Novel Approaches And Promising Perspectives For Enhancing Grapevine Editing And Regeneration

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Grapevine (*Vitis vinifera* L.) is a challenging plant species to transform and regenerate due to its complex genome and biological characteristics. This limits the development of cisgenic and gene-edited varieties. One hurdle is selecting the best starting tissue for the transformation process, much like isolating suitable tissue for protoplasts. One promising method involves delivering CRISPR/Cas components to protoplasts isolated from embryogenic calli, which are then induced to regenerate. However, this process is inefficient, time-consuming, and only applicable to a few genotypes. To enhance grapevine regeneration efficiency, the expression of developmental and plant growth regulators shows promise in escaping the recalcitrance encountered in traditional tissue culture methods. A strategy based on the *Bean yellow dwarf virus* (BeYDV) allows for the temporary expression of regulators while minimizing the risk of obtaining transgenic plants. Additionally, the high copy number replication of BeYDV allows for high CRISPR/CAS levels, thereby improving editing. The goal of this study is to evaluate the effects of individual or combined developmental regulators' expression on grapevine embryogenic tissues. We conducted preliminary assays using BeYDV-derived vectors for luciferase reporter gene expression to optimize delivery efficiencies. Assays were performed on 'Chardonnay' calli and protoplasts using both agrobacterium-mediated transformation and protoplast transfection approaches. The present study seeks to enhance the transformation protocols and regeneration processes, with the ultimate aim of realizing the full potential of editing technologies in grapevine.

Keywords: *Vitis vinifera*, genome editing, protoplasts, developmental regulators, BeYDV.