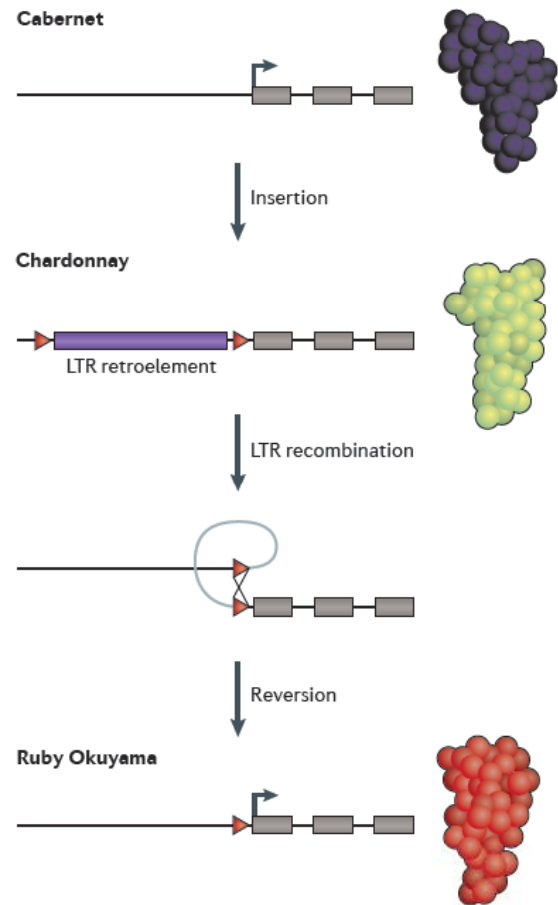


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Tarmo Puurand
Jclub in bioinformatics
01.04.2013

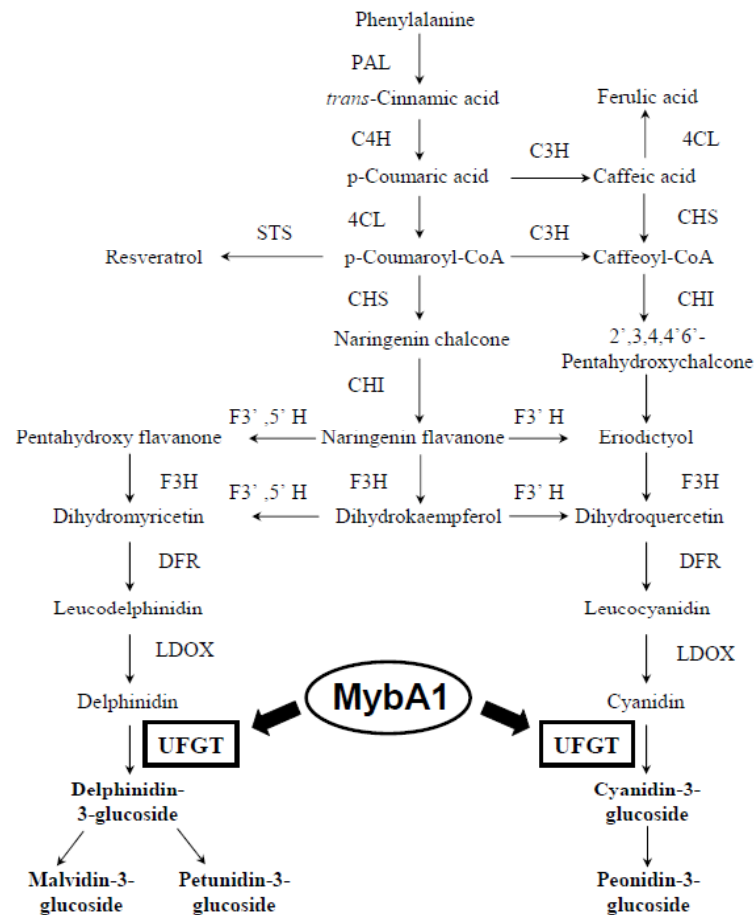


How important are transposons for plant evolution?

Lisch D.

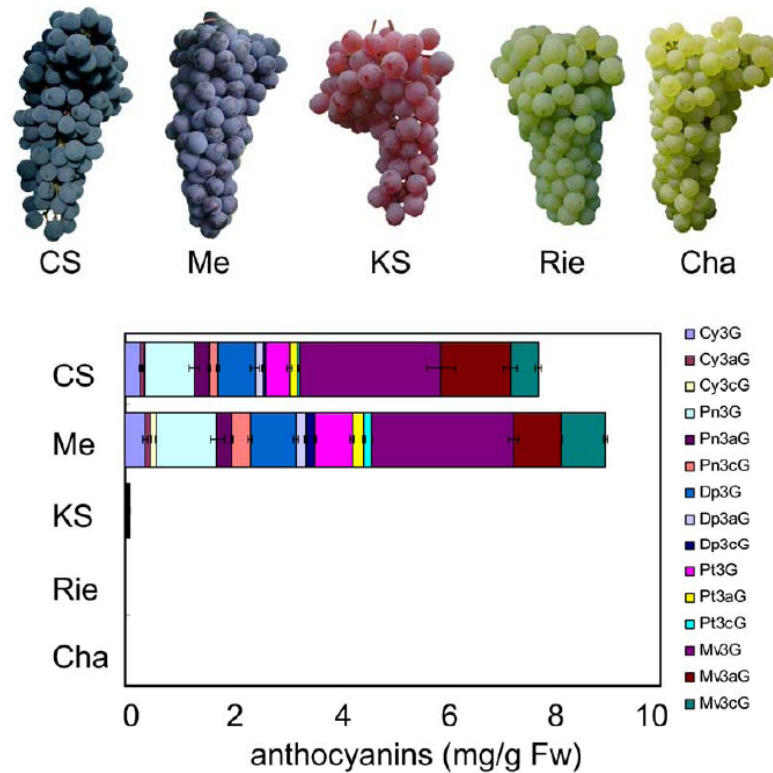
Nat Rev Genet. 2013 Jan;14(1):49-61.

