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Research Note

A retrotransposon-inserted *VvmybA1a* allele has been spread among cultivars of *Vitis vinifera* but not North American or East Asian *Vitis* species

N. Mitani[1)], A. Azuma[1)], E. Fukai[2)], H. Hirochika[2)] and S. Kobayashi[1)]

[1)] Grape and Persimmon Research Station, National Institute of Fruit Tree Science, Higashi-Hiroshima, Hiroshima, Japan
[2)] Molecular Genetics Department, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan

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

Introduction: The colour of grape skins is determined by the accumulation of anthocyanins. Previously, we showed that a *Myb*-related gene, *VvmybA1*, regulates anthocyanin biosynthesis in grapes (Kobayashi *et al*. 2004, 2005). Furthermore, Lijavetzky *et al*. (2006) and Azuma *et al.* (2007) showed that the colouring of grape skin depends on the genotype of *VvmybA1*. The allele *VvmybA1a* contains a retrotransposon, *Gret1*, upstream of the *VvmybA1* coding sequences, and the expression of *VvmybA1* is blocked. In contrast, the *VvmybA1c* allele is functional. *VvmybA1c* completely lacks *Gret1* and most likely represents the original sequence of *VvmybA1* before *Gret1* was inserted (*VvmybA1c* is dominant to *VvmybA1a*).

A strong association between the *VvmybA1a* allele and white-fruited phenotypes has been detected in many cultivars of the grapevine *Vitis vinifera*, although a small number of exceptions have been observed (Lijavetzky *et al*. 2006, This *et al*. 2007, Walker *et al*. 2007). In addition, Walker *et al.* (2007) showed that the locus houses two very similar adjacent genes (*VvMYBA1* and *VvMYBA2*), either of which could control berry colour.

It is thought that cultivation of *V. vinifera* began during the Neolithic era (6000-5000 BCE) along the eastern shores of the Black Sea, and the cultivated grape is believed to have been domesticated from the wild grape *V. vinifera* subsp. *sylvestris* (hereafter denoted as *sylvestris*) (Mullins *et al.* 1992). Since the *VvmybA1a* allele is widely distributed among cultivars of *V. vinifera*, we hypothesized that *Gret1* was originally inserted upstream of the *VvmybA1* coding sequence of a black-skinned ancestor, and that subsequently a white-skinned grape was produced by spontaneous crossing (Kobayashi *et al.* 2004). To date, the *VvmybA1a* allele has been detected in over 200 accessions of *V. vinifera* (Lijavetzky *et al*. 2006, This *et al*. 2007, Walker *et al*. 2007).

Determination of the presence and distribution of the *VvmybA1a* allele in the wild grape and other *Vitis* species would provide new information on the genomic relationships among these species and on the evolutionary differentiation of the genus *Vitis*. Here we report on the distribution of the *VvmybA1a* allele in typical cultivars of *V. vinifera* and in North American and East Asian *Vitis* species.

Material and Methods: Forty accessions (Table) were analysed. Cultivars of *V. vinifera* were eco-geographically classified into three proles: *pontica*, *occidentalis*, and *ori-*

T a b l e

Distribution of the *VvmybA1a* allele in *Vitis* species

	Species	Cultivar name	Skin color	*Gret1* insertion
	V. vinifera			
	subsp. *sylvestris* (♀)		black	+
	subsp. *sylvestris* (♂)		---	+
	pontica	Black Hamburg	black	+
		Alphonse Lavalée	black	-
		Furmint	white	+
		Plavaj	white	+
	occidentalis	Cabernet Sauvignon	black	+
		Merlot	black	+
		Pinot Noir	black	+
		Chardonnay	white	+
		Melon	white	+
		Riesling	white	+
	orientalis	Muscat Hamburg	black	+
		Rizamat	red	+
		Flame Tokaj	red	+
		Bajan Sirej	white	+
		Italia	white	+
		Thompson Seedless	white	+
		Niunai	white	+
NA[1)]	*V. aestivalis*		black	-
	V. cinerea		black	-
	V. doaniana		black	-
	V. labrusca		black	-
	V. longii		black	-
	V. palmata		black	-
	V. riparia	Gloire de Montpellier (♂)	---	-
				-
	V. rubra		black	-
	V. rupestris	Constantia	black	
EA[2)]	*V. amurensis*		black	-
	V. coignetiae (♀)		black	-
	V. coignetiae (♂)		---	-
	V. quingquangularis		black	-
	V. shiragai		black	-
	V. thunbergii		black	-
	V. vinifera x *V. labrusca*	Concord	black	+
		Steuben	black	-
		Bronx Seedless	red	+
		Urbana	red	+
		Golden Muscat	white	+
		Seneca	white	+

[1)] NA = North America; [2)] EA = East Asia.

