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Research Note

Genetic analysis of the *VvMybA1* gene in Armenian grapevines (*V. Vinifera* L.)

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K e y w o r d s : anthocyanins; *Gret1*; skin color.

Introduction: As a result of natural hybridization and human selection over centuries, the grape berry skin colour has become greatly diversified. The colour of grape skins is determined by the accumulation of anthocyanins (Harbone *et al*. 2000). Anthocyanin content is a trait of major interest in *Vitis vinifera* L. They have very important role in wine and grape industry, confer colour and contribute to other sensory characteristics (Boss *et al*. 1996). Molecular analyses of structural genes involved in the anthocyanin biosynthetic pathway showed that expression of the UDP glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) gene is a critical point in the control of berry colour (Kobayashi *et al*. 2001). It was shown that *Myb*-related gene, *VvMybA1,* regulates anthocyanin biosynthesis in grapes via UFGT gene expression (Kobayashi *et al*. 2004, 2005) and the colouring of grape skin depends on the genotype of *VvMybA1* (Lijavetzky *et al.* 2006, AZUMA *et al.* 2007). Recently, it has been shown that the presence of *Gret1*, a Ty3–*gypsy*-type retrotransposon, in the promoter region of *VvMybA1* gene, is associated with white-berry cultivars when present in a homozygous state (Kobayashi *et al*. 2004).

Coloured cultivars hold at least one allele at the *VvMybA1* locus not containing this large insertion: *VvMybA1b* or *VvMybA1c* (Kobayashi *et al*. 2004, Azuma *et al.* 2007, Yakushiji *et al*. 2006). It was revealed that the *VvMybA1b* and *VvMybA1c* alleles are functional. The *VvMybA1b* allele has a single long terminal repeat (LTR) of *Gret1* in the 5´flanking region near the coding region of *VvMybA1* gene (Azuma *et al.* 2007). *VvMybA1c*, the wild type allele, is the original sequence of *VvMybA1* gene before the insertion of *Gret1* (Yakushiji *et al*. 2006). In most examined white-skinned grapes, the colour locus is homozygous for *VvMybA1a,* whereas the genotype of colour skinned berries are heterozygous (*VvMybA1a*/*VvMybA1b* or *VvMybA1a*/*VvMybA1c*) or are homozygous (*VvMybA1c*) (Azuma *et al.* 2008).

Due to the lack of data for Armenian grapevines, the present study has the objective to analyze the role of *VvmybA1* gene in berry color variation. In order to ascertain the existent allelic composition for this gene and its association with berry color variation, thirty autochthonous Armenian grapevine cultivars were analyzed.

Material and Methods: Young leaves were collected from fifteen coloured ('Areni', 'Armenia', 'Agaraki', 'Anushahyut', 'Anush', 'Charenci', 'Earskheni', 'Kakhet', 'Karin', 'Karmrahyut', Karmir Kakhani', 'Meghrabuyr', 'Nalbandyani', 'Nerkeni', 'Nerkarat') and fifteen white-berry cultivars ('Arevar', 'Ararati', 'Chilar', 'Garan dmak', 'Itsaptuk', 'Mskhali', 'Muscat', 'Muscat TSKHA', 'Merdzavani vaghahas', 'Nazeli', 'Shahumyani', 'Spitak Araqseni', 'Tokun', 'Parvana', 'Voskehat',) selected as representatives of Armenian germplasm collection (Merdzavan, Armavir region, Armenia). Genomic DNA was extracted using Qiagen DNA Plant Mini Kit (Qiagen, Hilden, Germany) and used as a template for PCR. Two different primer combinations were used to analyze the *VvMybA1* locus. The first step was PCR detection of the *Gret1* insertion in the promoter region of *VvMybA1*gene. This allele, *VvMybA1a*, is considered as non-functional. The primers for detection of this allele were *a* and *d3* (Lijavetzky *et al.* 2006). The PCR was performed with 50 ng of genomic DNA using the following PCR conditions: 94 °C for 5 min, 39 cycles of 94 °C for 30 s, 58 °C for 45 s, and 72 °C for 1.5 min, followed by a final extension step at 72 °C for 10 min. PCR fragments were separated by electrophoresis in 1.0 % agarose gel in 1x TAE buffer stained with ethidium bromide and visualized under UV light.

