

## Differences of tropane alkaloids content, growth and morphology in metabolic engineered *Scopolia parviflora* hairy root lines.

Young Min Kang, Hee Young Jung, Seung Mi Kang, Yong-Duck Kim, Won Jung Kim,  
Ji Yun Min, Dong Jin Bahk, Dae Jin Yun<sup>1</sup>, Jung Dong Bahk<sup>1</sup>, Myung Suk Choi\*

Department of Forest Science, <sup>1</sup>Department of Applied Life Science,  
Gyeongsang National University, Jinju, 660-701, Korea

### Objectives

Tropane alkaloids (TA) are valuable anticholinergic drugs employed as antispasmodics and in the treatment of motion sickness. Although this usefulness of these compounds, the current supply is problematic. Genetic engineering can lead to increased yields of these compounds. We cloned different genes of TA biosynthesis and also took a genetic engineering 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

*S. parviflora* was provided by Gwangreung National Arboretum, Korea and induced the hairy roots and *in vitro* plants. Various TA biosynthetic genes (A: Wild type hairy root of *S. parviflora*, B: PMT gene of *Nicotiana sylvestris*, C: H6H gene of *Hyoscyamus niger*, D: PMT1 gene of *S. parviflora*, E: PMT2 gene of *S. parviflora*, F: H6H gene of *S. parviflora*) were transformed to *S. parviflora* by using *Agrobacterium rhizogenes* KCTC 2703. Induced hairy roots were cultured at B5 liquid medium with 0.1 mg/L IBA and 5% sucrose. Transgenic lines were screened kanamycin resistant assay, PCR, Northern and Western analysis. Growth was measured by the growth index (harvest weight - inoculum weight/ inoculum weight). Morphology of hairy root lines was measured by visual and light microscope. TA contents were quantified by HPLC.

### Results and Discussion

Most transgenic hairy root lines were well-developed, and lateral branching appeared (Fig. 1). Growth index of hairy root lines (D, E and F) were higher than those of others (A, B and C). Growth of transgenic root line E up to 4.4 times compared to wild type (Table 1). The levels of TA in transgenic lines varied greatly from 1.06 to 8.12 mg per g D.W. and were higher than that of wild type root (Table 1). Also, these roots were converted from hyoscyamine to scopolamine.

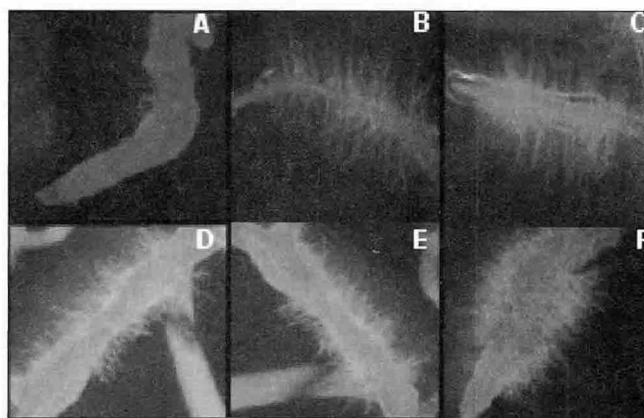


Fig.1. Phenotypic differences in various transgenic hairy root lines

Table 1. TA contents and Growth index in various hairy root lines

	Hairy root lines					
	A	B	C	D	E	F
<b>Growth Index</b>	5.12	5.27	5.52	15.26	22.40	19.13
<b>Content of scopolamine (mg/g D.W.)</b>	1.06	6.21	8.12	2.71	2.19	2.17
<b>Content of hyoscyamine (mg/g D.W.)</b>	2.85	4.51	4.83	2.78	2.76	3.04