

Verifying synonymies between grape cultivars from France and Northwestern Italy using molecular markers

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Summary

Thirty-one grape cultivars from France and Northwestern Italy, presumed to be synonymous, were analyzed using RAPD (Random Amplified Polymorphic DNA) and SSR (Simple Sequence Repeats or microsatellite) markers to verify 25 synonym hypotheses. RAPD analyses were performed with 8 selected decamer primers and the profiles of 7 microsatellite loci were used in order to confirm RAPD results, if required. Sixteen synonymies were confirmed, including the French cv. Verdesse with the Italian cv. Bianver, the French cv. Persan with the Italian cv. Biquet, the French cv. Chatus with the Italian cv. Neiret, the French cv. Gouais blanc with the Italian cvs Preveiral and Liseiret. Most of the investigated cultivars belong to the *Vitis vinifera* germplasm of both sides of the Western Alps and the occurrence of synonyms indicates the existence of a common pool of grapes grown under different names in this part of Europe.

Key words: *Vitis*, SSR, RAPD, DNA, fingerprinting, synonym.

Introduction

The number of grape varieties grown in countries with traditional and new viticulture has been estimated to be in the range of 5000 to 8000, under 14 000 to 24 000 different names (VIALA and VERMOREL 1901-1910; ALLEWELDT 1988). Besides the major and the minor varieties (around 300-400 cultivars), which account for most of the grape plantings in the world, a pool of local grape cultivars is grown marginally, often endangered and/or not officially registered. In the last few years new attention has been addressed to this almost forgotten source of grapevine diversity and germplasm investigation programs have been developed either for scientific purposes or for increasing wine flavour assortments.

Identifying old grape varieties, investigating their origins (SEFC *et al.* 2000), their parentage (BOWERS and MEREDITH 1997; SEFC *et al.* 1997; BOWERS *et al.* 1999 a; MEREDITH *et al.* 1999), their relationships with other grape cultivars (CUISSET 1997; SEFC *et al.* 1998; BISSON 1999), and listing their names in different areas (synonyms) has gained much interest with respect to the enhancement of our knowledge on genetic

resources of *Vitis vinifera* and on the historical development of viticulture. In most countries with traditional or modern viticulture wine regulations require accurate cultivar identification; *e.g.* in the European Union the use of winegrapes is strictly regulated: only registered and specifically authorized cultivars can be grown and the rules for the wine geographic appellations establish the grapes to be used. Thus, identifying grape varieties and verifying their synonyms has a practical significance.

For many reasons (inaccuracy in propagating woody cuttings, existence of synonyms, mislabelling, lack of reference descriptions, introduction into new growing areas) accurate grape identity often needs to be established (BOURSIQUOT and THIS 1996), especially for those grapes known only to a minor extent.

Random Amplified Polymorphic DNA (RAPD) are reasonably low-price markers that were proved to be successful in distinguishing grape varieties, confirming and/or rejecting synonymy hypotheses (GOGORCENA *et al.* 1993; JEAN-JAQUES *et al.* 1993; BÜSCHER *et al.* 1994; TSCHAMMER and ZYPRIAN 1994; GRANDO *et al.* 1995; MORENO *et al.* 1995; LOUREIRO *et al.* 1998). Microsatellite (or Simple Sequence Repeats, SSR) markers have superior reliability and are particularly suitable for both, DNA typing and parentage analysis studies, being codominant and inherited in a Mendelian way (THOMAS and SCOTT 1993; THOMAS *et al.* 1994; CIPRIANI *et al.* 1994; BOTTA *et al.* 1995; BOWERS *et al.* 1996; BOWERS and MEREDITH 1997; SEFC *et al.* 1997; BOWERS *et al.* 1999 b; SEFC *et al.* 1999).

In this work several cultivars from Italy and France presumed to be synonymous were analyzed using RAPD and SSR markers. Most of the investigated cultivars belong to local grape germplasm of the two sides of the Western Alps (Piedmont, Ain, Isère, Drôme, Savoie, Hautes Alpes) and/or Central France. Extensively grown before the phylloxera invasion, most of these grapes were discarded or less cultivated when vineyards were re-established. Nowadays these marginally grown varieties give locally appreciated wines; sometimes they are endangered.

