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 puta
tive father. ‘B.C. n°1A’ and ‘B.C. n°1B’ were shown to be distinct genotypes. ‘Ugni blanc’, and not ‘Colom
bard’, was discovered to be the *Vitis vinifera* father of ‘B.C. n°1B’.

Key words: microsatellites, rootstock, interspecific hybrid, parentage.


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.’ (*Ca
bernet-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 kid
ney-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.

No ampelographic similarity was found with ‘333 E.M.’ which is characterized by several traits of *Vitis vinifera* (*Cab
ernet-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 (RAVAT 1902, GALET 1988). For most of these artificial hy
brids, pedigree and parental information had been recorded by the breeders and reported throughout decades by amp
elographers, 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 MEREDETH 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. 2007).

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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 DNAeasy 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: VVM15, VVM16, VVM21, VVM24, VVM25, VVM27, VVM28, VVM32 (Bowers et al. 1996, 1999); VVIN16, VVIN67, VVIN73, VVIN52, VVIN60, VVIN54, VVIN61, VVIN31 (Merdingoulu et al. 2005); VVS2 (Thomas and Scott 1993); VMC1b11 (Zyprian and Toper, 2005, unpubl. data) and VMC463 (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 Ottenwalter (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 ampelographical 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 presumptive genitors. Allele size is given in base pairs. * represents loci with transmission of null allele |
| 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 |
| VVlb01 | 294 | 298 | 290 | 294 | 290 | 302 | 284 | 302 | 290 | 290 | 294 | 294 | 290 | 294 |
| VVlh54 | 163 | 163 | *167 | *167 | 143 | 143 | 143 | 143 | 161 | 155 | 179 | 163 | 167 | 165 | 167 |
| VVln16 | 147 | 149 | 147 | 149 | 147 | 149 | 147 | 149 | 151 | 155 | 149 | 149 | 149 | 149 | 149 |
| VVln73 | 263 | 263 | 263 | 263 | 254 | 254 | 254 | 254 | 254 | 254 | 254 | 254 | 254 | 254 | 254 |
| VVlp31 | 182 | 182 | 182 | 182 | 200 | 200 | 200 | 200 | 182 | 182 | 182 | 182 | 182 | 182 | 182 |
| VVlp06 | 311 | 324 | 311 | 330 | 307 | 311 | 307 | 315 | 309 | 311 | 324 | 330 | 330 | 303 | 319 |
| VVlq52 | *77 | 83 | 83 | 83 | 83 | 83 | 83 | 83 | 77 | 77 | 77 | 77 | 83 | 83 | 83 |
| VVlv37 | 159 | 167 | 153 | 167 | 151 | 153 | 145 | 151 | 147 | 159 | 159 | 167 | 159 | 167 |
| VVlv67 | 329 | 360 | 329 | 329 | *361 | *361 | 339 | 368 | 360 | 371 | 361 | 368 |
| VVM15D | 241 | 241 | *241 | *241 | 236 | 236 | 226 | 236 | 224 | 247 | 241 | 247 | 247 | 247 |
| VVM24 | 204 | 206 | 200 | 206 | 200 | 202 | 202 | 206 | 200 | 215 | 206 | 206 | 206 | 210 |
| VVM25 | 240 | 252 | 252 | 254 | 236 | 254 | 236 | 254 | 238 | 250 | 240 | 254 | 248 | 254 |
| VVM27 | 176 | 188 | 180 | 188 | 180 | 186 | 186 | 186 | 186 | 188 | 176 | 180 | 172 | 178 |
| VVM28 | 243 | 243 | 243 | 247 | 218 | 243 | 218 | 243 | 235 | 235 | 243 | 247 | 247 |
| VVM32 | 236 | 236 | 243 | 243 | 243 | 243 | 239 | 257 | 249 | 271 | 255 | 257 |
| VVM53 | 229 | 229 | 229 | 234 | 234 | 261 | 255 | 261 | 217 | 229 | 223 | 229 | 229 | 238 |
| VVM7 | 231 | 249 | 231 | 253 | 231 | 251 | 251 | 251 | 231 | 239 | 249 | 253 | 239 | 239 |
| VVS2 | 131 | 143 | 143 | 143 | 143 | 143 | 137 | 141 | 137 | 147 | 131 | 141 | 141 | 149 |
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 '31 Richter'. 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 Andrés 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), Dzhabakoza et al. (2007) and de Andrés 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). 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 vigneron 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...
calcareous soils (as ‘Rupestris du Lot’). ‘Novo-mexicana’ is a non well characterized variety of *Vitis longiss* (synonym *solonis*). This group of varieties originated from Texas, Arkansas and Oklahoma. Ravaż (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 Andrés 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.

### References


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