Pathway leading to the synthesis of anthocyanins



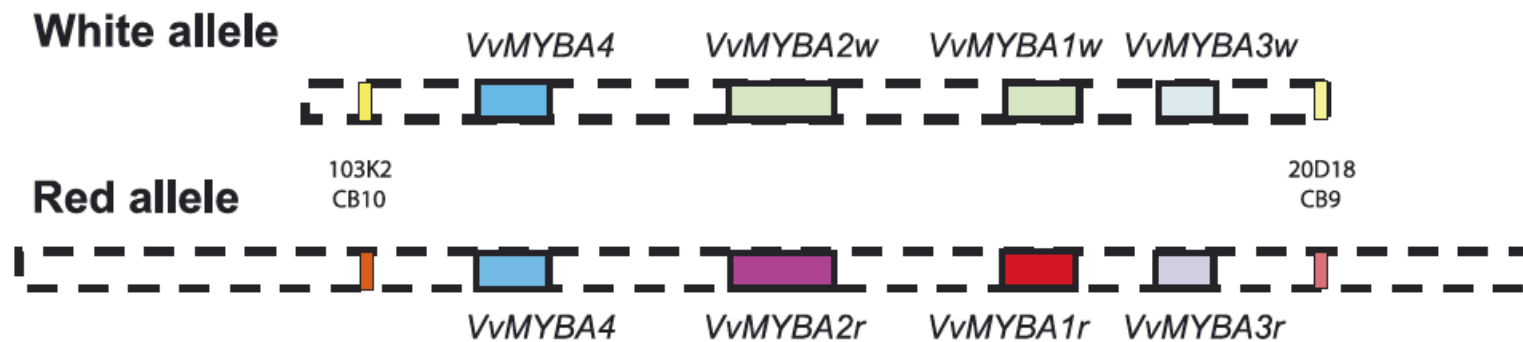
MybA1 is a transcription factor that regulates the transcription of the *UFGT* gene. PAL, phenylalanine ammonia lyase. C4H, cinnamate 4-hydroxylase. 4CL, 4-coumarate ligase. C3H, coumarate-3-hydroxylase. STS, stilbene synthase. CHS, chalcone synthase. CHI, chalcone isomerase. F3'H, flavonoid 3'-hydroxylase. F3',5'H, flavonoid 3',5'-hydroxylase. F3H, flavonone-3-hydroxylase. DFR, dihydroflavonol 4-reductase. LDOX, leucoanthocyanidin dioxygenase. UFGT, UDP-glucose:flavonoid 3-o-glucosyltransferase.

Total amounts of anthocyanins



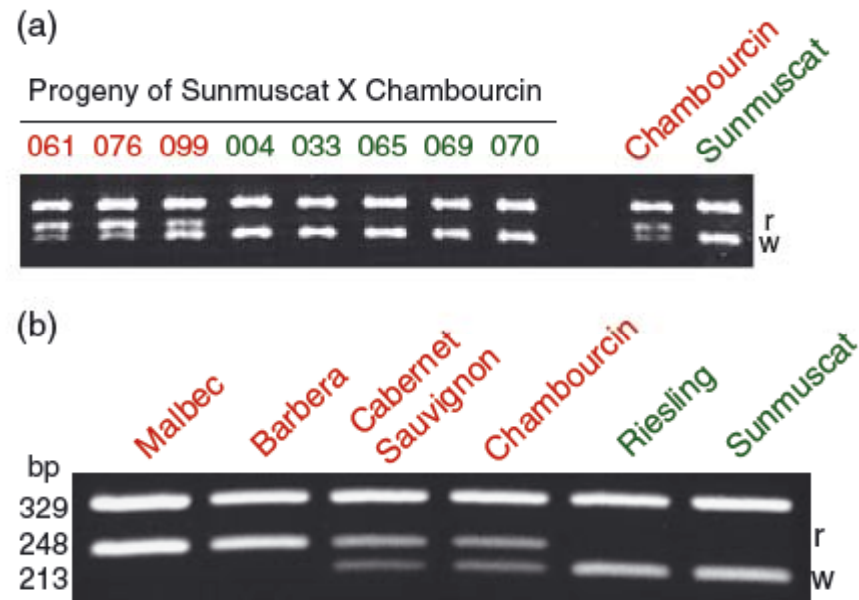
Anthocyanin (cyanidin, peonidin, delphinidin, petunidin, and malvidin) composition in berry skins was measured using reversed-phase high performance liquid chromatography. Berries were collected at 19 weeks post flowering. Bars indicate means \pm 6 standard deviations of duplicate experiments. CS: Cabernet Sauvignon, Me: Merlot, KS: Koshu, Rie: Riesling, Cha: Chardonnay.

