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Investigations on the identity of ‘Canaiolo bianco’ and other white grape varieties of central Italy

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Summary

During research for the rescue and description of grapevine germplasm in central Italy, 28 white accessions were collected, 10 of them sharing the name ‘Canaiolo bianco’ but having different morphological and yield characteristics. ‘Canaiolo bianco’ is listed as essential in the specifications for some D.O.C.G. and D.O.C. Tuscan wines, but its correct identity is not clear. With the aim to identify and characterize our samples and to pinpoint what the true-to-type ‘Canaiolo bianco’ is, we described these accessions using a multidisciplinary approach, encompassing DNA analysis, ampelographic, phyllometric, phenological and yield descriptions. Moreover we collected as much historical information as possible about them. The 28 accessions investigated were grouped in 8 varieties with very distinct traits. The research has provided useful information for clarifying various cases of synonymy and homonymy. Moreover, we hypothesize that the true to type ‘Canaiolo bianco’ corresponds to the so called ‘Drupeggio’.

Key words: *Vitis vinifera* L., microsatellite markers, phyllometric analysis, synonyms, true-to-type.

Introduction

Tuscan viticulture boasts a centuries-old tradition, characterized by high ampelographic variability that, as well as constituting an important source of biodiversity, has also given rise to various cases of synonymy and homonymy between similar grapevine varieties.

The change of variety assortment in the latter half of last century led to a marked genetic erosion with the consequent risk of loss of germplasm, which has been partially remedied by various programs of recovery and conservation of the minor grapevine varieties. As part of the work of recovery and description of germplasm, we investigated the identity of some white berry varieties coming from different viticultural areas and bearing different local names.

We focused particular attention on ‘Canaiolo bianco’, an ancient minor variety registered in the Italian catalogue. ‘Canaiolo bianco’ is part of the Tuscan viticultural tradition; the oldest description we found dates back to the first half of 18th century by Pier Antonio Micheli (in VERGARI and SCALACCI 2008). Numerous citations attest its histori-

cal use in wine-making, even if it is currently only grown on a total of 3.98 ha (ARTEA 2008) and has not been propagated by nurseries for at least a decade. ‘Canaiolo bianco’ is listed as an essential grapevine variety in the specifications for Carmignano D.O.C.G. (Controlled and Guaranteed Denomination of Origin), Barco Reale di Carmignano D.O.C. (Controlled Denomination of Origin) and Bianco della Valdinevole D.O.C. wines. Two clones have been registered in the Italian catalogue of grapevine varieties: USPIFI SCA 27 in 1987, later withdrawn, and the recent ‘Canaiolo bianco’ ARSIAL-CRA 402.

A first series of observations on accessions growing in Tuscan vineyards and sharing the name ‘Canaiolo bianco’ demonstrated that the morphological and yield characteristics were different, although some morpho-physiological traits of berry and bunch were in common, which is of medium-large size with not very firm flesh and a neutral flavor. We therefore set up a study to identify the true-to-type ‘Canaiolo bianco’, starting from the indications given by the viticulturists and conducting investigations on ten accessions from different sources, in particular from some farms in the Carmignano D.O.C.G. area (Prato, Tuscany) and in the Orvieto area (the Umbria region).

Moreover we added to this comparison eighteen accessions showing analogies with some of the recovered samples of ‘Canaiolo bianco’. Some of these vines belong to varieties recognizable as synonyms of ‘Canaiolo bianco’ from historical references.

Material and Methods

The work was conducted in 2007–2009 on the 28 accessions listed in Tab. 1. The following analyses and measurements were performed: a) DNA analysis with microsatellite markers (SSR). The DNA was extracted from leaflets at the tip of the shoot and analyzed using 11 SSR markers: the 6 loci suggested in the European project GenRes081 (THIS *et al.* 2004), plus 5 others routinely used at CRA-VIT for varietal identification, *i.e.* VVMD28 (BOWERS *et al.* 1999), ISV2, ISV3, ISV4 and VMCNG4b9 (CRESPIAN 2003, WELTER *et al.* 2007). The protocols of DNA extraction and analysis are the same as reported in CRESPIAN *et al.* (2006). b) Ampelographic descriptions according to the O.I.V. list (2007) on 40 characters. c) Phyllometric analysis, performed on samples of 20 mature leaves and SuperAmpelo software (SOLDAVINI *et al.* 2009).

