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Parentage of grapevine rootstock 'Fercal' finally elucidated

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Summary

Using a set of 20 microsatellite markers, 'B.C. n°1B' (mother) and '31 Richter' (father) were demonstrated to be the true parents of 'Fercal' rootstock. '333 Ecole de Montpellier' was definitively excluded as the putative father. 'B.C. n°1A' and 'B.C. n°1B' were shown to be distinct genotypes. 'Ugni blanc', and not 'Colombard', was discovered to be the *Vitis vinifera* father of 'B.C. n°1B'.

K e y w o r d s : microsatellites, rootstock, interspecific hybrid, parentage.

A b b r e v i a t i o n s : 'B.C. n°1': 'Berlandieri-Colombard n°1', '333 E.M.': '333 Ecole de Montpellier', 'SO4': 'Selection Oppenheim 4'.

Introduction

Most of the grapevine rootstocks used in France have been created and selected at the end of the 19th century while phylloxera spread throughout the European vineyard. Numerous hybrids were created at this time, and the most interesting ones have been vegetatively propagated since. In France, only two rootstocks were bred during the second half of 20th century, *i.e.* 'Fercal' and 'Gravesac'. 'Fercal' was obtained from a cross made in Bordeaux in 1959. At this stage, its parents were supposed to be 'B.C. 1 (*Vitis berlandieri* x Colombard) n°1' and '333 E.M.' ('Cabernet-Sauvignon' x *Vitis berlandieri*) (Pouget and Ottenwaelter 1978). Actually, two different genotypes were mixed up at the beginning under the name of 'B.C. n°1', and Galet (1988) separated them in 'B.C. n°1A' and 'B.C. n°1B'. There was no clear indication to determine which one of them was involved as the genitor of 'Fercal', even if Galet (1988) wrote that 'B.C. n°1A' was the mother of 'Fercal'.

'Fercal' is famous because of its very high tolerance to limestone chlorosis. It is considered to perform better than '41 B Millardet et de Grasset' and '140 Ruggeri', as it tolerates more than 40 % of active lime and is able to grow in soil where the index of chlorosing power is above 120 (Pouget and Ottenwaelter 1978). In 2006, it was at the fourth rank for grafting in France, behind 'SO4', '110 Richter' and '3309 Couderc'. Its other characteristics are a high tolerance to phylloxera and some tolerance to *Meloidogyne incognita* and *Meloidogyne hapla* (Boubals 1978). It has good rooting and grafting abilities. On the ampelographic point of view, it is characterized by very high density of prostrate hairs at the half open tip of the young shoot and on the young leaves which are green with bronze spots. The shoots, with elliptic section and ribbed surface, are also covered by a high density of prostrate and erected hairs. The mature leaves are wedge-shaped to kidney-shaped, entire and involute with short teeth and open and U shaped petiole sinus (Figure). Its flowers carried reflexed stamens and fully developed gynoecium. The berries are small, blue black and spherical.

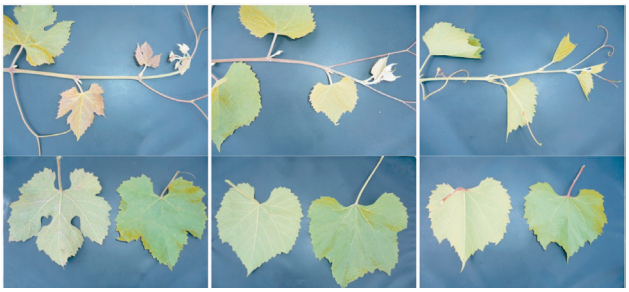

Figure: Shoot tip and adult leaf (lower face on the left, upper face on the right) of 'B.C. n°1B' (left), 'Fercal' (center) and '31 Richter' (right). Pictures were taken in Bordeaux germplasm repository.

