

P3 – *VviMybA1* and *VviMybA2* independent mutations trigger white to red berry skin color reversion in *Vitis vinifera* cv ‘Albariño’ and ‘Verdejo’

Ferradás, Yolanda^{1*}; Royo, Carolina¹; Carbonell-Bejerano, Pablo^{1,2}; Mauri, Nuria³; Rey, Manuel⁴; Yuste, Jesús⁵; Weigel, Detlef²; Motilva, María José¹; Martínez-Zapater, José Miguel¹

¹Instituto de Ciencias de la Vid y del Vino (ICVV, CSIC-CAR-UR), Logroño, Spain

²Max-Planck Institute for Biology, Molecular Biology, Tuebingen, Germany

³Center for Research in Agricultural Genomics, Barcelona, Spain

⁴Facultad de Biología – Universidad de Vigo, Departamento de Biología Vegetal y Ciencias del Suelo, Vigo, Spain

⁵Instituto Tecnológico Agrario de Castilla y León, Valladolid, Spain

*yolanda.ferradas@icvv.es

Abstract

Spontaneous somatic mutations within vegetative meristems may be related to changes in berry color of *Vitis vinifera*. Anthocyanin accumulation in the berry skin is triggered *VviMybA1* and *VviMybA2*, transcription factors, regulating the expression of genes encoding different enzymatic steps in the anthocyanin biosynthetic pathway. Null mutations in both *VviMybA1* and *VviMybA2* can lead to a white-skinned phenotype. The lack of berry color in white berry cultivars has been commonly associated with a recessive null allele harboring an insertion of *Gret1* retro-transposable element in the *VviMybA1* promoter region together with a two nucleotide deletion in *VviMybA2* coding sequence producing a null frameshift. On rare occasions, berry anthocyanin pigmentation is partially recovered in white-skinned cultivars giving rise to red-skinned variants. Here, we studied the origin of two spontaneous red somatic variants derived from white-skinned Verdejo and Albariño cultivars, through transcriptomic and targeted UPLC-QqQ-MS/MS metabolomic approaches. At veraison, RNA-seq analysis identified 386 and 84 differentially expressed genes in berry skin of Verdejo and Albariño variants, respectively, compared to their white-skinned ancestors (FDR < 0.05). In both red-skinned variants, flavonoid biosynthesis activation was detected, including the upregulation of *chalcone synthase*, *flavonone-3-hydroxylase*, *leucoanthocyanidin dioxygenase* or *UFGT*. Anthocyanin accumulation in berry skin was observed from veraison stage in both red-berried somatic variants, which was dominated by cyanidins. In addition, colored Albariño berries presented detectable amounts of delphinidins and peonidins. Anthocyanin accumulation was more pronounced at maturation stage. Besides, the Albariño red somatic variant showed greater anthocyanin concentration than the Verdejo variant in both stages. Additionally, targeted Nanopore sequencing and PCR validation identified molecular genetic alterations responsible of white to red skin color reversion. Colored Albariño recovered *VviMybA1* expression as consequence of the partial *Gret1* retrotransposon excision, leaving behind a solo LTR region. Colored Verdejo displayed a mitotic gene conversion between the two CR domains within *VviMybA2* exon 3, which restored the reading frame and protein function. This is the first time that a berry color recovery is related to a *VviMybA2* function regain, enabling to determine the specific role of the two major transcription factors regulating grape anthocyanin accumulations.

Keywords: Grapevine, Berry color, Anthocyanin, *VviMybA* genes, Somatic variation