al.* 1996), VVMD28, VVMD32, VVMD36 (Bowers *et al.* 1999 b). PCR was performed in a volume of 25 µl containing 50-100 ng of genomic DNA, 0.5 U Taq DNA polymerase (Bioline), 1X reaction buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris-HCl pH 8.8, 0.1 % Tween-20), 1.5 mM $MgCl_2$, 0.2 µM of each primer and 100 µM of each dNTP. One primer of each pair was fluorescently labeled with Dye Phosphoramidites (HEX, 6-FAM and TET). PCRs were carried out using a Gene Amp PCR System 9600 (Perkin Elmer) and two different annealing temperatures (50 °C for the loci VVS1, VVS2, VVS3, VVS4, VVMD5, VVMD6, VVMD7, VVMD8, VVMD27, VrZAG62 and VrZAG79 and 56 °C for the loci VVMD25, VVMD28, VVMD31, VVMD32 and VVMD36). Separation and sizing of SSR alleles were performed on a ABI 310 Genetic Analyzer and analysed with GeneScan software 2.1. The automated scoring process of alleles was controlled by using as size standards 4 out of the reference cultivars selected in the frame of the EU-project GENRES#081(Cabernet franc, Chardonnay, Moscato bianco and Pinot noir). As a consequence, comparison of the data reported here with those of others is possible.

Data analysis: The genotypes of all the accessions of this study were tested against a global database that combined (a) the database of University of California, Davis (C. Meredith, pers. comm.), (b) online databases (Grape Microsatellite Collection, http://www.ismaa.it/areabioav/gmc.html and Greek Vitis Database, http://www.biology. uch.gr/gvd), (c) recently published genotypes not included in the online databases and (d) data of Swiss, North Italian, Turkish, Armenian and Georgian cultivars (J. Vouillamoz, unpubl.). The database of University of California, Davis, containing the most important grape cultivars of the world (ca. 400) was used as a reference for every new set of entry. Harmonisation of allele sizes was made using three or more shared true-to-type cultivars, thus allowing pairwise comparisons. This global database currently contains microsat-ellite data for over 2,000 accessions corresponding to approximately 1,500 different cultivars.

The program Identity by Wagner and Sefc (1999) was used to calculate for each locus: the number of alleles (n), the allele frequencies, the expected (H_e) and observed (H_o) heterozygosity, the estimated frequency of null alleles (r) and the probability of identity (P. I.). We scored cultivars with only one allele per locus as homozygous (Sefc *et al.* 1998) rather than as heterozygous nulls (Thomas *et al.* 1994, Bowers *et al.* 1996). Expected heterozygosity was computed as 1- Σp_i^2 to measure gene diversity, where p_i is the frequency of the i^{th} allele in the sample studied (Nei 1987), while observed heterozygosity is the ratio between the heterozygous genotypes and the total analysed genotypes. Probability for the presence of null alleles was estimated from heterozygotes deficiencies as the ratio $(H_e-H_o)/(1+H_e)$ (Brookfield 1996). Probability of identity (Paetkau *et al.* 1995), defined as the probability that 2 randomly chosen individuals display the same SSR profile, was calculated as $\Sigma p_i^4 + \Sigma\Sigma(2p_ip_j)^2$ (where p_i and p_j are the frequencies of allele i and j, respectively). The software Identity was also used to detect identical genotypes.

Nei's *et al.* (1983) D_A genetic distance was calculated for pairwise comparison between individuals and a tree of individuals was constructed with the UPGMA method

Table 2

Allele sizes (bp) at 8 loci for the 56 *Vitis vinifera* cultivars analysed in this study. The reference varieties are indicated in bold

