Removal of a 10-kb *Gret1* transposon from *VvMybA1* of *Vitis vinifera* cv. Chardonnay

Yang, Yingzhen[1], Ke, John[1], Han, Xiaoyan[2], Wuddineh, Wegi A.[1], Song, Guo-qing[2], Zhong, Gan-Yuan[1*]

[1]USDA-Agricultural Research Service Grape Genetics Research Unit, Geneva, NY, USA
[2]Department of Horticulture, Michigan State University, East Lansing, MI, USA
*ganyuan.zhong@usda.gov

Abstract

Many white grape cultivars have a nonfunctional *VvMybA1* gene due to the presence of a 10-kb *Gret1* transposon in its promoter. In this study, we successfully demonstrated removal of the 10-kb *Gret1* transposon from a *VvMybA1* allele in *Vitis vinifera* cv. Chardonnay through transgenic expression of Cas9 and two gRNAs simultaneously targeting two junction sequences between *Gret1* LTRs and *VvMybA1*. We generated 80 and 106 *Agrobacterium*-and bombardment-transformed transgenic vines, respectively, and conducted molecular analyses of editing efficiencies in these vines and their progenitor calli. While the editing efficiencies were as high as 17% for the 5' target site and 65% for the 3' target site, simultaneous editing of both 5' and 3' target sites resulting in the removal of *Gret1* transposon from the *VvMybA1* promoter was 0.5% or less in most transgenic calli and vines, suggesting that these calli and vines had very few cells with their *VvMybA1* alleles simultaneously edited at both target sites. Nevertheless, two bombardment-transformed vines were found to have the *Gret1* successfully edited out from one of their two *VvMybA1* alleles. Precisely removing more than a 10-kb DNA fragment from a grape gene broadens the possibilities of using gene editing technologies in modifying various trait genes in grapes and other plants. Detailed molecular and sequencing analyses of the edited events in transgenic calli and vines revealed many interesting features of gene-editing, including large structural changes likely resulting from illegitimate recombination of highly homologous *VvMybA* genes in the *VvMybA* complex loci.

Keywords: CRSPR-Cas9, berry color, grapes, *Gret1,* large DNA fragment deletion, *MybA1*