Optimization of Factors Governing Agrobacterium-mediated Transformation of the Egyptian Tomato Cultivar (Edkawy)

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ABSTRACT

A reproducible transformation system for the Egyptian tomato cultivar (Edkawy) using Agrobacterium tumefaciens and cotyledon explants was developed. PBI 121 construct, in which the selectable marker gene β-glucuronidase (Gus) was replaced by nopaline synthase-phosphinothricin acetyl transferase (bar) was introduced into tomato cotyledon explants using the Agrobacterium tumefaciens strain LBA4404. Transformed explants were firstly selected on kanamycin and secondly on BASTA herbicide containing media. Regeneration of transformed shoots via organogenesis was achieved on MS medium containing 2 mg/l zeatin, 0.2 mg/l IAA and 50 mg/l kanamycin. Factors governing the efficiency of Agrobacterium-mediated transformation, including origin of explants, bacterial concentration, co-cultivation time, explants wounding and inclusion of acetosyringone were optimized. PCR analysis was used to confirm the insertion of neomycin phosphotransferase II gene (nptII) into tomato genome.

Key words: Tomato, transformation, tissue culture

INTRODUCTION

omato (Lycopersicon esculentum L.) ranks foremost among the important crops in the world. vegetable Egyptian farmers in Edco city, El-Behera governorate, Egypt, using traditional selection. developed tomato (Edkawy), which showed superiority in salt tolerant. The productivity and value of this cultivar could be greatly increased by the introduction of stably inherited traits such as resistance, herbicide and drought resistance. In addition, several workers are trying to improve tomato fruit quality by genetically engineering them to contain more solids, be thicker, and to contain more lycopene, which provide the red color. These traits are not available in the cultivar Edkawy. Development of A. tumefaciens-based transformation system for Edkway could contribute in the genetic improvement of this cultivar by allowing the introduction of useful genes. Agrobacterium-mediated transformation of various tomato cultivars has been reported (for review see Hamza and Chupeau, 1993). A limitation in the use of this transformation system is that it is usually genotype-dependent.

In this paper, the best conditions for the production of transformed tomato plants using Agrobacterium are reported. The procedure has been used to introduce the antibiotic and herbicide resistance genes and the successful recovery of transformed plants.