



### 基于抗草甘膦基因的棉花茎尖农杆菌介导转化方法的研究

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### Studies on the Genetic Transformation Method by Cotton Shoot Tip-Agrobacterium Medium Using the C

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摘要

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**摘要** 以中棉所49、珂字棉201和YZ-1为受体材料, 通过对培养基草甘膦浓度、农杆菌侵染时间和浓度、共培养时间以及恢复培养条件等因优化, 建立了基于抗草甘膦基因的棉花茎尖农杆菌转化技术体系, 并将抗草甘膦基因(EPSPS-G6)导入3个受体材料, 获得转基因抗草甘花植株。研究表明, 适合于棉花茎尖农杆菌介导的草甘膦筛选浓度为 $10 \text{ mg} \cdot \text{L}^{-1}$ ; 茎尖转化体系为农杆菌菌液 $\text{OD}_{600}$ 为 $0.9 \sim 1.0$ , 侵时间为 $20 \text{ min}$ , 共培养时间为 $48 \text{ h}$ , 选用SH培养基并加入适量活性炭( $0.5 \text{ g} \cdot \text{L}^{-1}$ )作为恢复培养基。用本研究创制的转化技术体系, 转处理3个陆地棉受体360个茎尖, 共获得60株抗性再生植株, 经PCR检测, 获得阳性抗性植株26株, 移栽成活23株。以茎尖外植体数计算, 体系的转化成功率6.4%。该转化体系适合于转化抗草甘膦基因或以抗草甘膦基因为筛选基因的外源基因, 具有转化频率高、嵌合体少、转化期短等优点。

**关键词:** 农杆菌 棉花 茎尖 转化 草甘膦

**Abstract:** A genetic transformation system via cotton shoot tip-agrobacterium medium based on a glyphosate resistant gene was established through the optimization of the glyphosate concentration during screening, infection period and infection concentration of the *agrobacterium*, period of co-culture with the agrobacterium, and the condition of recover culture etc., used CCRI 49, Coker 201, and YZ-1 as the materials, and their transgenic herbicide resistant cotton plants with EPSPS-G6 were obtained as well. As the results showed that, the optimal glyphosate concentration for screening transgenic cotton shoot tips was  $10 \text{ mg} \cdot \text{L}^{-1}$ , the optimal agrobacterium density was  $0.9 \sim 1.0$  in  $\text{OD}_{600}$ ; the infection period was about  $20 \text{ min}$ ; the co-culture period with agrobacterium was  $48 \text{ hours}$ ; and SH medium containing  $0.5 \text{ g} \cdot \text{L}^{-1}$  activated carbon was the best recovery medium for the transgenic shoot tip to grow. Using this transformation system, 60 glyphosate resistant plantlets were obtained from the 360 cotton shoot tips of three upland cotton cultivar or germplasms. From them, 26 plantlets were transgenic according to the PCR products, and 23 plantlets with the glyphosate resistant gene survived after transplanting. Based on explant numbers, the success rate of transformation in this experiment was  $6.4\%$ . The presented transformation system is an excellent method to transform the glyphosate gene, or an exogenous gene with glyphosate as a screening gene, with the advantages of high transformation rate, less chimera, and short transformation period etc.

**Keywords:** *agrobacterium* cotton shoot tip transgenic glyphosate

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