Enhancing Hydric Stress Tolerance by Editing the VviMYB60 Promoter with CRISPR/Cas9

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Abstract (250 words)

Climate change presents increasing challenges to viticulture, particularly with rising water stress contributing significantly to yield losses and damages. The identification of the MYB60 transcription factor, which regulates stomatal opening and closing in *Arabidopsis thaliana* and *Vitis vinifera*, offers potential solutions. Notably, knockout studies in Arabidopsis have shown reduced stomatal opening and increased drought tolerance in myb60 mutants. Additionally, the grapevine ortholog, *VviMYB60*, can restore the wild-type phenotype of Arabidopsis myb60 mutants. Further investigation of the Arabidopsis promoter region has revealed that mutations in DOF motifs lead to reduced expression of *AtMYB60*.

Utilizing the advancements in CRISPR/Cas9 genome editing, this project aims to modify the *VviMYB60* promoter region to lower gene expression, thereby reducing stomatal opening in grapevines. Binary vectors for genome editing were constructed to target two specific regions of the *VviMYB60* promoter. Agrobacterium-mediated transformation was performed on Chardonnay embryogenic calli, resulting in the successful regeneration of plants under selection conditions. Sanger sequencing analysis of the targeted region confirmed the occurrence of genetic edits in four of the six lines analyzed so far.

To further characterize the edited lines, next-generation sequencing will be utilized, providing a more comprehensive understanding of the mutations, as well as gene expression of *VviMYB60* will be evaluated to confirm that the editing reduces its expression. Morphological and physiological parameters will be measured after acclimatation in greenhouse and finally these edited lines will undergo drought tolerance testing to assess their performance.

Keywords: CRISPR/Cas9, VviMYB60, promoter, drought tolerance, stomatal regulation.