Agrobacterium tumefaciens-mediated transformation of *Papaver somniferum* L.

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The opium poppy (*Papaver somniferum* L.) is an ancient medicinal plant capable of producing several benzylisoquinoline alkaloids of pharmaceutical importance, including the analgesics morphine and codeine, and the antibiotic sanguinarine[1)]. Sanguinarine is benzo[c]phenanthridine alkaloid that has antimicrobial properties and is thought to be a part of the chemical defense systems of opium poppy. Although cell culture was initially believed to be a stable supply system immune to the environmental fluctuations, habitat limitations, and slow growth rates of field plants, the strict and native regulation of alkaloid synthesis has often resulted in low or nonexistent levels of the desired alkaloids. Most of the efforts, however, have not focused on genetically engineered cell cultures, tissue cultures, or plants but rather on non-engineered cell lines studied under elicitation, with precursor feeding, or in optimized media. While these classical methods have hastened characterization efforts, metabolic engineering offers the most promising method for improved product composition and increased alkaloid yield of plants and cultured cell systems[2)]. Genetic transformation would be a powerful tool for enhancing the productivity of novel alkaloid of limited yield [3)]. And transferring genes into plants has played an important role in plant[4)]. We conducted transformation of *Papaver somniferum* with *A. tumefaciens* with an aim of preparation to gene-insertion for increasing the sanguinarine productivity. Opium poppy (*Papaver somniferum* L.) callus were grown on solid culture medium at a temperature of 25℃ under no illumination. Solid culture was maintained on Gamborg B5 medium containing sucrose, Phytoagar (Duchefa), vitamins, 0.1mg/L 2,4-dichlorophenoxy acetic acid and 0.5mg/L 6-benzyladenine. For opium poppy callus transformation, pCAMBIA1304 was electroporated into *Agrobacterium tumefaciens* LBA4404. The plasmid vector, pCAMBIA1304 has a mgfp5-gusA-His6 fusion gene which

contains *hpt* gene, *mgfp* gene for green fluorescence protein expression(GFP) and β -glucuronidase(GUS) reporter gene. *Agrobacterium* growth medium was YEP medium containing rifampicin and kanamycin. Selection medium for callus was YEP medium containing hygromycin 50 mg/L. Transformation was monitored by GFP expression with a fluorescence microscope. Histochemical GUS activity was examined by using 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid (X-Gluc) as a substrate. PCR analysis was carried out on the same cell line used for GUS histochemical assay. The GUS-positive calli were verified for the presence of *hpt, gus, mgfp* genes by PCR. All the selected cell lines which were resistant to hygromycin showed the expected amplified fragment according to the position of primers on the DNA matrix. Amplified DNA bands of the expected length were identified, so we used these calli for southern blot analysis. As a result of analysis, it was clear that transformation of opium poppy callus was accomplished successfully under the above optimized conditions. Even if foreign plasmid was inserted to the opium cells, specific growth rate of transformants was still similar to that of untransformed cells and their morphology did not change. The concentration of sanguinarine, one of benzo[c]phenanthridine alkaloids from the transformed callus was checked by HPLC with photodiode array detector. There was no difference between sanguinarine concentration of untransformed callus and that of transformant. Therefore, the inserted foreign gene did not affect the gene transcription and the protein translation associated with the sanguinarine production from opium poppy. We will expect that the transformation with desired vectors will eventually increase sanguinarine production.

References

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