

Abstract

Protein-DNA interactions play a very important role in regulating several cellular processes in multicellular organisms. Similar interactions occur in plants as well. A gene regulatory network of transcription factors enriched in Shoot Apical Meristem(SAM), and promoters of cytokinin genes (biosynthesis, degradation, and signalling) has been constructed by yeast-1-hybrid(Y1H).Furthermore, validation needs to be carried out as yeast is a heterologous system. There are various experimental methods available to validate the protein-DNA interactions, but we have taken in planta approach to validate them using mesophyll protoplasts from Arabidopsis. Transient expression in mesophyll protoplasts (TEAMP) is an experimental method to validate these interactions in planta as Arabidopsis mesophyll protoplasts maintain many of the same physiological responses and cellular activities as present in intact plants. We have taken this approach and tried to standardize optimal conditions of protoplast isolation, protoplast cell culture, and transfection. We also constructed various expression clones to perform the transient reporter based (GFP, GUS, and LUC) assays.