Proceedings of the LXII SIGA Annual Congress
Verona, Italy – 25/28 September, 2018
ISBN **978-88-904570-8-1**

Poster Communication Abstract – 1.09

WHOLE PLANT REGENERATION FROM PROTOPLASTS OBTAINED FROM EMBRYOGENIC CALLI OF TWO ITALIAN GRAPEVINE VARIETIES

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protoplasts, embryogenic calli, plant regeneration, PEG-mediated transfection, genome editing

Plant protoplasts represent a useful tool for basic research and biotechnological approaches. Protoplasts can be exploited for physiological, biochemical and molecular studies, from functional analysis of gene and characterization of metabolic pathways to recent applications of genome editing. However, most of these studies require the regeneration of the entire plants from protoplasts. This phase represents the bottleneck of this technology, because, most agronomically important plant species, including grapevine, are recalcitrant to regeneration. Grapevine (*Vitis vinifera L.*) protoplasts were obtained from many sources of plant material (leaves, stems, roots, mesocarp) and used for many studies, but the regeneration of plants was successfully performed only from protoplasts isolated from embryogenic tissue. Here, we report the application of a modified previously reported protocol for protoplasts isolation and plant regeneration of two Italian cultivars, Garganega and Sangiovese. Protoplasts of both varieties were obtained from stamen-derived embryogenic calli. After isolation, protoplasts were cultivated in solid Nitsch's medium, supplemented with sugars, auxin and cytochinin. Within four months from the initiation of culture, well developed protoplasts-derived torpedo somatic embryos were transferred into medium supplemented with cytochinin under light in order to induce germination. Subsequently, germinated somatic embryos were moved in a rooting medium. Regenerated plants were transferred to the greenhouse and showed a normal morphology. Finally, protoplasts PEG-mediated transfection has been tested using a plasmid carrying GFP as marker gene. Fluorescence microscopic analysis showed that the GFP expression was initially low, but it took place after 24 hours and continued after 48 and 72 hours from the transfection. These results indicate that this system represents a useful tool for numerous applications in grapevine, including the genome editing.