World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:15, No:04, 2021

Establishments of an Efficient Platform for Genome Editing in Grapevine

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Abstract : Grapevine is an important agricultural fruit crop plant consumed worldwide and with a key role in the global economy. Grapevine is strongly affected by both biotic and abiotic stresses, which impact grape growth at different stages, such as during plant and berry development and pre- and post-harvest, consequently causing significant economic losses. Recently global warming has propelled the anticipation of the onset of berry ripening, determining the reduction of a grape color and increased volatilization of aroma compounds. Climate change could negatively alter the physiological characteristics of the grape and affect the berry and wine quality. Modern plant breeding can provide tools such as genome editing for improving grape resilience traits while maintaining intact the viticultural and oenological quality characteristics of the genotype. This study aims at developing a platform for genome editing application in grapevine plants with the final goal to improve berry quality, biotic, and abiotic resilience traits. We chose to directly deliver ribonucleoproteins (RNP, preassembled Cas protein and guide RNA) into plant protoplasts, and, from these cell structures, regenerate grapevine plants edited in specific selected genes controlling traits of interest. Edited plants regenerated by somatic embryogenesis from protoplasts will then be sequenced and molecularly characterized. Embryogenic calli of Sultana and Shiraz cultivars were initiated from unopened leaves of in-vitro shoot tip cultures and from stamens, respectively. Leaves were placed on NB2 medium while stamens on callus initiation medium (PIV) medium and incubated in the dark at 28 °C for three months. Viable protoplasts, tested by FDA staining, isolated from embryogenic calli were cultured by disc method at 1*105 protoplasts/ml. Mature well-shaped somatic embryos developed directly in the protoplast culture medium two months later and were transferred in the light into to shooting medium for further growth. Regenerated plants were then transferred to the greenhouse; no phenotypic alterations were observed when compared to non in-vitro cultured plants. The performed experiments allowed to established an efficient protocol of embryogenic calli production, protoplast isolation, and regeneration of the whole plant through somatic embryogenesis in both Sultana and Shiraz. Regenerated plants, through direct somatic embryogenesis deriving from a single cell, avoid the risk of chimerism during the regeneration process, therefore improving the genome editing process. As pre-requisite of genome editing, an efficient method for transfection of protoplast by yellow fluorescent protein (YFP) marker genes was also established and experiments of direct delivery of CRISPR–Cas9 ribonucleoproteins (RNPs) in protoplasts to achieve efficient DNA-free targeted mutations are in progress.

Keywords : CRISPR-cas9, plant regeneration, protoplast isolation, Vitis vinifera
Conference Title : ICTCTR 2021 : International Conference on Tissue Culture Technologies and Research
Conference Location : Boston, United States
Conference Dates : April 22-23, 2021