OB2. Efficient Transformation of Watermelon [Citrullus lanatus (thunb.) Matsum.& Nakai)] with Agrobacterium tumefaciens

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Objectives

Watermelon (Citrullus lanantus) is one of the important crops in Korea. This study was carried out to investigate an efficient plant regeneration method from cotyledon explants of watermelon and several factors that influence the efficiency of Agrobacterium-mediated transfer of the foreign gene into two watermelon cultivars.

Materials and Methods

- Materials: Seeds of F₁ hybrid watermelon (cv. Bog and Ilchul), Agrobacterium strain AGL1/pCAMBIA3301
- O Selection of transformants: Phosphinothricin 0.5 mg/L
- O Transformation: Agrobacterium-cocultivation method
- O Analysis of the putative transgenic plants: Histochemical assay for GUS-INT gene, PCR, RT-PCR, and Southern blot analysis

Results and Discussion

Adventitious shoot organogenesis from watermelon cotyledon was effective when 2.5 μ M zeatin was used compared with 2.5 ~ 10 μ M BA. After preculture of two days, the explants were inoculated with bacterial liquid suspension (O.D=0.8) and cocultivated for five days. This combination of treatments showed the highest frequency of shoot regeneration on selection medium. Transformation frequency was highest on cocultivation medium supplemented with 50 μ M acetosyringone at pH 5.2 under 16h light/8h dark photoperiod at 22°C. After 3 months, putative transgenic plants were regenerated on the selection medium containing PPT, and many of them showed GUS positive expression. The plantlets were again confirmed for gene insertion by PCR analysis with specific primer for bar and gusAint gene. PCR-positive watermelon plants were also confirmed by RT-PCR, Southern blot, and bioassay using 'Bastar' herbicide.

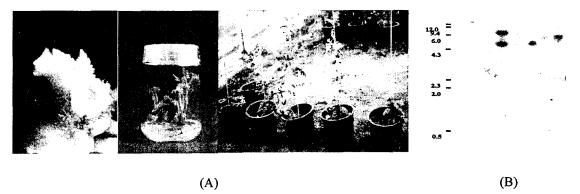


Figure. Regenerated plants (A) and Southern blot analysis (B) of transgenic watermelon. M: size marker, C: non-transformant, Lane 1-6: PCR-positive regenerated transgenic plants.

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