Cultivar	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VVMD25	VVMD25	VVMD27	VVMD27	VVMD31	VVMD31	VrZAG62	VrZAG62	VrZAG79	VrZAG79
Aglianico	148	152	229	243	237	237	247	261	181	187	209	209	188	188	243	245
Aglianico di Napoli	140	148	233	237	237	247	253	261	177	187	209	209	188	190	249	257
Aglianicone	148	152	225	243	237	247	247	261	177	181	209	209	188	196	245	257
Aleatico*	130	132	223	237	231	247	239	253	177	181	209	213	202	204	243	255
Barbarossa	130	132	223	223	247	251	237	253	183	187	209	209	192	200	241	257
Barbera del Sannio	140	146	223	235	241	249	237	237	183	192	201	221	188	204	245	249
Biancolella	130	152	229	229	237	245	239	247	177	179	209	213	188	192	253	257
Cacamosca	140	152	225	229	237	245	247	253	181	183	209	213	188	194	241	245
Calabrese Averno	130	132	223	229	237	237	247	253	177	183	207	213	194	202	241	245
Calabrese Montenuovo	130	132	223	229	237	237	239	253	177	177	207	209	196	202	241	245
Calabrese or Nero d'Avola	140	148	223	237	237	247	239	253	177	179	201	207	186	202	249	249
Caprettone	130	148	225	229	247	247	243	261	185	187	209	213	194	202	249	257
Casavecchia	130	152	235	235	241	247	239	239	183	192	201	207	188	200	249	249
Catalanesca	130	152	229	231	241	249	237	247	181	183	207	213	188	200	245	249
Cavalla Averno	130	148	223	229	245	261	247	261	177	179	201	207	194	194	257	257
Coda di pecora	140	148	225	237	237	237	247	261	183	192	213	221	196	204	247	249
Coda di volpe	130	142	223	233	245	245	239	239	179	192	207	209	194	204	249	257
Falanghina beneventana	132	152	223	225	245	247	247	253	177	179	209	213	188	194	257	257
Falanghina Campi Flegrei	148	152	223	233	237	247	247	261	183	183	209	209	188	196	241	249
Fenile	142	152	233	235	237	245	237	239	183	192	209	213	188	194	241	253
Fiano	152	152	223	225	237	237	247	253	181	187	201	209	188	194	243	249
Forastera	130	152	223	229	245	247	239	247	179	183	209	213	192	200	241	257
Ginestra	142	148	225	233	247	263	237	261	187	187	209	213	194	194	241	245
Greco di Tufo	130	152	225	229	245	247	237	239	183	187	209	213	202	204	241	249
Ianese	130	130	225	243	237	263	237	247	183	187	213	221	194	196	241	245
Livella Ischia	152	152	229	235	237	241	239	239	179	183	201	209	188	188	249	257
Livella Mirabella	130	148	231	243	237	247	247	261	183	187	207	209	188	200	243	249
Malvasia del Chianti	142	142	223	237	237	251	239	239	177	177	209	211	196	200	241	249
Mangiaguerra	142	152	229	235	237	241	239	253	183	183	207	209	188	188	249	249
Moscatello	148	148	223	225	241	247	247	247	179	179	211	213	188	204	237	241
Moscato di Baselice	130	130	225	233	231	247	239	247	177	192	209	213	186	196	249	253
Nerella	130	140	229	233	245	261	239	247	187	192	207	213	194	196	247	249
Pallagrello-AV	130	152	237	243	237	237	239	247	183	189	209	213	188	196	245	249
Pallagrello-CE	130	142	223	243	247	261	239	239	183	187	207	209	200	202	241	249
Palummina Mirabella	130	152	223	229	247	261	239	239	177	183	207	209	194	202	249	257
Pellecchiona	140	148	229	233	245	247	253	261	183	187	209	213	188	194	241	241
Pepella	130	148	225	233	247	247	239	239	177	187	207	209	194	200	241	257
Piedirosso avellinese	130	148	223	229	245	245	253	261	177	183	201	213	194	194	241	257
Piedirosso napoletano	140	148	229	233	247	261	237	261	187	187	207	209	194	202	257	257
Ricciulella	132	152	225	243	237	249	247	253	181	187	209	213	188	200	243	245
Ripolo	134	148	229	237	237	237	247	261	179	192	209	209	188	196	241	247

* not true to type.