Table 1

List of the 28 accessions studied, their origin and identity according to the obtained results

ID	Accession name	Identity	Origin
1	Unknown white (A2CUB)	unknown G2	Terranuova Bracciolini (Arezzo)
2	Bottaio bianco (A43CUB)	Drupeggio	Terranuova Bracciolini (Arezzo)
3	Bottaio bianco (SCP1)	Drupeggio	Scopetone (Arezzo)
4	Canaiole bianco (FI)	Drupeggio	Rufina (Firenze)
5	Canaiole bianco (LU)	unknown G3	Lucca
6	Canaiole bianco (Fornace)	unknown G1	Carmignano (Prato)
7	Canaiole bianco (104)	Zuccaccio	Rufina (Firenze)
8	Canaiole bianco (Calavria 1)	Vernaccia di San Gimignano	Carmignano (Prato)
9	Canaiole bianco (Calavria 2)	Vernaccia di San Gimignano	Carmignano (Prato)
10	Canaiole bianco (F 19)	Chasselas	Bibbiena (Arezzo)
11	Canaiole bianco (ISV 27)	Vernaccia di San Gimignano	Carmignano (Prato)
12	Canaiole bianco (POP 2)	Zuccaccio	Poppi (Arezzo)
13	Canaiole bianco (S. Felice)	Vernaccia di San Gimignano	Carmignano (Prato)
14	Drupeggio clone 10 (not still recorded)	Drupeggio	Orvieto (Terni)
15	Drupeggio clone 7 (not still recorded)	Drupeggio	Orvieto (Terni)
16	Grecia (152)	unknown G3	Montecarlo (Lucca)
17	Greco di Pitigliano (5)	Empibotte	Pitigliano (Grosseto)
18	Promaticcia (3)	Drupeggio	Castelnuovo Berardenga (Siena)
19	Promaticcia (B14)	Drupeggio	Vinci (Firenze)
20	Riminese (157)	Empibotte	Pitigliano (Grosseto)
21	Vernaccia di San Gimignano (21)	Vernaccia di San Gimignano	San Gimignano (Siena)
22	Vernaccia di San Gimignano (S. Felice)	Vernaccia di San Gimignano	San Gimignano (Siena)
23	Vernaccia di San Gimignano (Tofanari)	Vernaccia di San Gimignano	San Gimignano (Siena)
24	Volpicchio (F2)	Drupeggio	Bibbiena (Arezzo)
25	Zuccaccio (A1CUB)	Zuccaccio	Terranuova Bracciolini (Arezzo)
26	Zuccaccio (A37CUB)	Zuccaccio	Terranuova Bracciolini (Arezzo)
27	Zuccaccio (CARR 9)	Zuccaccio	Terranuova Bracciolini (Arezzo)
28	Zuccaia bianca (accession n. 3227)	Zuccaccio	University of Florence

On the basis of the genotyping results obtained through SSR profiles, the phenological phases, average bunch and berry weight, as well as analytical data on the macrostructure of the must were recorded on just one accession of each of the identified cultivars, selected from those held in the Arezzo CRA-VIC collection.

Results and Discussion

Molecular analyses: Genotyping with SSR markers produced 8 molecular profiles (Tab. 2). In order to identify the corresponding varieties, they were compared with the CRA-VIT molecular database (unpubl. data) and only two of them corresponded to varieties registered in the Italian National Catalogue, 'Vernaccia di San Gimignano' and 'Chasselas'.

