题　　目：橡胶草 TkENOD2 启动子克隆与表达载体构建

摘要

在基因工程中，研究外源基因在受体细胞中的表达一直是一个热点问题，研究者通常会在体外构建一个能高效表达外源蛋白的表达载体。启动子作为基因转录中的重要顺式调控因子，在转录调控中扮演重要角色，对其功能的研究有助于实现基因的高效特异性表达。在植物基因工程研究领域中，组成型启动子在植物的不同部位都能够启动基因的转录翻译，一旦插入外源基因，可能会打破植物在原有环境下的正常生理代谢，造成植物正常生长受损。而组织特异型启动子可在特定的植物器官中驱动基因的表达，从而减少这一现象的发生。本课题本课题克隆了橡胶草中组织特异型启动子 TkENOD2，构建克隆载体 BluntZero-pTkENOD2。采用同源重组的方法将 pCAMBIA1303 中花椰菜病毒 35S 强启动子替换为组织特异性启动子 pTkENOD2。用电击法将 pCAMBIA1303-pTkNOD2 表达载体转入农杆菌感受态细胞中。为后期利用 GUS 报告基因进行检测来确定组织特异性启动子的活性及遗传转化实验提供基础。

关键词：组织特异型启动子；TkENOD2;启动子克隆；表达载体构建

Abstract

In gene engineering, it has been a hot issue to study the expression of foreign genes in receptor cells..Promoters, as important cis-regµlatory factors in gene transcription, play an important role in transcriptional regµlation.The study of its function is helpfµl to achieve efficient and specific expression of genes.Promoters can be divided into three categories according to their functions: constitutive promoters, inducible promoters and tissue-specific promoters.In the field of plant genetic engineering, constitutive promoters can initiate the transcription and translation of genes in different parts of plants.Once inserted, exogenous genes may destroy the normal physiological metabolism of plants in the original environment, resµlting in impaired normal growth of plants.Tissue-specific promoters can drive gene expression in specific plant organs, thereby reducing the occurrence of this phenomenon.In this study, we cloned tissue-specific promoter TkENOD2 from Rubber grass and