Diagram of the berry colour locus from Cabernet Sauvignon



VvMYBA sequences are indicated by the heavily outlined boxes in order along the chromosome. The smaller coloured boxes indicate the markers 20D18CB9 and 103K2CB10. Green and yellow boxes indicate the white allele version of polymorphic sequences; red and purple boxes indicate the red allele versions; blue boxes indicate sequences that are the same in both alleles. Not to scale.

Berry colour segregates with CAPS marker 20D18CB9



(a) Gel showing that berry colour segregates as predicted with the marker in progeny of a cross between Sunmuscat (white) and Chambourcin (red). (b) Marker differentiates between homozygous red (Malbec and Barbera), heterozygous red (Cabernet Sauvignon and Chambourcin) and white (Riesling and Sunmuscat) cultivars. Red-berried plants named in red, white-berried plants in green. 'r' indicates band present in red allele, 'w' band from the white allele.

Genotype of white grape cultivars assessed by markers and sequence analysis

	Grape cultivar	Group no. ^a	20D18CB9 ^b	103K2CB10 ^b	MYBA2 ^c	CA deletion ^d	Retro mark
1	Aligote	9	W	W		Yes	W
2	Auxerrois		W	W		Yes	W
3	Biancone		W	W		Yes	W
4	Calmeria		W	W		Yes	W
5	Canada Muscat		W	W		Yes	W
6	Canocazo		W	W		Yes	W
7	Chaouch	2	W	W		Yes	W
8	Chardonnay	9	W	W	W	Yes	W
9	Chasselas		W	W		Yes	W
10	Chenin Blanc		W	W		Yes	W
11	Clairette Blanche		W	W		Yes	W
12	Colombard	11	W	W		Yes	W
13	Crouchen		W	W		Yes	W
14	Dawn Seedless		W	W		Yes	W
15	Doradillo	13	W	W	W	Yes	W

Red-berried cultivars scored with two CAPS markers

Cultivar	Use	20D18CB9 ^a	103K2CB10 ^{a,c}
1 Aleatico	Wine	W	R1 ^d W
2 Alicante	Wine	R	R2 ^e
3 Baco Noir	Wine	RW E ^b	W
4 Barbera	Wine	R	R2R3 ^f
5 Barlinka	Table	R	R2W
6 Bastardo	Wine	RW	R2W
7 Beauty Seedless	Table	RW	R2W
8 Black Frontignac	Table	W	W
9 Blush Seedless	Table	RW	W
10 Bonvedro	Wine	R	R1R2
11 Brown Frontignac	Table	W	W
12 Cabemet Franc	Wine	RW	R1W
13 Cabemet Sauvignon	Wine	RW	R1W
14 Cardinal	Table	RW	W
15 Carignan	Wine	RW	R2W
16 Carina	Table	R	W
17 Carolina Blackrose	Table	RW	R2W
18 Chambourcin	Wine	RW	R2W
19 Christmas Rose	Table	RW	W
20 Cinsaut	Wine	RW	R2W
21 Concord	Table	RW E	R3W
22 Criolla Negra	Wine	RW	W
23 Dolcetto	Wine	RW	R2W
24 Durif	Wine	R	R2W
25 Emperor	Table	RW	W
26 Flame Seedless	Table	RW	W
27 Foch	Wine	RW E	W
28 Gamay	Wine	RW	R2W
29 Grenache	Wine	RW	R2W
30 Kadarka	Wine	RW	R2W

Cultivar	Use	20D18CB9 ^a	103K2CB10 ^{a,c}
31 Kyoho	Table	RW E	R1W
32 Malbec	Wine	R	R2
33 Marroo Seedless	Table	RW	R2W
34 Mataro	Wine	RW	R2W
35 Merlot	Wine	RW	R1W
36 Mondeuse	Wine	RW	R2W
37 Monukka	Table	R	W
38 Muscat Hamburg	Table	RW	W
39 Nebbiolo	Wine	RW	W
40 Petit Verdot	Wine	RW	R2W
41 Pinot Gris	Wine	RW	R2W
42 Pinot Meunier	Wine	RW	R2W
43 Pinot Noir	Wine	RW	R2W
44 Raboso	Wine	RW	R2W
45 Raboso Piave	Wine	R	R3
46 Red Globe	Table	RW	W
47 Ribier	Table	R	R2W
48 Rubired	Wine	RW	R2W
49 Ruby Cabernet	Wine	R	R1W
50 Ruby Seedless	Table	RW	W
51 Sangiovese	Wine	RW	R3W
52 Shiraz	Wine	RW	W
53 Souzao	Wine	R	R1R2
54 Tarrango	Wine	W	R1W
55 Terret Noir	Wine	RW	R2W
56 Tinta Amarella	Wine	RW	R2W
57 Tinta Cao	Wine	W	R1W
58 Touriga	Wine	W	R1W
59 Valdiguie	Wine	RW	R2W
60 Zinfandel	Wine	RW	R2W

^aPCR results with markers; W indicates white allele; R indicates red allele.