The eight accessions nos. 2, 3, 4, 14, 15, 18, 19 and 24 had the same profile. They were found under various names: 'Bottaio bianco', 'Canaiole bianco', 'Promaticcia', 'Volpicchio', with only the accessions from the Umbria region being called 'Drupeggio'. We believe that this is the true-to-type 'Drupeggio' and propose this genotype as reference on the basis of i) the knowledge that 'Drupeggio' or 'Duropeggio' was common in the Orvieto area (Umbria) from the beginning of 1800 until now (BALDINI 1995), and ii) the available ampelographic description of 'Drupeggio'

(CARTECHINI and MORETTI 1989) we used to compare our accessions matches that of our samples.

Seven accessions (nos. 8, 9, 11, 13, 21, 22 and 23) were identified as 'Vernaccia di San Gimignano'. In this case, the original names were 'Canaiole bianco' (four samples, all from the same area of Carmignano) and 'Vernaccia di San Gimignano'.

The six accessions nos. 7, 12, 25, 26, 27 and 28 had the same profile, not ascribable to any of those in the CRA-VIT molecular archive. Of these, two were called 'Canaiole bianco' and the others were known as 'Zuccaccio' from the area where they were found. This group included the 'Zuccaia' from CRA-VIT collection, a name that sounds very similar to 'Zuccaccio'.

Two accessions (17 and 20) matched with an 'Empibotte' sample analysed at CRA-VIT and coming from the Marche region. Accession n. 10 had the profile of 'Chasselas blanc' and was an obvious error of designation.

Four accessions were not identified because their molecular profiles did not find any match with the CRA-VIT database. Because the names of these accessions could not be used to identify them, they were defined as unknown G1 (no. 6), G2 (no. 1) and G3 (nos. 5 and 16). In the case of both G1 and G3, the original names once again correspond to 'Canaiole bianco'.

Ampelographic and phylometric characterization: The accessions referable to

Table 2
SSR profiles of the 8 genotypes found

Variety	Drupeggio	Chasselas	Empibotte	Vernaccia di San Gimignano	Zuccaccio	unknown G1	unknown G2	unknown G3	
Microsatellite loci	VVS2	133-145	133-143	133-143	135-143	133-137	143-155	145-155	133-133
	VVMD5	228-240	228-236	226-232	226-226	226-246	228-240	226-246	226-226
	VVMD7	239-249	239-247	249-253	239-249	239-247	247-253	247-249	239-239
	VVMD27	181-183	185-189	183-194	183-189	185-189	181-191	179-191	185-189
	VVMD28	247-261	221-271	239-251	247-261	239-239	237-261	251-261	237-261
	VrZAG 62	187-189	193-203	199-203	187-189	187-203	191-203	189-203	185-195
	VrZAG 79	246-258	250-258	246-250	238-244	250-250	246-258	246-256	244-250
	ISV2	161-165	141-165	141-157	141-141	141-165	169-169	141-165	137-165
	ISV3	139-139	133-139	133-139	139-145	133-145	133-139	133-139	133-145
	ISV4	187-197	169-177	169-187	169-197	169-177	177-177	177-187	177-187
VMCNG4b9	150-158	158-162	158-176	150-166	150-158	158-158	158-166	158-164	

the four varieties identified by the molecular analyses, *i.e.* 'Drupeggio', 'Vernaccia di San Gimignano', 'Zuccaccio' and 'Empibotte' ('Chasselas' was excluded being a well-described variety and only found occasionally in the surveyed areas), were compared for their morphological traits and the results of the DNA analysis were confirmed.

Tab. 3 shows the most relevant ampelographic traits (20 out of 40) that identify the four genotypes for each variety observed in the same environmental and cultural conditions (CRA-VIC collection), while the Figure shows the standard profiles of the mean leaf shape.

