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COMPARATIVE ANALYSIS OF STABLE GENETIC TRANSFORMATION METHODS FOR KNOCKING OUT VVIAGL11 IN 'MICROVINE' GRAPEVINE CULTIVAR

BELLON O.*, BERTINI E.*, FORLEO L. R.**, D'AMICO M.**, CARDONE M. F.**, BERGAMINI C.**, ZENONI S.*, AMATO A.*

*) Department of Biotechnology, University of Verona, Strada Le Grazie 15, 37134 Verona, Italy
**) Research Centre for Viticulture and Enology, Council for Agricultural Research and Economics (CREA), Turi, Bari, Italy

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Italy has recently resumed genetic improvement activity in viticulture to promote the most appreciated and representative varieties of the Made in Italy, modifying only the characteristics of interest. In table grapes, a character that the consumers increasingly appreciate is seedlessness. Several studies demonstrated the central role of the MADS-type transcription factor VviAGL11 in stenospermocarpy, a form of seedlessness in which correct fertilization of the ovule is followed by premature abortion of the semen. A VviAGL11 working mechanism has been proposed and partially validated, however, a complete in planta demonstration is still needed. Grapevine cv. 'microvine' represents a powerful model system for functional gene characterization due to its dwarf stature, short generation cycles, and continuous flowering. Nevertheless, procedures for its stable transformation need to be better reported.

In this study, with the primary goal to stable transform 'microvine' plants for producing a truncated or inactivated VviAGL11 protein, we are setting two different genetic transformation procedures suitable for 'microvine': stable transformation mediated by Agrobacterium and CAS9-mediated genome-editing.

As first, we demonstrated the ability of embryogenic calli, obtained from microvine anthers collection and culture, to regenerate embryos capable of germinating and sprouting into a new plant, an ability that is often cultivar dependent. We therefore transformed 'microvine' embryogenic calli

with a construct for the GFP overexpression to define the susceptibility of the 'microvine' material to the Agrobacterium infection as well as the successful integration of the genetic sequence of interest. Based on the promising results obtained so far, we are engineering the vectors for the VviAGL11 knock-out.

’Microvine' embryogenic calli were also used to isolate protoplast for CAS9-mediated genome-editing. We demonstrated for the first time in ‘microvine’ the ability to regenerate an entire plant after the complete development of an embryo after protoplast cultivation. We isolated and sequenced the coding sequence of VviAGL11 in microvines and synthesize specific guide RNAs. The guide RNA, combine with CAS9, have been used for protoplast transfection and possibly regenerating plant will be screened for the VviAGL11 mutation.

The use of 'microvine' as a model system offers promising outcomes for functional gene characterization, benefiting Italy's viticulture genetic improvement and seedless table grape cultivation. Comparing two stable transformation methods is also crucial to attaining our objective of generating a truncated VviAGL11 protein. Moreover, the remarkable achievement of regenerating whole plants from 'microvine' protoplasts paves the way for future research and possible CAS9-mediated genome-editing.