Since most of these cultivars are not officially registered in Italy and thus are not authorized, their identification and synonym confirmation with other grown varieties could mean for some of them legal acceptance and consequently commercial exploitation. In addition, a better knowledge of the cultivar distribution across the Western Alps will provide general information on their origin, features and history of grape genetic resources in this area.

Material and Methods

Ampelographic data: Thirty-one wine grapes were analyzed (Tab. 1) in order to verify 25 synonym hypotheses. Fifteen cultivars from Italy are mostly grown either in the alpine valleys or at the foot of the Western Alps in the provinces of Turin (TO) and Cuneo (CN). Sixteen grape varieties originate from Eastern and Central France.

Most of the synonymy assumptions are based on vine appearance and on phenological and agronomic features of the cultivars grown in Italy compared with published ampelographic descriptions of the French varieties; visual observations of assumed synonyms were made for the culti-

vars *Avanà/Hibou noir* and *Hibou rouge*, *Neiret/Chatus*, *Canari/Luverdon*.

DNA analysis: For the DNA extraction, leaf samples were harvested from plants in field collections located in Grinzane Cavour (Piemonte, Italy) and at Domaine de Vassal (Montpellier, France). Italian samples were extracted following the procedure described by THOMAS *et al.* (1993). French samples were extracted using the method described by BOWERS *et al.* (1993).

For the RAPD analysis 8 oligonucleotide decamer primers, OPA-2, OPA-7, OPA-10, OPA-11, OPO-2, OPO-5, OPO-7 and OPO-19 (Operon technologies, Alameda, California), were chosen on the basis of their polymorphism in grape

Short description of presumed synonymous cultivars (in bold the names used in this work)

Abondance: extremely generous bearer as the name indicates; ordinary wine; its identity with a grape erroneously called **Nebbiolo di Dronero** in Piedmont was assumed on the basis of morphological and agronomical features reported by GALET (1990).

Avanà: traditionally grown in the Italian Susa Valley, here the major grape; rather early ripening, fruity, soft wines; regarded as a tool to revitalize local viticulture and oenology. The synonymy with **Hibou** mentioned by ROUGIER (VIALA and VERMOREL 1901-1910) was rejected by GALET (1990), who even considered **Hibou noir** to be different from **Hibou rouge**.

Biquet (or Becouet, Becouette, Becuette, Pousse de chèvre, Berla 'd crava cita): rich in colour and extract, traditionally blend in the Susa Valley with the weaker *Avanà*; the supposed synonym **Persan** was in the past widely planted in the French Savoy (TOCHON 1868).

Bianco: local curiosity in some old vineyards in Piedmont; generous yield, moderate must acidity; supposed to correspond to the popular Spanish Palomino fino or **Listan** in France.

Bian ver: small greenish clusters, powerful and flavoured wines; nearly disappearing from Piedmont; the assumption of *Bian ver* being identical with **Verdesse** (in the past widely grown in Isère near Grenoble) was based on the ampelographic description reported by GALET (1990) and oenological features (MAS and PULLIAT 1874-1879; VIALA and VERMOREL 1901-1910); *Verdesse* occasionally occurs in Suisse vineyards too (MAIGRE, pers. comm.).

Blanchet: little white one, obscure origin, blended with other white grapes in Piedmont alpine valleys; wines of local interest; assumed to be synonym of **Roussette d'Ayze** described by ENTAV-INRA-ENSAM-ONIVINS (1995).

Borsè: fruitful, early ripening variety; wine or table grape; common in the Piedmont foothills before the phylloxera invasion; also called *Montanera* in the surrounding of Saluzzo (CN); suggested to be identical with **Cardin**.

Canari: mentioned in late 1800, originally in the Ariège and Haute Garonne area (Pyrenean) (ODART 1874; DI ROVASENDA 1877), by now almost disappeared; its identity with **Luverdon** (also named *Gamay Luverdon*, erroneously *Gamay*) from the alpine area of Piedmont is questionable. Marginally grown at high altitude in the alpine valleys of Susa and Chisone (Piedmont); abundant yield, early ripening, prone to bunch rot, poor quality.

Cardin: *Cardino* and the area *Mondovi* (CN) and *Tadone* South of *Ivrea* (TO); assumed to be identical with the French **Cornet**, an early table grape (MAS and PULLIAT 1874-1879; VIALA and VERMOREL 1901-1910; GALET 1990).

