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Research Note

Origin of 'Csillám', a promising source for black rot resistance

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K e y w o r d s : microsatellite (SSR) analysis; parentage; berry colour; *VvMybA1* and *VvMybA2* genes.

Introduction: French-American grape hybrids and their derivatives produced in the last 100 years are important resistance gene sources against powdery, downy mildews and black rot. Previously we reported on the parentage analysis of Hungarian grapevine cultivars of 'Seibel-Seyve-Villard' origin among them 'Csillám', lineage of which ('Csabagyöngye' x 'Seyve Villard 12375') has been disproven (Tóth-Lencsés *et al.* 2015). Deciphering the true origin of varieties is interesting in itself, but in the case of 'Csillám' another remarkable observation made it even more stimulating; in a survey aiming at the comparison of the resistance against black rot - (BR) [(*Guignardia bidwellii* (Ellis) Viala & Ravaz, anamorph *Phyllosticta ampelicida* (Engleman)] a new severely threatening grapevine disease - 'Csillám' displayed a surprisingly high level of tolerance among 74 accessions, exceeding all the 'Seyve Villard' descendents, only 'Seibel 7053', 'Merzling' and 'Börner' (*Vitis riparia* Gm x *Vitis cinerea* Arnold) gave similar excellent results (Roznik *et al.* unpubl.).

This directed our attention to decipher the true parentage of 'Csillám'. For this purpose, we applied two approaches: SSR genotyping at 31 loci and determining the genetic background of berry pigmentation both in the white berried 'Csillám' and its "novel" candidate black berried parents. The white grapes arose from the ancient black ones by independent mutations causing the inhibition of anthocyanin biosynthesis. The insertion of the *Gret1* retrotransposon in the promoter of the *VvMybA1* transcription factor gene is one of the reasons for colourless phenotype (Kobayashi *et al.* 2004); two SNPs in the coding region of *VvMybA2* gene also block the pigment biosynthesis (Walker *et al.* 2007; Carrasco *et al.* 2015).

Material and Methods: P l a n t m a t e r i a l : Young leaves of the cultivars listed in the Table and reference varieties for berry colour analysis, 'Barbera', 'Chardonnay', 'Pinot noir' were collected from the gene bank of the Institute of Viticulture and Enology of Pécs (PTE) and Eger.

T a b l e

SNP genotypes of the three reference cultivars ('Barbera', 'Chardonnay', 'Pinot noir') and the 'Seibel 4643', 'Kékfrankos' and 'Csillám' in the *VvMybA2* gene

Varieties	SNP *in VvMybA2* gene	
	VvMybA2R44 / K980	*VvMybA2C22*
References		
Barbera	G/G	G/G
Chardonnay	T/T	T/T
Pinot noir	G/T	G/T
Cultivars of interest		
Seibel 4643	G/T	G/T
Kékfrankos	G/T	G/T
Csillám	T/T	T/T

Explanation: G/G, homozygous black: 'Barbera'; T/T, homozygous white: 'Chardonnay'; two loss-of-function alleles; G/T, heterozygous black: 'Pinot noir', one functional allele.

P C R , S S R a n d C A P S a n a l y s i s : DNA isolation and microsatellite analyses were carried out according to Halász *et al.* (2005). First, 9 nuclear SSR primer pairs VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62 and VrZAG79 were chosen according to the GrapeGen06 EU project. For parentage analysis, additional 22 primers were involved into microsatellite profile determination shown in the supplemental Table.

A CAPS marker 20D18CB9 linked to the *VvMybA1* gene coding a transcription factor which regulates the anthocyanin biosynthesis was applied. The PCR amplicons generated with the 5'-GAT GAC CAA ACT GCC ACT GA-3' forward and the: 5'- ATC ACC TTG TCC CAC CAA-3' reverse primers were digested with *DdeI* enzyme as it was described by Walker *et al*. (2006). The same protocol was applied for the allele-specific primers of *VvMybA1* gene and *VvMybA2* locus linked CAPS marker. Fragments amplified with *VvMybA1* specific primers were separated in 1.5 % ethidium-bromide stained (0.5 $\mu g \cdot mL^{-1}$) agarose gel.

S N a P s h o t a n a l y s i s : Two SNPs (*VvMybA2R44/K980, VvMybA2C22)* found in the *VvMybA2* gene were examined with SNaPshot method (ABI PRISM SNaPshot Multiplex kit) according to the manufacturer's manual (Kerekes *et al*. 2015).