^bE, extra band.

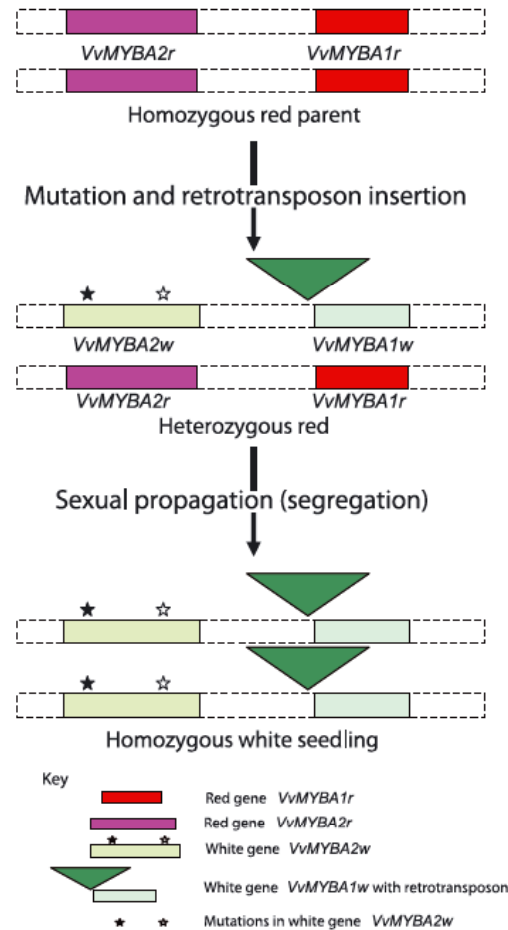
^cThree distinct banding patterns of red alleles:

^dR1, like Cabernet Sauvignon;

^eR2, like Chambourcin;

