

研究论文

转抗虫基因三倍体毛白杨植株体内农杆菌残存与逃逸

杨敏生¹, 米丹¹, D. Ewald², 王颖³, 梁海永¹, 甄志先¹

1. 河北农业大学, 保定071000

2 Institute for Forest Genetics and Tree Breeding, BFH, Germany

3. 中国热带农业科学院热带生物技术研究所, 海口571101

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摘要 用部分改造的 BtCry1Ac 基因与慈菇蛋白酶抑制剂(API-A) 基因构建的双抗虫基因表达载体, 通过农杆菌介导法对三倍体毛白杨进行了转化, 对转化后植株体内残存农杆菌在继代培养和移栽过程中进行了跟踪检测。结果表明: 通过对转化再生植株的分子生物学检测, 42 个株系中, 33 个株系为阳性, 阳性率达到 80%; 用 Bt 毒蛋白抗血清进行 ELSA 检测结果表明, 7 个转基因株系都有 Bt 杀虫蛋白表达; 基因转化后, 可采用附加 50 mg/L 卡那霉素, 300 mg/L 羧苄青霉素的筛选培养基消除细菌并进行抗性芽筛选。对 28 个转基因株系叶片、茎段和根段在含有卡那霉素 50 mg/L YEB 培养基上进行细菌培养, 通过在 T-DNA 区、质粒 Vir 区和农杆菌基因组设计引物, 进行 PCR 检测, 证明有 3 个株系 (33、37、5 号) 检测到残存工程农杆菌, 并在组培瓶中存活 24 个月。将带菌的 3 个株系组培苗移栽到花盆中, 室内培养 1 个月后, 在 33 号株系根际土壤中检测到了目的农杆菌。

关键词 [双抗虫基因](#); [三倍体毛白杨](#); [残存农杆菌](#); [根际土壤](#)

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The survival and escape of *Agrobacterium tumefaciens* in triploid hybrid lines of Chinese white poplar transformed with two insect-resistant genes

YANG Min-Sheng¹, MI Dan¹, D. Ewald², WANG Ying³, LIANG Hai-Yong¹, ZHEN Zhi-Xian¹

1. Agricultural University of Hebei, Baoding 071000, China;

2. Institute for Forest Genetics and Forest Tree Breeding, BFH, D-15377 Walsdorf, Germany;

3. Institute of Tropical Biological sciences, State key Laboratory of Tropical Crops Biotechnology, Haikou 571101, China

Abstract Two partly modified insect-resistant genes (BtCry I Ac gene [Bt gene toxin against Lepidopteran insects] and API gene [arrowhead proteinase inhibitor]) were transferred to the triploid hybrid of Chinese white poplar ((*Populus tomentosa* Carr. × *Populus bolleana* Louché) × *Populus tomentosa* Carr.) mediated by *A. tumefaciens*. An examination was made concerning the survival of *Agrobacterium* in transgenic plants during the process of transplanting and subculturing on the nutrient medium. Results suggested that 80% of plants, which we got by repetitive selection on media added with 50 mg/L kanamycin and 300 mg/L carbenicillin showed positive reactions after examination with molecular methods. The ELISA test indicated that the Bt toxin protein was expressed in 7 of the transgenic sub-clones. Leaves, stems and roots of all the 28 transgenic plants were cultured on the YEB medium added with 50 mg/L kanamycin and the survival of *Agrobacterium* was detected in 3 sub-clones (33, 37, 5) which could have existed for 24 months in the bottle. Those 3 transgenic sub-clones were transplanted and cultivated for one month in the room, and then the target *Agrobacterium* was found in rhizosphere of the sub-clone 33.

Key words [two partly modified insect-resistant genes](#) _ [triploid hybrid of Chinese white poplar](#) _ [Agrobacterium](#) _ [rhizosphere](#)

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通讯作者 杨敏生 dueloo@yahoo.com.cn