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DEVELOPMENT AND APPLICATION OF THE PLASTID TRANSFORMATION TECHNOLOGY IN POTATO

D. GARGANO, V. VALKOV, P. LENZI, N. SCOTTI, S. GRILLO, T. CARDI

CNR-IGV, Institute of Plant Genetics, Via Università 133, 80055 Portici, Italy

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Plastid transformation in higher plants allows precise transgene insertion by homologous recombination, high and stable gene expression and protein accumulation, gene coexpression in operons as well as gene containment through maternal inheritance of cytoplasmic organelles. So far, potato plastid transformation has been achieved mostly with reporter genes and the maximum efficiency was 10 times lower than that reported for tobacco. Herein we describe the development and application of the plastid transformation technology in potato for the expression of *S. commersonii* Δ9 desaturase and HPV16 L1 transgenes. Δ9 desaturase gene controls the insertion of double bonds in fatty acid chains; a high content of unsaturated fatty acids in plant cells can have a positive effect for tolerance to abiotic stresses as well as for nutritional purposes. VLPs made from the major capsid protein L1 of carcinogenic HPVs have been shown to induce protective immunity in animal models; the expression of the L1 gene in plant cells is an alternative approach for the development of a prophylactic vaccine.
The cv. Desiree was selected for transformation experiments based on preliminary regeneration data, while optimal conditions for gene delivery using the biolistic approach were set up using the nuclear vector pGUS-HYG (Craig et al. 2005).
In the process of improving the procedure for plastid transformation we tested three different regeneration protocols, using transformation vectors which target the inverted repeats or the large single copy of the chloroplast genome. In first experiments, one transplastomic shoot every 30 bombardments was regenerated on a medium containing 400 mgl^{-1} spectinomycin, confirming the transformation efficiency reported in the literature. Later modifications of the procedures for the preparation of gold particles and for the tissue culture of explants allowed to obtain up to one transplastomic shoot every 2-5 bombardments.

Using the improved protocol, we have transferred the gene coding for Δ9 desaturase, isolated from the wild potato species *Solanum commersonii* to potato plastome. All transplastomic plants showed a high production of the desaturase transcript. Transplastomic plants with the L1 gene fused to a His6 tag also showed a strong signal after Northern analysis, but Western analysis carried out with anti-L1 antibodies did not detect the expected protein, although preliminary results wiith anti-His antibodies had showed the presence of two bands only in transplastomic plants.

Experiments with vectors containing different promoters and terminators are under way. Further, work in our laboratory is aiming to develop improved vectors for potato transformation. The complete sequence of the potato plastome has been obtained and will be used to isolate species-specific flanking and regulatory sequences. Finally, genomic approaches are being used to compare the expression of plastidial genes in chloroplasts and amyloplasts with the aim to identify regulatory sequences supporting high gene expression in non-green plastids.

Craig W, Gargano D, Scotti N, Nguyen TT, Lao NT, Kavanagh TA, Dix PJ, Cardi T 2005 Direct gene transfer in potato: a comparison of particle bombardment of leaf explants and PEG-mediated transformation of protoplasts. Plant Cell Rep, in press