

# Pedigree Reconstruction of the Italian Grapevine Aglianico (*Vitis vinifera* L.) from Campania

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**Abstract** A total of 41 accessions of Aglianico belonging to three different biotypes (Taburno, Taurasi, and Vulture) and 9 accessions of Sirica grapes were sampled from diverse areas of Campania (Italy). All accessions were first genotyped using 21 microsatellite markers (SSR) to evaluate possible homonymies, synonymies, and the genetic structure of each group. A larger dataset was then constructed adding Italian and International cultivars. On the basis of results obtained analyzing the first dataset, further investigations were carried out enlarging the number of investigated loci (up to 43). The addition of 22 SSRs was useful in the definition of likely genetic relationships linking Aglianico biotypes, Sirica and Syrah. According to their SSR allelic profiles, the monophyletic origin of the three Aglianico biotypes was confirmed. Among Aglianico Taburno accessions, eight samples (called Aglianico like-to-type) performed a different SSR allelic profile from Aglianico true-to-type. Sirica and Syrah proved to be synonyms. This work allowed to determine the genetic relationship between Aglianico and the cultivars supposed to be related. The parentage analysis was investigated. The most likely pedigree has been reconstructed; revealing a second-degree relationship between the worldwide cultivated Syrah from the Rhone Valley and Aglianico. Aglianico like-to-type appeared related to Aglianico in a parent-offspring fashion.

**Keywords** Landraces · Molecular markers · Pedigree · Relatedness · SSR · *Vitis vinifera*

## Introduction

Campania is a region located in Southern Italy whose capital city is Naples, one of the main cultural and artistic towns in Italy since the 9th century BC. Viticulture in this region is one of the most important and variegated of Italy. Campania's wines, celebrated since the Roman age, can rely on a large and regional specific varietal assortment, are supposed to have a local origin maintained up to the present days. The main autochthonous cultivars of this region are: "Aglianico," "Asprinio," "Biancolella," "Coda di volpe," "Falanghina," "Fiano," "Forastera," "Greco di Tufo," "Piedirosso," and "Sciascinoso" [1].

Aglianico is a red grape cultivar widespread in Southern Italy and renowned for the quality of its wines. This variety is grown in several Italian Regions, but it is mainly cultivated in Campania and Basilicata, and particularly in the provinces of Benevento, Avellino, and Potenza. Its origin is supposed to be very ancient and traditionally its introduction is connected to the foundation of the Greek Campanian colonies [2]. Following this hypothesis, the name "Aglianico" could derive from the word "Hellenica" [3]. However, the first written references of the cultivation of Aglianico date back to the 16th century. Anyway, Aglianico has been grown in Southern Italy for many centuries. Traditionally, the main growing areas for Aglianico are Taburno and Taurasi, in Campania, and the Vulture district, in Basilicata. Aglianico is characterized by a great intra-variety phenotypic variability originating from the conscious or unconscious selection operated over the centuries by viticulturists and growers [4]. Thus, three main biotypes

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were selected, one for each of the main cultivation areas: Taurasi (from the province of Avellino), Taburno (from the province of Benevento), and Vulture (from the province of Potenza) [3]. The monophyletic origin of these biotypes was confirmed by DNA fingerprinting [1].

Sirica is supposed to be a minor grape variety belonging to the Campania traditional germplasm platform even if from the phenotypical point of view it seems close to the most famous Syrah grapes.

SSR (simple sequence repeats) marker-based DNA fingerprinting [5] allows cultivar identification [6–9] and their co-dominant Mendelian inheritance allows the reconstruction of possible crosses among varieties [8, 10–12]. Thanks to this approach, parent cultivars and their offspring can be recognized and pedigrees describing the genetic history of grapevine cultivars can be reconstructed [13–17].

In this paper, the hypothesis of a parentage linking the three Aglianico biotypes from Campania and Basilicata and Sirica was analyzed. In order to perform this work a two step approach, commonly used in the identification of pedigrees in grapevine [12, 15, 18, 19], was carried out. As a first step a dataset, including Aglianico and Sirica specimens and other Italian and International varieties (for a total of 179 samples) was defined and genotyped at 21 SSR loci. Basing on the results, a second dataset was defined, by maintaining just the most interesting varieties from the first one, and adding other accessions for a total of 45 varieties, some of them supposed to be involved in the relative relationships. The number of SSRs was increased to 43 loci and the Aglianico pedigree was provided. The microsatellite loci were selected among the most polymorphic and the coverage of the genome was guaranteed by having at least one SSR for each chromosome.