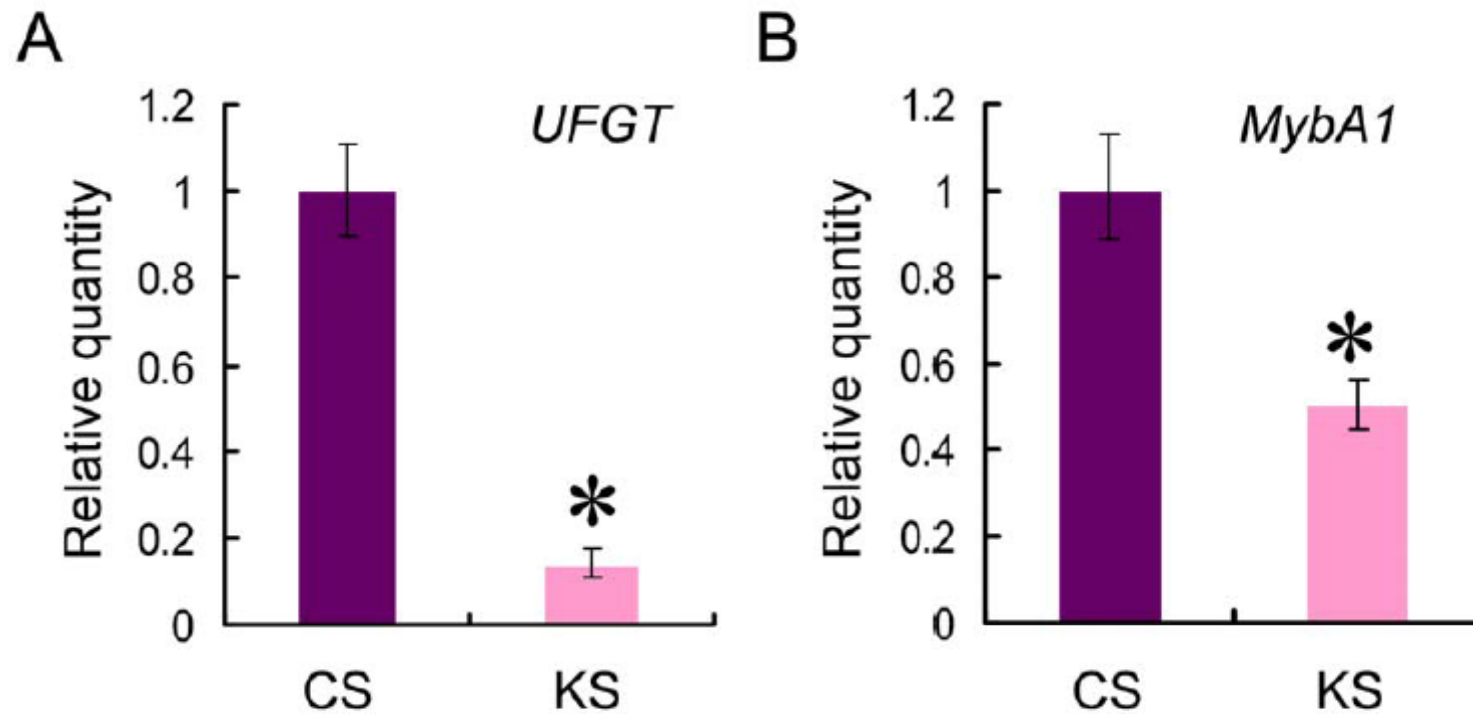
^fR3, like Concord

Model for the origin of the white grape genes

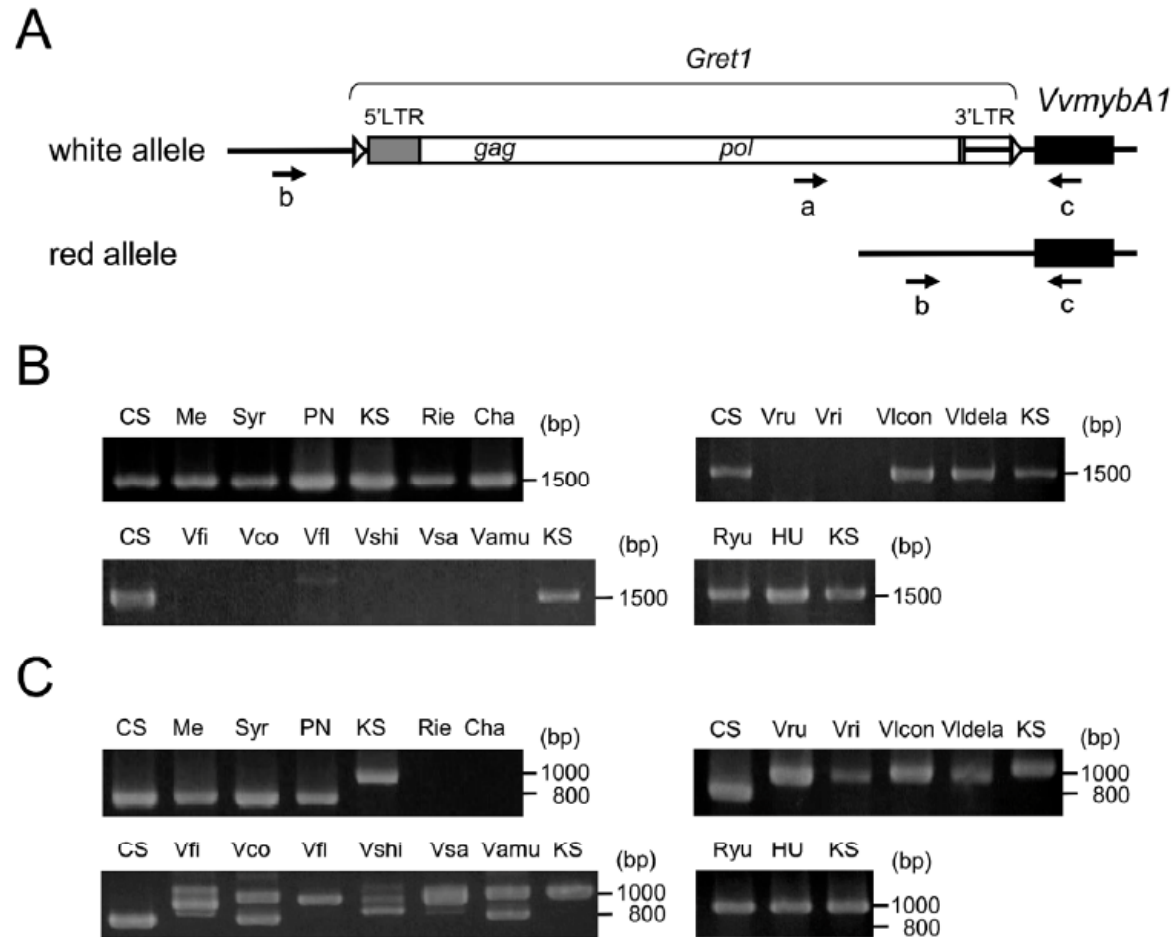


After duplication of the *VvMYBA* genes, *VvMYBA2r* and *VvMYBA1r* were both active resulting in a red grape phenotype. The accumulation of two mutations in *VvMYBA2w* and the insertion of a retrotransposon in the promoter of *VvMYBA1w* results in the inactivation of both these genes; however, the phenotype of the berries would still be red until sexual reproduction resulted in a homozygous genotype when white-fruited grapes would finally be observed.

