TRANSGENIC SUGARCANE (Saccharum officinarum L.) PLANTS ARE TOLERANT TO STEM BORER (Diatraea saccharalis F.) ATTACK DESPITE THE LOW EXPRESSION LEVELS OF cryIA(b) GENE FROM B. thuringiensis var. kurstaki HD-1

Ariel Arencibia, Roberto Vázquez, Dmitri Prieto, Elva R. Carmona, Pilar Téllez, Alberto Coego, Lázaro Hernández, Gustavo de la Riva and Guillermo Selman-Housein

Plant Division, Centre for Genetic Engineering and Biotechnology, P.O. Box 6162, C.P. 10600, Havana, Cuba.

Introduction

Sugarcane (*Saccharum officinarum L.*) is a widely spread and economically important monocot plant. Sugarcane stem borer (*Diatraea saccharalis F.*) is the most important pest of this crop, causing agricultural and industrial yield losses estimated in more than 200 million dollars annually for the producers of American continent (1). *Bacillus thuringiensis* is a Gram-positive bacterium producing a parasporal body during sporulation mainly composed by delta-endotoxins, which are active against several insects. The truncated gene *cryIA(b)* encode for active region of CryIA(b) delta-endotoxin from *B. thuringiensis* var. *kurstaki* HD-1 was expressed under the control of CaMV 35S promoter (2) in transgenic sugarcane plants transformed by intact cell electroporation. The levels of recombinant toxin in transgenic plants were established and biological activity test were performed against neonate sugarcane stem borer larvae. Transgenic sugarcane plants showed remarkable larvicidal activity despite the low expression level of *B. thuringiensis* delta-endotoxin gene *cryIA(b)*.

Materials and Methods

Sugarcane cell suspension from Cuban commercial variety Ja 60-5 were transformed by intact cell electroporation (3). The biological activity of putative transgenic plants was assayed "*in vitro*". Two stem borer larvae (24 hours after hatch) were carefully feeded on 12 weeks old transgenic plants during 7 days. Infected plants were scored for plant damaged and larvae size. The determination of recombinant CryIA(b) toxin was established by immuno-radiometric assay (IRMA). Immunopurified anti-CryIA rabbit polyclonal antibodies were iodinated by the chloramine T method (4). Specific activity of iodinated antibodies were 8.3 mCi/mg determined in a Clini-Gamma-ô-counter (LKB Bromma, Sweden). IRMA assay was performed in microtiter plates (Titertek, FlowLaboratories, The Netherlands). Wells were coated with 100 µL of a solution of 10 mg/mL immunopurified antibodies in Carbonate-bicarbonate buffer pH 9.6 at 37 °C for 2 hours. Then, the plates were washed two times with PBST buffer and stored to 4 °C. In coated plates, 50 µL

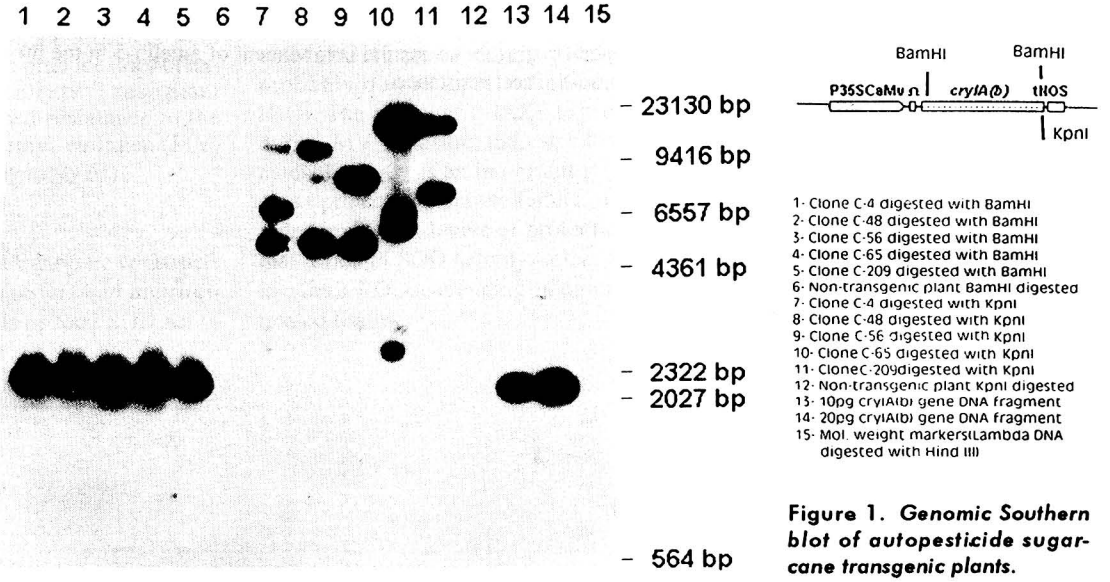

Figure 1. ***Genomic Southern blot of autopesticide sugarcane transgenic plants.***

Table 1. *Tolerance to borer attack and the level of recombinant toxin in transgenic plants.*

Clone	CrylA(b) toxin (ng/mg total protein)	Plant Damage
4	0.61	+
48	1.59	+
56	0.48	+ +
65	0.55	+ +
209	0.66	+
(-)Control	not detected	+ + + + +

(+) each represents 20 % of the total damage observed in negative control plant line.

neutralized extract (25 mg total soluble proteins from standard and samples) and $5x10^6$ cpm of iodinated polyclonal antibodies were mixed into each well and incubated at 4 °C for 12 hours. Then, the plates were washed five times and radioactivity bound to each well was determined after cutting each well from the plate.

Results and Discussion

By intact cell electroporation sugarcane transgenic plant lines of commercial variety Ja 60-5 were regenerated from transformed histochemically stained calli. By biological activity test 22 putative transgenic lines showed no significative plant damage and remarkable larvicidal activity. The selected plant lines were adapted to natural soil conditions and firstly in green house and then in experimental field conditions. After three months the transgenic plant lines were evaluated for their resistance to a super-infestation borer attack: two larvae per plant. Four weeks later were evaluated the number of dead stem hearts, the total number of stem segments and the total number of stem damaged. The expression in transgenic sugarcane plants is dramatically low because of the use of both unmodified bacterial *cryIA(b)* gene and CaMV 35S promoter in a monocot plant. Despite this fact, selected transgenic sugarcane plant lines are tolerant to borer attack (Figure 1, Table 1). However, higher expression levels are required for the effective control of this agronomically important pest. Taking into account these facts elite transgenic sugarcane plant lines are in progress.

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