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High efficiency *Agrobacterium rhizogenes*-mediated transformation of Kenaf. (*Hibiscus cannabinus L*).

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Objectives

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 *H. cannabinus* root cultures by using cotyledon leaves and hypocotyl infected with different *A. rhizogenes* strains which containing the binary vector pBI121 for generating CaMV 35S::GUS. This protocol will be useful for studying and applying for the production of valuable metabolites such as phenolic compounds from *H. cannabinus* hairy root cultures.

Materials and Methods

○Optimization of hairy root culture conditions

For the selection of optimal *A. rhizogenes* strain, the effects of strains 13333, R1000, R1200, R15834 and R1601 on the induction of hairy roots from *H. cannabinus* cotyledon and hypocotyl explants were tested. For the selection of optimal antibiotics concentration for hairy root growth, the various concentration of kanamycin was tested.

○GUS staining analysis

Hairy roots were stained for GUS activity in staining solution. Hairy roots were soaked directly in staining solution, placed under vacuum for 10 min and incubated at 37 oC for overnight. After staining, the solution was changed with 70 % ethanol until tissue was cleared.

○PCR analysis for rol genes

The amplification cycle consisted of denaturation at 95^0C for 1min, primer annealing at 55^0C for 1min and primer extension at 72^0C for 1min. After 30 repeats of the thermal cycle and final extension 72^0C for 10min.

Results

We found that the R1000 strain was most efficient in inducing transgenic hairy root formation in *H. cannabinus*. Hairy root were induced from cotyledon and hypocotyl explants of *H. cannabinus*. After two days of co-cultivation with *A. rhizogene* strains, explant tissues were transferred to ager-solidified 1/2MS containing 500 mg/l cefotaxime, to effect killing of *A. rhizogenes*. Young hairy roots emerged from

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woundedsites. To our knowledge, ours is the first report describing efficient transformation protocols for this species.

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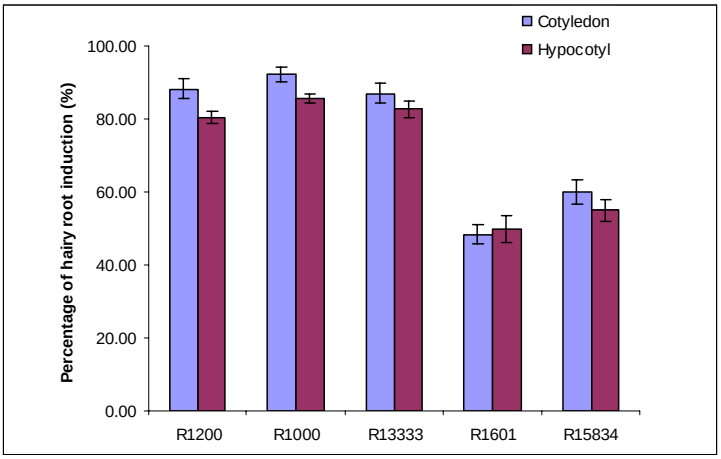
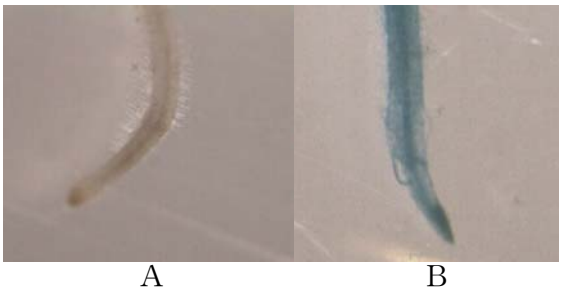


Fig. 1 Efficiency of transformation to hairy roots by *A. rhizogenes* strains under standard conditions. Each value are presents the mean of three different experiments.



A B

Fig 2. GUS histochemical analysis of transgenic hairy roots of *H. cannabinus* (A) wild type hairy root (B) Transgenic hairy root with pBI121 containing GUS gene.