Kanjana Saetiew 2006: Transformation of VP1 Gene from Foot and Mouth Disease Virus into Stylosanthes hamata. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Assistant

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The VP1 gene which encodes protein that can induce immunity against the foot and mouth disease virus (FMDV) was transferred to Stylosanthes hamata calli via Agrobacterium -mediated transformation. The A. tumefaciens strain AGL1 possessed plasmid pCABVP1 which contained the VP1 gene and a selectable marker gene, hpt. An Agrobacterium suspension at OD₆₀₀ in the range of 0.6-1.0 and a co-cultivation period of 2 days was employed. Only 131 out of 2,200 calli survived in the MS selective medium containing 5 mg/l 2,4-D, 30 mg/l sucrose and 8mg/l agar at pH 5.7 and supplemented with 50 mg/l hygromycin. Surviving calli were regenerated on MS medium containing 15 mg/l BA and rooted on MS medium supplemented with 1mg/l IBA. Of all 300 regenerated plantlets, 93 lines showed PCR positive for only the VP1 gene, 97 lines showed PCR positive only for the hpt gene, while another 75 lines showed positive for both genes. Six lines that showed PCR positive for both, the VP1 and hpt genes, were subjected to Southern blot analysis to confirm the integration of the VP1 transgene. In some lines, a single copy of the VP1 gene was found to be inserted into the S. hamata genome, however, two copies of the VP1 gene were also found in the other lines. Two out of six Southern blot positive lines were then subjected to an analysis for the transcription of the VP1 gene using RT-PCR techniques, and the subsequent result showed positive. In addition the VP1 protein was detected in these 2 positive lines, using western blot analysis with serum from a FMDV infected pig. Only one line showed the correct expression of the VP1 gene.

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