Ⅱ-27

Enhanced Digitoxin Production in Transgenic Hairy Root Cultures of *Digitalis purpuria* by Repeated Elicitation

Department of Biology, College of Natural Sciences, Chonnam National University, Gwang-ju 500-757

Sung Jin Hwang

Objectives

The objective of the current study was to establish the hairy root cultures in *D. purpurea* and their efforts towards enhance digitoxin production in various culture conditions. Furthermore, a strategy of repeated elicitation was developed to improve digitoxin production.

Materials and Methods

Plant materials : Seeds of *D. purpurea* were surface sterilized with 70% ethanol for 5 min and 0.4% sodium hypochlorite for 5 min. They were then rinsed three times in sterile distilled water and placed on a half-strength MS medium. After the seeds were germinatedunder darkness at 25±1℃, the plantlets were transferred to a solid MS medium containing 2% sucrose.

Genetic transformation: To induce hairy roots, leaves from in vitro grown seedlings were cut into small pieces and co-cultured with *A. rhizogenes* strains for 24 h in the dark. The induced adventitious roots were excised and cultured on a 1/2MS medium containing 300 mg/L cefotaxime to eliminate the bacteria. Three transformed root clones obtained after the excision of single roots were maintained and propagated in the dark on a hormone-free MS medium supplemented with 3% sucrose.

Cultures of hairy root clones: Four different media were tested for their effects on biomass yield : 1/2MS, MS, B5, and WPM. All experiments were carried out in 100 ml Erlenmyer flask containing 40 ml of the liquid medium, inoculated with ca. 0.05 g f.w tissue. The flask were incubated on a rotary shaker at 100 rpm at 25±1℃ under darkness. After 6 weeks of culture, biomass dry weight and digitoxin contents determined. MJA was added to the hairy root culture once (one day 14) or repeatedly(on days 14, 21 ,and 28).

PCR analysis: For confirmation, isolation of total DNA from bacterium-free *D. purpurea* hairy roots and non-transformed root s was conducted using established methods. PCR identification of the rooting locus rol genes was

Corresponding author (E mail) jimhwang@jnu.ac.kr (Phone) +82-62-530-3416

performed using DNAs from the hairy roots as template and non-transformed roots as control, respectivery. The PCR products were fractionated by electrphoresis on a 0.8% agarose gel using TAE buffer and photographed under a UV lamp at wavelength of 260 nm.

Digitoxin analysis: Dried hairy roots were extracted with 70% methanol under reflux on a water bath for 10 min. Supernatant after the centrifugation was used for the determination of digitoxin. Filtered samples were injected into HPLC system (Young-in Sci. Co., Korea) with UV detector. Curosil G column was used for the analysis. The mobile phase was a mixture of acetonitrile and water (35:65). Flow rate was 1 ml/min and measuring wavelength was 220 nm. Standard digitoxin for HPLC analysis was purchased from Sigma Chem Co.

Results

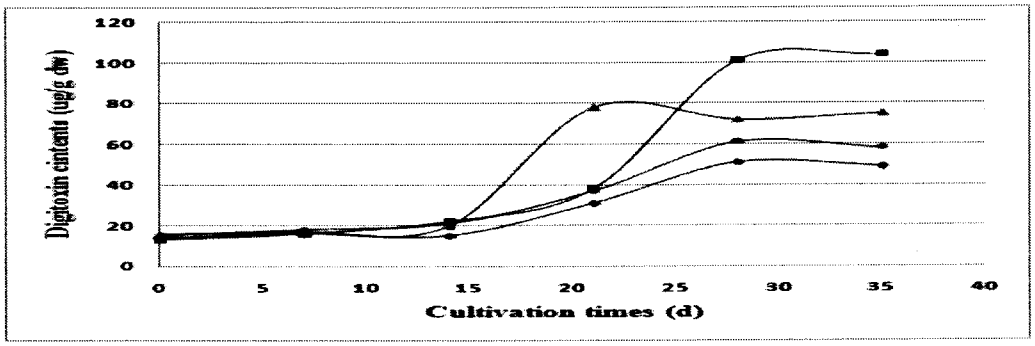

Fig. 1. Time course of digitoxin contents in hairy root cultures of *D. purpurea* treated with 100 μM MJA once one day 14 (circle), on days 14, 21, 28 (squre), and 300 uM MJA on day 14 (triangle) in shake flasks. The cultures without any treatment were taken as control (rhombus).

Table 1. Hairy root induction rate after co-cultivation of leaf explants with various *A. rhizogenes strains*

Bacterial strains	Hairy roots induction rate(%)
A4	43.1
15834	10.3
43057	98.2

Table 2. Comparison of biomass and digitoxin contents in hairy root clones of *D. purpurea*

Clones	Biomass (g dw/flask)	Digitoxin contents (ug/g dw)
DG-01	0.71 ± 0.05	54.3 ± 0.5
DG-02	0.68 ± 0.05	51.3 ± 0.6
DG-03	0.78 ± 0.05	42.0 ± 0.6