(05-1-69)

Transient Expression of Foreign Gene in Transgenic Lily Pollen through *Agroinfiltration*

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Objectives

We have made an effort to establish a transient expression system using lily (*Lilium longiflorum*) pollen through agroinfiltration method and the transgenic pollen was verified for its foreign protein synthesis and antigenecity during *in vitro* and mouse experiment, respectively.

Materials and Methods

1. Material:

Pollen source - *Lilium longiflorum* cv Georgia

Agrobacterium strain - LBA4404/pBI121 and LBA4404/pBIHBsAg

2. Methods:

Mature pollen grains were collected from lily flowers. Pollen was agroinfiltrated and grown for 24 hrs at 27°C in dark condition. Elongated pollen samples were analyzed for their GUS expression by molecular techniques. Pollen cultures transformed with HBsAg DNA construct were fed to mouse to examine immune reaction in serum against HBsAg.

Results and Discussion

Lily pollen was vacuum infiltrated for 20 min with *Agrobacterium tumefaciens* LBA4404 harboring gene for β-glucuronidase(GUS) or Hepatitis B virus surface antigen (HBsAg). Pollen transgenesis was confirmed by GUS histochemistry, RT-PCR and Southern hybridization. This transgenic pollen system for transient gene expression was applied to the production of edible pollen vaccine against HBV. DNA fragment encoding HBsAg adw subtype was engineered to construct pBIHBsAg, which was then introduced into the pollen through the agroinfiltration method. Transgenic pollen was analyzed to confirm its HBsAg expression by Southern blot hybridization for RT-PCR product, western blotting and ELISA. Transgenic pollen samples were fed to BALB/c mouse, which resulted in a relatively low level of immune reaction to HBsAg from the mouse serum.

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