## Materials and Methods

### Plant Material

One hundred and seventy-nine Italian and International grape varieties were analyzed in this study. The kinship analysis was focused on 41 accessions of Aglianico (Taburno, Taurasi e Vulture biotypes) and nine accessions of Sirica (Table 1), collected from plants over 100-year old. The Aglianico accessions were sampled in vineyards located in the main cultivation areas (Campania, Italy), while the Sirica accessions were collected in the same vineyards of Aglianico Taurasi. Pinot Noir was analyzed as reference variety.

### DNA Extraction

For each sample, young leaves (1–2 cm of diameter) were collected and dehydrated with silica gel. DNA was extracted

**Table 1** List of 41 Aglianico and 9 Sirica analyzed accessions in a set of 179 grapevine samples

Samples	Origin
Aglianico Taburno 01	Taburno-BN
Aglianico Taburno 02	Taburno-BN
Aglianico Taburno 03	Taburno-BN
Aglianico Taburno 04	Taburno-BN
Aglianico Taburno 05	Taburno-BN
Aglianico Taburno 06	Taburno-BN
Aglianico Taburno 07	Taburno-BN
Aglianico Taburno 08	Taburno-BN
Aglianico Taburno 09	Taburno-BN
Aglianico Taburno 10	Taburno-BN
Aglianico Taburno 11	Taburno-BN
Aglianico Taburno 12	Taburno-BN
Aglianico Taburno 13	Taburno-BN
Aglianico Taburno 14	Taburno-BN
Aglianico Taburno 15	Taburno-BN
Aglianico Taburno 16	Taburno-BN
Aglianico Taburno 17	Taburno-BN
Aglianico Taburno 18	Taburno-BN
Aglianico Taburno 19	Taburno-BN
Aglianico Taburno 20	Taburno-BN
Aglianico Taburno 21	Taburno-BN
Aglianico Taburno 22	Taburno-BN
Aglianico Taburno 23	Taburno-BN
Aglianico Taurasi 01	Taurasi-AV
Aglianico Taurasi 02	Taurasi-AV
Aglianico Taurasi 03	Taurasi-AV
Aglianico Taurasi 04	Taurasi-AV
Aglianico Taurasi 05	Taurasi-AV
Aglianico Taurasi 06	Taurasi-AV
Aglianico Taurasi 07	Taurasi-AV
Aglianico Taurasi 08	Taurasi-AV
Aglianico Taurasi 09	Taurasi-AV
Aglianico Taurasi 10	Taurasi-AV
Aglianico Taurasi 11	Taurasi-AV
Aglianico Taurasi 12	Taurasi-AV
Aglianico Vulture 01	Rionero in Vulture-PZ
Aglianico Vulture 02	Rionero in Vulture-PZ
Aglianico Vulture 03	Rionero in Vulture-PZ
Aglianico Vulture 04	Rionero in Vulture-PZ
Aglianico Vulture 05	Rionero in Vulture-PZ
Aglianico Vulture 06	Rionero in Vulture-PZ
Sirica 01	Taurasi-AV
Sirica 02	Taurasi-AV
Sirica 03	Taurasi-AV
Sirica 04	Taurasi-AV
Sirica 05	Taurasi-AV
Sirica 06	Taurasi-AV
Sirica 07	Taurasi-AV
Sirica 08	Taurasi-AV
Sirica 09	Taurasi-AV

using the Qiagen DNeasy Plant Mini Kit (Qiagen—Hilden, Germany), starting at 0.020 g of dry lysis tissue.

## Microsatellite Analysis

For genotyping, the samples were analyzed by 21 nuclear SSR loci. Twenty out of the 21 SSR primers combinations (except VVIV37), used for molecular characterization were the same used in genotyping the Vassal grapevine collection [9], VrZag62 and VrZag79 [20]. This set of SSRs was chosen according their position in the linkage groups of *V. vinifera*, to cover the whole genome [9]. In order to verify and define the assignments to the proper relationship categories, the accessions of Aglianico, Sirica, and other ten Italian varieties were further analyzed increasing the number of loci to 43. Twenty-two additional SSR loci were investigated: VMC1c10, VMC1e8, VMC2b3, VMC2b5, VMC2h4, VMC4c6, VMC5a1, VMC5c1, VMC5c5, VMC5g8, VMC5h2, VMC5h5, VMC6e1, VMC6e10, VMC8f10, VMC9b5 (*Vitis* Microsatellite Consortium, Agrogen), VrZag29, VrZag83, VrZag93 [22], VVMD17, VVMD34, and VVMD36 [14, 21].