Tab. 2, continued

Cultivar	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VVMD25	VVMD25	VVMD27	VVMD27	VVMD31	VVMD31	VrZAG62	VrZAG62	VrZAG79	VrZAG79
Rosso antico	132	148	223	225	247	251	239	253	183	183	207	209	192	202	249	249
S.Antonio	130	130	229	233	237	241	237	247	177	183	207	209	188	188	247	249
S.Pietro	132	148	233	237	231	237	253	261	183	183	209	209	196	204	249	249
Sanginella Salerno	130	142	231	233	237	241	237	237	177	183	207	209	188	196	241	249
Santa Sofia	142	148	223	225	237	247	239	261	179	183	207	209	188	202	241	249
Sciascinoso	140	148	233	237	237	237	253	261	177	177	201	209	188	196	257	257
Summariello	140	148	223	229	241	251	261	265	187	192	207	211	188	200	249	249
Suppezza	130	148	225	233	237	237	247	261	183	183	207	209	188	196	249	249
Tintiglia	152	152	229	235	237	241	239	247	187	187	201	209	188	188	243	249
Tintore di Tramonti	140	152	235	243	237	241	237	247	181	183	201	209	188	188	245	249
Trebbiano toscano	130	140	223	229	247	251	239	253	177	181	207	209	194	200	243	249
Tronta	148	152	223	243	237	237	247	261	183	187	209	213	188	188	243	249
Uva rosa	130	142	223	233	245	247	237	239	179	187	213	213	194	204	241	257
Uva strone	140	148	225	237	245	245	253	261	177	183	209	213	194	194	241	257
Zagarese	142	148	223	233	247	247	239	261	177	179	209	209	190	202	257	257
Cabernet franc	136	144	223	237	237	261	203	213	179	187	237	253	194	204	245	257
Chardonnay	134	140	231	235	237	241	211	213	179	187	237	253	188	196	241	243
Moscato bianco	130	130	225	233	231	247	209	213	177	192	239	247	186	196	249	253
Pinot noir	134	148	225	235	237	241	213	213	183	187	237	247	188	194	237	243

(Unweighted Pair Group Method with Arithmetic Mean) using the program Populations (version 1.2.28) (Langella 2002). The dendrogram was displayed with Treeview (Page 1996).

Results and Discussion

Microsatellite analysis: The 114 accessions produced 56 distinct allelic profiles when analysed at 8 SSR loci. The probability to encounter different individuals with the same profile at all loci turned out to be low (P. I. = 6.95 x 10^{-8}) in our sample, so that identical genotypes can be considered as synonyms. Tab. 2 reports allele sizes for all the varieties investigated in this study, along with the genotypes of the 4 reference cultivars as size standards.

Genetic parameters: Number of alleles (n), allele size range, expected (H_e) and observed (H_o) heterozygosity, estimated frequency of null alleles (r) and probability of identity (P. I.) of the 56 unique genotypes are listed for each locus in Tab. 3. Total number of alleles per locus ranged from 6 to 9, which is comparable to the number reported in previous works (Bowers *et al.* 1996, 1999 b, Sefc *et al.* 1999, 2000). Heterozygosity values obtained with our set of 56 cultivars were high, being comprised between 0.714 and 0.946 and turned out again to be comparable to the results obtained in the above mentioned papers. Mean observed heterozygosity averaged over loci (0.817) was a bit higher than expected by the random union of gametes (0.790). Estimated frequency of null alleles (r) was negative for 4 loci and positive for the 4 others. However, a positive value for r doesn't necessarily imply the presence of null alleles, but only indicates this possibility (Sefc *et al.* 1998). It should be underlined that the algorithm developed by Brookfield (1996) estimates null allele frequency in the case of panmictic, natural populations, not subjected to the same breeding constraints as grapevine (Sefc *et al.* 1998). Probability of identity values ranged between 0.084 and 0.216; then turned out to be higher than the threshold of 0.05 at which a microsatellite is considered hyperpolymorphic in grape (Sefc *et al.* 2001). Observed allelic frequencies are displayed for each locus in Fig. 1. Twenty-one (32.8 %) among the 64 alleles detected in this study had a frequency lower than 0.05.

