

## 아그로박테리움을 이용한 최적의 병풀 모상근 생산

작물과학원 인삼약초연구소<sup>1</sup>, 강원대학교 바이오산업공학부<sup>2</sup>, 전남대학교 생물학과<sup>3</sup>  
 김옥태<sup>1</sup>, 김선희<sup>3</sup>, 이현용<sup>2</sup>, 황백<sup>3</sup>

### Optimal Production of *Centella asiatica* Hairy Roots by *Agrobacterium rhizogenes*-Mediated Transformation

<sup>1</sup>Ginseng and Medicinal Plant Research Institute, NICS, RDA; <sup>2</sup>Kangwon National University; <sup>3</sup>Chonnam National University  
 Ok-Tae Kim<sup>1</sup>, Sun-Hee Kim<sup>3</sup>, Hyun-Yong Lee<sup>2</sup>, Baik Hwang<sup>3</sup>

#### Objectives

The primary objective of the current study was, first, to establish a stable genetic transformation system for *C. asiatica* using the R1000 strain of *A. rhizogenes*. A protocol was established to increase the yield of *C. asiatica* hairy roots via the control of the co-cultivation period and the inoculation region of the explants. We have also described the use of hygromycin phosphotransferase (hpt) and green fluorescence protein (mgfp5) as selection markers for the generation of antibiotic-resistant hairy roots.

#### Materials and Methods

##### ○ Plant Materials

Four node segments per petri dish (90×20 mm) were cultured on MS basal medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 0.8% agar at 23±2°C under light conditions. After 2 weeks of cultivation, the leaves and petioles were used as explants for the induction of hairy roots.

#### Results and Discussion

Transformed root ("hairy roots") cultures have been shown to be a good model for the study of many secondary metabolites. In order to obtain suitable materials for our understanding of triterpene biosynthesis, the first step in an efficient transformation system for *Centella asiatica* (L.) Urban was established using the *Agrobacterium rhizogenes* strain R1000, which harbors pCAMBIA1302 encoding the hygromycin phosphotransferase (hpt) and green fluorescence protein (mgfp5) genes. The formation of hygromycin-resistant *C. asiatica* hairy roots was influenced by the origin of the explant. Hairy roots were obtained at a frequency of up to 14.1% from a connector (B region) between the leaf and petiole (Table1). When the length of co-cultivation

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\*Corresponding author: (TEL)+82-62-530-3392, (E-mail): bhwang@chonnam.ac.kr

with bacteria was adjusted to 7 days, the most abundant hairy root formation was observed (Table 2). Transformation was confirmed by polymerase chain reaction and Southern blot analyses.

Table 1. The effect of shoot explant regions on induction of *C. asiatica* hairy roots

Explant regions	n (A) <sup>a</sup>	Hmr (B) <sup>b</sup>	PCR positive/negative	Efficiency (%) (B/A)
A	189	6	6/0	3.2
B	198	28	27/1	14.1
C	141	2	2/0	1.4

<sup>a</sup>Numbers of explants after infection with *Agrobacterium*

<sup>b</sup>Numbers of hygromycin-resistant hairy roots after 3-month culture on selection medium containing 20 mg l<sup>-1</sup> hygromycin

Table 2. The effect of the period of co-cultivation with *A. rhizogenes* on induction of *C. asiatica* hairy roots

The period of co-cultivation (days)	n (A) <sup>a</sup>	Hmr (B) <sup>b</sup>	PCR positive/negative	Efficiency (%) (B/A)
3	165	8	8/0	4.8
7	155	56	54/2	36.1
14	145	41	40/1	28.3
21	132	11	11/0	8.3
28	130	9	8/1	6.9

<sup>a</sup>Numbers of explants after infection with *Agrobacterium*

<sup>b</sup>Numbers of hygromycin-resistant hairy roots after 3-month culture on selection medium containing 20 mg l<sup>-1</sup> hygromycin