Grec rouge: ancient variety (Middle Ages) mainly grown as table grape; one of the most attractive grapes due to its bright red colour of berries and big bunches; also for wine production; disappeared due to the phylloxera invasion (MAS and PULLIAT 1874-1879; VIALA and VERMOREL 1901-1910). We assumed its identity with *Ivernasso* and **Grisa rousa** (lit. red grey) used for table grape or wine production.

Grosse Jacquère: also called *Cacaboué*; not to be confused with *Jacquère*, a vine of higher quality in Savoy. Some visual features of *Grosse Jacquère* suggested its identity with the Italian **Blanchet**.

Gouais blanc: ancient variety (Middle Ages) called *Weisser Heunisch* in Central Europe; extremely fruitful and resistant to winter frost; ordinary, acidic wine; cultivation no longer allowed in France. Its features described by VIALA and VERMOREL (1901-1910), GALET (1990, 2000) and AMBROSI *et al.* (1997) suggested *Gouais blanc* being identical with **Preveiral**, a fruitful, vigorous vine found in the upper hills of several alpine valleys in Piedmont, where it is called *Blancio* (white) or **Liseiret**.

Neiret (or *Bourgnin*): planted along the foothill area in Piedmont; different local names (**Nebbiolo di Dronero**, *Bolgnino*, *Brunetta*, *Brachèt*); grapes rich in colour, tannins and acidity. The supposed synonym **Chatus** (described by VIALA and VERMOREL 1901-1910, GALET 1990, ENTAV-INRA-ENSAM-ONIVINS 1995), well adapted to the acidic soils, in the past widely grown in France, disappeared until recently rediscovered in the Ardèche area.

Plant rouge femelle (female red vine): vine rich in red pigmentation, but typically more vigorous, less colour and sweeter grapes than *Teinturier mâle*, to which it has often been compared.

Plant rouge mâle: also called *Teinturier mâle*, *Gros noir* or *Teinturier de Cher*; vigorous; very rich in anthocyanin pigmentation of shoots, leaves and berries (red flesh); mentioned in the Renaissance (VIALA and VERMOREL 1901-1910); specific growing areas in Cher, Loiret, Loir-et-Cher and Indre-et Loire (around Tours, Bourges and Orléans); also found sporadically in most of the Central European vineyards; introduced to Hérault and used by BOUSCHET for crossings with Mediterranean grape varieties. Although not found in Italy, it looks similar to the Italian **Tinturié** or **Tenjin**, having berries with red flesh.

Troyen: ancient fruitful wine grape, well adapted to humid marly soils, planted in the past over much of North eastern France (Yonne, Aube, Meuse and Moselle); introduced to the Ardèche area as *Liverdun* (VIALA and VERMOREL 1901-1910); today almost disappeared; due to its name and some ampelographic and cultural features reported by GALET (1990) we verified its synonymy with the so-called **Luverdon**.

Table 1

Investigated grape cultivars and their berry colour (N=black, B=white, R=red), country of origin of the analyzed material, growing area and the area where cultivation is allowed according to the EU rules

Grape cultivars	Berry colour	Country of origin	Growing area	Cultivation allowed in:
Abondance	N	France	Central France	Not allowed
Avanà	N	Italy	Susa Valley (TO ¹)	TO
Biquet (or Becouet)	N	Italy	Susa Valley, Pinerolo (TO)	Not allowed
Bianco	B	Italy	Bergamasco (AL ²)	Not allowed
Bian ver	B	Italy	Susa Valley, Pinerolo (TO)	Not allowed
Blanchet	B	Italy	Pomaretto (TO)	Not allowed
Borsè	N	Italy	Boves (CN ³)	Not allowed
Canari	N	France	Ariège (Southwestern France)	Not allowed
Cardin	N	Italy	Monastero Vasco (CN)	Not allowed
Chatus	N	France	Alpes, Savoie, Ardèche	Ardèche
Cornet	N	France	Drôme (Southeastern France)	Not allowed
Gouais blanc	B	France	Northern, Central, Eastern France	Not allowed
Grec rouge	R	France	Provence (Southeastern France)	Not allowed
Grisa rousa	R	Italy	Susa Valley (Turin province)	Not allowed
Grosse Jacquère	B	France	Savoie (Southeastern France)	Not allowed
Hibou rouge	N	France	Vassal collection (France)	Not allowed
Hibou noir	N	France	Savoie (Southeastern France)	Not allowed
Liseiret	B	Italy	Bormida Valley	Not allowed
Listan (or Palomino fino)	B	France	France, Spain, South Africa	Spain, France, Portugal
Luverdon	N	Italy	Pomaretto (TO)	Not allowed
Nebbiolo di Dronero	N	Italy	Dronero, Costigliole S. (CN)	Not allowed
Nebbiolo di Dronero (false)	N	Italy	Pinerolo (TO)	Not allowed
Neiret (or Bourgnin)	N	Italy	Pinerolo (TO)	Not allowed
Persan	N	France	Savoie, Isère (Southeastern France)	Savoie, Haute Savoie
Plant rouge femelle	N	France	Central France	Not allowed
Plant rouge mâle	N	France	Central France	Not allowed
Preveiral	B	Italy	Pinerolo (TO)	Not allowed
Roussette d'Ayze	B	France	Haute Savoie	Haute Savoie
Tinturié (or Tenjin)	N	Italy	Scattered all over Piedmont	Not allowed
Troyen	N	France	Northeastern France	Not allowed
Verdesse	B	France	Isère (Southeastern France)	Isère, Savoie, Haute Savoie