Homonymies and synonymies: Fifty-six out of the 69 putative cultivars tested were distinguished on the basis of a unique genotype at SSR loci. As a consequence, a number of suspected as well as unsuspected synonyms and homonyms were demonstrated (Tab. 4). The following cultivars showed identical SSR profiles and are considered synonyms:

- Aglianico is known since the XVIth century (Vitagliano 1991); it represents today the most widespread black variety in the Southern part of Italy. Wines like Aglianico Taurasi and Aglianico del Vulture are produced of it. We analysed some accessions having slightly different names with regard to their sensorial (Aglianico amaro, with must acidity), morphological (Aglianichello, with small sized clusters) and topographical (Aglianico Pannarano, from the place of origin) characteristics. They all turned out to be synonyms of Aglianico.

T a b l e 3

Genetic parameters of the 8 SSR loci analysed with 56 grapevine varieties

Locus	n	Allele size range (bp)	H_e	H_o	r	P. I.
VVS2	8	130-152	0.809	0.857	-0.027	0.118
VVMD5	8	223-243	0.842	0.946	-0.057	0.084
VVMD7	9	231-263	0.780	0.714	+0.037	0.135
VVMD25	7	237-265	0.793	0.821	-0.016	0.139
VVMD27	8	177-192	0.801	0.786	+0.008	0.123
VVMD31	6	201-221	0.702	0.839	-0.081	0.216
VrZAG62	9	186-204	0.811	0.804	+0.004	0.104
VrZAG79	9	237-257	0.785	0.768	+0.010	0.134
All loci	64	-	-	-	-	6.95×10^{-8}
Mean	8	-	0.790	0.817	-	0.132

n = number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity;
r = expected frequency of null alleles; P. I. = probability of identity.

