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## Cloning and characterization of the tropane alkaloids biosynthesis genes from *Scopolia parviflora*, a new family of proteins transport into transgenic hairy root lines

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### Objectives

Two pharmacologically important tropane alkaloids (TA), hyoscyamine and scopolamine, were produced by Solanaceae plants. Many plants containing these alkaloids have been used for their medicinal, hallucinogenic, and poisonous properties. Metabolic and genetic engineering can lead to increased yields of these compounds. We cloned three key enzymes of TA biosynthesis in *Scopolia parviflora* and also took the technic approach to increase productivity. Thus, this study reported that differences of TA contents, root growth and morphology among the obtained various transgenic hairy root lines.

### Materials and Methods

#### 1. Materials:

Plant- *S. parviflora* was provided by Gwangreung National Arboretum in Korea and induced the hairy roots and *in vitro* plants.

*Agrobacterium rhizogenes* strain- KCTC (Korean Collection for Type Cultures) 2703

#### 2. Methods:

Construction of cDNA library- *Sppmt1*, *Sppmt2* and *Sph6h* was isolated in the root of *S. parviflora*. Poly(A)+RNAs were obtained by using mRNA purification kit. cDNA library was constructed by using a ZAP-cDNA Synthesis Kit and known probes.

Plant culture and transformation- TA biosynthesis genes were transformed to *S. parviflora* by using *A. rhizogenes* harboring *pEB* vector. Induced hairy roots were cultured at B5 liquid medium with 0.1 mg/l IBA and 5% sucrose. Transgenic hairy root lines were

screened kanamycin resistant assay, PCR, Northern, and Western analysis.

Growth index- (harvest weight - inoculum weight / inoculum weight).

Morphology observation- Light microscope and Energy Filtering Transmission Electron Microscopy (EF-TEM) × 5000.

Contents of TA- It was quantified by HPLC. Mobile phase: 50 mM dipotassium phosphate/acetonitrile (78:22), Column: TSK gel ODS (4.6 mm, 5 μm), Flow rate: 0.8 ml/min, Detection: UV detector (215 nm)

### Results and Discussion

Most transgenic hairy root lines were well-developed and lateral branching appeared. Growth index of transgenic hairy root lines were higher than wild type. Growth of transgenic root lines up to 3 times more compared to wild type. Northern blotting showed the presence of the corresponding mRNA in the transgenic hairy root lines tested that carried 35S promoter-genes. The levels of key enzyme transcripts in the transgenic hairy root lines were higher than the wild type root. The protein extracts prepared from transgenic hairy root lines were subjected to SDS-PAGE for western blotting. The levels of *h6h* expression were arbitrarily placed between 2-3 fold against wild type root. The best of TA contents in transgenic line had 3.00 hyoscyamine and 3.43 scopolamine mg per g D.W. It were higher than that of wild type root. Also, these roots were converted from hyoscyamine to scopolamine. A high concentration of scopolamine had accumulated in the transgenic hairy root lines which constitutively expresses the enzymes. Thus, we cloned three key enzymes of TA biosynthesis in *Scopolia parviflora* and produce transgenic hairy root lines in high TA content through the *Sppmt1*, *Sppmt2* and *Sph6h*.