Correspondence to: Dr. S. Kobayashi, Grape and Persimmon Research Station, National Institute of Fruit Tree Science, Akitsu 301-2, Higashi-Hiroshima, Hiroshima 739-2494, Japan. Fax: +81-846-45-5370; E-mail: skobaya@affrc.go.jp

entalis. We selected several cultivars with different skin colours from these proles. All these species and cultivars are part of the germplasm collection maintained in the vineyards of the Grape and Persimmon Research Station, National Institute of Fruit Tree Science, Japan. (The *sylvestris*, the American species, and the East Asian species came from the collections of the Turkmenistan Experimental Station for Plant Genetic Resources, the US Department of Agriculture, and the University of Osaka Prefecture, respectively.)

Total DNA was extracted from young leaves of the plants as described (Kobayashi *et al.* 2005) and used as a template for the polymerase chain reaction (PCR). The primers for the 3′LTR region of *Gret1* were '*a*' and '*c*' (Figure, Kobayashi *et al*. 2004), and PCR reactions and electrophoresis were performed as reported (Kobayashi *et al.* 2004). The 5′LTR regions of *Gret1* were amplified by using primers '*b*' (Figure, Kobayashi *et al*. 2004) and '*d*' (5′-CACAGACAGCGCCAATGTTGT-3′) as above, and sequenced directly. Then the sequences of the two LTRs were compared in each cultivar.

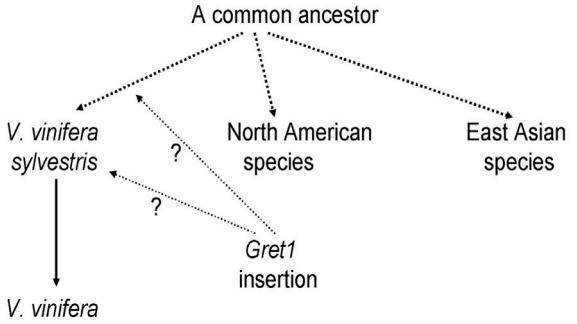

Figure: A model of evolutional differentiation of *Vitis* species.

Results and Discussion: The *VvmybA1a* allele, which contains *Gret1*, was detected in almost all of the cultivars of *V. vinifera* that we analysed, including *sylvestris* and the interspecific hybrids (*V. vinifera* × *V. labrusca*), with the exception of 'Alphonse Lavallée' and 'Steuben' (Table). In contrast, it was not detected in any of the North American or East Asian *Vitis* species. Most recently, Cadle-Davidson and Owens (2008) showed that the *VvmybA1a* allele was not detected even in white-fruited wild *Vitis* species (*V. aestivalis* and *V. riparia*).

The two LTRs of a retrotransposon are usually identical at the time of its insertion into the host genome (Lewin 1997). Therefore, the date of insertion can be estimated from the sequence divergence between the two LTRs. We analysed the two LTR sequences of *Gret1* in the *VvmybA1a* alleles of the above cultivars. The sequences of the 5′LTR differed from those of the 3′LTR at only five bases in each cultivar; however, no differences were observed within either sequence among cultivars, including *sylvestris*. Although we earlier reported that the sequences of the two LTRs of *VvmybA1a* differed at four bases (Kobayashi *et al.* 2004), five is correct. That is, in the sequence data of clone *It22* registered as *VvmybA1a* in the DNA Data Bank of Japan (accession no. AB111100), the nucleotide at position 4904 in the 5′LTR region is a T, but the nucleotide in this position in the genome of 'Italia' was revealed to be a C by direct sequencing of the PCR product of Italia in this study. From the above sequence data, the MEGA4 software estimated the *Gret1* insertion date to be roughly 0.20 ± 0.09 (s.d.) million years ago by the method described by Tamura *et al*. (2007). In this estimation, we used the rate of 1.5×10^{-8} nucleotide substitutions per synonymous site per year, which was estimated by analysis of sequence variation in chalcone synthase and alcohol dehydrogenase loci in dicots (Koch *et al.* 2000).

Our findings suggest that *Gret1* was inserted upstream of the *VvmybA1* coding sequences in *sylvestris* or an unknown ancestor of *sylvestris* after the North American and East Asian species had diverged from the common ancestor (Figure). The *VvmybA1a* allele that appeared in interspecific hybrids was presumably transferred from *V. vinifera* by crossing. We analysed only the *VvmybA1a* allele and only in a limited number of species or cultivars. Further analyses using the other *VvmybA1* alleles and a wide range of species and accessions in the genus *Vitis*, and especially in *sylvestris*, will be necessary to clarify the genomic relationships and evolutionary differentiation of *Vitis* species.

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