P-80

Beet curly top virus를 이용한 replicating 벡터의 개선

경희대학교 : 김경일, 정호용, 정하영, 이현호, 정인식*

Modification of a Replicating Vector Based on Beet Curly Top Virus

Graduate School of Biotechnology and Plant Metabolism Research Center,
Kyung Hee University
Kyung-Il Kim, Ho-Yong Chung, Ha-Young Chung, Hyun-Ho Lee, and In-Sik Chung*

Objective

Members of the Geminiviridae amplify their 2.5-3.0 kb circular, single-stranded DNA (ssDNA) genomes in the nuclei of infected cells. Replication proceeds by a rolling circle mechanism that employs double-stranded DNA (dsDNA) replicative form intermediates that are amplified to a high copy number and serve as both replication and transcription templates. Only a single viral protein, the replication initiator protein (Rep), is required to direct this process from the viral origin of replication. Thus, it is possible to construct minimal viral replicons from which expression of foreign genes can be controlled by heterologous promoter and terminator signals. Because of these unique features, the potential of the DNA-containing geminiviruses as extrachromosomal gene amplification vectors for the expression of useful proteins in plants has long been recognized. While reports of *mastrevirus* and *begomovirus* based expression vectors are numerous, the vector potential of curtoviruses has remained for the most part unexplored. In this study, we demonstrate the feasibility of a BCTV-based, transient expression vector system. In addition, we confirm that the vector is compatible with the use of heterologous viral and cellular promoters to drive passenger gene expression.

Materials and Methods

The recombinant plant expression vectors were introduced into *A. tumefaciens* GV3101 by electroporation, as described by Ainsworth *et al.* Recombinant *A. tumefaciens* GV3101 was used to transform leaf-disks derived from *N. benthamiana* plants as described by Ainsworth *et al.* Leaf-disk experiments and infiltration assays were performed to examine feasibility and effectiveness of different viral and cellular promoters.

*Corresponding author : In-Sik Chung E-mail : ischung@khu.ac.kr Tel : 031-201-2436

Results

Recombinant green fluorescent protein (GFP) with a molecular mass of 29 kDa was transiently expressed in *Agrobacterium*-inoculated leaf-disks prepared from *Nicotiana benthamiana* plants. Expression of GFP from the Cauliflower mosaic virus (CaMV) 35S promoter within a replicating vector based on the geminivirus Beet curly top virus (BCTV) was more than 3 times higher than from a control, non-replicating vector. Use of the Cassava vein mosaic virus (CsVMV) promoter in the BCTV replicating vector increased the expression of recombinant GFP 320% at the transcript level, compared to use of the control CaMV 35S promoter.

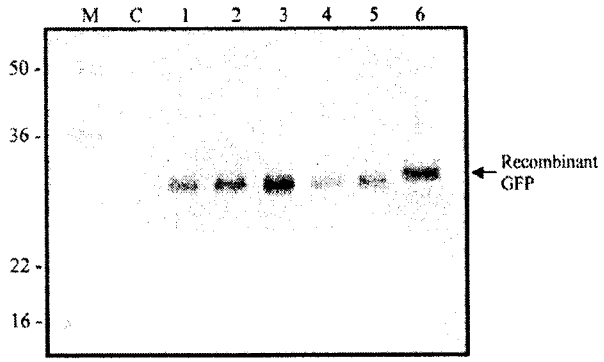

Fig. 1. Comparison of GFP accumulation obtained with BCTV vectors containing different heterologous promoters.