Direct Inoculations of Plant Cell Cultures with a Plant Viral Vector

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In vitro plant cell culture expression systems offer the advantages of whole plant expression systems and can be advantageous in situations where small to medium scale production of high value human therapeutics at high purity is a requirement. The advantages that can offset the production cost differences between the two systems result from the production of heterologous proteins using bioreactor technology. Plant cell culture in bioreactors offers containment of the production process in a controlled environment when compared to open-field transgenic crops. Also, the production time in plant cell cultures using transient expression methods is potentially short (on the order of 1 to 2 weeks) when compared to stable or even transient expression in whole plants where the host growth cycle is relatively large (on the order of months). Plant virus expression vectors for transient heterologous protein production have been developed and successfully used in whole plant host expression systems. However, there is currently no published work concerning the development or characterization of plant virus expression vectors in plant cell culture and in particular in plant cell suspension cultures of dedifferentiated plant cells.

The ability to directly inoculate callus cultures with a tobacco mosaic virus (TMV)-based expression vector would show the potential for a rapid, high-level heterologous protein expression system in plant cell culture. We present a method for inoculating *Nicotiana benthamiana* callus cultures with a TMV-based expression vector, 30Bcycle3GFP. The method merges the inoculation procedures for TMV into tobacco callus and analogous DNA delivery techniques that utilize silicon carbide fibers along with intense mixing to wound callus cells and allow entry of the viral vector into the cells. We achieved successful infections of *Nicotiana benthamiana* callus cultures with the 30Bcycle3GFP based on reporter gene expression (Cycle3GFP) and the presence of viral vector RNA. Evidence for replication of the viral vector along with quantitative analysis of the viral vector RNA and the expressed reporter gene product, Cycle3GFP, are presented. Cycle3GFP could be found at levels as high as 0.7% of the total soluble protein extracted in directly inoculated cultures.