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OPTIMIZATION AND OVEREXPRESSION OF PYRROLINE-5-CARBOXYLATE SYNTHETASE TO INCREASE COLD STRESS TOLERANCE IN THREE COMMERCIAL PETUNIAS

Maryam Jamshidnia, Department of Plant Breeding Biotechnology, Sari Agricultural Sciences , and Natural Resources University SANRU, Sari, Iran; Maryam.Jamshidnia58@gmail.com (Presenting author)
Assist. Prof. Cyrus Ghobadi, Department of Agricultural Biotechnology, Isfahan University of Technology, 84156-83111 Isfahan, Iran; cyrus@cc.iut.ac.ir
Prof. Badraldin E. Sayed Tabatabaei, Department of Agricultural Biotechnology, Isfahan University of Technology, 84156-83111 Isfahan, Iran; sayedt@cc.iut.ac.ir
Assist. Prof. Ahad Yamchi, Department of Plant Breeding Biotechnology, Gorgan University of Agricultural Science, Gorgan, Iran; yam12001@yahoo.com

Pyrroline-5-carboxylate synthetase is a bifunctional enzyme catalyzing two steps of proline biosynthesis in plants. The potential benefit of this gene is to confer water stress resistance (drought, salt, cold) due to the increased intracellular levels of proline that work like an osmoprotectant. As an attempt to examine the relationship between proline and cold stress in petunia, *p5cs* gene was transferred to petunia plants through *Agrobacterium*-mediated transformation. The major modifications were more practical regeneration and transformation protocols. In order to select the best shooting media and explants, shoot apices and leaf discs were examined in a completely randomized design in different media. Efforts were made to improve transformation steps by introducing the best explants and medium for direct regeneration and efficient transformation. Therefore, this procedure was described and applied to transform petunia with a *p5cs* gene. *Agrobacterium* strain LBA4404 harboring a plasmid pBI121 was used as the binary vector system for transformation. PBI121 contained β-glucuronidase *gus* gene as a reporter gene, Δ^1-pyrroline-5-carboxylate synthetase *p5cs* gene and neomycin phosphotransferase *npt*II gene as a selectable marker. After co-cultivation, explants were cultured on a selective medium containing kanamycin (200 mg/l) and then they were transferred to the proliferation medium (modified MS with 1 mg/l BAP). Shoot regeneration occurred within 3 weeks. Regenerated plants were subjected to polymerase chain reaction (PCR) and GUS histochemical assay in order to confirm transformation. GUS and proline assay confirmed the presence of the *gus* and *p5cs* genes in the genome of all transformants in stress and non-stress conditions and the shoot apex was the best explants for transformation of petunia. Transgenic plants accumulated up to 13.2 times more proline than the wild type in Supercascaide cultivar. Moreover, the transformed plants were more tolerant than the wild types in stress conditions.

Keywords: Agrobacterium, Gene transformation, gus gene, p5cs gene, Shoot apex