¹ TO = province of Turin; ² AL = province of Alessandria; ³ CN = province of Cuneo.

analysis (BOTTA *et al.* 1999; AKKAK and BOTTA 1998). The Polymerase Chain Reaction (PCR) was performed in a Perkin-Elmer/Cetus 480 DNA Thermal Cycler, using 20 µl of a mixture containing 10 ng DNA, 0.5 U of Taq DNA-polymerase (HT Biotechnology, UK), 10 mM Tris-HCl pH 9, 50 mM KCl, 2 mM MgCl₂, 0.01 % gelatine, 0.1 % Triton X-100, 0.28 µM of primer and 200 µM of each dNTP. PCR conditions were: 3 min at 95 °C, followed by 45 cycles of denaturation (1 min at 94 °C), annealing (1 min at 36 °C) and extension (2 min at 72 °C); a final elongation step was done at 72 °C for 7 min. Each amplification was performed at least twice using different DNA dilutions or extractions for each sample. 12 µl of sample were loaded on a 1.5 % agarose gel and run for 4 h in TAE (Tris-Acetic acid-EDTA) 1X buffer at 2.5 V·cm⁻¹. The gel was then stained for 30 min in ethidium bromide 0.5 µg·ml⁻¹ aqueous solution. A 100 bp DNA ladder was used as molecular weight marker.

Following the RAPD analysis, 11 samples were also examined using the microsatellite *loci* VVS2, VVS5 (THOMAS and SCOTT 1993) and VVMD5 (BOWERS *et al.* 1996) using the chemiluminescence detection. PCR was performed on 20 µl of a mixture containing 50 ng DNA, 0.5 U Taq DNA polymerase (AmpliTaq Gold, Perkin Elmer), with the supplied reaction buffer, 1.5 mM MgCl₂, 0.5 µM of each primer and 200 µM of each dNTP. PCR conditions were: 3 min at 95 °C, then 26 cycles of denaturation (50 s at 94 °C), annealing (40 s at 50-52 °C) and extension (1.5 min at 72 °C); a final elongation step was done at 72 °C for 7 min. 2 µl of each sample were run, after denaturation, on a 50 cm sequencing gel (6 % polyacrylamide, 8 M urea, 1x TBE buffer). For the chemiluminescence detection, the DNA was blotted on a Zeta Probe (Bio-Rad, USA) positively charged nylon membrane and hybridized overnight in 15 ml hybridization buffer (1 M EDTA, 7 % SDS, 0.25 M disodium phosphate pH 7.2) con-

taining 20 pmol of probe. The biotin labelled oligonucleotide probes were (GA)₁₃ for VVS2 and VVMD5 and (GT)₁₃ for VVS5 at a hybridization temperature of 45 °C. Chemiluminescence detection was performed using the Phototope-Star Detection Kit by New England BioLabs (USA) following manufacturer's instructions.

In addition, microsatellite *loci* VVMD7 (BOWERS *et al.* 1996), VVMD27 (BOWERS *et al.* 1999 b), VrZAG62 and VrZAG79 (SEFC *et al.* 1999) were analysed at ENSAM-INRA. PCR was performed as described (LOUREIRO *et al.* 1998); the amplified *loci* were separated on denaturing 6 % polyacrylamide sequencing gel and visualized by silver staining with a commercial kit (Promega).