PCR amplifications were carried out in 25  $\mu$ l final volume reaction mixtures in a PTC100 (MJ Research—Waltham, USA). The PCR mix used for SSR amplifications was as follows: 10 ng of template DNA was added to a 24  $\mu$ l reaction mix containing 1.5 mM  $MgCl_2$ , 0.4 U Taq polymerase (Invitrogen-Life Technologies, Foster City, USA), 200  $\mu$ M dNTP, 1 $\times$  Taq polymerase buffer (Invitrogen), and 0.16 pM of labeled/unlabeled primers. Forward primer of each locus was labeled with a fluorescent dye (FAM, NED, PET, or VET). The following thermal cycling protocol was applied for SSR amplification: 4 min at 94  $^{\circ}C$  followed by 36 cycles of 1 min at 94  $^{\circ}C$ , 45 s at 56 or 60  $^{\circ}C$ , 1 min at 72  $^{\circ}C$  followed by a final step of 20 min at 72  $^{\circ}C$ . The products were resolved on 2 % agarose gel in 1 $\times$  TAE (10 $\times$ : 0.4 M Tris-acetate, 0.01 M  $Na_2EDTA$ , pH 8), and ethidium bromide staining.

PCR product analysis was carried out on ABI PRISM 310 Genetic Analyser (Applied Biosystems-Life Technologies, Foster City, USA) using a 36-cm capillary filled with POP-4<sup>TM</sup> Polymer (Applied Biosystems). Alleles were sized with the software GENEMAPPER 1.0 (Applied Biosystems). Amplification products were diluted in water according to different concentrations of PCR products detected by agarose gel. One microliter of each dilution was added to a mix of 20  $\mu$ l of deionised formamide (Applied Biosystems) and 0.3  $\mu$ l of GeneScan<sup>TM</sup> 500 LIZ<sup>®</sup> Size Standard (Applied Biosystems). The mix was denatured for 4 min at 94  $^{\circ}C$  and the capillary electrophoresis was carried out at 60  $^{\circ}C$ .

## Data Analyses

The SSR allele lengths were collected in bp and transformed in European Vitis Database format (EVD, [www.eu-vitis.de/index.php](http://www.eu-vitis.de/index.php)) to verify the trueness-to-type. GenAIEx 6.2

software [22] was used to identify the number of alleles ( $N_a$ ), the number of effective alleles ( $N_e$ ), number of private alleles ( $N_p$ ), observed heterozygosity ( $H_o$ ), and expected heterozygosity ( $H_e$ ) and to discover possible synonymous accessions or varieties.

Kinship analysis was carried out on Aglianico and Sirica accessions. For each pair of cultivars, the number of shared alleles to determine any possible parent-offspring (PO) relationship was estimated. To share at least one allele at each SSR locus is a pre-requisite for demonstrating a PO relationship. In order to assign properly the relationship categories (first, second, third degree and unrelated), COANCESTRY 1.0 software [23] was used to estimate three relatedness coefficients:  $\Delta_7$ ,  $\Delta_8$ , and  $r$  [24, 25]. The estimation of the 95 % confidence intervals of relatedness coefficients was carried out adopting 1,000 bootstrapping samples. Kinship analysis was estimated twice, the first time on 21 SSR profiles and the second one on 43 SSR profiles. The  $\Delta_7$ ,  $\Delta_8$ , and  $r$  coefficients were estimated basing on allelic frequency, so the assignment to the most likely relationship category was performed expanding the analysis to 12 Italian cultivars genotyped with 43 SSR markers and other varieties (up to 45 samples) reported in literature [12, 15, 26–28], to have a more precise prediction. The samples reported in literature (Arvine grande, Calabrese di Montenuovo, Cascarolo bianco, Chasselas, Cilieggiolo, Completer, Cornalin d'Aoste, Cornalin du Valais, Dureza, Freisa, Goron, Gropello di Revò, Humagne, Lafnetscha, Lagrein, Madeleine Royale, Marzemino, Mayolet, Monduese blanche, Nebbiolo, Negrello, Nosiola, Petit rouge, Plantscher, Rèze, Roussin de Morgex, Sangiovese, Schiava grossa, Teroldego, and Vuillermin) were chosen because their molecular characterization was carried out using the major number of the 43 SSR markers and belonging to Italian and International germplasm. In order to provide a better validation, the kinship analysis was extended to Dureza, Mondeuse blanche, and Teroldego cultivars, using the genotyping reported in Vouillamoz and Grando [15]; due to the fact that these varieties are linked by known relationships and are related to Syrah.

