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Expression of Human Amyloid-β Peptide in Transgenic Potato Plants

YOUM, Jung-Won[1] · JEON, Jae-Heung[1] · JOUNG, Jae-Ryul[1] · LEE, Byoung-Chan[1] · KANG, Won-Jin[1] · KIM, Mi-Sun · MUK, In-Hee · JOUNG, Hyouk[1] · KIM, Hyun-Soon[1*]

[1]*Plant Cell Biotechnology Lab., KRIBB, P. O. Box 115, 300-600, Taejon, Korea*

[2]*Brain Disease Research Center/Department of Anatomy, Ajou University, Suwon, Korea*

Objectives

Plant expression systems have advantages over other in vitro systems in terms of low production costs and low risk of contamination by animal viruses or bacterial sources. In this study, cDNA encoding human amyloid-β peptide were introduced into potato plants using Agrobacterium-mediated transformation, and analysed the expression of β-amyloid gene by PCR, Southern and Northern blot analysis.

Materials and Methods

A VP6 fragments was cloned with BamHI in the binary pMBP vector under the control of the CaMV 35S promoter, with KpnI/SacI in the pAT vector under the control of the patatin promoter and with SalI/XbaI in the pE35S vector under the enhanced CaMV 35S promoter (Figure 1). Each plasmids were mobilized into *Agrobacterium tumefacience* LBA4404 by the freeze-thaw method and potato (*Solanum tuberosum* L. cv Desiree) was transformed by modified leaf-disc cocultivation methods using the *Agrobacterium* strains described above. Kanamycin resistant transformants were screened for β-amyloid gene expression by PCR, Southern and Northern blot analysis.

Results and Discussion

Shoots were generated from transformed callus selected on medium containing 0.1mg/ml of kanamycin and 1.0mg/ml of carbenecillin. DNA amplification of plants regenerated in the presence of kanamycin was analysed by PCR for the co-integration of β-amyloid gene. By using internal β-amyloid primers for DNA amplification, the presence of the 126bp fragment was demonstrated in genomic DNA from each transformants containing β-amyloid. In order to identify the copy number of β-amyloid in potato plant, total genomic DNA was isolated from transgenic potato and cutted with SacI. As the result of Southern analysis, we confirmed the insertion of β-amyloid gene into the potato genome as one to three copies. To determine the expression patterns of β-amyloid, we carried out northern blot analysis. No signal could be detected in the non-transgenic plant, different levels of transcripts were observed per each constructs. Finally, we selected 3 transformants of pMBPAβ, 8 transformants of pE35SAβ and 3 transformants of pATAβ, respectively. Genomic DNA and mRNA analyses demonstrated the incorporation of the foreign gene into the potato genome, as well as their transcription. Continuously, we are carrying out the identification of β-amyloid protein in leaf and tuber tissues of transformed potato plants for immunoblot analysis.

*Corresponding author. Tel 042-860-4492 E-mail jeonjh@kribb.re.kr