DNA-Free genome editing confers disease resistance in grapevine

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Abstract

Grapevine (*Vitis Vinifera* L.), one of the most important cultivated fruit crops, is facing significant challenges due to climate change. Specifically, increasing temperatures negatively impact the physiological traits and disrupt plant phenology. Additionally, increased virulence in pathogen attacks and pests leads to significant yield loss, requiring widespread application of plant protection products. Traditional agronomic practices offer only partial mitigation, requiring the development of precise and effective intervention strategies. The economic worth of viticulture has prompted continuous efforts in grapevine genetic improvement programs, traditionally involving conventional breeding and clonal selection that, however, are complex and time-consuming approaches. Instead, the advent of New Breeding Techniques, especially genome editing via the CRISPR/Cas9 system, presents a promising avenue for the development of tools suitable to mitigate the current viticulture challenges, including fungal diseases. We report the application of a DNA-free genome editing approach to induce targeted mutations in the VviMLO17, a gene associated with powdery mildew susceptibility in grapevine. CRISPR/Cas9 ribonucleoparticles were introduced into protoplasts isolated from embryogenic calli. Through protoplast regeneration, a homozygous edited grapevine plant mutated in the VviMLO17 gene was obtained. This mutation confers resistance to *Erysiphe necator*, as evidenced by phenotypic analyses that demonstrated a reduced susceptibility to pathogen attack. The success of DNA-free CRISPR/Cas9 application for the improvement of target traits establishes a foundation for promoting viticulture sustainability yet preserving the identity of the grapevine cultivars. This advancement aligns with market and legislative demands, paving the way for a resilient and environmentally conscious winegrowing system.

Keywords: genome editing DNA-free, CRISPR/Cas9 system, protoplast regeneration, powdery mildew resistance, sustainable viticulture.