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超声波辅助处理农杆菌介导大豆胚尖转化转基因植株的获得和分子鉴定

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Title: Molecular Characterization of the Transgenic Soybean Plants by Sonication-assisted Agrobacterium-mediated Transformation Using the Embryonic Tips

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摘要： 以大豆Williams 82成熟胚尖为试验材料，建立了超声波辅助处理的大豆胚尖农杆菌转化体系，研究了不同超声波处理时间对报告基因UidA(GUS) 瞬间表达的影响和不同草铵膦浓度对大豆转化效率的影响。结果表明：在超声波处理2 min时，报告基因GUS的瞬时表达效率达到最高；在0.3～0.6 mg.L^{-1}草铵膦梯度筛选浓度下，阳性转基因植株的数量最多。对于所获得的转基因株系进行了PCR检测和除草剂Basta涂抹抗性检测；实时荧光定量PCR检测显示转基因植株的目的基因的表达量达到野生型的1.7～42倍,Southern 杂交检测表明外源基因已经整合到大豆基因组中。

Abstract: Agrobacterium-mediated transformation as a practical and common method for introducing specific DNA fragments into plant genomes is well established and the number of transgenic plants produced using this method is increasing.Despite the popularity of the method,low efficiency of soybean transformation is a major challenge for scientists.Modification of transformation method may lead to better understanding of the system and result in high efficiency transformation.In this paper,the sonication-assisted Agrobacterium-mediated transformation (SAAT)system of soybean(Glycine max)was set up using embryonic tips of soybean cultivar of Williams 82.The effects of different SAAT duration and glufosinate concentration on transformation efficiency were investigted.The results showed that the transient expression of UidA(GUS)reporter gene reached the hightest with 2 minutes sonication treament among various time durations.The gradient seletion of glufosinate concentration from 0.3 to 0.6 mg.L^{-1}produced the highest transformation rate.Transgenic plants were verified by PCR analysis and herbicide paint assay.Real-Time Quantitative PCR showed the expression of target genes in transgenic plant was about 1.7 to 42 times than that in wild type.Southern blot result proved the target gene was integrated into the soybean genome of transgenic plants.This research will provide new information for soybean transformation improvement.

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