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# High efficiency of genetic transformation and regeneration of *Codonopsis lanceolate* Trautv via *Agrobacterium tumefecience*-mediated procedure

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## Objectives

We focused on exploring the factors influencing *A. tumefecience*-mediated transformation of *Codonopsis lanceolate* to optimized the transformation system and different combination of hormone concentration to optimize the regeneration system

## Materials and Methods

○ Plant material

- *Codonopsis lanceolate* : The leaves and cotyledones from Seedling of *C. lanceolate* were collected from National institute Crop Science

○ Regeneration system

- Plant regeneration

o Medium and Hormone concentration : MS, Shenk and Hildebrant Medium(SH), Gamborg B5 medium with the concentration of auxin (2,4-D, NAA), cytokinins(BA), GA3, 3% sucrose, and 0.8%(w/v) agar pH 5.8

o Carbone source concentration : sucrose (1, 3, 6%)

o Polyamine concentration : spermidine, spermine, putrescine (respectively 0.1, 1mg/ℓ)

o Sensitivity to Kanamycin : MS(Sucrose 3%, agar 0.8%)+ NAA 2mg/ℓ + BA 2mg/ℓ + Kanamycin (0, 20, 30, 50, 70, 100mg/ℓ)

○Transformation system

- Gene construct

o γ-tocopherol methyltransferase inserted to pYB1130 (SacI/SmaI)

o *Agrobacterium tumefecience* LBA4404

- Transformation efficiency

o Innoculation time (0, 1, 3, 5, 8, 10 min)

o Cocultivation period (1, 2, 3, 4, 5 days)

- PCR analysis

oN-1 primer (5'-GAG-GCT-ATT-CGG-CTA-TGA-CTG-3'); N-2 primer (5'-ATT-CTC-GTG-ATG -GCC-TTG-3'); TMT-1 primer(5'-GAATTCATGAAAGCAACTCTAGC-3'); TMT-2 primer(5'-TAA TCG ATTA GAC TTA GAG TGG CTT C-3')

- Southern blot analysis

o Restriction enzymes : Sam Ⅰ / Sac Ⅰ

o Probe : DIG DNA labeling and detection kit

## Results and Discussion

- ○ The shoot induction frequency and shoot length of *C. lanceolate* was the highest at NAA 2mg/ℓ and BA 2mg/ℓ Yellow-white embryogenic calli formed cut ends of cotyledon on MS medium supplemented with 2,4-dichlorophenoxyacetic acid(2,4-D 0.1, 0,5, 1, 2mg/l) after 3-4 weeks. Then somatic embryos were produced by subculturingmultiple shoots induced on MS medium containing 2mg/ℓ GA3 after 3 weeks cotyledonary explants culture.
- ○ The sucrose concentration was optimized at 3% sucrose for shoot induction and callus formation
- ○ In the polyamine effects, the shoot induction frequency and shoot length of *C. lanceolate* was the highest at spermidine 0.1mg/ℓ, in this condition shoot induction frequency was high and more over shoots apperared in the early time and grew vigorously
- ○ Shoot induction was completely inhibited at 100mg/ℓ , when the concentration of Kan 50 mg/ℓ or higher there were no induced shoot and no developed callus either
- ○ The innoculation time was optimized at 5minute and the optimum co-cultivation period was 2 days
- ○ PCR and Southern blot analysis was confirmed that the γ-tocopherol methyltransferase introduced to plant genome and a system of high efficiency of genetic transformation and regeneration of *C. lanceolate* was established.

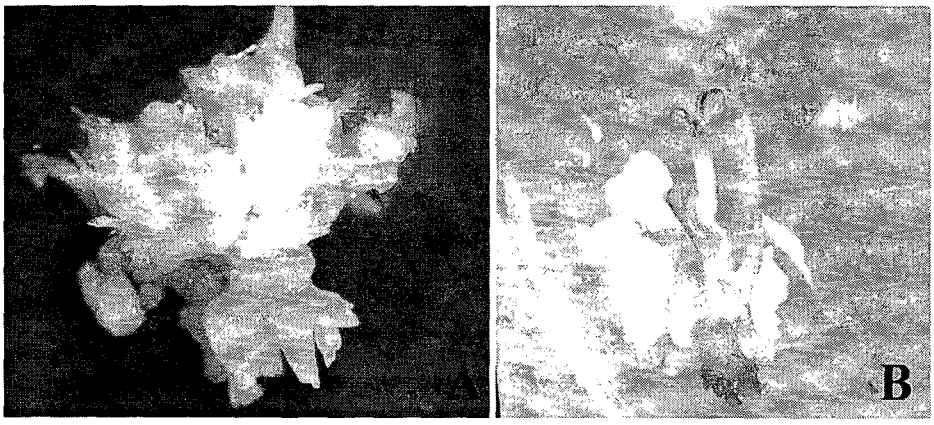


Fig. 1 Somatic embryogenesis and plant regeneration in transgenic *C. lanceolate*

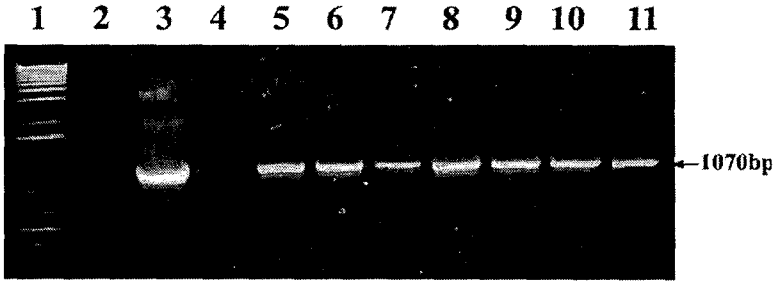


Fig. 2. Detection of γ-TMT gene in transgenic plants by using TMT-1 and TMT-2 primer. Lane 1 : pGEM marker DNA ; Lane 2 : PCR amplication of non- transgenic plant ; Lane 3 : PCR product of γ -TMT gene fragment; Lane 4-11 : PCR amplication of transgenic plant