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植物诱变育种·农业生物技术

农杆菌介导球孢白僵菌转化体系的建立及突变体筛选

杨丽荣, 全鑫, 黄莹, 梁慎, 薛保国

1. 河南省农业科学院植物保护研究所/ 河南省农作物病虫害防治重点实验室, 农业部华北南部作物有害 生物综合治理重点实验室, 河南 郑州 450002;
2. 河南省农业科学研究院园艺研究所, 河南 郑州 450002

摘要： 以球孢白僵菌(*Beauveria bassiana*)YB-06为受体菌株, 采用农杆菌介导的遗传转化方法,实现了农杆菌AGL1(pATMT1)介导的球孢白僵菌的遗传转化,并研究了转化介质和pH对转化效率的影响。结果表明:在采用28℃、200μmol/L的乙酰丁香酮(AS)、农杆菌浓度OD_{600}=0.8、孢子浓度为1×10^6个/ml、pH5.1~5.8时可实现T-DNA的插入转化。当转化体系pH5.3时,转化效率最高,达586个转化子/10^6分生孢子;不同共转化介质对转化效率影响明显, 玻璃纸和硝酸纤维素膜转化效率较高。对3000个转化子生长速度和产孢量等性状分析获得46个性状特异突变体。本文结果为进一步研究球孢白僵菌的生长发育、致病机理和毒力相关基因功能奠定了基础。

关键词： 球孢白僵菌 农杆菌AGL1 T-DNA插入转化 转化效率

GENETIC TRANSFORMATION OF *Beauveria bassiana* USING *Agrobacterium tumefaciens-MEDIATED* TRANSFORMATION AND MUTANTS SCREENING WITH SPECIAL TRAITS

YANG Li-rong, QUAN Xin, HUANG Ying, LIANG Shen, XUE Bao-guo

1. Plant Protection Institute, Henan Academy of Agricultural Science, Henan Key Laboratory for Control of Crop Diseases and Insect Pests, IPM Key Laboratory in Southern part of North China for Ministry of Agriculture, Zhengzhou, Henan 450002;
2. Institute of Horticulture, Henan Academy of Agricultural Sciences, Zhengzhou, Henan 450002

Abstract: In this study, *Beauveria bassiana* YB-06 was successfully transformed using *Agrobacterium tumefaciens-mediated* transformation approach, and T-DNA insertion mutants were further obtained. The effects of pH and cultivation media on genetic transformation were studied under the condition of 28℃, 1×10^6 spores/ml, *A. tumefaciens* (AGL1) OD_{600}=0.8, 200μmol/L acetosyringone and 48h co-cultivation in the presence of induction medium. The results showed that the transformants could be obtained in specific pH value ranging from 5.1 to 5.8 and the transformantion efficiency reached the highest level at pH5.3 with about 586 transformants per 10^6 spores. The results also revealed that transformation efficiency was higher on cellophane and NC millipore filter compared to filter paper. HPH^+ resistance and PCR assay of the transformants showed that the T-DNA had been successfully integrated into the genome of the fungus. A few mutants were identified by colony growth, colony color, and conidium output of the 3,000 transformants. Altogether, this transformation system provided a basis for the study of development, pathogenicity mechanism and functional genetics study of the fungus.

Keywords: *Beauveria bassiana* *Agrobacterium tumefaciens* AGL1 T-DNA insertion transformation transformation efficiency

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通讯作者: 薛保国(1957-),男,河南汝南人,博士,研究员,研究方向为微生物分子生物学研究。Tel: 0371-65852150;E-mail: xuebbb@gmail.com

作者简介:

作者Email: xuebbb@gmail.com

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