Results and Discussion

The 8 primers used in the RAPD analysis produced amplified fragments between 300 and over 2600 bp in size. 72 bands were scored between 300 and about 1600 bp, the size range of the most reliable and reproducible amplicons (VIDAL *et al.* 1999). Primers OPA-2 (Fig. 1), OPA-07, OPA-10, and OPA-11, showed the highest polymorphism. RAPD pro-

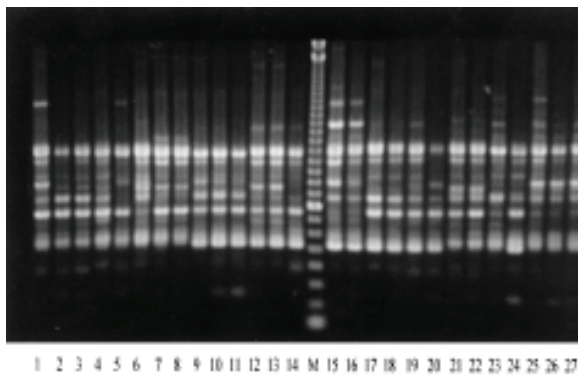


Fig. 1: RAPD patterns obtained using primer OPA-02. From left to right: 1) Cornet, 2) Cardin, 3) Borsè, 4) Roussette d'Ayze, 5) Blanchet, 6) Troyen, 7) Canari, 8) Luverdon, 9) Chatus, 10) Neiret, 11) Nebbiolo di Dronero, 12) Plant rouge femelle, 13) Plant rouge mâle, 14) Tinturié, M = molecular marker (100 bp ladder), 15) Grec rouge, 16) Grisa rousa, 17) Verdesse, 18) Bian ver, 19) Listan, 20) Bianco 21) Persan, 22) Biquet, 23) Abondance, 24) Nebbiolo di Dronero (false), 25) Gouais blanc, 26) Liseiret, 27) Preveiral.

files obtained with primers OPO-7 and OPO-2 on Avana, Hibou rouge, Hibou noir, Grosse Jacquère and Blanchet are shown in Fig. 2: both primers allowed to discriminate Grosse Jacquère from Blanchet for a strong polymorphic band. On the contrary, results suggested that Avana, Hibou noir and Hibou rouge, showing identical profiles for all the examined primers, are synonym varieties.

RAPD results are summarized in Tab. 2, where all supposed synonymous cultivars are confronted in pairs. The DNA profiles obtained by the selected primers easily allowed to distinguish varieties which were not synonyms. All primers were able to discriminate Blanchet from Roussette d'Ayze and Grosse Jacquère, Tinturié from Plant rouge mâle and Plant rouge femelle, Abondance from Nebbiolo di Dronero (false) and only one primer displayed identical pattern between Troyen and Luverdon on one side and Troyen

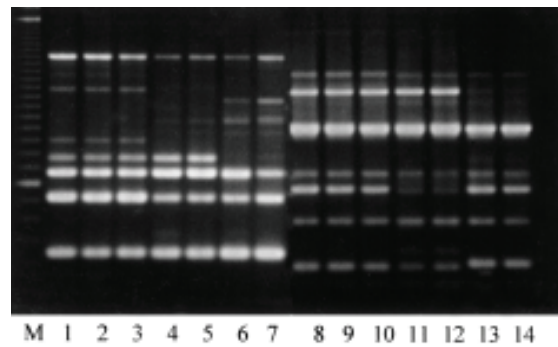


Fig. 2: RAPD patterns obtained using primers OPO-02 (lanes 1-7) and OPO-07 (lanes 8-14). From left to right: M = molecular marker (100 bp ladder), 1) Avana, 2) Hibou rouge, 3) Hibou noir; 4,5) Grosse Jacquère; 6,7) Blanchet, 8) Avana, 9) Hibou rouge, 10) Hibou noir; 11,12) Grosse Jacquère; 13,14) Blanchet.

and Canari on the other. Cornet and Cardin, on the one hand and Cornet and Borsè on the other, showed identical patterns for primers OPO-2, OPO-5 and OPO-7, but were different with the other 5 primers, rejecting the synonymy.

