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Analysis of Coat Protein Expression Cassettes in Protoplasts

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1. Introduction

Transient gene expression studies of plant virus genes or parts of genes can be studied using plant protoplasts. Such studies are dependent on a number of important factors:

1. The method for isolating plant cells or protoplasts. Methods are now well-established for the isolation of protoplasts from a wide range of plant species ***(1)***.
2. Vector requirements or a gene construct that will be expressed in the protoplast under the culture conditions. It is important to use a vector that enables the expression of the insert gene. Genes lacking a plant promoter are expressed using the cauliflower mosaic virus (CaMV) 35S gene promoter. CaMV, one of the best-characterized plant DNA viruses, was used in early plant transformation studies, and the CaMV 35S promoter is recognized in a wide range of plants. For this reason, it is the most commonly used promoter element to express foreign genes during transformation. Gene expression cassettes consist of a multiple cloning site placed between a CaMV 35S promoter, or other strong promoter, and a poly(A) addition site or nopaline synthase (nos) terminator ***(2,3)***. Inserting the gene of interest into the multiple cloning site will drive the expression and allow transient studies.
3. An efficient method for introducing the nucleic acid into the isolated cells. A number of successful techniques exist in order to transfect nucleic acid into protoplasts, such as polyethylene glycol (PEG) treatment, electroporation, microinjection, and particle bombardment. PEG is a more common choice for protoplast transformation, because it does not require more specialized equipment associated with electroporation or biolistics. The precise function that PEG has during transfection is unknown; however, it is believed that PEG precipitates nucleic acid onto the protoplast plasma membrane and then stimulates its uptake by endocytosis ***(4)***.

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