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Expression of Porcine Epidemic Diarrhea Virus Gene in Transgenic Carrot Plants

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Objectives

Porcine epidemic diarrhea virus (PEDV) is classified as a member of the Coronaviridae and causes an acute enteritis in pigs. This study was conducted to express PEDV gene from transgenic carrot (*Daucus carota*) using agrobacterium-mediated transformation system.

Materials and methods

1. Transformation of carrot with *A. tumefaciens* strain LBA4404, containing a binary vector which carries the PEDV gene.
   a. Embryogenic callus (EC) induction from hypocotyl on MS medium with 2,4-D 1mg/L, 50 mg/L kanamycin and 300 mg/L cefotoxime
   b. Suspension culture of EC and somatic embryo induction on MS medium without 2,4-D
   c. Plantlet formation from SE on selection medium (MS medium with 50 mg/L kanamycin and 300 mg/L cefotoxime)

2. Genomic DNA isolation and PCR analysis with primer set (5′ CTC ATT CAA TCA CAC GAT TGG-3′ and 5′ -CAC CAC TAC CGA ACG CAG GGT AAC-3′) of PEDV gene

3. Total RNA isolation and Northern blot analysis

Results and Discussion

Porcine epidemic diarrhea virus (PEDV) is classified as a member of the Coronaviridae and causes an acute enteritis in pigs. The death rate of swine caughted by PEDV is over than 95% but there is no effective vaccine of this disease. Transgenic plants transformed with antigenic epitope were proposed and have been developed as edible vaccines. Compared with injection vaccine, plant-based vaccine system has several advantages; cheaper, longer shelf-life and safer. We carried out this study to express PEDV coat protein from transgenic carrot (*Daucus carota*) using agrobacterium-mediated transformation system.

Putative transgenic embryos were selected on MS medium with 50 mg/L kanamycin and 300 mg/L cefotaxime. Regenerated plantlet from somatic embryo were induced after 1 month of culture on MS medium containing 50 mg/L kanamycin.

Genomic PCR and Northern blot analysis demonstrated the integration into plant nuclear genome and the expression of PEDV gene in transgenic carrot.

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