There are appreciable differences in the shoot tip with regard to the intensity of anthocyanin coloration and the density of prostrate hairs. Clear differences are observed in the color of the upper side and in the hairiness of young leaf. In addition mature leaves differ very clearly in the profile of the blade, shape and dimensions of the teeth, and in the degree of petiole sinus opening. From the ampelometric analysis (data not shown) 'Zuccaccio' has the smallest leaf, while 'Drupeggio' has the deepest upper lateral sinuses. Calculation of the biometric values shows that 'Zuccaccio' has a circular shaped leaf, 'Drupeggio' and 'Empibotte' are wedge-shaped, while the 'Vernaccia di San Gimignano' leaf has a mixed shape, wedge and pentagonal, and an open V shaped petiole sinus. On the contrary, the bunch and the berries have rather similar characteristics. Interestingly, 'Drupeggio' has pinkish tinges on the skin when ripe, whereas the others are generally green-yellow. Finally, 'Empibotte' and 'Drupeggio' are the most vigorous, with shoots of larger diameter and long internodes (data not shown).

Phenological and yield aspects: Tab. 4 reports the phenological description of the 4 identified genotypes and some information on bunch and berry weight, sugar and acidity contents computed as a mean of the period 2005-2009.

Based on the above results, the ten accessions named 'Canaiolo bianco' encompassed 6 different varieties. So, the question is: which one is the true-to-type 'Canaiolo bianco'? We tried to give an answer by assembling the puzzle of the molecular, morphological and the available historical information.

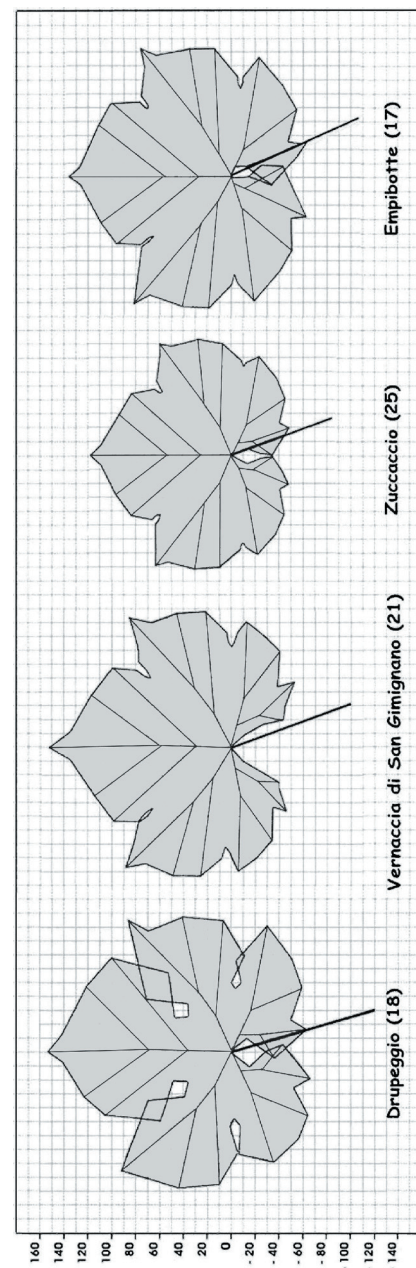


Figure: Standard leaf profiles of 'Drupeggio', 'Vernaccia di San Gimignano', 'Zuccaccio' and 'Empibotte'.

Table 3

Ampelographic description of ‘Vernaccia di San Gimignano’, ‘Drupeggio’, ‘Zuccaccio’ and ‘Empibotte’

