(05-1-33)

Transgenic Tobacco BY2 Cells Expressing a PEDV Spike Protein

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Objectives

Porcine epidemic diarrhea virus (PEDV) is an infectious and highly contagious swine virus, which belongs to the *Coronaviridae* family. PEDV causes enteritis in swine of all ages, and is fatal in neonatal piglets. Hence, it is important to develop an effective vaccination against PEDV infection. The spike protein of PEDV is a primary target antigen for developing an effective vaccine against coronaviruses, since it mediates essential biological functions. In this study, in order to produce an effective plant-based vaccine against PEDV, we constructed the transformation vectors expressing antigen from the spike protein of PEDV and generated the transgenic tobacco cultured cells.

Materials and Methods

1. Material;
   - Plant: Tobacco (cv. BY2) suspension cells
   - Vectors: 35Spro::PEDV P1/Myc/pCAMBIA2300/EHA105 (M-P1 vector)
     35Spro::PEDV P2/Myc/pCAMBIA2300/EHA105 (M-P2 vector)
     35Spro::PEDV P3/Myc/pCAMBIA2300/EHA105 (M-P3 vector)
     35Spro::PEDV P4/Myc/pCAMBIA2300/EHA105 (M-P4 vector)
2. Methods: *Agrobacterium*-mediated transformation, PCR analysis, dot-blot analysis, western blot analysis

Results and Discussion

High expression of foreign gene in plant systems is necessary for its use as a vaccine. In order to develop the efficient high expression vector, we constructed 4 kinds of transformation vectors that harboring P1 (0.5 kb), P2 (1.7 kb), P3 (1.4 kb) and P4 (2.6 kb) fragment of PEDV spike protein. Each fragment was synthesized by PCR and then fused to Myc-tag sequences at its C-terminal end. Transformed tobacco (cv. BY2) cultured cells were generated following *Agrobacterium tumefaciens*-mediated transformation. Individual kanamycin-resistant calli were transferred to fresh selection medium with 100 mg/L kanamycin every 3 weeks, and transformed cell lines were selected by PCR analyses. Transgenic tobacco cell lines that express high levels of PEDV antigen were screened and confirmed by dot-blot and western blot analyses. The further characterization of these cell lines in terms of biological activity and immunogenicity are under study.

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