## Results

### Identity

The genotyping of 179 Italian and International varieties by a set of 21 SSR loci represented the first step of the research work. In the pool of samples, 41 Aglianico and 9 Sirica accessions were included. Two hundred and eighty-two different alleles and an average of 13.4 alleles per locus were revealed (Table 2). The  $N_a$  detected among the Aglianico and Sirica accessions was 66. The  $N_a$  value per

locus ranged from 7 (VVIN16 and VVIN73 loci) to 23 (VVMD28 locus) and the allele size ranged from 69 (VVIQ52 locus) to 382 bp (VVIV67 locus). The  $N_e$  value per locus ranged from 2.48 (VVIN73 locus) to 11.62 (VVMD28 locus). The  $N_p$  value was 47, ranging from 0 (VrZag79 and VVIN16 loci) to 5 (VVIV67, VVMD25, and VVMD28 loci). No private allele was detected among Aglianico and Sirica accessions.  $H_o$  value was 0.753 (ranging from 0.216 for VVIN73 locus to 0.944 for VVMD32), while the  $H_e$  value was 0.810 (ranging from 0.597 for VVIN73 locus to 0.914 for VVMD28).

Tree different genotypes were identified in the frame of Aglianico and Sirica samples: (i) Aglianico Taburno, Taurasi, and Vulture biotypes (called Aglianico true-to-type) showed the same allelic profile; (ii) eight accessions of Aglianico Taburno (called Aglianico like-to-type) performed a different fingerprint from Aglianico true-to-type; and (iii) one unique SSR profile for all the Sirica accessions. The same three genotypes were confirmed after increasing the number of analyzed loci up to 43, during the second step of the work. The trueness-to-type for Aglianico and Sirica was attributed basing on the comparison of these results with the fingerprints reported in previous works [1, 29]. The identity between Sirica accessions and Syrah [15] was checked and confirmed; just a discrepancy of

10 bp in the VMC2b5 locus was found. Table 3 presents allele sizes at 43 loci per each identified genotype.

### Relationship Category Assignment

In order to assign the proper relationship category, in the three identified genotypes, the number of loci with at least one shared allele in a pairwise fashion was estimated. Among all possible pairs, only the couple Aglianico like-to-type and Aglianico true-to-type showed at least one shared allele for all analyzed loci (26 shared alleles, 100 % of the 21 analyzed loci). In the other two pair combinations, 76.2 % of shared loci for Sirica vs Aglianico true-to-type and vs Aglianico like-to-type was highlighted. Basing on these results, a PO relationship category was assigned to Aglianico like-to-type and Aglianico true-to-type genotypes.  $\Delta_7$ ,  $\Delta_8$ , and  $r$  coefficients were calculated for each pair of genotypes among the three identified. The values obtained using COANCESTRY 1.0 software were compared with theoretical values suggested in the COANCESTRY 1.0 User's manual. The relatedness coefficients for the pair Aglianico like-to-type—Aglianico true-to-type showed the typical values of PO relationship (0; 1; 0.5):  $\Delta_7 = -0.066$ ,  $\Delta_8 = 0.939$ , and  $r = 0.399$ . Aglianico like-to-type and Sirica showed the typical values of halfsibs/avuncular/grandparent-grandchildren (HS/AV/GP-GC) values: 0; 0.5; 0.25 vs 0.092; 0.442; 0.313. While  $\Delta_7$ ,  $\Delta_8$ , and  $r$  coefficients values for Aglianico true-to-type—Sirica pair (0.008; 0.372; 0.178) were similar to double first cousins relatedness values (0; 0.375; 0.25).  $\Delta_7$ ,  $\Delta_8$ , and  $r$  coefficients confirmed the PO relationship between Aglianico like-to-type and Aglianico true-to-type highlighted detecting the number of share alleles per locus.