No ampelographic similarity was found with '333 E.M' which is characterized by several traits of *Vitis vinifera* ('Cabernet-Sauvignon', mother) and especially open tip of the young shoot, red young leaves, null density of prostrate and erected hairs on the shoots, circular mature leaves with five lobes and overlapped petiole sinus with base limited by vein.

Specific genetic diversity and initial efforts to identify rootstock cultivars were based on ampelographic traits (Ravaz 1902, Galet 1988). For most of these artificial hybrids, pedigree and parental information had been recorded by the breeders and reported throughout decades by ampelographs, without any way to check these data.

The first tools that were useful to check the pedigree appeared with the development of biochemical markers (like isozymes) useful as genetic markers (Pasteur *et al.* 1987). Today, molecular markers, especially microsatellites, have proven to be useful in DNA fingerprinting and parentage analysis of grape cultivars (Thomas *et al.* 1994, Sefc *et al.* 2001). They have been exploited in a number of countries for identification, verification of synonyms and parentage analysis for *Vitis vinifera* cultivars (Bowers and Meredith 1997, Lacombe *et al.* 2007, Vouillamoz *et al.* 2007). The data about genotyping rootstocks are scarce (Lin and Walker 1998, Sefc *et al.* 1998, de Andrès *et al.*

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2007, Dzhambazova *et al.* 2007). For rootstocks, pedigree reconstruction was attempted by de Andrès *et al.* (2007), using 9 microsatellite loci. Among 16 hybrids, genetic and bibliographic information was in agreement for only two of these cases. In the above-mentioned study, the genotype of the considered rootstock and the genotype of one cited parent was consistent for seven more accessions, including 'Fercal'. According to this work, 'B.C. n°1B' was given to be one parent of 'Fercal'. However '333 E.M.' was excluded to be the other parent, in agreement with the first data based on isozyme analysis published by Boursiquot and Parra (1992) and the data published later by Lin and Walker (1998). The aim of our work was to check these results and to identify the other parent of 'Fercal', in order to reconstruct its pedigree.

Material and Methods

Plant material was harvested in the french repository of INRA at Domaine de Vassal, Herault, France. It consists of five rootstocks: 'Fercal', 'B.C. n°1A' and 'B', '333 E.M.', '31 Richter' and two *Vitis vinifera* cultivars, 'Ugni blanc' and 'Colombard'.

DNA was extracted from 50 mg of young leaves that had been freeze-dried 24 h at 0.370 mbar and -55 °C. Extraction was made according to the Qiagen DNeasy plant mini kit protocol (Qiagen, Hilden, Germany) with minor modifications: addition of 1% w/v of PVP-40 to the AP1 solution, addition of 180 µl AP2 and 10 minutes centrifugation at 6000 rpm.

Twenty microsatellite loci (SSR) were genotyped: VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32 (Bowers *et al.* 1996, 1999); VVIn16, VVIv67, VVIv37, VVIq52, VVIp60, VVIh54, VVIb01, VVIn73, VVIp31 (Merdinoglu *et al.* 2005); VVS2 (Thomas and Scott 1993); VMC1b11 (Zyprian and Töpfer, 2005, unpubl. data) and VMC4f3 (Di Gaspero *et al.* 2000).

PCR were performed as previously described by Adam-Blondon *et al.* (2004) with slight modifications: amplifications were run in 20 µl reaction mix. PCR products were then diluted 5 or 10 times before separation of the fragments.

Electrophoresis was carried out in an ABI Prism® 3100 Genetic Analyser (Applied Biosystems, Foster, CA) using GENESCAN HD 400 ROX (Applera) as internal size standard. GENESCAN® and GENOTYPER® 2.5 software were used to size the fragments.

Results were compared to a large database comprising 2853 accessions (271 hybrids, 168 rootstocks, 2414 *Vitis vinifera*) of the Vassal germplasm repository which were genotyped with the same 20 SSRs.

