Research Note

VvmybA1 genotype determines grape skin color

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K e y w o r d s : anthocyanin, *myb*, retrotransposon, grape, skin color.

Introduction: The skin color of grapes is determined by the quantity and composition of anthocyanins. Black and red cultivars accumulate anthocyanins in their skins, whereas white cultivars do not synthesize them. In grape, *Myb*-related genes such as *VlmybA1-1* of *Vitis labruscana* regulate anthocyanin biosynthesis by controlling expression of the gene for UDP-glucose: flavonoid 3-*O*-glucosyltransferase (Kobayashi *et al*. 2002). A homolog of *VlmybA1-1*, *VvmybA1*, has the same function in *V. vinifera* (Kobayashi *et al*. 2004, 2005). These *Myb* genes appear to enhance the expression of all genes in the anthocyanin biosynthesis pathway, because the transcription of all anthocyanin biosynthesis genes examined by Kobayashi *et al*. (2002) appears to be slightly activated by the introduction of VlmybA1-1 into the somatic embryos of grapevine. In addition, the expression of *VvmybA1* coincided with that of the genes for anthocyanin biosynthesis enzymes and the accumulation of anthocyanins in grape skins (Jeong *et al*. 2004). However, the expression of *VvmybA1* is blocked in the *VvmybA1a* allele, which contains a retrotransposon, *Gret1*, upstream of the *VvmybA1*-coding sequences (Fig. 1). In contrast, the alleles *VvmybA1b* and *VvmybA1c* are functional. *VvmybA1b* has a single copy of LTR (solo LTR) of *Gret1* in the 5′-flanking region near the coding sequences of *VvmybA1* and is expressed (Kobayashi *et al*. 2004, 2005). *VvmybA1c* lacks *Gret1* completely and is most likely the original sequence of *VvmybA1* before the insertion of *Gret1* (Yakushiji *et al*. 2006). White-skinned cultivars that we examined are homozygous for *VvmybA1a*, whereas the red- or black-skinned cultivars examined are heterozygous for *VvmybA1a* and *VvmybA1b* or *VvmybA1c* (Kobayashi *et al*. 2004).

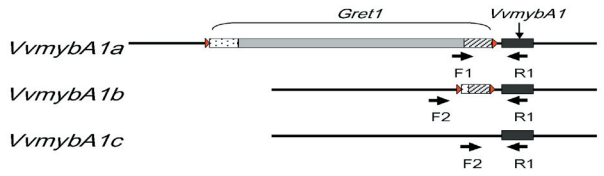

Fig. 1: Structures of *VvmybA1a*, *VvmybA1b,* and *VvmybA1c.* Primer positions are indicated below the maps. F1, F2, forward primers. R1, reverse primer.

These findings suggest that *VvmybA1* is a major gene determining the coloring of grape skin. However, it is not clear whether *VvmybA1* is the sole determinant, as other genes related to anthocyanin biosynthesis might be involved. To examine whether the coloring of grape skin is determined by *VvmybA1*, we investigated the relationship between the skin color and *VvmybA1* genotype using cross seedlings.

Materials and Methods: We used 22 three-year-old seedlings of an Akitsu21 (*V. labruscana*; red-skinned) × Iku82 (*V. labruscana*; red-skinned) cross, 29 seedlings of a 617-14 (*V. labruscana*; white-skinned) × Iku82 cross, and 41 seedlings of an Iku71 (*V. labruscana*; red-skinned) × Iku91 (*V. vinifera*; white-skinned) cross. Segregation of skin color of the seedlings was visually assessed (as white or red/black) at harvest time. Chi-square tests were used to determine the agreement of observed and expected skin color ratios.

Total DNA was extracted from young leaves of all parents and seedlings as described previously (Kobayashi *et al.* 2002) and was used as a template for PCR. The primers for *VvmybA1a* were F1 (5′-AAAAAGGGGGGCAATGTAGGGACCC-3′) and R1 (5′-GAACCTCCTTTTTGAAGTGGTGACT-3′) and those for *VvmybA1b* and *VvmybA1c* were F2 (5′-GGACGTTAAAAAATGGTTGCACGTG-3′) and R1 (Fig. 1; Kobayashi *et al*. 2004). PCR reactions were performed in a total volume of 10 µl comprising 5 ng DNA, 200 µm dNTPs, 0.2 µm of each primer, and 0.5 units of ExTaq polymerase (Takara, Kyoto, Japan). The PCR cycling conditions were an initial 95 °C for 3 min; 34 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s; and a final 72 °C for 5 min. PCR fragments were separated by electrophoresis in 1.2 % agarose gel in TAE buffer and photographed under UV light.

Results and Discussion: The skin color segregated to 17 red/black-skinned and 5 white-skinned in the Akitsu21 × Iku82 cross, 12 red/black-skinned and 17 white-skinned in the 617-14 × Iku82 cross, and 24 red/black-skinned and 17 white-skinned in the Iku71 × Iku91 cross (Table). Chi-square tests revealed that the segregation ratios fit the expected distributions.