Characters	Code OIV	Variety name (ID number of the accession described from Tab. 1)	Vernaccia di San Gimignano (21)	Drupeggio (18)	Zuccaccio (25)	Empibotte (17)
Shoot	3	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	high	none or very low	none or very low	low
	4	Density of prostrate hairs on the shoot tip	medium	low	medium-high	medium
	15-2	Intensity of anthocyanin coloration on the bud scales	none or very weak	medium	none or very weak	none or very weak
Young leaf	51	Color of upper side of blade	yellow-bronze	green-yellow	yellow	green
	53	Density of prostrate hairs between main veins on lower side of blade	medium	high	high	high
	68	Numbers of lobes	three-five	five	three-entire leaf	five
	74	Profile of blade in cross section	flat; V-shaped	V-shaped; involute	revolute; twisted	involute
	75	Blistering of upper side of blade	medium; weak	medium	weak	weak
	79	Degree of opening/overlapping of petiole sinus	open	open-closed-overlapped	open-closed-overlapped	overlapped
	80	Shape of base of petiole sinus	V-shaped	brace-shaped	V-shaped	V-shaped
	81-1	Teeth in the petiole sinus	none	none	none	present
	84	Density of prostrate hairs between main veins on lower side of blade	low	high	high	medium
87	Density of erect hairs on main veins on lower side of blade	none or very low	low	high	none or very low	
Woody shoot	103	Main color	grey-brownish	brownish-red on nodes	brownish-red	brounish
Bunch	202	Length	short	medium	medium	medium; long
	204	Density	medium; dense	dense	medium, loose	dense
	206	Length of peduncle of primary bunch	short	short	medium	medium
	208	Shape	conical	conical	conical	cylindrical
Berry	225	Color of skin	green-yellow	green-yellow-pink	green-yellow	green-yellow
	235	Firmness of flesh	soft	soft	slightly firm	soft

At first, the four accessions called ‘Canaiolo bianco’ found in the Carmignano D.O.C. production area have the molecular profile of ‘Vernaccia di San Gimignano’. San Gimignano is a little ancient town in the Siena province, where the homonymous ‘Vernaccia’ has been cultivated since the 13th century (in VERGARI and SCALACCI 2008).

Secondly, we observed that the official ampelographic descriptions of ‘Canaiolo bianco’ (BREVIGLIERI and CASINI, 1962) and ‘Vernaccia di San Gimignano’ (BRUNI *et al.* 1962), edited by the Ministry of Agriculture, look very similar and are ascribable to the same variety: ‘Vernaccia di San Gimignano’, as confirmed by the DNA analyses reported by TORELLO MARINONI *et al.* (2009). These authors showed that the Ligurian variety ‘Piccabòn’, known also as ‘Bervedino’ in Emilia Romagna, ‘Vernaccia di San Gimignano’ and the Tuscan ‘Canaiolo bianco’ have the same molecular profile. However, the hypothesis that ‘Canaiolo bianco’ and ‘Vernaccia di San Gimignano’ were once the

same grapevine is denied by some observations. In the old descriptions ‘Canaiolo bianco’ is reported as having dense hairs on the lower leaf blade (GALLESIO 1817 and 1839), while ‘Vernaccia di San Gimignano’ has sparse hairs (descriptor 84 in Tab. 3). Moreover ‘Primaticcio’, alias ‘Promaticcia’, is a historical synonym of ‘Canaiolo bianco’ (GALLESIO, 1817 and 1839), as ‘Uva vecchia’ (RACAH 1932). ‘Primaticcia’ and ‘Uva vecchia’ look like the genotype we identified as ‘Drupeggio’ and not ‘Vernaccia di San Gimignano’: from our data, the molecular profile of ‘Promaticcia’ is different from that of ‘Vernaccia di San Gimignano’ and its ampelographic traits match that of ‘Uva vecchia’ described by SCALABRELLI *et al.* (2008), which in turn clearly corresponds to ‘Drupeggio’. Finally, ‘Drupeggio’ is the only official synonym of ‘Canaiolo bianco’ reported in the Italian catalogue, while ‘Vernaccia di San Gimignano’ is clearly different from ‘Drupeggio’ (Tabs 2 and 3). For all these reasons we believe that ‘Vernaccia di San Gimigna-

Table 4

Phenological phases (mean of 5 years), qualitative and quantitative parameters of 'Vernaccia di San Gimignano', 'Drupeggio', 'Zuccaccio' and 'Empibotte'