S t a t i s t i c a l a n a l y s i s : Parentage was analyzed by the Identity 1.0 statistics (Wagner and Sefc 1999).

Results and Discussion: The 'Seyve-Villard 12375' x 'Csabagyöngye' pedigree of 'Csillám' (CSFT194) published in the literature (Kozma 1986, Kozma jr. 2002, Bényei and Lőrincz 2005), has already been disproven by applying the 9 GrapeGen SSR primer pairs (Tóth-Lencsés *et al*. 2015).

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Analysis and evaluation of 383 genotypes (data not shown), including 45 interspecific hybrids of 'Seibel' and 'Seyve Villard' origin at the same 9 loci made it possible to predict that 'Kékfrankos' and 'Seibel 4643' are the putative parents of 'Csillám'.

To confirm the new parentage, we used more 22 SSR primer pairs and genotyped 'Csillám' together with its disputed and "novel" parents at 31 microsatellite loci (supplemental Table). Additional 7-7 loci excluded the 'Seyve-Villard 12375' x 'Csabagyöngye' pedigree and all the 31 loci verified the 'Kékfrankos' x 'Seibel 4643' origin of 'Csillám'.

'Csillám' is a white berried cultivar, while the varieties we found to be its new "candidate" parents ('Kékfrankos' and 'Seibel') bear black fruits (Figure).

For providing further proof that 'Kékfrankos' x 'Seibel 4643' is the real pedigree of 'Csillám' we tested the varieties of interest for the presence or absence of *Gret1* retrotransposon in the promoter of the *VvMybA1* gene.

The CAPS analysis proved that 'Kékfrankos' is a heterozygous black genotype (restriction digestion resulted in three DNA fragments 329, 248, 213 bp), but the also black berried 'Seibel 4643' showed homozygous white genotype (329, 248 bp) for the *Gret1* insertion similarly to the really white 'Csillám' (supplemental Figure).

Since the blackness of the 'Seibel 4643' cannot be explained by the lack of *Gret1* we examined the allele profile of *VvMybA*2 transcription factor gene with SNaPshot method based on reference varieties. We determined that

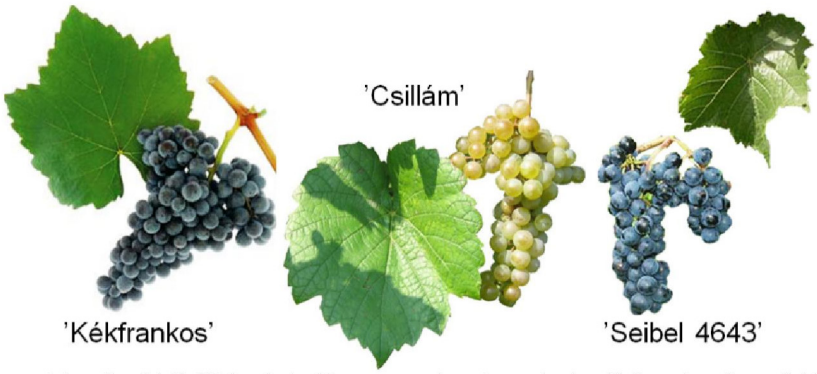

Figure: Bunch and leaf of 'Csillám' (Pál Kozma's photo), 'Kékfrankos' and 'Seibel 4643' (http://lescepages.free.fr/roi_noirs.html).

'Seibel 4643' carries functional SNP in heterozygous form in both SNP positions, therefore the anthocyanin biosynthesis operates even if it is homozygous for the *Gret1* insertion in the *VvMybA1* locus (Table). Our results not only confirm the true origin of 'Csillám' from the berry colour side, but also prove that it inherited the *VvMybA1+Gret1* and *VvMybA2* SNP (*VvMybA2R44* / K980; *VvMybA2C22* mutant 'white' alleles from both parents ('Kékfrankos'/'Blaufränkisch' and 'Seibel 4643'/'Roy de noirs'/'Pannonhalmi kék').

Astonishingly excellent behaviour of 'Csillám' in black rot resistance survey may be due to the 'Seibel 4643' parent. This fact calls our attention to find those cultivars which also derive from crosses including 'Seibel 4643'. 'Csillám' itself is a promising BR resistant cultivar so we think that it is well worth taking it into breeding programs as a perspective BR resistance source.

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