In order to deeply investigate these relationships and to verify their significances, the number of analyzed SSR loci was increased to 43 for the three identified genotypes and for a set of 12 additional samples as previously reported. The number of samples was increased up to 45 adding allelic profile of varieties characterized in literature [12, 15, 26–28], reporting data for the most part of the 43 SSR markers. The number of shared alleles per locus estimated for 43 SSR allelic profiles confirmed the results obtained by the genotyping with 21 SSR loci: Aglianico like-to-type and Aglianico true-to-type genotypes are linked by PO relationship. The 27 additional samples were included to validate the algorithm used to calculate  $\Delta_7$ ,  $\Delta_8$ , and  $r$  values and to investigate new relationships. Among these, three genotypes with known genetic relationships (Dureza  $\times$  Mondeuse blanche = Syrah and Dureza–Teroldego = full-sibling relationship) were included. Table 4 reports only the genotype combinations with significant attributions to relationship category. As expected, the established PO pairs Dureza–Syrah and Mondeuse blanche–Syrah were confirmed (0; 1; 0.5). Different from Vouillamoz and Grando [15], the Dureza–Teroldego

**Table 2** Number of alleles ( $N_a$ ), number of effective alleles ( $N_e$ ), number of private alleles ( $N_p$ ), observed and expected heterozygosity ( $H_o$  and  $H_e$ ) values of an 179 samples set genotyped with 21 SSR loci

Locus	$N_a$	$N_e$	$N_p$	$H_o$	$H_e$
VMC1b11	14	5.764	4	0.686	0.827
VMC4f3	17	7.321	1	0.732	0.863
VrZag62	13	6.755	3	0.696	0.852
VrZag79	13	8.412	0	0.752	0.881
VVIB01	11	4.763	2	0.808	0.790
VVIH54	14	4.913	2	0.798	0.796
VVIN16	7	3.386	0	0.732	0.705
VVIN73	7	2.484	1	0.216	0.597
VVIP31	15	10.232	1	0.848	0.902
VVIP60	14	3.964	1	0.768	0.748
VVIQ52	8	3.584	1	0.848	0.721
VVIV67	16	6.839	5	0.803	0.854
VVMD5	14	6.712	4	0.824	0.851
VVMD7	15	7.526	1	0.712	0.867
VVMD21	10	2.802	1	0.664	0.643
VVMD24	9	3.972	2	0.792	0.748
VVMD25	17	6.410	5	0.752	0.844
VVMD27	11	7.051	2	0.800	0.858
VVMD28	23	11.617	5	0.864	0.914
VVMD32	18	7.622	4	0.944	0.869
VVS2	16	8.597	2	0.768	0.884

**Table 3** SSR profiles at 43 loci of 3 identified genotypes among 41 Aglianico and 9 Sirica accessions

Locus	Aglianico like-to-type	Aglianico true-to-type	Sirica	Pinot Noir
VMC1b11	170	168	168	166
	184	184	188	172
VMC1c10	147	159	159	156
	171	171	171	156
VMC1e8	209	209	229	225
	229	229	231	229
VMC2b3	185	176	167	162
	185	185	176	168
VMC2b5	202	190	190	189
	215	202	198	193
VMC2h4	201	210	218	203
	210	210	218	203
VMC4c6	162	162	156	162
	174	162	174	162
VMC4f3	166	166	172	172
	205	166	205	178
VMC5a1	154	154	165	154
	165	165	169	164
VMC5c1	146	146	146	146
	146	146	172	165
VMC5c5	118	118	118	116
	118	118	118	118
VMC5g8	312	312	312	312
	316	312	316	316
VMC5h2	192	192	192	192
	208	208	208	208
VMC5h5	168	176	176	168
	194	194	192	178
VMC6e1	167	141	162	151
	167	167	167	165
VMC6e10	97	97	105	107
	116	105	109	109
VMC8f10	220	233	211	195
	236	236	233	197
VMC9b5	247	247	247	243
	251	251	261	247
VrZag29	113	113	113	113
	115	115	117	117
VrZag62	187	187	187	187
	192	187	192	193
VrZag79	242	242	242	236
	255	244	248	242
VrZag83	194	194	194	194
	200	200	200	206
VrZag93	191	191	191	189
	199	191	199	189