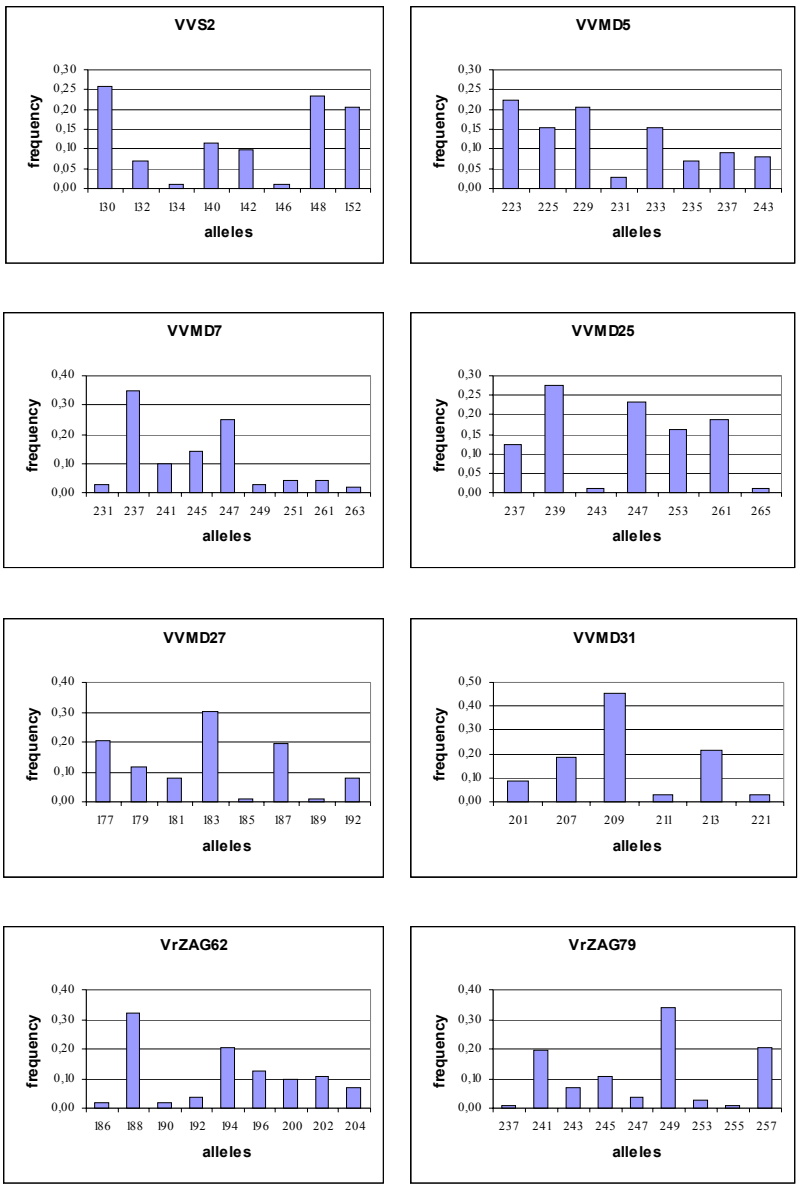

Fig. 1: Distribution and relative frequency of microsatellite alleles in 56 grape cultivars from Campania analysed at 8 SSR loci.

Table 4

Cases of synonymy and homonymy found among the analysed cultivars

Synonymies	Homonymies
Aglianichello, Aglianico amaro, Aglianico Pannarano and Aglianico	Aglianico di Napoli and Aglianichello /Aglianico amaro / Aglianico Pannarano /Aglianico
Asprinio and Greco di Tufo	Calabrese Averno and Calabrese Montenuovo and Calabrese o Nero d'Avola
Biancatenera , Biancazita and Ginestra	Falanghina beneventana and Falanghina Campi Flegrei / Falanghina pigna piccola
Biancolella and S. Nicola	Livella Battipaglia and Livella Ischia and Livella Mirabella
Calabrese Averno and Castiglione	Pallagrello-AV and Pallagrello-CE
Falanghina Campi Flegrei and Falanghina Pigna Piccola	Palummina and Palummina Mirabella
Livella Battipaglia and Sciascinoso	Piedirosso avellinese and Piedirosso beneventano / Piedirosso napoletano
Palummina , Piedirosso beneventano and Piedirosso napoletano	
Tintiglia and Uva anonima nera	

- Asprinio and Greco di Tufo (Ferrante 1927) are registered on the National Catalogue as two distinct varieties producing different wines. The genotypes of 4 accessions of Asprinio and 11 accessions of Greco di Tufo are identical at a total of 16 microsatellite loci, which demonstrates the synonymy of these 2 varieties, as was indicated by ampelographic descriptions (Boselli *et al.* 2000 a).
- The synonymy between Ginestra, named after the broom flavour of its grape, and Biancazita was suspected by Froio (1875), while it is likely that the term Biancatenera has been recently introduced to indicate a variation of Ginestra. This synonymy explains the small distance observed between Biancatenera and Biancazita in the ampelographic evaluation of these varieties reported by Boselli *et al.* (2000 a).
- Biancolella and San Nicola are cultivated respectively on the islands of Ischia and Capri where it is likely that the same genotype received different names.
- The identity between Calabrese (Averno) and Castiglione may result from their wide cultivation in the past along the same coast of Campi Flegrei in the province of Napoli.
- Falanghina Campi Flegrei is the most widespread white cultivar in the province of Napoli while Falanghina Pigna Piccola has almost disappeared because of its limited production due to small sized clusters.
- Livella and Sciascinoso have been described as different varieties since the XIXth century (Gasparrini 1844). However, as they share the same olive-like berry shape, they have also been confused (Bordignon 1965). We found a genetic identity between Sciascinoso and Livella (Battipaglia), one of the three Livella accessions included in our study (Tab. 1).
- Piedirosso beneventano and Piedirosso napoletano are toponyms named after their provinces of cultivation. The synonymy of Piedirosso with Palummina explains the number of Piedirosso synonyms referring to the pigeon claw-like red colour of their berry peduncles (Per'e Palummo, Piede di Colombo, Palombina).
- Tintiglia and Uva anonima nera, cultivated in the province of Avellino. This cultivar has a strong red colour of berries and a diffuse red hue on the whole plant, but it is distinct from other *teinturier* cultivars such as Tintore di Tramonti in this study or the genetically heterogenous dark red juice cultivars Teinturier du Cher, Pinot fin teinturier, Saperavi, Ancellotta and so on in our database.