UFGT and MybA1 gene expression in Koshu grape berry skin

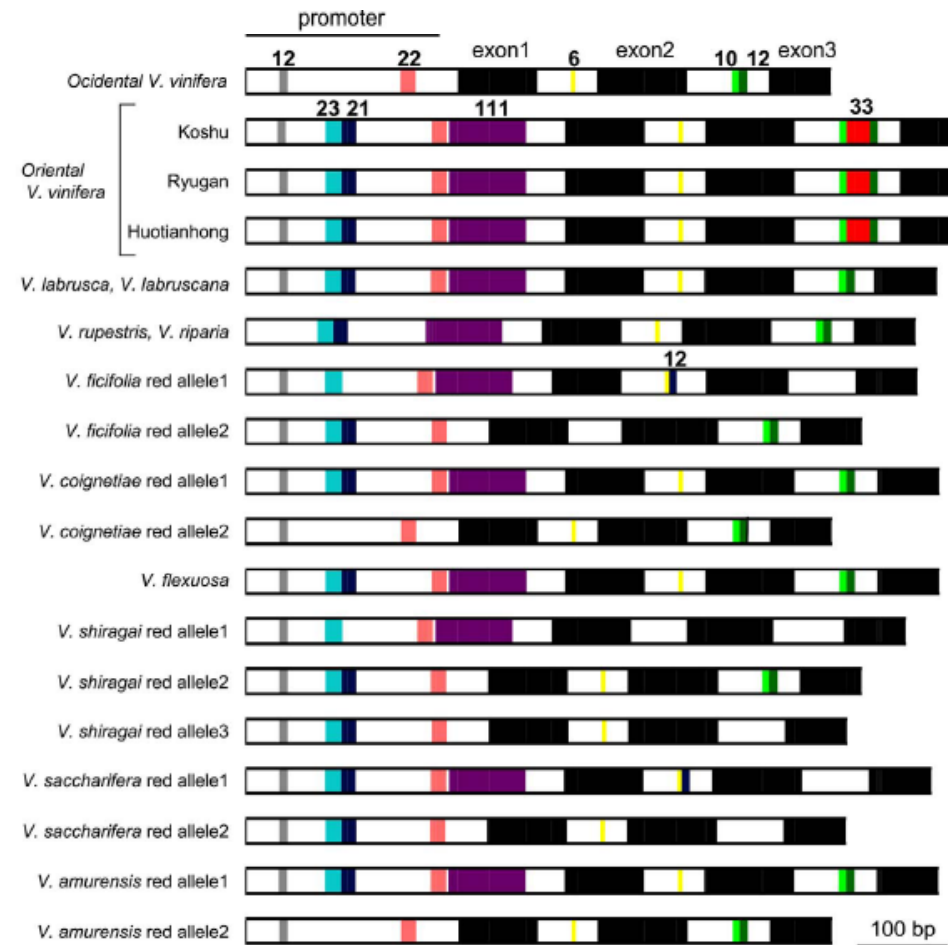


Analysis of MybA1 gene structure in grapes

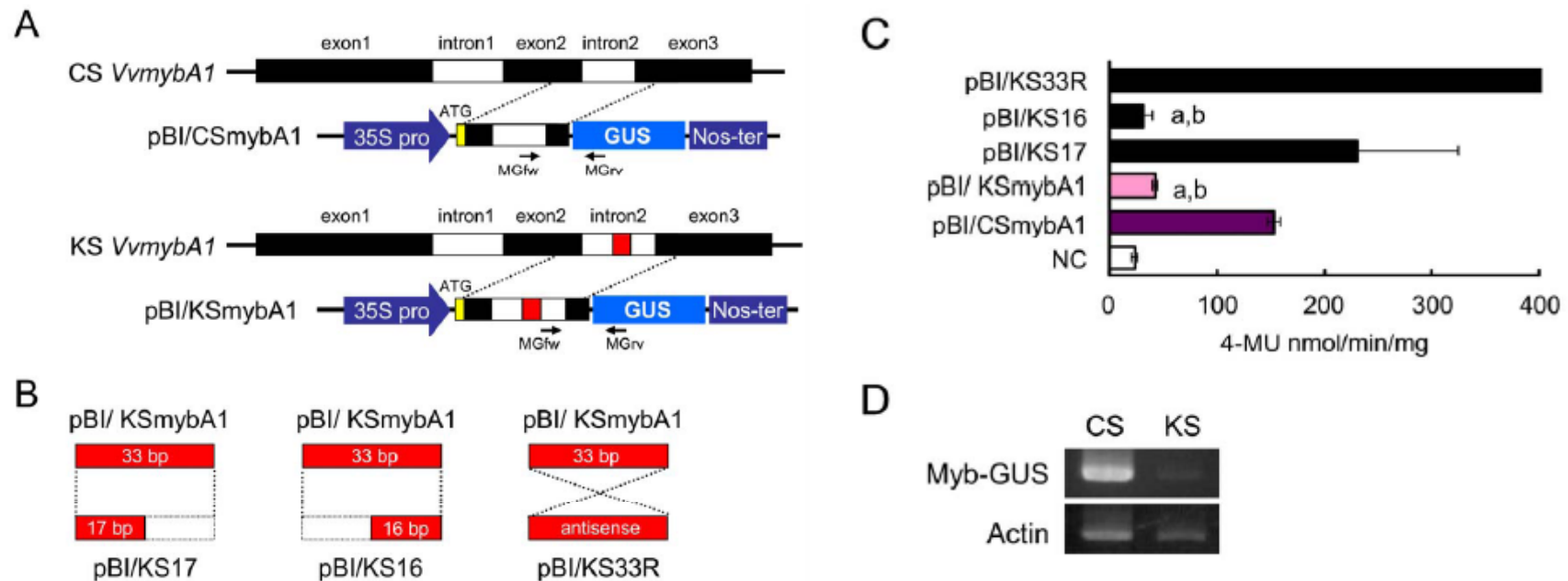


(A) Primer maps used for PCR analysis. (B) PCR analysis of white alleles in *Vitis* species using primer set a and c. (C) PCR analysis of red alleles in *Vitis* species using primer set b and c. Numbers on the right indicate the positions of the molecular size markers. These results represent reproducible data from three independent experiments. CS, Cabernet Sauvignon. Me, Merlot. Syr, Syrah. PN, Pinot Noir. KS, Koshu. Rie, Riesling. Cha, Chardonnay. Ryu, Ryugan. HU, Huotianhong. Vru, *V. rupestris*. Vri, *V. riparia*. Vlcon, *V. labrusca* cv. Concord. Vldela, *V. labruscana* cv. Delaware. Vfi, *V. ficifolia*. Vco, *V. coignetiae*. Vfl, *V. flexuosa*. Vshi, *V. shiragai*. Vsa, *V. saccharifera*. Vamu, *V. amurensis*.