**Table 3** continued

Locus	Aglianico like-to-type	Aglianico true-to-type	Sirica	Pinot Noir
VVIB01	289	287	289	287
	293	293	293	293
VVIH54	162	167	162	167
	167	167	167	171
VVIN16	148	148	148	148
	152	152	152	157
VVIN73	262	262	260	262
	262	262	262	264
VVIP31	182	174	182	176
	196	182	188	180
VVIP60	303	303	313	317
	329	319	315	319
VVIQ52	79	79	81	77
	84	84	84	77
VVIV67	352	352	356	358
	352	352	374	366
VVMD5	228	228	222	224
	234	242	228	234
VVMD7	238	238	238	238
	260	238	238	242
VVMD17	222	222	210	210
	222	222	222	219
VVMD21	240	240	244	244
	244	240	262	244
VVMD24	206	210	206	214
	210	210	210	216
VVMD25	236	246	246	244
	246	260	246	254
VVMD27	187	181	185	183
	187	187	187	187
VVMD28	228	228	218	218
	258	258	228	236
VVMD32	250	250	240	240
	256	256	272	272
VVMD34	223	240	240	240
	240	240	240	240
VVMD36	261	267	251	251
	291	291	291	251
VVS2	139	149	129	133
	153	153	129	147

*Reference variety* Pinot Noir

pair showed the typical values of half-sibship. This result could explain the coefficients for Dureza–Teroldego laid in-between the values of FS and the second degree relatives found in Vouillamoz and Grando [15]. The HS relationship between Dureza and Teroldego was supported by AV values

found between Syrah and Teroldego. Values close to PO theoretical values for Aglianico like-to-type—Aglianico true-to-type were found. Aglianico true-to-type and Syrah showed a combination of  $\Delta_7$ ,  $\Delta_8$ , and  $r$  values near to AV theoretical values. Syrah appeared to be related to Aglianico like-to-type by FC (first cousin: 0; 0.25; 0.125) relationship. Among genotypes with known genetic relationships, we found an HS (half-sibs) relationship between Aglianico true-to-type and Dureza genotypes (theoretical values: 0; 0.5; 0.25) and an AV relationship between Aglianico like-to-type and Dureza. Aglianico true-to-type and Teroldego appeared to be the third degree or more distant relatives, supporting the second degree relative relationship for Dureza and Teroldego.

### Pedigree Reconstruction

A pedigree illustrating the reconstruction of the most likely relationships among these varieties was constructed (Fig. 1). The reconstruction was consistent with the genotypes having known genetic relationships (Dureza  $\times$  Mondeuse blanche = Syrah). A common origin was highlighted between Dureza and Aglianico true-to-type. Based on this suggestion, we hypothesized that Dureza and Aglianico could share the same unknown parent. A first-degree relationship (PO) was identified between Aglianico like-to-type—Aglianico true-to-type pair, strongly supported by the number of loci with at least one shared allele (43),  $\Delta_7$ ,  $\Delta_8$ , and  $r$  values ( $\Delta_7 = 0.082$ ,  $\Delta_8 = 0.955$ , and  $r = 0.547$ ). Our data also highlighted Syrah as the second degree relative (nephew) of Aglianico true-to-type. These main identified relationships were supported by FC relationship between Aglianico like-to-type and Syrah ( $r =$

0.156) and AV relationship between Aglianico like-to-type and Dureza ( $r = 0.241$ ).

### Discussion

The SSR molecular marker method is the most widely used approach to identify grapevine material, to confirm true-ness-to-type, and to search and/or to confirm parents of grapevine varieties [8, 13, 30]. Starting from the genotyping of 179 grapevine samples with 21 SSR markers, a parentage analysis of Aglianico cultivar was investigated. In order to measure the genetic diversity of this set, Na, Ne, Np, Ho, and He per each locus were estimated. The values of these statistical parameters are typical for a species with high genetic variability, as reported in Laucou et al. [9].