The following cultivars sharing the same name showed distinct SSR profiles and are thus considered homonyms:

- Aglianico di Napoli, a variety cultivated in the province of Napoli and Aglianico, as reported in the ampelographic study of Boselli *et al.* (2000 b).
- Calabrese (from Averno), Calabrese (from Montenuovo) and Calabrese or Nero d'Avola.
- Falanghina beneventana and Falanghina Campi Flegrei are widely grown respectively in the provinces of Benevento and Napoli. They were already reported to show different morphology (Boselli *et al.* 2000 a).
- Livella (from Battipaglia), Livella (from Ischia) and Livella (from Mirabella) are distinct cultivars originating from different places and belonging to the heterogeneous group of "Olivelle" based on the olive-shape of their berry (Froio 1875).
- Pallagrello (AV) and Pallagrello (CE), respectively cultivated in the provinces of Avellino and Caserta, are distinct cultivars. This homonymy probably dates back to the end of the XIXth century, when the name Pallagrello was used to indicate several distinct vines now called Coda di volpe, Trebbiano or Malvasia (Rasetti 1904, Carlucci 1909).
- Palummina and Palummina (from Mirabella) are distinct cultivars distributed along the coast around Napoli.
- Piedirosso avellinese, especially grown in the province of Avellino, differs both in leaf and cluster shape from Piedirosso beneventano (syn.: Piedirosso napoletano). The homonymy is likely due to the common red colour of berry peduncles.

The 56 individual genotypes of Campania were compared to a global database containing approximately 1,500 cultivars from all over the world (including a large number from Italy): no case of synonymy was found, thus confirming the uniqueness of these genotypes.

Genetic relationships: NEI's unbiased D_A genetic distance was calculated between each pair of cultivars. According to TAKEZAKI and NEI (1996), the D_A genetic distance is more efficient than NEI's standard genetic distance (*Ds*), NEI's minimum genetic distance *(Dm)* or Roger's distance (*Dr*) in obtaining the correct tree topology with microsatellite analysis, either under the infinite-allele model (IAM) or the stepwise mutation model (SMM) of microsatellites evolution.

A dendrogram drawn from the pairwise distance matrix (data not shown) is presented in Fig. 2. Three main groups were distinguished (1, 2 and 3 in Fig. 2), suggesting at least three different origins for the Campania germplasm. In general no correlation was found between clustering and modern geographic distribution of cultivars. Of some interest is the cluster including Mangiaguerra, Livella Ischia, Tintiglia and Tintore di Tramonti, which are all characterised by a strong red colour of the berries and the diffuse red hue of the entire plant. However, only pairs of genetically very closely related cultivars were consistent with (a) geographic origin (Falanghina Campi Flegrei-Suppezza; Sciascinoso-Aglianico di Napoli; Pellecchiona-Cacamosca; Coda di Pecora-S. Pietro; Piedirosso napoletano-Caprettone; Coda di volpe-Uva rosa), (b) morphology (Tronta-Aglianico; S. Antonio-Sanginella Salerno), (c) both geographic origin and morphology (Piedirosso avellinese-Uva strone; Calabrese Montenuovo-Calabrese Averno). In addition, several closely related pairs did not show any common feature (Falanghina beneventana-Biancolella; Pepella-Trebbiano toscano; Forastera-Barbarossa; Pallagrello-CE-Palummina

