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ISOLATION AND TRANSFECTION OF STRAWBERRY PROTOPLASTS FOR GENE EDITING

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Abstract: Strawberry is the most economically important soft fruit. The improvement of the organoleptic qualities of ripe fruit and the postharvest shelf life are main objectives of strawberry breeding programs. Fruit softening is mainly due to the disassembly of cell walls and the dissolution of middle lamella. In strawberry, functional analyses of genes encoding polygalacturonases (PGs) indicate that these enzymes play a key role in fruit softening, i.e. the antisense downregulation of PG genes *FaPG1* or *FaPG2* increased fruit firmness and postharvest shelf life (Paniagua et al., 2020). These results suggest that PG encoding genes are excellent targets for gene editing to improve strawberry fruit quality. Transfection of protoplasts with CRISPR/Cas9 ribonucleoprotein complexes is currently being explored in many species to produce DNA-free edited plants. In this research, a protocol for strawberry protoplasts transfection has been optimized with the final goal of producing non-transgenic strawberry plants with the *Fa*PG1 gene edited. Protoplasts were isolated from 9 weeks old *in vitro* grown plants of *Fragaria* x *ananassa*, cv. 'Chandler', micropropagated in Murashige and Skoog (MS) medium supplemented with 2 mg/L of BA. Protoplast extraction and purification was performed as described by Barceló et al. (2019). Using this protocol, a yield of 1 x 10^5 protoplast/g fresh tissue was obtained and nearly 50-70% of them were viable. Protoplasts were transfected with the plasmid pHBT-sGFP(S65T)-NOS using a PEG-mediated transformation system, as reported by Yoo et al. (2007). To improve the efficiency of protoplast transfection, different variables were evaluated: PEG concentration, time of incubation on PEG and DNA concentration. At 48 h after transfection, the highest percentage of protoplasts showing GFP expression, 18%, was obtained with 15 minutes incubation in 20% of PEG and 5 µg of DNA. This optimized protocol is currently being used to transfect strawberry protoplasts with CRISPR/Cas9 ribonucleoproteins to edit *FaPG1* gene.

Key words: *Fragaria* x *ananassa*, strawberry, protoplasts, transfection.

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