Parentage analyses were performed using Famoz software (Gerber *et al.*, 2003) adapted to grapevine (Di Vecchi Staraz *et al.* 2007) as previously described by Lacombe *et al.* (2007).

Results and Discussion

'Fercal' was initially considered by Pouget and Ottenwaelter (1978) as an hybrid between *Vitis berlandieri* and *Vitis vinifera* varieties, 'B.C. 1 n°1' and '333 E.M.'. Its agronomical performance, *i.e.* its very high limestone tolerance, was considered to result from its putative pedigree. However ampelographic data were not very consistent with the given parents. More recently, isozyme analysis and molecular data led also to question this announced parentage (Boursiquot and Parra 1992, Lin and Walker 1998). Microsatellite data for the 20 markers analysed in this study are presented Tab. 1.

Table 1

Microsatellite alleles of 'Fercal' and its presumtive genitors. Allele size is given in base pairs.
* represents loci with transmission of null allele

	'B.C. n°1A'		'B.C. n°1B'		'Fercal'		'31 Richter'		'333EM'		'Ugni blanc'		'Colombard'	
VMC1b11	184	196	184	196	169	196	169	169	184	192	184	184	169	184
VMC4f3	164	185	164	185	185	220	177	220	171	177	171	185	171	171
VVIb01	294	298	290	294	290	302	284	302	290	290	294	294	290	294
VVIh54	163	163	*167	*167	143	143	143	161	155	179	163	167	165	167
VVIn16	147	149	147	149	147	149	147	149	151	155	149	149	149	149
VVIn73	263	263	263	263	254	263	254	254	261	263	256	263	263	263
VVIp31	182	186	182	182	182	200	200	206	182	188	182	188	178	182
VVIp60	311	324	311	330	307	311	307	315	309	311	324	330	303	319
VVIq52	77	77	83	83	83	83	83	83	77	77	77	83	83	83
VVIv37	159	167	153	167	151	153	145	151	147	159	159	167	159	167
VVIv67			329	360	329	329	*361	*361	339	368	360	371	361	368
VVMD21	241	241	*241	*241	236	236	226	236	224	247	241	247	247	247
VVMD24	204	206	200	206	200	202	202	206	200	215	206	206	206	210
VVMD25	240	252	252	254	236	254	236	254	238	250	240	254	248	254
VVMD27	176	188	180	188	180	186	186	186	186	188	176	180	172	178
VVMD28	243	243	243	247	218	243	218	241	235	235	243	247	245	247
VVMD32	236	236	243	249	243	243			239	257	249	271	255	267
VVMD5	229	254	229	234	234	261	255	261	217	229	223	229	229	238
VVMD7	231	249	231	253	231	251	251	251	231	239	249	253	239	239
VVS2	131	143	141	143	141	141	137	141	137	147	131	141	141	149

Table 2

Parentage analysis using Famoz software on the 2853 accessions of Vassal repository

Kid	Putative parent	Putative parent pair	LOD Score	Loci contributing in LOD score	Missing loci	Matching loci	Mismatching loci
‘Fercal’	‘31 Richter’		37.12	17	3	16	1
	‘B.C. n°1B’		17.34	14	0	18	2
		B.C. n°1B X 31 Richter	41.16	17	-	17	3
‘B.C. n°1B’	‘Picolin’		20.13	20	0	20	0
	‘Fercal’		17.34	14	0	18	2
	‘Ugni blanc’		15.27	20	0	20	0

According to those data, there is no doubt that ‘B.C. n°1A’ and ‘B.C. n°1B’ are different. Indeed, only 5 markers (VMC1b11, VMC4f3, VVIn16, VVIn73, VVMD21) over the 20 studied ones gave similar size for both alleles within these two genotypes.