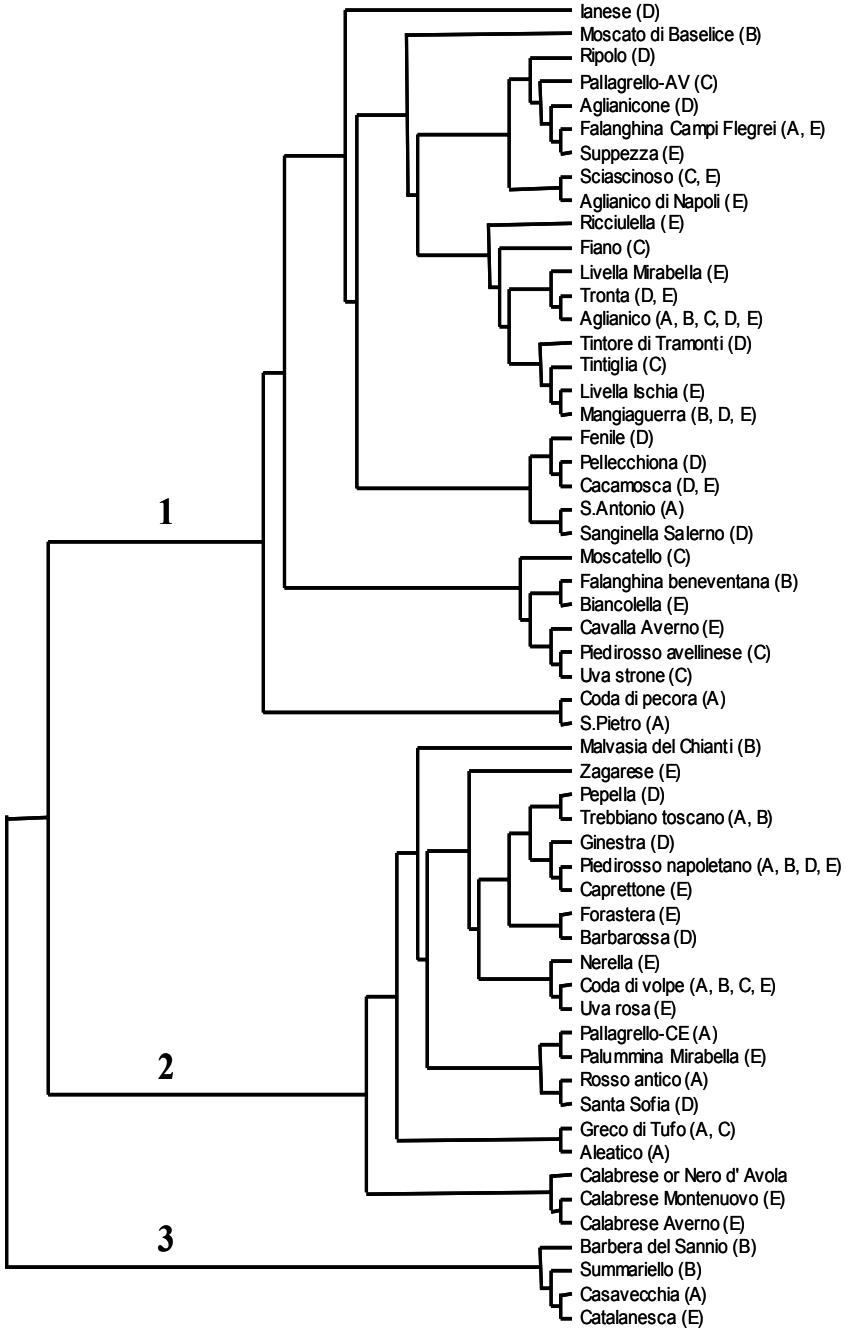

Fig. 2: Dendrogram of genetic relationships among the 56 grapevine varieties investigated in this study calculated with NEI's *et al.*(1983) D_A genetic distance. Letters in brackets refer to the cultivation areas reported in Tab. 1 and Fig. 3.

Mirabella; Rosso Antico-Santa Sofia; Greco di Tufo-Aleatico; Casavecchia-Catalanesca). Although sampled in Campania, Calabrese Montenuovo and Calabrese Averno are supposed to originate from Calabria (South Italy), as suggested by their names. Calabrese (or Nero d'Avola) is also the name of the most widespread cultivar in Sicily. Since all three are clustered together in group 2, this supports a Calabrian or Sicilian origin for Calabrese Montenuovo and Calabrese Averno. Campania grape varieties might have been introduced from at least three distinct sources and then were subject to internal transfers within Campania, as suggested by the relative lack of correlation between genetic and geographic distribution. The group 3 (Barbera del Sannio, Summariello, Casavecchia and Catalanesca) is completely separated from other Campanian cultivars and might represent recent introductions.

