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植物遗传学

水稻双元细菌人工染色体载体系统转化体系的建立

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摘要

普通双元载体已被广泛应用于农杆菌介导的植物转化，但这类载体通常只能转移5～20 kb的外源DNA片段，而双元细菌人工染色体（BIBAC）载体可以容纳普通双元载体的不足，通过它已在烟草、番茄等双子叶植物中实现了大片段DNA（150 kb）的转移。BIBAC载体在单子叶植物转化中的应用尚未见报道。由于单、双子叶植物间以及大、小片段转化间的转化体系存在明显差异，常规的农杆菌介导的水稻转化体系不能适应BIBAC系统转化的要求。因此，建立适于BIBAC系统的水稻转化体系是十分必要的。通过比较不同的受体材料，不同的去除农杆菌及选择抗性愈伤的方式等对转化效率的影响，建立了适合水稻BIBAC系统的转化体系。该体系的技术要点包括：以水稻品种H1493为转化受体，以含毒性辅助质粒pCH32的LBA4404菌株(HP4404)为侵染菌株。预培养的培养基pHL 6；UNA代替AM基浮农杆菌；侵染菌液浓度为OD600=1.0；共培养温度为21℃；采用过渡（Resting）培养除去农杆菌；采用二步法进行选择等。基于PCR检测、Southern印迹分析的结果表明，BIBAC载体所携带的插入片段及标记基因已整合到转化植株的基因组中。这个体系的建立为在水稻中利用BIBAC系统进行大片段DNA转化奠定基础。

关键词

水稻; 农杆菌; 双元细菌人工染色体; 植物大片段DNA转化

分类号

Development of Transformation System of Rice Based on Binary Bacterial Artificial Chromosome (BIBAC) Vector

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Abstract

<P>An Agrobacterium-
mediated transformation protocol using binary bacterial artificial chromosome (BIBAC) vector system in rice (Oryza sativa L.) was developed. Calli derived from mature embryos of japonica rice cv. H1493 were used as target tissues. Various aspects in transformation and regeneration processes including callus induction and culture, Agrobacteriu
m concentration and duration of co-cultivation, bacterial elimination and transformant selection were examined in order to improve the transformation efficiency. An optimized transformation conditions was established including: using an Agrobacterium strain, LBA4404(HP4404), which carries a super-
virulent helper plasmid pCH32, for the infection; a modified N6 medium system for callus induction and culture; pH 5.6 for media in pre-cultivation and co-cultivation; Agrobacterium concentration at OD600 = 1.0 for 3 days co-
cultivation and 7 days for a resting period of the infected calli. Based on PCR and Southern blot analysis, it was demonstrated that insert DNA and marker genes carried by BIBAC2 were integrated into the rice genome.</P>

Key words

Agrobacterium tumefaciens; binary bacterial artificial chromosome (BIBAC); rice (Oryza sativa L.); plant transformation of large DNA fragment

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