The parentage analysis (Tab. 2) performed with Famoz software on the 2853 accessions of Vassal repository gave a strong LOD score (41.16) for the cross 'B.C. n°1B' x '31Richter'. No other possibilities were given. ‘B.C. n°1B’ shares 18 alleles over the 20 tested microsatellites and could be confirmed as the mother of ‘Fercal’. Indeed, the 2 other loci looked like homozygotes. Most probably, these 2 loci were characterized by a null allele transmitted to ‘Fercal’ (VVIh54 and VVMD21). Recent work by de Andrès *et al.* (2007) with 6 additional loci (VVS5, ssrVrZAG47, ssrVrZAG62, VVS1, VVS29, ssrVrZAG79) supported also this conclusion. ‘B.C. n°1A’ was definitively discarded from ‘Fercal’ parentage. ‘31 Richter’ shares 18 alleles and was proposed as the father of ‘Fercal’. No amplification occurred for 1 microsatellite locus whereas 1 locus could be considered with a null allele (VVIv67). ‘333 E.M.’ was excluded to be the parent of ‘Fercal’. Thirteen loci were not matching if it was considered alone, and 19 if ‘B.C. n°1B’ was considered as the mother. Data reported by Lin and Walker (1998), Dzhambazova *et al.* (2007) and de Andrès *et al.* (2007) with other sets of microsatellites clearly showed that the profiles obtained for ‘333 E.M.’ were never consistent with the possibility of this rootstock being closely related to ‘Fercal’.

To confirm these findings, 15 other microsatellite markers were tested on ‘B.C. n°1B’, ‘31 Richter’ and ‘Fercal’ (Tab. 3). ‘Fercal’ shared 14 alleles with ‘B.C. n°1B’ and 15 alleles with ‘31 Richter’. One locus (VrZAG62) of ‘B.C. n°1B’ transmitted a null allele. Those results confirm ‘B.C. n°1B’ and ‘31 Richter’ as parents of ‘Fercal’. Finally, all these data are consistent with ‘Fercal’ being a hybrid from ‘B.C. n°1B’ (mother) and ‘31 Richter’ (father), and not 'B.C. n°1A' x 333 E.M', as it was described previously (Galet 1988). The origin of the mistake for the father was probably more an error in pollen collection rather than a contamination. Indeed ‘333 E.M.’ and ‘31 Richter’ vines were in two adjacent rows in the germplasm repository where the cross for ‘Fercal’ had been made 48 years ago. ‘B.C. n°1’ vines were 7 rows further. Considering the training system in such repository, with no trellis and shoots lying on the floor, such a mistake is not surprising.

‘B.C. n°1B’ is supposed to be a hybrid between ‘Berlandieri Lafont n°9’ and ‘Colombard’ (Galet 1988).

Table 3

Complementary set of microsatellite alleles for ‘Fercal’ and its presumptive parents. Allele size is given in base pairs.
* represents locus with transmission of null allele

	‘B.C. n°1B’		‘Fercal’		‘31 Richter’	
VMC2f10	98	122	100	122	82	100
VMC3b9	95	104	95	102	102	102
VMC3d8	36	57	36	57	36	82
VMC5h11	183	201	183	193	183	193
VMC6b11	91	107	97	107	97	110
VMC7g3	113	115	115	118	118	118
VMC7g5	158	164	158	161	159	161
VrZAG62	200*	200*	182	182	182	188
VrZAG79	243	247	243	255	255	257
VVC6	108	112	112	114	114	118
VVIn52	77	77	77	87	87	91
VVIn83	236	236	236	236	228	236
VVIp22	352	367	336	352	336	336
VVMD17	210	232	218	232	218	218
VVMD31	205	211	205	207	207	209

