DNA-free editing to improve stress resilience of wine grape genotypes recalcitrant-to-regeneration

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Abstract (250 words)

Wine viticulture, being firmly linked to the vine-terroir relationship, has always encountered significant bottlenecks to genetic innovation. Nonetheless, the development of new breeding strategies leading to the selection of stress resilient genotypes is urgent, especially in viticulture, where it would allow reducing the use of chemical treatments adopted to control fungal diseases. Genome editing represents an extremely promising breeding technique. Unfortunately, the well-known recalcitrance of several wine grape cultivars to *in vitro* regeneration strongly limits the exploitation of this approach, which to our knowledge has so far been developed on table grape genotypes with high regeneration potential. By targeting the phytoene desaturase gene as visual editing proof, we developed a genome editing and regeneration protocol to produce transgene-free grapevine plants exploiting the lipofectamine–mediated delivery of CRISPR–Cas9 ribonucleoproteins into protoplasts. We regenerated edited grapevines of *Vitis vinifera* 'Nebbiolo', a cultivar extremely recalcitrant to *in vitro* regeneration and at the basis of outstanding quality wines, such as 'Barolo' and 'Barbaresco'. Successful editing was confirmed by a combination of approaches: HRM, Sanger and amplicon deep sequencing, phenotype visualization. We then exploited the method to silence two micro(mi)RNAs involved in biotic stress responses: vv-miR482, which is conserved in different species, and the grapevine-specific vv-miR3623. Since *NBS-LRR* disease-resistance genes are the targets of those miRNAs, the objective is to regenerate vines with a broad-spectrum level of plant tolerance/resistance to different pathogens. The developed strategy could be extended to other important wine grape varieties and recalcitrant woody species.

Keywords: genome editing, protoplast regeneration, lipofectamines, microRNAs, biotic stress