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Efficient Transient Expression and Transformation of PEG-Mediated Gene Uptake into Mesophyll Protoplasts of Pepper

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Objectives

We described efficient transformation method based on PEG treatment for transient expression studies using GUS expression in pepper protoplasts. We also showed results in the production of stably transformed tissue derived by pepper protoplasts with maize activator element (*Ac*) and GFP gene.

Materials and Methods

1. Material

Plant – pepper (*Capsicum annuum, C. chinense, C. frutescens, C. pubescens, C. baccatum, C. chacoens*)

2. Methods

Mesophyll protoplasts were isolated from 47 of various *Capsicum* lines and cultured on protoplasts culture medium. The protoplasts were also transformed with maize activator (*Ac*) and GFP gene using PEG-mediated gene uptake method.

Results and Discussion

The optimal condition for protoplast isolation was obtained that leaf tissue was incubated with enzyme mixture consisting of 1.2% cellulase and 0.3% macerozyme in CPW solution for 12 hours. As far as the level of transient gene expression is concerned, higher concentration of PEG and lower molecular weight of PEG favoured higher gene expression – the optimum being at 40% of concentration and molecular weight 6,000 of PEG solution having 30 min of incubation time. The signal strength of GUS expression increased with the amount of added plasmid DNA. To test whether heterologous *Ac* transposable element can be mobilized in pepper genome, freshly isolated protoplasts were transfected with pCAMBIA::Ac vector, which also contains CaMV 35S-GFP gene, in PEG solution. The protoplasts expressing GFP were shown under the fluorescent light microscope. The protoplasts-derived microcolonies of approximately 8 cells formed after 21 days of culture in solid culture medium and, at this stage, they also expressed GFP gene. Visible colonies by naked eye were obtained from the protoplasts after 2 months of culture and expressed GFP. The callus also expressed GFP and, however, failed to produce shoots on MS-based media with any of the growth regulator combination tested. Maize activator (*Ac*) element was highly mobile in transformed pepper callus confirmed by Southern blot analysis.

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