Searching for its *vinifera* origin, it was shown that ‘Colombard’ was not related to this hybrid (Tab. 1). Seven alleles were not shared between ‘Colombard’ and ‘B.C. n°1B’ (VMC4f3, VVIp60, VVIv67, VVMD21, VVMD27, VVMD32, VVMD7). Famoz proposed 5 other putative parents, but two of them missed a lot of data (not shown) and one is 'Fercal', the offspring of ‘B.C. n°1B’. Cultivars ‘Picolin’ and ‘Ugni blanc’ remained putative parents of ‘B.C. n°1B’ (Tab. 2). ‘Picolin’ is reported to be an offspring of ‘Ugni blanc’ from a cross made in the 1960s. ‘B.C. n°1B’ was obtained at the beginning of 20th century by a vinegrower from the Cognac area, Mr. Blanchard (Galet 1988). Consequently, ‘Ugni blanc’, which was grown in the same area than ‘Colombard’, is the only possible parent. It shares 20 alleles over 20 microsatellites with ‘B.C. n°1B’. ‘Colombard’ is not closest from ‘B. C. n°1A’, as both genotypes share only 8 alleles. ‘Berlandieri Lafont n°9’ which is supposed to be the mother of ‘B.C. n°1B’ has not been analysed yet.

The main properties of ‘Fercal’s parents and of its still hypothetical grand-parents have been summarized in Tab. 4. ‘B.C. n°1B’ is described as adapted to clay-lime soils (Galet 1988) and tolerant to phylloxera. ‘31 Richter’ is given to be a hybrid between *Vitis berlandieri* ‘Rességuier n°2’ and ‘Novo-mexicana’ (Galet 1988). It is highly tolerant to phylloxera, but not so well adapted to

Table 4

Main caracteristics of 'Fercal' genitors

Genotype	Breeder	Genetic background	Properties	Source
'Berlandieri Lafont n° 9'	Ravaz	*Vitis berlandieri*	Highly tolerant to limestone chlorosis and to phylloxera Vigorous	Ravaz 1902 Galet 1988
'Ugni blanc'		*Vitis vinifera*		
'Berlandieri Rességuier n°2'	Rességuier	*Vitis berlandieri*	Tolerant to limestone chlorosis and highly tolerant to phylloxera Vigorous	Ravaz 1902 Galet 1988
'Novo mexicana'	Munson	Candicans hybrid (hypothetical) Riparia x Rupestris x Candicans	Tolerant to phylloxera, Earliness equivalent to Riparia	Galet 1988
'B.C. n°1B'	Blanchard et Vidal	Berlandieri x Vinifera	Adaptated to clay-limestone soils Tolerant to phylloxera	Galet 1988
'31 Richter'	Richter	Berlandieri x [Riparia x Rupestris x Candicans]	Highly tolerant to phylloxera Sensitive above 14% active lime	Galet 1988

calcareous soils (as 'Rupestris du Lot'). 'Novo-mexicana' is a non well characterized variety of *Vitis longii* (synonym *solonis*). This group of varieties originated from Texas, Arkansas and Oklahoma. Ravaz (1902) considered them as *Vitis riparia - Vitis arizonica* hybrids, but Galet (1988) did not support this hypothesis and classified them as *Vitis riparia - Vitis rupestris - Vitis candicans* hybrids. Up to now, there is no molecular evidence that *Vitis berlandieri* 'Lafont n°9', *Vitis berlandieri* 'Rességuier n°2' and 'Novo-mexicana' are the true grand-parents of Fercal (de Andrès *et al.* 2007).

Conclusions

Microsatellite analysis was very efficient to determine the true direct parentage for 'Fercal'. The parentage 'B.C. n°1B' x '31 Richter' is strongly supported by profile analysis. This study also demonstrated that 'B.C. n°1A' and 'B.C. n°1B' are two different genotypes, and that 'Ugni blanc' is the *Vitis vinifera* parent of 'B.C. n°1B'. Of course, this new information about 'Fercal' parentage does not change the agronomical performances of this rootstock. Parentage studies for grapevine rootstocks are not numerous. Our study shows that, even for a recently bred rootstock, the pedigree is not obvious. The origin of most rootstocks should be checked in the same way, in order to improve our knowledge on genetic relationships within this type of material.

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