A Chemically Inducible Cucumber Mosaic Virus Amplicon Expression System for Production of Recombinant Human Therapeutics in Transgenic Plant Cell Cultures

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Plant cell cultures are entering a new phase of application and playing an increasingly important role in production of human therapeutics. However, efficient gene expression systems are currently lacking in transgenic plant cell cultures. In this study, we established a novel *Cucumber mosaic virus* (CMV) inducible viral amplicon (CMViva) expression system to inducibly and efficiently produce a human blood protein, alpha-1-antitrypsin (AAT), in transgenic plant cell cultures. The CMViva expression system is constructed from the genome of *Cucumber mosaic virus*, which is a tripartite plant virus composed of three positive-sense, single-stranded RNAs. CMV RNA1 and RNA2 encode 1a and 2a protein that are essential components of the viral replicase for viral replication and transcription and 2b protein that is a potent silencing suppressor. RNA3 encodes the 3a protein and coat protein that are required for efficient cell-to-cell spread movement of CMV. 2b protein and coat protein is translated from a subgenomic mRNA.

To overcome the potential problem of post-transcriptional gene silencing (PTGS) in transgenic plant cells, we modified the CMV RNA1, 2 and 3 by integrating different promoters for controlling the transcriptions of mRNA. We genetically engineered a chemically inducible promoter to regulate the transcription of RNA1 and CaMV 35S constitutive promoter to control the transcription of RNA 2 and RNA 3, and replace coat protein by target protein AAT. As a result, AAT mRNA would be amplified by the viral replicase and AAT protein will only be expressed following the addition of inducer.

We achieved higher levels of extracellular functional recombinant AAT and higher ratio of functional recombinant AAT to total recombinant AAT production in bioreactor using the CMViva system compared with either a *Cauliflower mosaic virus* (CaMV) 35S constitutive promoter expression system or a chemically inducible promoter expression system (an estrogen receptor-based, estradiol-inducible promoter system, XVE). We investigated the effects of timing of

induction (TOI) and concentration of inducer (COI) on recombinant AAT production in inducible expression systems (XVE and CMViva), and medium exchange and pH on the recombinant AAT production using above three expression systems in transgenic *Nicotiana benthamiana* suspension cell cultures in bioreactors. A rational induction strategy is proposed for improving the functional recombinant AAT production yield.