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# Agrobacterium-mediated co-transformation of Resveratrol synthase gene in transgenic *Codonopsis lanceolata* transformed with γ-TMT.

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**Objectives:** The objective of our research was to establish the genetic co-transformation system of Resveratrol synthase gene in transgenic *Codonopsis lanceolata.*

**Materials and methods**

*Codonopsis lanceolata* Benth transformed with γ-TMT.

*Agrobacterium tumefaciens* strain LBA 4404/ binary vector pYBI121

**Regeneration of transgenic shoots:** MS medium supplemented with 0.1 mg/ℓ NAA and 1 mg/ℓ BAP, 3 % sucrose and 0.8 % agar at pH 5.8.

***Agrobacterium* cell density** $OD_{600}$ between 0.8 and 1.0

**Infection:** 5 minutes

**Co-cultivation:** co-cultivated for 3 days in the dark at 24-26 ℃, in MS medium supplemented with 0.1 mg/ℓ NAA and 1 mg/ℓ BAP, 3% sucrose and 0.8% agar at pH 5.8.

**Selection medium:** MS salt containing 0.1 mg/ℓ NAA and 1 mg/ℓ BAP, 3 % sucrose and 0.8 % agar at pH 5.8, supplemented with 100 mg/ℓ kanamycin for selection and 250 mg/ℓ cefotaxime.

**Shooting medium:** ½ MS

**DNA isolation and Polymerase chain reaction:** DNA was extracted from young leafs excised from kanamycin resistant shoots. Two primers used for PCR amplification of the 700 bp of the *npt* II gene were N-1 (5″- GAA-GCT-ATT-CGG-CGG-CTA-TGA-CTG - 3″) as a sense primer and N-2 (5″-ATC-GGG-AGC-GGC-GGC-GAT-ACC-CTA-3″) as a anti-sense primer.

Amplification conditions were 35 cycle each consisting 1min, at 94 ℃, 1 min at 60 ℃and 1 min 30 s at 72 ℃ and with a final extension at 72 ℃ for 10 min. The primers for a 1070 bp fragments of the TMT were:

TMT-1 primer (5-GAA-TTC-ATG-AAA-GCA-ACT-CTA-GC-3) as a sense primer and TMT-2 primer (5-TAA-TGG-ATT-AGA-CTT-AGA-GTG-GCT-TC-3)as a anti-sense primer. Amplification condition for -TMT gene fragment were 35 cycle each consisting 50 s at 94 ℃, 50 s at 57 ℃, 1min at 72 ℃, post elongation 72 ℃ for 10 min and with a final extension at 72 ℃ for 10 min.

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The AhRS3-specific primer pair, RS3-1 (5'-AGGCACCGTCGTTGGATGCAAGG-3') and RS3-2 (5'-GGCCACACTGCGGAGAACAACGG), was used for *AhRS* transgene detection.For AhRS3, sequence amplification,annealing was performed at 59 ℃. After 35 cycles, a final extension step was performed at 72 ℃ for 5 min.

**Result and Discussion:**

Adventitious shoots regenerated 3 weeks after *Agrobacterium* infection on regeneration medium containing 0.1 mg/ℓ NAA and 1 mg/ℓ BAP, 100 mg/ℓ kanamycin 250 mg/ℓ cefatoxime. Numerous adventitious shoot inductions of putative transformants were observed from the cut surface of explants which initially resembled knob like structure and later developed into new plant. Among the regenerated plants, most shoots gradually turned white and died about 4-5 weeks after infection, where as, putative transforming shoots continued to survive and grow normally. PCR analysis of showed the expected bands of *npt* II gene. PCR analysis was carried out to confirm the insertion of the *npt* II g, γ-TMT, *AhRS3* genes in the genome of transformed plant. The expected amplified *npt* II fragments of size 700 bp, 904 bp fragment of AhRS3 gene and 1070 bp fragment of γ-TMT genes were found in the T0 transformed plants, indicating the successful co-transformation of gene in the *Codonopsis lanceolata.*


Fig. 1. Regeneration of putative transgenic plants of *Codonopsis lanceolata* via *Agrobacterium* mediated transformation of leaf explants.(a) Embryogenic callus developed from the leaf explants on kanamycin containing medium 4 weeks after inoculation with *Agrobacterium tumefaciens*.(b) Regeneration of putative transgenic shoots from the embryogenic callus of leaf explants.(c) Rooting of elongated putative transgenic plantlets on a selection medium.(d) Transgenic plants growing in the greenhouse.

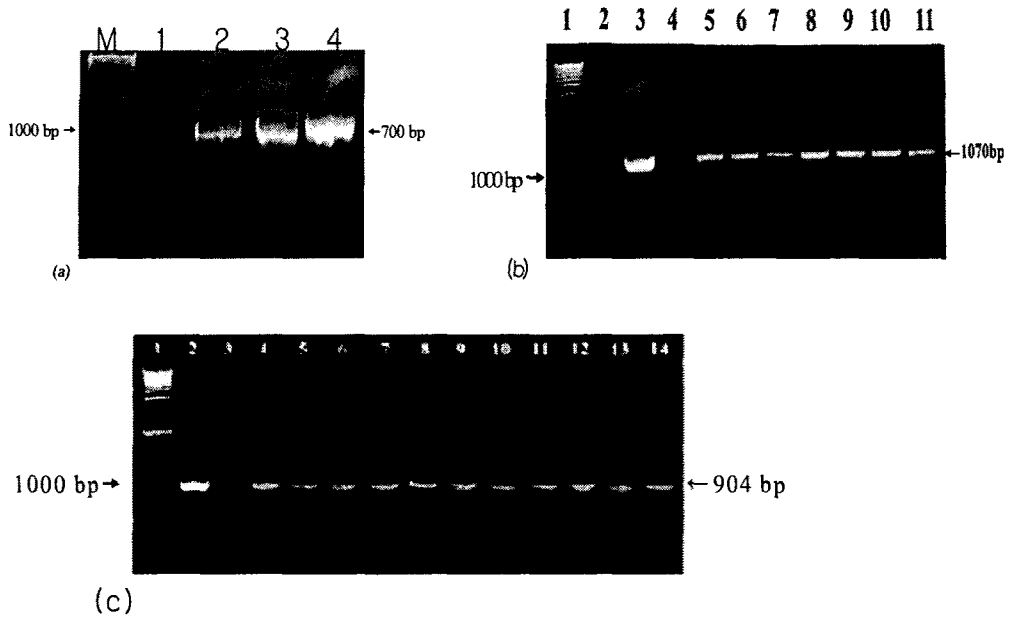


Fig. 2. (a) PCR analysis from leaves of transgenic *Codonopsis lanceolata* using specific primers of amplification of 700 bp *npt* II gene in agarose gel. M, Molecular marker; lane 1, the genomic DNA from untransformed plants (Negative control); lane 2-4, the genomic DNA of T0 transformants. (b) PCR analysis of the 1070 bp fragment of γ-TMTgene. Lane 1, Molecular marker; lane 2, the genomic DNA from untransformed plants (Negative control); lane 3, the plasmid pYBI121 as a positive control; lane 4-11, the genomic DNA of T0 transformants. (c) PCR analysis of the 904 bp fragment of AhRS3 gene. Lane 1, Molecular marker; lane 2, the genomic DNA from untransformed plants (Negative control); lane 3, *AhRS3* expression vector DNA as a positive control; lane 4-14, the genomic DNA of T0 transformants.

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