A Rapid and Highly Efficient Method for Transformation of Sugarcane Callus

Dwi Andreas Santosa,[*,1] Roy Hendroko,[2] Abdelazim Farouk,[3] and Ralf Greiner[4]*

Abstract

Modern sugarcane cultivars have complex genetic characteristics and low fertility that render their genetic improvement through traditional breeding difficult. Genetic engineering methodology to introduce foreign genes provides new opportunities for the genetic improvement of sugarcane cultivars. One of prerequisites for successful insertion of a gene cassette into the plant genome is the availability of an efficient transformation protocol. An improved protocol for *Agrobacterium*-mediated transformation of sugarcane is described. Between 85 and 100% of calli transformed using this procedure produced new calli, and 100% of them were positive for the inserted gene. The whole procedure permitted the production of transgenic calli in a short time (1.5 mo). The transformed calli can be cultured further for the production of the inserted gene-encoded enzyme by using cell culture, or they can be regenerated into transgenic plants. This protocol may be implemented also for the generation of transgenic plants from other species.

Index Entries: Agrobacterium; regeneration; sugarcane; transformation; transgenic plant.

1. Introduction

Sugarcane is cultivated in large areas in Indonesia and other tropical and subtropical countries and annually provides 60 to 70% of sugar worldwide *(1)*. Most modern sugarcane cultivars are interspecific hybrids, have complex genetic characteristics, and low fertility. This makes genetic improvement through traditional breeding difficult and makes these cultivars prime candidates for improvement through genetic engineering *(2)*. A few authors have reported the successful transformation of sugarcane by electroporation *(3)*, *Agrobacterium*-mediated transformation *(4)*, and particle bombardment *(2,5)*. Apart from these, there are no other reports on successful genetic transformation of sugarcane.

Direct transformation procedures, such as particle bombardment and electroporation, have several disadvantages because of poor reproducibility, variable transgene copy number, and the instability of the gene construct in the new host. These limitations make *Agrobacterium*-mediated transformation an increasingly common procedure for transferring foreign genes into plants, especially for monocot plants *(4,6–11)*.

First, we tried to transform meristematic explants and callus in the presence of antioxidants to prevent necrotic processes by the method described previously *(4,12)*, but after 1.5 yr of efforts, we got neither transformed callus nor plants. *Agrobacterium* growing on the explants or callus could not be eliminated completely, and the tissue became necrotic and died. This led us to develop another pro-

*Author to whom all correspondence and reprint requests should be addressed. [1]Department of Soil Science, Faculty of Agriculture, Research Center for Biotechnology, Research Center for Environment, Bogor Agricultural University, Indonesian Center for Biodiversity and Biotechnology, Bogor, Indonesia. E-mail: dsantosa@indo.net.id; [2]PT Rajawali Nusantara Indonesia, RNI Bldg., Jl. Denpasar Raya Kav. DIII, Kuningan, Jakarta 12950, Indonesia; [3]International Islamic University Malaysia, Kulliyyah of Engineering, Department of Biotechnology Engineering, Jalan Gombak, 53100 Kuala Lumpur, Malaysia; [4]Federal Research Center for Nutrition and Food, Center for Molecular Biology, Haid-and-Neu-Strasse 9, 76131 Karlsruhe, Germany.