On the contrary, all the other pairs or triplets analyzed showed the same profiles for all 8 RAPD primers. Avana & Hibou rouge & Hibou noir, Chatus & Neiret & Nebbiolo di Dronero, Verdesse & Bian ver, Listan & Bianco, Persan & Biquet, Gouais blanc & Liseiret & Preveiral respectively are thus synonyms.

At a first stage of this study primer OPO-2 originated a 650 bp fragment which distinguished the French cultivars Chatus, Canari, Listan, Grec rouge, and Verdesse from the supposed Italian synonym cultivars. The band was thought to be an artefact probably due to the different extraction procedures carried out in Italy and in France. A second extraction was thus performed in France, followed by a purification in Italy using the mentioned procedure by Thomas *et al.* (1993). In addition, DNA from Chatus was extracted and purified in Italy from woody material obtained from France. Analysing the samples obtained in this way, the 650 bp fragment was only recovered as a faint band in Grec rouge (*d?* in Tab. 2), while for the other cultivars the hypothesis of synonymy was confirmed (Fig. 3). DNA extraction and purification methods thus appear to be factors affecting RAPD results. If comparison of data between laboratories is required, these procedures should be standardized using, for example, a commercial DNA extraction kit.

For further confirmation of RAPD results, SSR analyses were performed on several cultivars and on the corresponding presumed synonyms. The results obtained at *loci* VVS2, VVS5, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG 79 on these 11 cultivars (Tab. 3) indicated that compared to the Italian cvs Luverdon, Bianco, Grisa rousa and Bian ver respectively the French cvs Canari, Listan, Grec rouge and Verdesse showed the same profile. The French cv. Chatus had the same genotype as Italian Neiret and Nebbiolo di Dronero, thus RAPD analyses and synonymy were confirmed. The results obtained with SSR markers were not affected by extraction and purification methods.

The confirmation of the synonymy of the French cv. Gouais blanc with two cultivars of the Italian side of Western Alps, Liseiret and Preveiral, is rather significant:

Table 2

RAPD profile comparison of the supposed synonyms: *d* different RAPD profiles; = identical RAPD profiles; *d?* ambiguous interpretation (see text)

Compared grape cultivars		Primer							
		OPA-2	OPA-7	OPA-10	OPA-11	OPO-2	OPO-5	OPO-7	OPO-19
Cardin	Cornet	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	=	=	=	<i>d</i>
Borsè	Cornet	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	=	=	=	<i>d</i>
Borsè	Cardin	=	=	=	=	=	=	=	=
Blanchet	Roussette d'Ayze	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
Blanchet	Grosse Jacquère	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
Hibou rouge	Avanà	=	=	=	=	=	=	=	=
Hibou noir	Avanà	=	=	=	=	=	=	=	=
Hibou noir	Hibou rouge	=	=	=	=	=	=	=	=
Luverdon	Troyen	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	=
Luverdon	Canari	=	=	=	=	=	=	=	=
Canari	Troyen	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	=
Neiret (or Bourgnin)	Chatus	=	=	=	=	=	=	=	=
Nebbiolo di Dronero	Chatus	=	=	=	=	=	=	=	=
Nebbiolo di Dronero	Neiret (or Bourgnin)	=	=	=	=	=	=	=	=
Tinturié	Plant rouge femelle	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
Tinturié	Plant rouge mâle	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
Plant rouge mâle	Plant rouge femelle	=	=	=	=	=	=	=	=
Grisa rousa	Grec rouge	=	=	=	=	<i>d?</i>	=	=	=
Bian ver	Verdesse	=	=	=	=	=	=	=	=
Bianco	Listan	=	=	=	=	=	=	=	=
Biquet (or Becouet)	Persan	=	=	=	=	=	=	=	=
Nebbiolo di Dronero (false)	Abondance	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>
Liseiret	Gouais blanc	=	=	=	=	=	=	=	=
Preveiral	Gouais blanc	=	=	=	=	=	=	=	=
Preveiral	Liseiret	=	=	=	=	=	=	=	=

