

Research Note

## Allelic variation observed at one microsatellite locus between the two synonym grape cultivars Black Currant and Mavri Corinthiaki

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**Summary:** Black Currant and Mavri Corinthiaki are considered synonyms for Corinthe Noir, a parthenocarpic seedless raisin cultivar. No differences between them were found by RAPD analysis with 11 primers. They also have the same genotype in 15 out of 16 microsatellite loci, demonstrating the Black Currant and Mavri Corinthiaki are essentially identical. Nevertheless, a difference was observed in one allele at one locus (VVMD7): the Black Currant genotype is 240:246 while the Mavri Corinthiaki genotype is 240:248. A mutation in the microsatellite sequence might be responsible for that difference. Possible consequences of such mutations are discussed.

**Key words:** DNA typing, parentage analysis, STMS, RAPD.

**Introduction:** Sequence-Tagged Microsatellite Sites (STMSs) are excellent markers for grapevine characterization (THOMAS *et al.* 1994, BOWERS *et al.* 1996, SEFC *et al.* 1999). Their wide use would ideally require that all plants belonging to the same variety (from a monoclonal origin) show identical genotypes in all microsatellite loci. Microsatellite sequences have a relatively high mutation rate, which gives place to an increased number of alleles and, consequently, to a high level of polymorphism. This is one of the outstanding characteristics of STMS markers, but such a high mutation rate could lead to different plants of the same variety, or even different parts of the same plant showing different genotypes for a given STMS. This could happen if a somatic mutation occurs in the microsatellite sequence of a given plant which is vegetatively propagated. The STMS technique has been widely used for grapevines and, as far as we know, a mutation in the microsatellite sequence itself has not yet been described supporting its convenience for grapevine genetic studies. Nevertheless, it is theoretically possible especially taking into account that many cultivars used in viticulture are very old and somatic mutations are cumulative.

Black Currant and Mavri Corinthiaki are ancient cultivars considered synonyms for Corinthe noir in Cyprus and Greece, respectively (OTV 1987). They produce parthenocarpic seedless grapes, which are normally consumed as raisins. In this paper we describe the comparative analysis performed with both cultivars using RAPD and STMS techniques.

**Material and Methods:** Plant material was obtained from the Vitis Germplasm Bank (BGV) at the Finca El Encin, Alcala de Henares (Spain). The accession Black Currant came to BGV from Limassol (Cyprus) in 1958 and the accession Mavri Corinthiaki from Thessalonica (Greece) arrived at BGV in 1962.

**RAPD analysis:** DNA was extracted from young leaves following the CTAB procedure described by LODHI *et al.* (1994). Two different plants per accession were independently analysed; 11 different primers from Operon Technologies (Alameda, CA) were used: D03, D04, D05, D19, E02, E06, E07, H13, H19, T11 and T20. These primers were randomly chosen from those available in the laboratory. Amplifications were performed in a reaction volume of 25  $\mu$ l containing 10 mM Tris-HCl pH 8.3; 50 mM KCl; 200  $\mu$ M of each dNTP; 5 mM MgCl<sub>2</sub>; 0.4  $\mu$ M primer; 2 U of Amplitaq DNA Polymerase, Stoffel Fragment (Perkin Elmer) and 25 ng of genomic DNA. The amplification program was: 1 cycle (94 °C, 6 min) and 40 cycles (94 °C, 1 min; 35 °C, 1 min; ramp 35 °C to 72 °C 1.5 min; 72 °C 6.5 min). Amplified fragments were separated by electrophoresis in 2 % agarose gels.

