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## Expression of a Sweet Protein Monellin Gene in Transgenic Strawberry

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### Objectives

The goal of this study is to improve the sweetness value of strawberry. Toward this goal, a single-chain monellin gene placed under a CaMV 35S promoter, encoding both polypeptide, was used to transform strawberry leaf-disc. Transgenic strawberry plants expressing sweet protein monellin have been generated.

### Materials and Methods

Leaf explants from 8 week-old aseptic seedlings were placed in 50 ml tubes, inoculated with an overnight grown *Agrobacterium* culture diluted 1/10 in MS basal medium and gently shaken for 20 mins. The infected explants were blotted dry on sterile filter paper and cocultivated on the organogenic callus induction medium for 3 days. Explants were then transferred to the organogenic callus induction medium ( $3.2 \text{ mg}^{-1}$  trans zeatin and  $0.5 \text{ mg}^{-1}$  IBA) and following the shoot regeneration medium ( $3.2 \text{ mg}^{-1}$  BAP and  $0.5 \text{ mg}^{-1}$  IBA) supplemented with  $20 \text{ mg}^{-1}$  kanamycin and  $500 \text{ mg}^{-1}$  carbenicillin, respectively. Shoots

regenerated after 14 weeks of culture were rooted in MS basal medium. PCR and northern blot analysis were performed to conform whether the monellin gene was incorporated into and expressed in the genome of the strawberry plants, respectively.

### Results and Discussion

Explants co-cultured with *Agrobacterium* were transferred to the organogenic callus and shoot regeneration selection medium containing  $20 \text{ mg}^{-1}$  kanamycin and  $500 \text{ mg}^{-1}$  carbenicillin. Kanamycin-resistance shoots were formed on organogenic calli that had been formed on cutting edge of the explants and approximately 33% of total tested explants after 14 weeks of cultures produced. Fifty-six lines of the kanamycin-resistance plants transformed with monellin gene were established. Analysis of polymerase chain reaction (PCR) showed that at least forty plants transformed with the monellin gene were positive. Northern blot analysis revealed that the foreign monellin gene expressed at the transcriptional level in three plant lines (M1, M2, M3). Transgenic strawberry were rooted in MS basal medium and then were subjected to acclimation, transferred to potting soil.

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