In terms of biodiversity, the present distribution of Campania germplasm seems to reflect well the viticultural evolution in the 5 areas of this region. According to our data, the province of Napoli turned out to be the richest with 30 cultivars, followed by Salerno (18), Caserta (14), Benevento and Avellino (13), as shown in Tab. 1 and Fig. 3. A possible explanation is that the province of Napoli was not subjected to the same decrease of cultivar diversity that took place in the other areas in the last 30 years. This is mainly due to the orographic characteristics of the territory making this area suitable for more remunerative crops and to the wide fragmentation of properties which limits the expansion of viticulture. Cultivar abundance varies also according to the distance from the sea of the different areas of cultivation. Today the coast in the Napoli and Salerno provinces is characterised by the highest number of varieties probably due to the intensive trade by land and sea in the past. Some of them are widely grown, while others are represented by a few individuals only (*e.g.* Aglianico di Napoli, Cacamosca and Zagarese). On the other hand, the inner hilly part, including Avellino, Benevento, Caserta and the hinterland of Salerno, shows a more limited variability, but a specialized cultivation of main varieties such as Fiano, Greco di Tufo, Aglianico, Trebbiano toscano and Barbarossa (Barbera piemontese). Finally, in the most interior areas of the provinces of Avellino, Benevento, Caserta and Salerno some cultivars that are restricted to small niches can be found. Among them are Summariello, Ripolo, Moscatello, Ianese, Barbera del Sannio, Moscato di Baselice and Malvasia del Chianti which in most cases share a lower percentage of SSR markers with the other regional varieties (Fig. 2).

Conclusions

It can be concluded that Campania grape germplasm might have been introduced from at least three distinct geographic areas. While the wines from few local varieties are now emerging to the international market, our results point out the wide genetic diversity of grapevines which are still unexploited in this region.

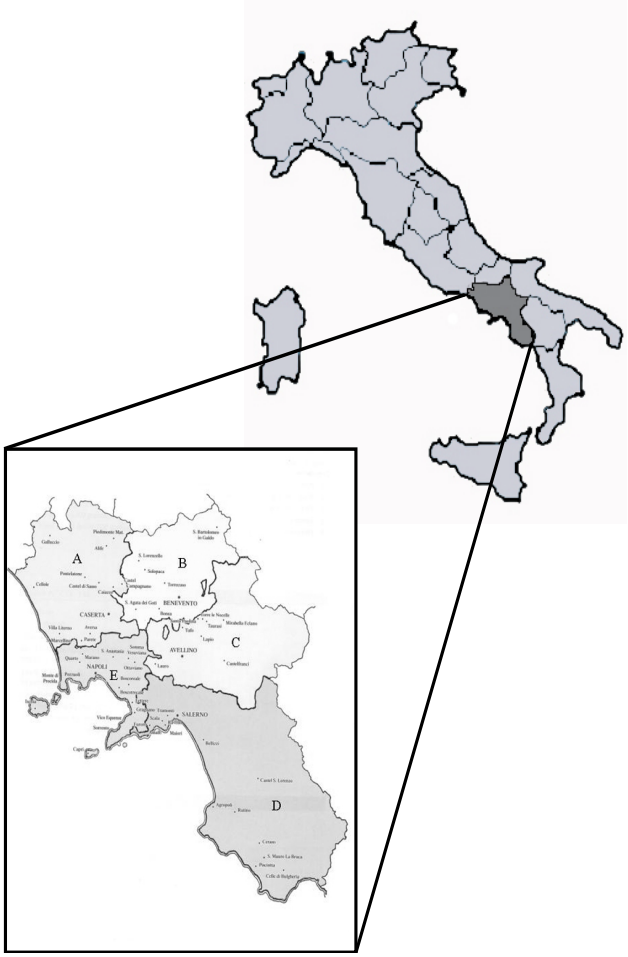

Fig. 3: The Campania region and its provinces: Caserta (A), Benevento (B), Avellino (C), Salerno (D), Napoli (E).

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