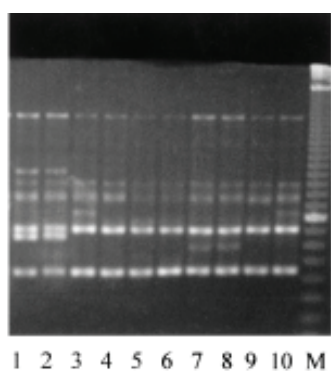


Fig. 3: RAPD patterns obtained using primer OPO-02. From left to right: 1, 2) Chatus extracted and purified in France; 3, 4) Chatus extracted and purified in Italy; 5, 6) Chatus extracted in France and purified in Italy; 7, 8) Neiret extracted and purified in Italy; 9, 10) Nebbiolo di Dronero extracted and purified in Italy, M = molecular marker (100 bp ladder).

cv. Gouais blanc, which several centuries ago was one of the most common grapes in Eastern France and Central Europe and parent, together with cv. Pinot, of numerous French varieties (BOWERS *et al.* 1999 a), is thus present at the Southern side of the Alps, too. Probably its plantings had covered a wide area in Piedmont especially in the cooler areas, where

this vine nowadays remains up hills in different alpine valleys as a "relic" of the past.

As to the synonymy of Canari = Luverdon, the reason for this cultivar of Southwestern France to be found in the Alps remains obscure.

Although the variety from Piedmont Tinturié with red berry flesh was found to be different from the French Teinturiers, our findings show that cv. Plant rouge mâle cannot be distinguished from cv. Plant rouge femelle with the set of markers used. The varietal distinction of these two vines has been debated for decades (VIALA and VERMOREL 1901-1910; GALET 1990, 2000), although some ampelographers recently doubted that they are different (LACOMBE, pers. comm.).

Conclusions

Thirty-one Italian and French grape varieties were analyzed with DNA markers in order to verify 25 synonym hypotheses: 9 synonym hypotheses were rejected, 16 were confirmed. These synonymies were mainly based on bibliographical data, and therefore comparisons were difficult. The fact that the major part of the hypotheses proved to be true is however a clear demonstration of the power of

Table 3

SSR profiles of 5 cultivars and their synonyms analyzed for 7 loci (the allele size is expressed in bp; - either homozygosity or the presence of a null allele)

Cultivar	VVS2		VVS5		VVMD5		Locus VVMD7		VVMD27		VrZAG62		VrZAG79	
	Alleles		Alleles		Alleles		Alleles		Alleles		Alleles		Alleles	
Canari	143	151	96	146	232	238	240	254	179	189	190	202	244	250
Luverdon	143	151	96	146	232	238	240	254	179	189	190	202	244	250
Listan	133	145	89	118	228	240	240	250	185	194	190	196	250	256
Bianco	133	145	89	118	228	240	240	250	185	194	190	196	250	256
Grec rouge	133	143	114	146	228	236	248	254	185	-	190	196	238	250
Grisa rousa	133	143	114	146	228	236	248	254	185	-	190	196	238	250
Verdesse	133	151	102	118	228	232	240	258	179	189	190	196	244	248
Bian ver	133	151	102	118	228	232	240	258	179	189	190	196	244	248
Chatus	133	137	89	122	224	228	240	250	179	194	190	196	250	-
Neiret	133	137	89	122	224	228	240	250	179	194	190	196	250	-
Nebbiolo di Dronero	133	137	89	122	224	228	240	250	179	194	190	196	250	-

ampelography. Furthermore, the synonymies based on actual comparison of the cultivars were always correct, but were unquestionable only if the accessions were present in the same collection. Molecular analysis can also solve cases where direct comparison between vines cannot be done in the same field. In this investigation it was a decisive tool for cultivar identification.

The cultivars investigated belong to the *Vitis vinifera* germplasm of both sides of the Western Alps and the occurrence of synonyms indicates the existence of a common pool of grapes grown under different names in this part of Europe. Nowadays marginally grown, they represent a portion of the grape genetic diversity that survived phylloxera invasion and other events responsible for genetic erosion of *Vitis* resources. Some of them are interesting grapes with regard to their high wine quality, such as Neiret (or Nebbiolo di Dronero), Bian ver and Biquet, synonyms of the authorized varieties Chatus, Verdesse and Persan, respectively. A common exploitation on both sides of the Alps, including exchange of oenological technologies and of outstanding clonal material, might be considered for these cultivars.

Acknowledgments

The authors sincerely thank THIERRY LACOMBE from Domaine de Vassal (INRA, France) for the helpful assistance and useful discussion visiting the collection.

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Received April 10, 2001