Variety name (ID number of the accession described from Tab. 1)	Vernaccia San Gimignano (21)	Drupeggio (18)	Zuccaccio (25)	Empibotte (17)
Bud Burst (decade)	April, II nd	April, I st	April, II nd	April, III th
Full Bloom (decade)	June, I st	June, I st	June, I st	June, I st
Veraison (decade)	August, I st	August, I st	August, II nd	August, II nd
Harvest (decade)	September, II nd	September, II nd	September, II nd	October, I st
Yield (Kg/mq)	1.63	1.83	1.07	1.89
Bunch Weight (g)	322.8 ± 72.2	423.6 ± 95.2	229.1 ± 25.8	448.2 ± 123.6
Berry Weight (g)	2.47 ± 0.12	2.12 ± 0.55	2.67 ± 0.46	2.62 ± 0.30
Sugar Content (°Brix)	19.7 ± 1.6	20.7 ± 1.4	18.4 ± 1.3	19.1 ± 1.4
Titrate acidity (g/L)	5.4 ± 1.1	6.9 ± 1.2	5.3 ± 0.6	7.7 ± 1.6

no' and 'Canaiolo bianco' can not be synonyms. Moreover, the very recent 'Canaiolo bianco' clone ARSIAL-CRA 402 (*i.e.* Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricoltura del Lazio and Consiglio per la Ricerca e la Sperimentazione in Agricoltura) has the molecular profile of 'Drupeggio' (data not shown). Putting together all the above information we conclude that the true-to-type 'Canaiolo bianco' corresponds to 'Drupeggio'.

Accessions nos. 7 and 12 wrongly bear the name 'Canaiolo bianco' as they have been identified as 'Zuccaccio', an ancient variety known in different areas of Tuscany, mentioned by SODERINI (1600), and recently described by ARMANNI and LEPRINI (2008).

'Greco di Pitigliano' and 'Riminese', both from the Pitigliano area (Grosseto), match with an 'Empibotte' accession analyzed at CRA-VIT. We are aware that 'Empibotte' is a generic name and refers to different varieties (Oriana Silvestroni, personal communication), therefore it is not surprising that our Empibotte is morphologically different from that described by BRUNI (1960). Nevertheless our genotype matches at four SSR loci with an Empibotte accession analysed by FILIPPETTI *et al.* (2001).

Lastly, no results have been obtained for the accessions belonging to the three unknown genotypes (nos. 1, 5, 6 and 16), two of which named 'Canaiolo bianco', due to the lack of historical and local information on them. The characterization of these varieties is under way.

It may therefore be concluded that in Tuscany 'Canaiolo bianco' lost its original identity over time, both because it was confused with 'Vernaccia di San Gimignano' and because, although found in many geographical areas, it is sparsely cultivated under many different local names. 'Primaticcio', 'Uva vecchia' and 'Drupeggio', which are the same variety by crossing molecular and ampelographic data, are well known historical synonyms of 'Canaiolo bianco'. In this research additional synonyms never reported before have been found, such as 'Bottaio bianco' and 'Volpicchio'.

Finally, given that old and present ampelographic descriptions, and accession names too, support the correct identification of 'Zuccaccio', we propose our SSR profile as the reference for this ancient variety.

Conclusions

This research showed the occurrence of synonymy and homonymy cases in a group of 28 white grape accessions cultivated in central Italy. They have been assigned to 8 varieties, with distinct morphological, genetic, phenological and yield characteristics. Of these, three have been distinguished by their wider diffusion and numbers of accessions traced, *i.e.* 'Drupeggio', 'Vernaccia di San Gimignano' and 'Zuccaccio'.

On the basis of historical and morphological data, we hypothesize that the true-to-type 'Canaiolo bianco' matches 'Drupeggio' and not 'Vernaccia di San Gimignano'. To verify this assumption, we plan to extend the study by searching for additional accessions both in Tuscany and also in other regions of central Italy.

This work may therefore be considered as a first step towards the definition of the identity of 'Canaiolo bianco'.

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