**STMS analysis:** DNA was extracted using the Plant DNeasy Mini Kit from Qiagen. Amplifications were performed in a reaction volume of 20  $\mu$ l containing: 10 mM Tris-HCl pH 8.3; 50 mM KCl; 200  $\mu$ M of each dNTP; 1.5 mM MgCl<sub>2</sub>; 0.2  $\mu$ M upper primer, with an attached fluorescent dye; 0.2  $\mu$ M lower primer; 0.5 U of Biotools DNA Polymerase and 50 ng of genomic DNA. The amplification program was: 1 cycle (95 °C, 5 min) and 40 cycles (94 °C, 45 s; 50 °C, 1 min; 72 °C, 1.5 min). Sixteen different STMSs have been analysed: VVS1 (labeled with the fluorochrome TET), VVS2 (HEX), VVS5 (6-FAM), VVS29 (HEX) (THOMAS and SCOTT 1993), VVMD5 (6-FAM), VVMD7 (TET) (BOWERS *et al.* 1996), ssrVrZAG 15 (HEX), ssrVrZAG 21 (TET), ssrVrZAG 25 (TET), ssrVrZAG 29 (HEX), ssrVrZAG 30 (6-FAM), ssrVrZAG 62 (TET), ssrVrZAG 64 (TET), ssrVrZAG 79 (HEX), ssrVrZAG 83 (6-FAM) and ssrVrZAG 112 (6-FAM) (SEFC *et al.* 1999). The separation of amplified fragments was carried out in an ABT PRTSM 310 Genetic Analyzer (PE Biosystems). The fragments were sized with the GENESCAN software, using TAMRA 500 as an internal marker.

**Results and Discussion:** Black Currant and Mavri Corinthiaki showed identical RAPD profiles with the 11 primers used (Figure, A). These primers detect a high level of polymorphism in grapevine, since only two of the 11 primers were sufficient to distinguish 45 different seedless table grape varieties, including several parthenocarpic seedless grape varieties (TBANEZ, in prep.).

The Table shows the GENESCAN values obtained for each STMS. Both accessions showed identical genotypes in 15 out of the 16 microsatellite loci analysed. There was only a difference in the largest allele of the VVMD7 locus: About 246 for Black Currant and about 248 for Mavri Corinthiaki. This locus was analysed in 5 different plants of each accession and the result was always the same. Figure, B shows the profile obtained for three out of 5 plants.

The coincidence in 15 STMS loci and in 11 RAPD profiles shows clearly that the two accessions are synonyms

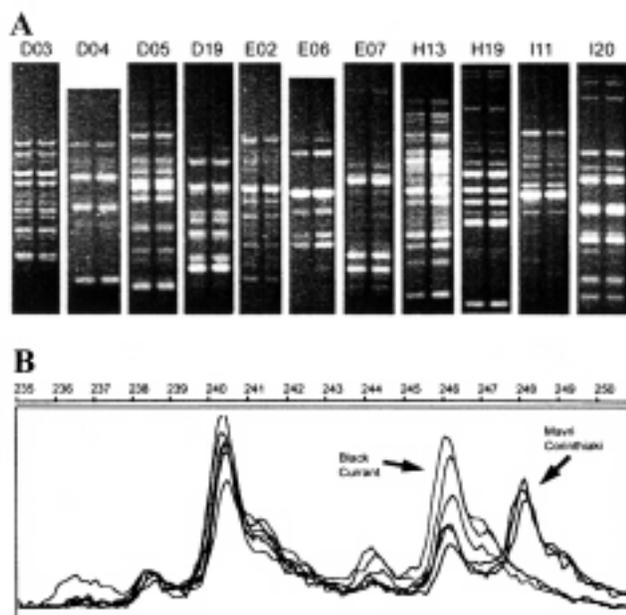


Figure: (A): RAPD profiles obtained with 11 different primers. For each primer, the band profile at the left corresponds to Black Currant, and the band profile at the right to Mavri Corinthiaki. (B): GENESCAN electrophoretograms obtained for VVMD7 locus with three plants of Black Currant and three plants of Mavri Corinthiaki. Arrows point the different alleles detected.

