03-1-37

Genetic Transformation of a *Lactuca sativa* with the bro Gene Coding Cysteine Proteinase Inhibitor from Pineapple

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Objectives

We are interested in the roles of proteinase inhibitor in plants, particularly with respect to the protection of crops from fungal, bacterial and virus attack. We have tried to make transformation of *Lactuca sativa* with the *bro* gene coding cysteine proteinase inhibitor from pineapple and investigate gene expression.

Materials and Methods

1. Materials

Lettuce variety : Chungchima (*Lactuca sativa* L. cv Green Skirt)

Agrobacterium strain : LBA4404/pBI121

2. Methods

Vector construction, *Agrobacterium*-mediated transformation, PCR analysis, Northern blot analysis, Infection

Results and Discussion

The recombinant DNA using gene coding cysteine proteinase inhibitor from pineapple have constructed with the 35S promoter, to be transformed into lettuce plant. Explants of *Lactuca sativa* cultivar, Chungchima, were co-cultivated with *Agrobacterium tumefaciences* LBA4404 strains containing *nptII* gene and *bro* gene coding cysteine proteinase inhibitor from pineapple for transformation. Through initial selection of regenerated explants by culturing on a kanamycin and carbenicillin containing MS medium, multiple shoots were obtained after 2 months of culture. For a complementary step of selection, putative transgenic shoots were transferred to 1/2 MS basal medium supplemented with 100mg/L kanamycin and 500mg/L carbenicillin. The selected shoots were confirmed by PCR and Southern blot analysis. Nothern blot showed that transcripts of *Bro* gene were detected in the various tissues of transgenic plants. Also, the leaves of transgenic plants were shown resistance to *Xanthomonas Campestris* PV. Vitianus. Our preliminary results show improved resistance to *Bremia lactucae* by blocking its digestive cysteine proteinases in the transgenic plants.

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