Comparisons of Gene Expression Systems for Production of Recombinant Human Therapeutics in Transgenic Plant Cell Cultures

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Abstract

Plant cell cultures have being developed to express recombinant human therapeutics. One of keys for establishing a successful production platform of transgenic plant cell culture is to design an efficient gene expression system. In this study, we developed and compared three different gene expression systems, including a *Cauliflower mosaic virus* (CaMV) 35S constitutive promoter expression system and a chemically inducible promoter expression system (an estrogen receptor-based, estradiol-inducible promoter system, XVE), and a novel *Cucumber mosaic virus* (CMV) inducible viral amplicon (CMViva) expression system for production of a recombinant human therapeutic protein, alpha-1-antitrypsin (AAT), in transgenic *Nicotiana benthamiana* suspension cell cultures. For comparisons of different gene expression systems, we (a) constructed transgenic plant cell lines transformed with different expression systems, (b) established a novel total and functional AAT ELISA method, (c) developed a competitive ELISA for determining the concentration of 17-beta estradiol, an inducer for inducible expression systems, (d) evaluated the human AAT stability and degradation in conditioned cell cultured medium (free of cells), and (e) studied the effects of medium exchange and pH on the recombinant AAT production in production phase. The novel chemically inducible viral amplicon system (CMViva) resulted in higher yield of functional extracellular rAAT and higher ratio of functional rAAT to total rAAT (20 %~40%) in transgenic *Nicotiana benthamiana* suspension cultures. A rational induction strategy was proposed for improving the functional rAAT production yield. These results lay the foundation for developing scaleable transgenic plant cell cultures in bioreactors for production of human therapeutics.