T a b l e

GENESCAN values corresponding to allele sizes for the different STMS loci in the cultivars studied

	Black Currant		Mavri Corinthiaki	
VVS1	178.54	185.23	178.70	185.45
VVS2	132.59	139.12	132.65	139.12
VVS5	148.18		147.94	
VVS29	168.02		168.23	
VVMD5	231.82	235.89	231.95	235.88
VVMD7	240.41	246.20	240.40	248.18
ssrVrZAG 15	163.88	165.93	163.85	165.88
ssrVrZAG 21	204.65		204.53	
ssrVrZAG 25	244.77		244.81	
ssrVrZAG 29	111.47		111.42	
ssrVrZAG 30	148.41		148.41	
ssrVrZAG 62	188.00	199.55	188.01	199.45
ssrVrZAG 64	140.16		140.04	
ssrVrZAG 79	244.87	248.93	244.88	248.66
ssrVrZAG 83	189.39		189.46	
ssrVrZAG 112	232.11		232.25	

belonging to the same variety, as was concluded from literature. A visual inspection of morphological characters did not reveal any difference between both accessions (data not shown). A possible explanation for the difference would be a mutation in the microsatellite sequence. The VVMD7 locus has a dinucleotide repeat (CT)<sub>n</sub> and the consecutive sized alleles generally differ in 2 bp (BOWERS *et al.* 1996), as was observed in our experiments.

There are examples where unexpected differences have been reported, *e.g.* CRESPIAN *et al.* 1999 observed a difference at the VVMD8 locus between Sultanina (145:157) and Thompson Seedless (145:-) (BOWERS *et al.* 1996) which is considered to be its synonym. A mutation of the 157 bp allele to a null allele (no amplification) would explain this discrepancy. The presence of null alleles in this locus has been deduced from cultivars that failed to amplify, which were considered homozygous null genotypes (BOWERS *et al.* 1996). However, in this case the mutation would affect the one or two annealing targets for the primers, and not the microsatellite sequence itself. The same occurred in the genotyping of locus VVS3 for Marroo Seedless carried out by SANCHEZ-ESCRIBANO *et al.* (1999): 216:222, and THOMAS *et al.* (1994): 216:-.

Some possible mutations have been deduced from parentage analysis, *e.g.* Carolina Black Rose (92:110) (THOMAS *et al.* 1994) and Alphonse Lavalée (110:118) are considered the parents of Cardinal (118:-) (SANCHEZ-ESCRIBANO *et al.* 1999). A mutation to null allele in the transmission of the allele inherited from Carolina Black Rose would explain the discrepancy.

None of the examples found in literature illustrates the type of mutation that is supposed to generate most alleles in microsatellite loci. A mutation in the microsatellite sequence should give rise to a new allele longer or shorter than the original one. The difference in size between them should be directly proportional to the length of the microsatellite motif, 2 bp in the case of VVMD7.

For several reasons STMSs are the marker of choice for grapevine characterization. Nevertheless, the results presented and discussed here show clearly that a difference in one allele is not always sufficient evidence to claim that two cultivars are different. Moreover, the possibility of mutations should be taken into account for parentage analysis, especially if no previous information is available, because incompatibility in one locus is not sufficient for a parental exclusion.

- BOWERS, J. E.; DANGL, G. S.; VIGNANI, R.; MEREDITH, C. P.; 1996: Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* **39**, 628-633.
- CRESPIAN, M.; BOTTA, R.; MILANI, N.; 1999: Molecular characterization of twenty seeded and seedless table grape cultivars (*Vitis vinifera* L.). *Vitis* **38**, 87-92.
- LODHI, M. A.; YE, G. N.; WEEDEN, N. F.; REISCH, B. I.; 1994: A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Mol. Biol. Repr.* **12**, 6-13.
- OIV; 1987: Synonyms of the varieties of table grapes and raisins. *Bull. OIV* **60** (671-672), 44-82.
- SANCHEZ-ESCRIBANO, E. M.; MARTIN, J. P.; CARRENO, J.; CENIS, J. L.; 1999: Use of sequence-tagged microsatellite site markers for characterizing table grape cultivars. *Genome* **42**, 87-93.
- SEFC, K. M.; REGNER, F.; TURETSCHER, E.; GLÖSSL, J.; STEINKELLNER, H.; 1999: Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome* **42**, 367-373.
- THOMAS, M. R.; CAIN, P.; SCOTT, N. S.; 1994: DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. *Plant Mol. Biol.* **25**, 939-949.
- THOMAS, M. R.; SCOTT, N. S.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theor. Appl. Gen.* **86**, 985-990.