The primers for detection of functional *VvMybA1c* allele and other putative functional alleles were F2 and R1 (Azuma *et al.* 2008). The PCR was performed using the same condition listed above increasing the annealing temperature up to 60 °C. PCR fragments were separated by electrophoresis in 1.7 % agarose gel in 1x TAE buffer stained with ethidium bromide and visualized under UV light.

Results and Discussions: To estimate the extent of berry colour variation explained by *VvMybA1* gene in grape cultivars, we have analysed the allelic polymorphism for this locus in fifteen coloured and fifteen white Armenian grape cultivars. Two different primer combinations were used to detect functional and non-functional alleles of *VvMybA1* gene. The identified alleles arranged based on the berry color are listed in the Table.

As expected, for all analyzed white cultivars, using *a* and *d3* primers combination, we detected the presence of the null *VvMybA1a* allele. The expression of *VvMybA1* gene is blocked in the sample holding the *VvMybA1a* allele, which contains a retrotransposon, *Gret1*, upstream of the *VvMybA1*-coding sequences (Azuma *et al.* 2007). Among the colored grapevines, six cultivars were heterozygous

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Table

VvMybA1 haplotypes and colour phenotypes observed in 30 Armenian grapevine cultivars

Haplotypes	*VvMybA1* alleles				Color phenotypes		Total
	VvMybA1a	*VvMybA1c*	*VvMybA1*^{SUB}	*VvMybA1e*	W	C	
1	+				15		15
2		+	+			1	1
3			+			2	2
4		+	+	+		1	1
5				+		1	1
6	+			+		1	1
7		+		+		2	2
8	+	+				5	5
9		+				2	2
Total					15	15	30

Berry skin colour: W - white, C - colored.

for the non-functional allele, showing both *VvMybA1a* allele and other functional alleles. These data confirmed that the trait coloured berries is dominant (KOBAYASHI *et al.* 2004).

The F2 and R1 primers combination allowed the detection of three putative functional alleles *VvMybA1c* (wild type allele), *VvMybA1*^{SUB} (containing two 44 and 111 bp insertions) and *VvMybA1e* (a similar allele to *VvMybA1*^{SUB} without 44 bp insertion), with *VvMybA1c* being the most frequent in colour grape cultivars (73.3 %).

The same allele frequencies were found in the data reporting the allelic polymorphism of *VvMybA1*gene in other cultivated and wild grapevine accessions (CARRASCO *et al.* 2014). The *VvMybA1e* allele, discovered for the first time in wild samples coming from the Iberian Peninsula and in cultivated and wild samples from Georgia (CARRASCO *et al.* 2014), was detected also in the Armenian germplasm.

Considering together the alleles detected using both primer combinations, nine different haplotypes for *VvMybA1* were identified (Table). Among coloured cultivars, the haplotype showing the highest frequency was the haplotype 8, holding both functional and non-functional alleles (*VvMybA1c* and *VvMybA1a*). The haplotype 4, held by only one cultivar, showed a triple band, due to probably a putative new duplication event near the *VvMybA1* locus (CARRASCO *et al.* 2014).

Thus, in all of the tested cultivars the berry color phenotype can be explained on the basis of *VvMybA1* genotyping, being white when no functional allele can be amplified and colored when at least one functional allele is detected.

Conclusions: Grape berry colour composition is a highly complex trait. It displays a high genetic diversity, which means variability among genotypes and a large phenotypic plasticity. The obtained data indicate that variation in *VvMybA1* gene has generated an allelic polymorphism strongly associated with berry colour variation in cultivated grapevine. Therefore, undiscovered functional alleles and haplotypes may exist in various *Vitis* species. Further analyses, using the *VvMybA1* functional and non-functional alleles, a broader range of accessions, *sylvestris* accessions, as well as *VvMybA2* polymorphism (another *Myb*-related gene involved in the berry color variation), need to fully understand these alleles and haplotypes. Detailed analysis of the diversity of alleles and haplotypes at the region of berry colour locus is needed to clarify the genomic relationships and evolutionary differentiation of *Vitis* species regarding this important grape quality trait.

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