P-169

High efficiency *Agrobacterium rhizogenes*-mediated transformation of bitter melon (*Momordica charantia* L.)

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Objective

To understand the molecular biological mechanism that regulates the synthesis of secondary metabolites, it is necessary to establish an efficient protocol for stable genetic transformation. In this paper, we describe how to transform *M. charantia* root cultures by using cotyledon, hypocotyl and first leaf explants infected with different *A. rhizogenes* strains which containing the binary vector pBI121 for generating CaMV35S::GUS. This protocol will be useful for studying and applying for the production of valuable metabolites such as phenolic compounds from *M. charantia* hairy root cultures.

Materials and methods

- Plant Material: *M. charantia* seeds were harvested in experiment green house of Chungnam National University (Deajeon, Korea) and keep in 4° C until was used. Unhull seeds were geminated aseptically and grown in incubator.
- Optimization of hairy root culture conditions
- GUS staining analysis
- PCR analysis for *rol* B and GUS gene
- Real-time polymerase chain reaction analysis

Results

Five different *A. rhizogenes* strains (R1200, R1000, 13333, R1601 and R15834) were used for their ability to induce the formation of hairy roots on three explant types of *M. charantia*. Strains R1200, R1000 and 13333 infected more than 80% of all seedling explant types. Among these strains, R1000 strain infected with the highest frequency on all three different explants examined, being 85.00, 91.67 and 93.33 % for cotyledon, hypocotyl and first leaf explants, respectively. To determine the appropriate antibiotics and concentration to use for plant selection, kanamycin and geneticin were used to test. The result shown that geneticin 30 mg L^{-1}completely inhibited the development of hairy roots. Hystochemical staining of GUS and molecular analysis of transgenic hairy roots was confirmed GUS expression and its integration into plant genome.
Quantitative RT-PCR RNA analysis revealed the higher levels of GUS transcripts than those in wild type hairy roots.

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Table 1. Effect of different strains of *A. rhizogenes* on hairy root induction of *M. charantia* explant after 4 week in culture.

A.*rhizogenes*strains	Induction efficiency (%)		
	Cotyledon	Hypocotyl	First leaf
R1200	82.66 ± 5.0	89.33 ± 4.1	89.00 ± 3.5
R1000	85.00 ± 3.1	91.67 ± 3.4	93.33 ± 2.5
13333	81.65 ± 4.1	89.00 ± 4.9	84.33 ± 5.5
R1601	41.60 ± 4.1	49.67 ± 3.7	49.00 ± 5.0
R15834	41.00 ± 4.0	45.67 ± 5.3	57.67 ± 4.1

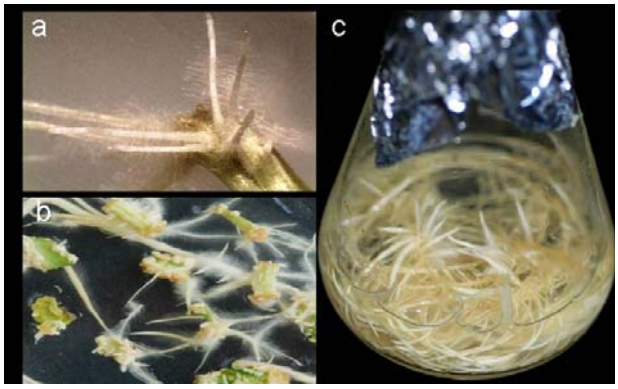

Fig 1. Development of hairy root from a hypocotyl explant of *M. charantia* after inoculation with *A. rhizogenes* strainR1000 (a), rapidly hairy root culture in phytagel-solidified medium (b) and liquid culture (c).

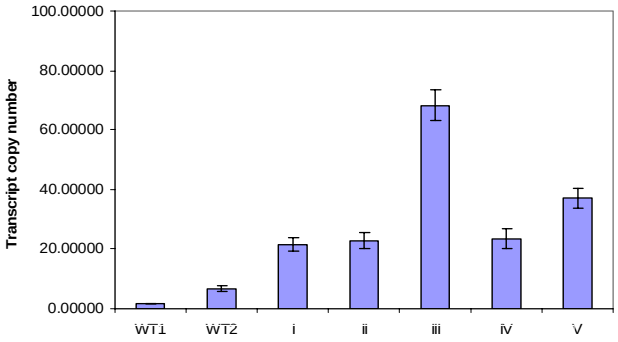

Fig 2. Quantitative RNA analysis of GUS gene expression in wild type roots (WT1 and WT2) and hairy roots transformed with *A. rhizogenes* R1000 (pBI121)(I-V). The value for each line is the mean of 3 replication ± SD.