The red-skinned parents (Akitsu21, Iku82, and Iku71) each contained both *VvmybA1a* and *VvmybA1c*, and the white-skinned parents (617-14, Iku91) contained only *VvmybA1a*. In the Akitsu21 × Iku82 cross, all white-skinned seedlings contained only *VvmybA1a*, 14 red/black-skinned seedlings contained both *VvmybA1a* and *VvmybA1c*, and 3 red/black-skinned seedlings contained only *VvmybA1c* (Fig. 2). In the 617-14 × Iku82 and Iku71 × Iku91 crosses, all white-skinned seedlings contained only *VvmybA1a* and all red/black-skinned seedlings contained *VvmybA1a* and *VvmybA1c* (data not shown). These results suggest that

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T a b l e

Skin color segregation in the seedlings of Akitsu21 × Iku82, 617-14 × Iku82 and Iku71 × Iku91 crosses

Cross combination	Total number of progeny	Number of progeny		Expected ratio	χ^2 value	*P*-value
		red/black	white			
Akitsu21 (red) × Iku82 (red)	22	17	5	3:1	0.061	0.806
617-14 (white) × Iku82 (red)	29	12	17	1:1	0.862	0.353
Iku71 (red) × Iku91 (white)	41	24	17	1:1	1.195	0.274

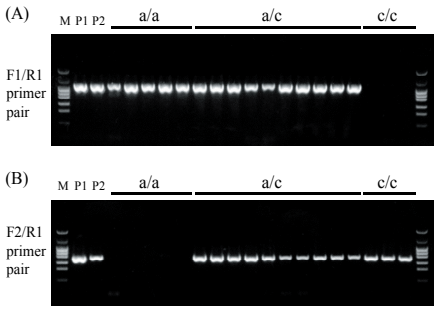

Fig. 2: PCR analysis of the *VvmybA1* genotype in Akitsu21 × Iku82 seedlings. (**A**) PCR analysis of *VvmybA1a*. (**B**) PCR analysis of *VvmybA1c*. P1: Akitsu21 (red-skinned). P2: Iku82 (red-skinned). a/a: white-skinned seedlings (*VvmybA1a*/*VvmybA1a*). a/c: red/black-skinned seedlings (*VvmybA1a*/*VvmybA1c*). Ten out of 14 samples were loaded into the well. c/c: red/black-skinned seedlings (*VvmybA1c*/*VvmybA1c*). M: size markers.

coloring of grape skin depends on the genotype of *VvmybA1* (*VvmybA1c* is dominant to *VvmybA1a*).

There are a few reports about inheritance of grape skin color. BARRITT and EINSET (1969) proposed that skin color is controlled by two pairs of genes. They suggested that a gene for black skin color (B---) was dominant to those for red and white skin colors, and that for red skin color (bbR-) was dominant to that for white skin color (bbrr). However, DOLIGEZ *et al*. (2002) and FISCHER *et al*. (2004) showed that the presence or absence of anthocyanin segregated as a monogenic trait determined by a locus on linkage group 2. The present study revealed that *VvmybA1* is the major gene for the determination of grape skin color. Furthermore, we earlier showed that *VvmybA1* transcripts were not present in any of the white cultivars examined, but were present in all the colored cultivars examined (KOBAYASHI *et al*. 2004). Thus, the presence or absence of anthocyanin and the quantity and composition of anthocyanins should be considered separately. Our results suggest that the expression of *VvmybA1* is essential for the coloring of grape skin, and that the color (red or black) would be influenced by the expression of anthocyanin biosynthesis pathway genes such as those for flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′5′-hydroxylase (F3′5′H). JEONG *et al*. (2006) demonstrated that the transcription levels of F3′H and F3′5′H genes likely control the ratio of cyanidin-based anthocyanins and delphinidin-based anthocyanins, respectively.

Recently, LIJAVETZKY *et al*. (2006) also indicated that coloring of grape skin depends on the genotype of *VvmybA1* in an analysis of self-cross seedlings of the colored cultivar Ruby Seedless, and they reported two additional alleles of *VvmybA1*. Other alleles have also been reported. YAKUSHIJI *et al*. (2006) and WALKER *et al*. (2006) showed that skin color mutations of 'Pinot Noir' and 'Cabernet Sauvignon', from black-skinned to white-skinned, are caused by deletion of the functional *VvmybA1* allele; they named the null allele in 'Pinot Noir' *VvmybA1d* (YAKUSHIJI *et al*. 2006). Furthermore, WALKER *et al*. (2006) reported that the berry color locus of 'Cabernet Sauvignon' is composed of two very similar and adjacent genes (*VvMYBA1* and *VvMYBA2*), either of which could control berry color. Therefore, further analysis of different *VvmybA1* genotype crosses is necessary to verify and expand the present hypothesis.

Based on our results, in cross-breeding programs it should be possible to detect the skin color of seedlings at a very young stage by examining the genotype of *VvmybA1* with PCR, thus cutting the time and expense of raising plants to maturity.

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