Leaf shape changed by heterologous express of *ROTUNFOLIA* 3(*ROT* 3) in perilla (*Perilla frutescens*)

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Objectives

An efficient regeneration protocol for perilla is a prerequsits for successful transformation of genes with desirable traits. The objective of this study was to establish a reproducible *Agrobacterium*- mediated protocol for efficient genetic transformation in perilla. This protocol is being applied to develop the transgenic perilla with ROT3 gene

Materials and Methods

o. Plant materials· cotyledon and hypocotyl of tissues from *in vitro* culture of perilla

o. *Agrobacterium* strain/plasmids : EHA 101/pIG121Hm

o. Culture condition : hormone-free MS medium, 26±1℃

Results and Discussion

A protocol was developed for *Agrobacterium*-mediated genetic transformation of perilla, *Perilla frutescens*, using hypocotyl and cotyledon explants. Hypocotyls and cotyledons obtained from 7-day-old seedlings were co-cultivated with *Agrobacterium,* EHA 101/pIG121Hm. Following co-cultivation, the hypocotyl and cotyledon explants were cultured on MS medium containing 0.1 mg/L NAA, 1 0 mg/l BA and 50 mg/L kanamycin for 3 days in the darkness Subsequently, hypocotyl and cotyledon explants were transferred to selective medium with 50 mg/L kanamycin and 250 mg/L cefotaxime. After 5 weeks, cotyledon and hypocotyl produced adventitious shoot were subcultured to MS medium containing 50 mg/L kanamycin and 250 mg/L cefotaxime. Transgenic plants were confirmed by PCR and RT-PCR analysis(Fig. 1). The leaf shape of transgenic perilla plants was compared with the donor plants(Fig. 2).

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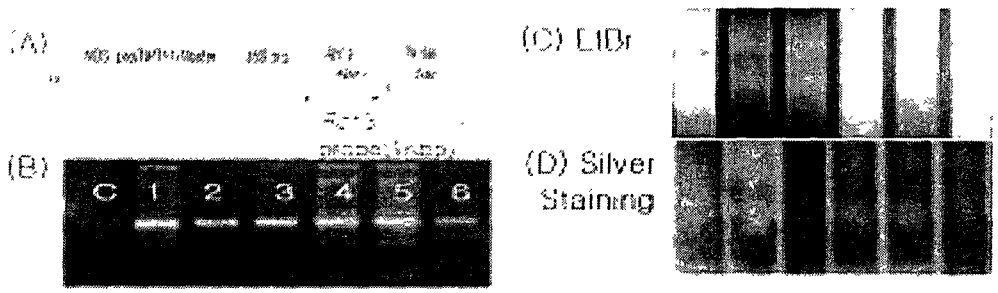

Fig 1. DNA gel-blot RT-RNA analysis. (A) Schematic diagram of part of the T-DNA region of vector Rot3. Nos-pro, promotor of nopaline synthase gene; NPT II, neomycin phosphotransferase II gene; Nos-ter, terminator of nopaline synthase gene; 35S promotor, (B) PCR analysis of genomic DNA from control and T_0 perilla leaf tissues. PCR products amplified from putatively transformed tissues were visualized by electrophoresis on a 1.0% agarose gel stained with ethidium bromide, (C) RNA products amplified from putatively transformed tissues were visualized by electrophoresis on a 1.0% agarose gel stained with ethidium bromide, (D) RNA products amplified from putatively transformed tissues were visualized with Silver Staining.

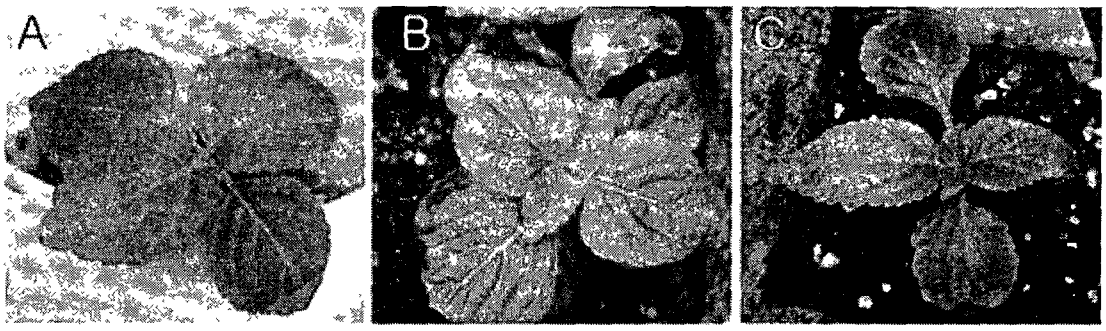

Fig 2. Comparision of leaf shapes between wild type(A) and transgenic plants(B & C). B: transgenic plants with round type leaf, C: transgenic plants with long-round type leaf.