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Co-transformation Using a Negative Selectable Marker Gene for the Production of Selectable Marker Gene-Free Transgenic Plants

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Objectives

This study aims at development of practical way to remove selectable marker genes as a solution for 1) escaping from social issues caused by environmental dispersal of antibiotics and herbicides selectable marker genes and direct or indirect harms of their products 2) eliminating numerical limits of usable selectable marker genes.

Materials and Methods

1. Plant materials : *Nicotiana tobacum* Samsun NN
2. Methods: Agrobacterium-mediated transformation, PCR, negative selection, southern blotting

Results and Discussion

The pNC binary vector contained a T-DNA harboring the *codA* gene next to the *nptII* gene, while a second binary vector, pHG, contained a GUS reporter gene. Tobacco plants (*Nicotiana tabacum* Samsun NN) were co-transformed via the mixture method with *Agrobacterium tumefaciens* LBA4404 strains harboring pNC and pHG, respectively. Seeds harvested from the co-transformants were sown on germination media containing 5-fluorocytosine (5-FC)(Fig.1A). Analysis of the progeny by GUS staining and PCR amplification revealed that all of the 5-FC-resistant R1 plants were *codA*-free, and that the *codA* gene segregated independently of the GUS gene(Fig.2B). Because *codA*-free seedlings developed normally on 5-FC-containing medium, we suggest that co-transformation with negatively selectable markers is a viable method for the production of easily distinguished selectable marker gene-free transgenic plants.

(A) (B)

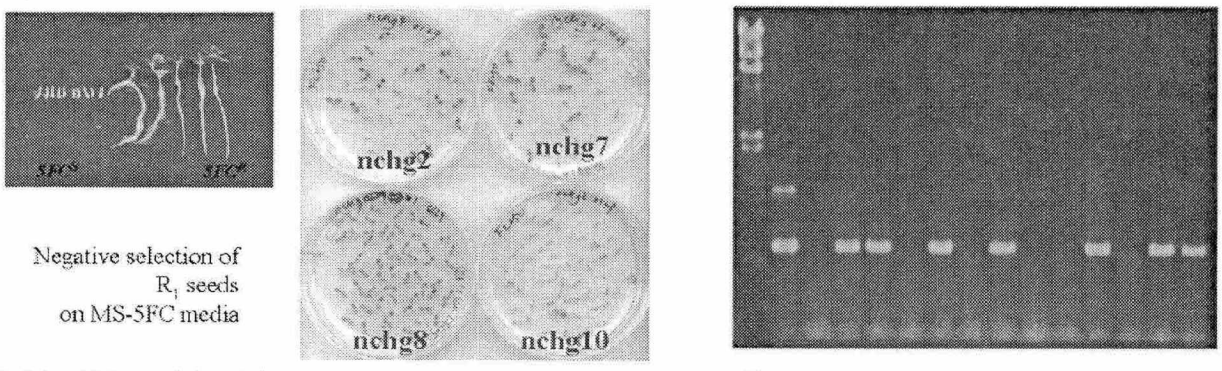

Fig. 1. (A) Discrimination of R1 seedlings germinated on negative selection medium containing 5-FC (250mg/L). (B)Genomic DNA from arbitrary 5-FC^{R} R1 plants was used as templates for *codA*- and GUS-specific PCR amplification. Lane 1: DNA size marker lane 2, PCR products of each R0 plant; lane 3 to end, those of R1 plants. Open arrowheads indicate *codA*-specific amplification and closed ones indicate GUS-specific amplification.

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