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Overexpression of the gamma-TMT gene in *Codonopsis lanceolata* induces genes related to ROS signaling

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Objectives

In the present study, we identified genes whose expression levels change with the overexpression of *γ-TMT* in transgenic *Codonopsis lanceolata*. We examined all of the deoduck-related genes available in the GenBank database and designed primers accordingly. We produced deoduck plants overexpressing *Arabidopsis thaliana γ-TMT* and conducted RT-PCR experiments to identify the marker genes related to biotic and abiotic stresses that showed altered expression levels in the transgenic deoduck leaves.

Materials and Methods

○ Materials

Invitro-grown *Codonopsis lanceolata* leaf explants of 3 - 4 weeks of age were used.

○ Methods

Agrobacterium tumefaciens strain LBA4404 harboring the binary vector pYB1130 was used. Transgene expression was confirmed by PCR and RNA gel blot analyses. The SOD-like activity in the *γ-TMT*-overexpressing deoduck transgenic plants, compared with the control plants.

Results and Discussion

The mixture method, which uses *Agrobacterium* strain LBA4404 to deliver multiple T-DNAs to plant cells, was used to introduce the pYB1130 binary vector into deoduck plants (Fig. 1). Regeneration of shoots from calli was induced using regeneration medium, and the shoots were transferred to rooting medium (Fig. 2A). PCR was performed to confirm whether the *npt II* and *γ-TMT* genes were integrated into the genomes of the T2 transgenic plants, using primer pairs specific for these genes and designed to produce 0.7-kb and 1.07-kb amplification fragments. No DNA band was amplified from control or non-transformed plants. The SOD-like activities of deoduck leaf extracts ground in ethanol were 61% in the γ-TMT-overexpressing deoduck transgenic lines and only 50% in control plants (Fig. 3D).

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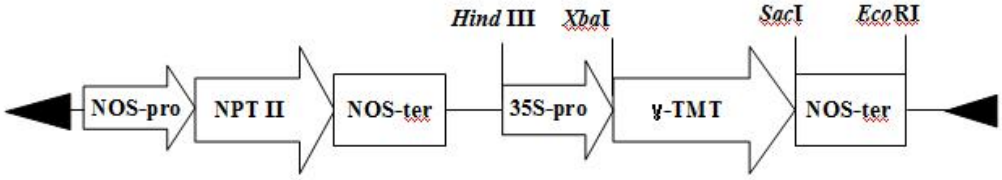

Fig. 1. Construction of the plant expression vector. Diagram of the Arabidopsis thaliana *γ-TMT* gene inserted into the plant gene expression vector pYB1130 (a modified version of pBI121).

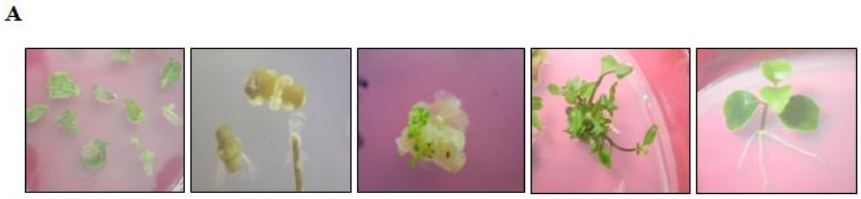

Fig. 2. Regeneration and T2 generation of transgenic Codonopsis lanceolata. (A) Regeneration of putative transgenic shoots from embryogenic callus of leaf explants on selection medium.

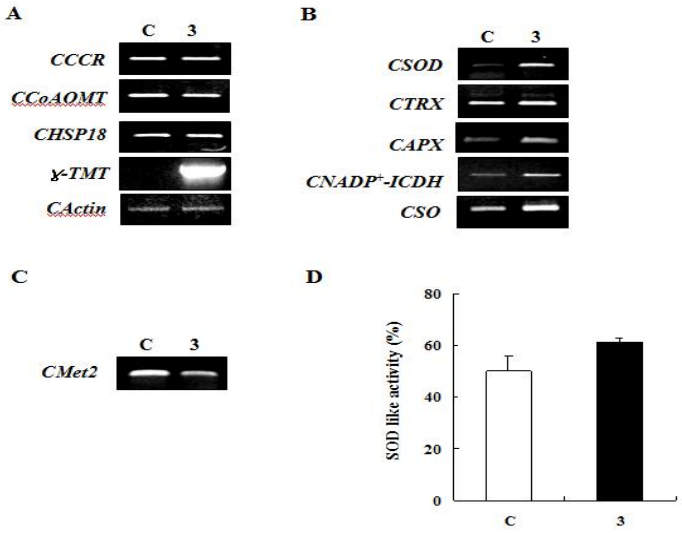

Fig. 3. RT-PCR analysis of genes encoding enzymes involved in plant defense, lignin biosynthesis, and ROS signaling metabolism in control and *γ-TMT*-overexpressing deoduck T2 progeny. Total RNA was extracted from leaf tissue of control (C) and transgenic (3) plants. The expression of the pathogen- and lignin biosynthesis-related genes *CCCR* (AB243011), *CCoAOMT* (AB243012), and *CHSP18* (BAE48789) (A) and the antioxidant-related genes *CSOD* (AY833718), *CTRX* (AB223035), *CAPX* (AB243015), *CNADP+-ICDH* (AB243085), and *CSO* (AB243086) (B) were compared by RT-PCR. (C) The expression level of the *CMet2* (AAV97748) gene was downregulated in transgenic deoduck plants. *CActin* was used as a positive control. (D) Pyrogallol autooxidation in leaf extracts of transgenic Codonopsis lanceolata.