Regarding the Aglianico accessions analyzed in this work, the variety true-to-type [1, 29] and the monophyletic origin of its biotypes (Taburno, Taurasi, and Vulture), suggested by Costantini et al. [1], were confirmed. Aglianico biotypes are clonal lines selected in different growing areas noticeable for morphological and physiological traits. The differences among the 3 biotypes regard mainly the morphology of the bunches and the timing of veraison and ripening. In detail, Taurasi and Taburno biotypes have a conic-pyramidal cluster, often with a lateral wing, while the Vulture biotype has a short stemmed, more compact, smaller, conic-cylindric cluster, only occasionally with a lateral wing. The Taburno biotype is more vigorous, higher in yield potential, and later in the timing of ripening than the other two biotypes.

In Campania, there are several local accessions having the word “Aglianico” in their names. Genetic characterization showed that Aglianico amaro, Aglianichello, and Aglianico Pannarano are synonyms of Aglianico true-to-type, while Aglianico di Napoli and Aglianicone are different from Aglianico true-to-type [1].

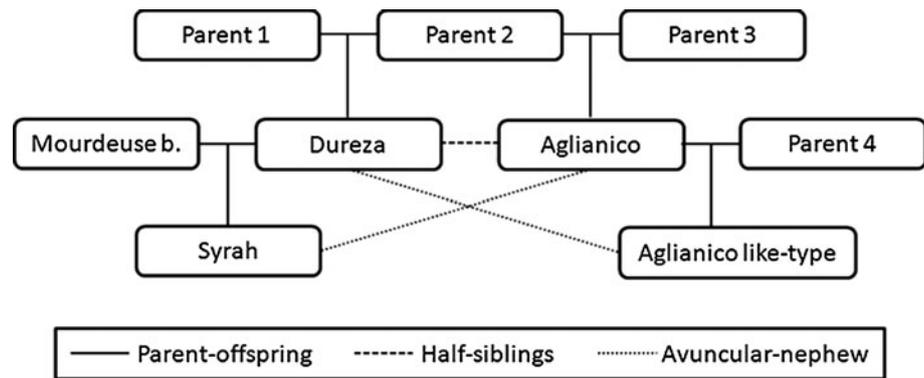
For the eight accessions of Aglianico Taburno (Aglianico like-to-type) having a different allelic profile from Aglianico true-to-type, no matches were found with varieties reported in EVD or with other local cultivars from Campania [1]. No information is available about the growing and spreading of this Aglianico-related putative cultivar in Campania region or in other wine-growing areas. Probably, it was just considered a variant of Aglianico whose importance declined after the spreading in Campania of Phylloxera (*Daktulosphaira vitifoliae* Fitch). Phylloxera is an insect (Homoptera: Phylloxeridae), introduced in Europe from Northern America, attacking grapevine roots. In the second half of the 19th century, this insect destroyed a significant portion of the European vineyards, as a result, a reduction of the diversity occurred in cultivated grapevines. After the introduction of Phylloxera, the vineyards were replanted: *Vitis vinifera* cultivars were

**Table 4** Two-gene ( $\Delta_7$ ), four-gene ( $\Delta_8$ ), and relatedness ( $r$ ) coefficients values for three individual pairwise analyzed with 43 SSR loci on a set of 31 varieties

Pairs of genotypes	$\Delta_7$	$\Delta_8$	$r$	RCA
1–2	0.082 (0.043)	0.955 (0.047)	0.547 (0.039)	PO
1–3	0.044 (0.078)	0.233 (0.052)	0.156 (0.065)	FC
1–4	−0.075 (0.062)	0.430 (0.114)	0.241 (0.100)	AV
2–3	−0.037 (0.040)	0.541 (0.065)	0.264 (0.056)	AV
2–4	−0.046 (0.038)	0.452 (0.049)	0.221 (0.019)	HS
3–4	−0.072 (0.115)	0.941 (0.012)	0.438 (0.088)	PO
3–5	0.011 (0.092)	0.872 (0.111)	0.466 (0.043)	PO
3–6	0.022 (0.083)	0.539 (0.040)	0.289 (0.073)	AV
4–6	0.010 (0.120)	0.428 (0.065)	0.200 (0.061)	HS

Dureza (4), Mondeuse blanche (5), and Teroldego (6), having known genetic relationships, were used to validate the analysis. Only the significant relationships was reported. Brackets: standard deviation, calculated with 1,000 bootstraps over loci

1 Aglianico like-to-type, 2 Aglianico true-to-type, 3 Sirica, RCA relationship categories assignment, AV avuncular, FC first cousins, FS full-sibling, HS half-sibling, PO parent-offspring

**Fig. 1** Aglianico pedigree reconstruction

grafted onto Phylloxera-resistant rootstocks selected from American *Vitis* species and hybrids. This management was applied mainly to the varieties showing the most interesting agronomic and enological potential, resulting in the emergence of a limited number of cultivars, that still nowadays dominate European viticulture, causing at the same time the disappearance of minor local cultivars [31].

