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HISTOLOGY, HISTOCHEMISTRY AND SEM ARE USEFUL TOOLS TO STUDY REGENERATION PROCESSES IN PLANT TISSUE CULTURE

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Tissue cultures *in vitro* are used for the multiplication of plants via direct and indirect (via callus) regeneration. This approach is commonly applied in the protection of endangered species by the introduction of regenerated in vitro plantlets to botanical gardens and to the nature (so called *ex situ* plant conservation). *In vitro* conditions, especially the supplementation of tissue culture media with plant growth regulators, cause a somaclonal variation, resulting in genetic differences among regenerated plants. To analyze callus structure, including cell shapes and sizes, cell differentiation (e.g. the presence of xylem vessels) and regeneration processes (organogenesis, somatic embryogenesis), the histological, histochemical and SEM techniques are applied.

In this study, to obtain regeneration of plants in culture conditions, we have used three *Viola* species (*V. epipsila* Ledeb., *V. stagnina* Kit. and *V. uliginosa* Besser), indicated to be critically endangered according to Polish Red Book of Plants (Kazmierczakowa & Zarzycki 2001) and two genotypes of a model plant *Arabidopsis thaliana* (L.) Heynh. (Columbia-0 and an insertional *cdkg;2* mutant line). An *Arabidopsis* homozygous *cdkg;2* knock-out originated from a T_3 generation of T-DNA insertional line SALK_090262 (Alonso *et al.* 2003) and has been selected from a subsequent T_4 generation based on PCR analysis using primers complementary to flanking positions of full-length cDNA of *CDKG;2* gene product (a clone isolated by Seki *et al.* 2002). The aims of the study were: 1) to select the most convenient method to obtain regenerated *Viola* plants with maternal genotype i.e., via direct organogenesis or somatic embryogenesis; 2) to determine the effect of mutation in *CDKG;2* gene on the explant response to *in vitro* conditions, including callus proliferation and regeneration.

In three *Viola* species organogenesis was induced on MS (Murashige and Skoog) basal medium supplied with thidiazuron (TDZ) in concentrations 0.5 mg $\cdot$ l^{-1} and 1 mg $\cdot$ l^{-1}. Callus proliferated on MS with equal concentrations of cytokinin and auxin (Kin+2,4-D). Histological analysis indicated two pathways of adventitious shoot formation: an indirect one from callus and a direct one from the explants (petiole, leaf fragments).

The regeneration of *A. thaliana* has been found to be genotype dependent. Histological analysis of hypocotyl-derived callus of Columbia-0 genotype regenerated on MS medium supplemented with TDZ showed the meristematic centers forming shoot apices, as well as sporadically embryo-like structures and organs clearly visible on transverse sections. Hypocotyl-derived callus of *cdkg;2* mutant was a heterogeneous tissue, enriched with parenchymatous cells, differentiated xylem elements, large, vacuolized cells at the periphery of callus tissue and groups of small meristematic cells scattered within the callus

tissue. The surface of hypocotyl- and cotyledon-derived calluses of Columbia-0 and mutant was covered with membranous structure similar to extracellular matrix (ECM).

Conclusions

1. Direct organogenesis is the most convenient method to obtain genetically uniform regenerants of *Viola* with maternal genotype;

2. *Arabidopsis* cyclin-dependent kinase gene *CDKG;2* is a regulator of plant organogenesis *in vitro*.

References

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