Comparison of MybA1 gene structure in *Vitis* grapes

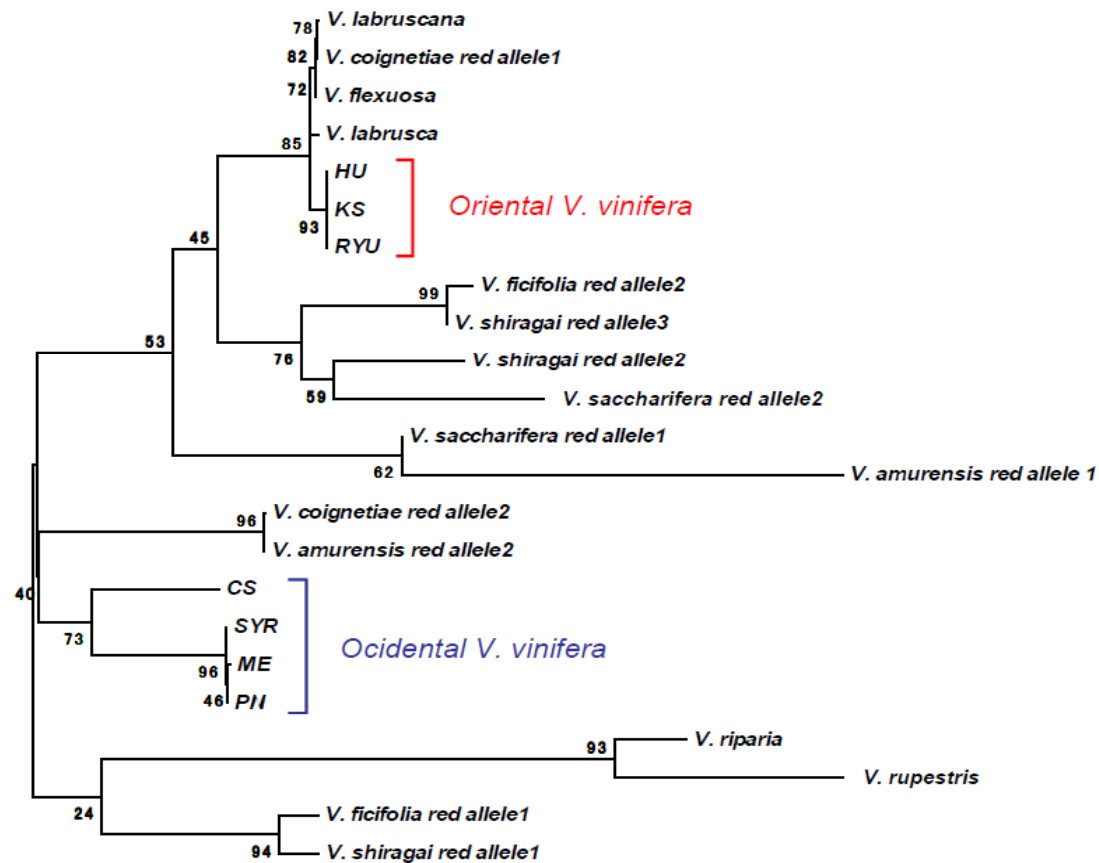


Effect of 33 bp short insertion on the transcription of a reporter gene



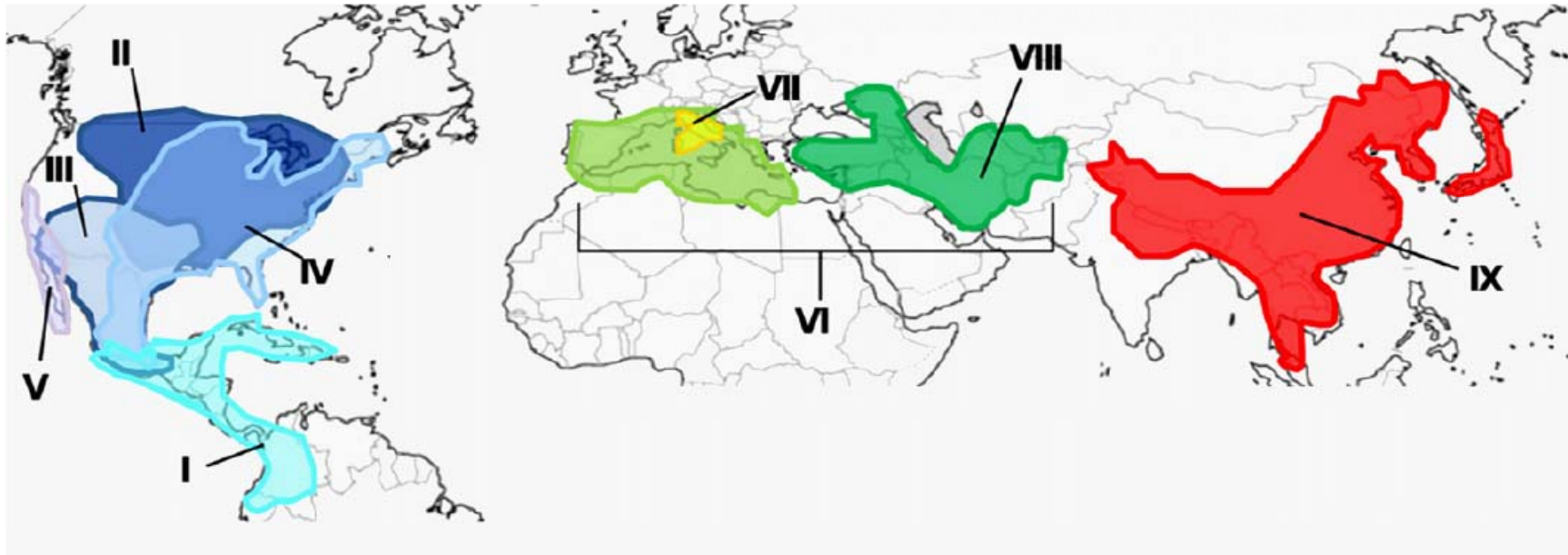
(A) Transformation constructs. Gene fragments from the second exon to the third exon, including the second intron, of Cabernet Sauvignon or Koshu MybA1 gene were inserted in front of the GUS reporter gene, resulting in pBI/CSmybA1 or pBI/KSmybA1 plant expression plasmids, respectively. Red boxes indicate the 33 bp short insertion in the second intron of Koshu MybA1. Yellow boxes indicate the ATG start codon. 35S pro, 35S promoter. Nos-ter, Nos terminator. (B) A schematic representation of the deletion (pBI/KS17 and pBI/KS16) and antisense (pBI/KS33R) mutants for 33 bp short insertion. The plant expression vectors having these mutants were constructed by procedures detailed in Methods. (C) GUS activity. BY-2 cells transformed by the plant expression vectors were used. GUS activity in the graph is expressed as the amount of 4-MU produced in one minute by one mg of protein extracted from the cells. a, $p < 0.01$ as compared with pBI/CSmybA1. b, $p < 0.05$ as compared with non-transformant cells (NC). (D) RT-PCR analysis. MybA1-GUS transcripts were detected by RT-PCR analysis using MGfw and MGrv primers. CS, pBI/CSmybA1. KS, pBI/KSmybA1. Myb-GUS, MybA1-GUS mRNA. Actin, an internal control.

Phylogenetic tree of *MybA1* genes of the red allele among grapes



Bootstrap values are indicated on the branches. CS, Cabernet Sauvignon. ME, Merlot. SYR, Syrah. PN, Pinot Noir. KS, Koshu. RYU, Ryugan. HU, Huotianhong.

Geographic distribution of wild grapes (subg. *Vitis*)



The timing and the mode of evolution of wild grapes (*Vitis*).
Zecca G, Abbott JR, Sun WB, Spada A, Sala F, Grassi F.
Mol Phylogenet Evol. 2012 Feb;62(2):736-47.

References

- White grapes arose through the mutation of two similar and adjacent regulatory genes. Walker AR, Lee E, Bogs J, McDavid DA, Thomas MR, Robinson SP. *Plant J.* 2007 Mar;49(5):772-85.
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- Pink-colored grape berry is the result of short insertion in intron of color regulatory gene. Shimazaki M, Fujita K, Kobayashi H, Suzuki S. *PLoS One.* 2011;6(6):e21308. doi: 10.1371/journal.pone.0021308. Epub 2011 Jun 17.