The Sirica SSR profile matched with the Syrah variety reported in Vouillamoz and Grando [15], except for VMC2b5 locus, where we found a discrepancy of 10 bp in the highest allele. This difference can be accepted if we consider the age of Sirica plants, about 100-year old (DNA was extracted by apical leaves). The frequency of mutations at microsatellite sites is directly proportional to the age of the variety. The probability to detect a mutation is also related to the number of microsatellite loci analyzed [32]. It was accredited that the most likely cause of mutations resulting in the length variation of microsatellite repeats is the DNA polymerase slippage [33].

The parentage analysis was carried out by estimating the number of shared alleles per locus and the relatedness coefficients ( $\Delta_7$ ,  $\Delta_8$ , and  $r$  values), for the PO relationship, and just the relatedness coefficients for the second and the third degree of kinship.  $\Delta_7$ ,  $\Delta_8$ , and  $r$  values suggested that: (i) Dureza and Aglianico share an unknown parent; (ii) Aglianico like-to-type genotype is the progeny of “Aglianico true-to-type  $\times$  unknown variety”; and (iii) Sirica is related to Aglianico true-to-type by AV relationship. Aglianico like-to-type, proposed as a seedling progeny of Aglianico true-to-type, was underlined by the number of shared alleles and confirmed by relatedness coefficients data. These evidences highlight that Aglianico has had a significant impact for the generation of new genotypes by sexual reproduction to develop and enhance the viticulture of Campania by breeding a pool of local varieties. Demonstrations of spontaneous and non-spontaneous crossing events between varieties are very frequent in the grapevines Italian varietal assortment, as noticed by Cipriani et al. [8]. Moreover, other observations suggest that grapevines breeding has been restricted to a relatively small number of parent cultivars, as reported by Crespan et al. [17] for two

Italian examples, Sangiovese and Garganega, and that only a small number of the possible genetic combinations have been explored [34].

Our data and pedigree reconstruction suggest that Aglianico true-to-type and Sirica (synonymous of Syrah) are related by AV relationship. This result provides the evidence of an unexpected genetic relationship between these two varieties, geographically placed in two different and distant growing areas, Rhone Valley and Campania. Indeed, Syrah is a famous Rhone Valley red grape cultivar [35], one of the noblest wine grape cultivars, planted worldwide; progeny of Dureza  $\times$  Mondeuse blanche [36], sibling of Viognier [34], and the third degree relative of Pinot [15]. The experimental data show that Aglianico is more ancient compared to Syrah. Historical information confirm these results, the first evidences of Aglianico in Campania are dated in the first half of the 16th century (land registry document), while Syrah is dated after the 18th century [37].

Actually, we do not have enough elements to understand the association between the geographic and the genetic origin of these two varieties to explain the viticultural migrations. There is no historical information about migration of Aglianico from Italy to France and vice versa and there is little information about the history of Dureza (Syrah parent); it is a minor, ancient, and endangered variety cultivated in the Rhône Alpes area [38]. The discovery of a shared parent between Dureza and Aglianico could allow identifying the processes leading these two cultivars to spread in two of the most important viticultural areas.

## Conclusions

SSR molecular markers are a useful instrument to investigate the identification of genetic resources and to estimate and establish the relatedness between genotypes when pedigree information is unknown. The aim of this work was to investigate the genetic relationship of Aglianico cultivar, the most important Campanian grapevine cultivar. Our evidences allow reconstructing the Aglianico pedigree. The

unexpected result obtained by these data is the AV (avuncular) relationship between Aglianico and Syrah, validated by the genetic analysis of 43 loci SSR, confirming the intensive and successful breeding work of viticulturists in rising progeny by sexual reproduction. The connection point between these two varieties is Dureza, a parent of Syrah and a half-sibling of Aglianico. The absence of information about shared parents between Aglianico and Dureza will open the way for future investigations to know migrations of genetic resources between two main viticultural areas, Campania (Italy) and Rhône Alpes (France).

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