

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

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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 Cline-Gamma-δ-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

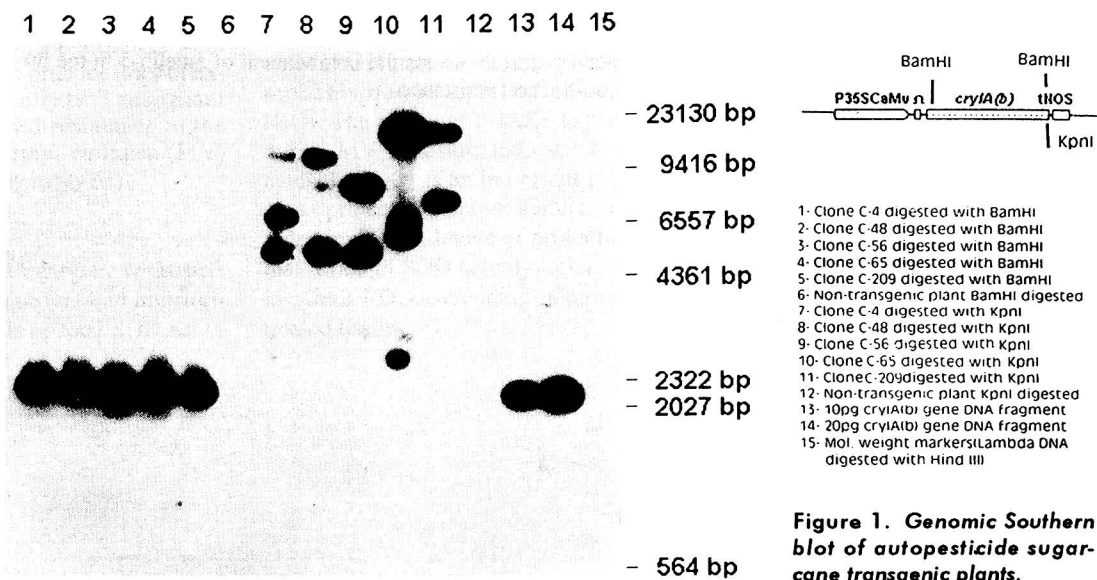


Figure 1. Genomic Southern blot of autopesticide sugarcane transgenic plants.

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

Clone	CryIA(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 5×10^6 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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2. Gutiérrez C et al., Biotecnología Habana'92. Book of Short Reports 1992;14.3.
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4. Greenwood F. Nature 1962;194: 495-496.



Curso Internacional Teórico-Práctico Inmunología y Patogénesis de *Neisseria meningitidis*

2-13 de Diciembre de 1996

Centro de Ingeniería Genética y Biotecnología,
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El Centro de Ingeniería Genética y Biotecnología (CIGB) se complace en invitarle a participar en el curso internacional teórico-práctico de inmunología y patogénesis de *Neisseria meningitidis*, que se impartirá del 2 al 13 de diciembre de 1996 en las instalaciones del CIGB, C. Habana, Cuba. Este curso (40 h de clases teóricas y 20 h de clases teórico-prácticas) pretende dar una visión actualizada del estado de las investigaciones en el tema de la meningitis meningocócica, así como de las técnicas de biología molecular avanzada que sirven de herramienta fundamental en este trabajo. Además, propiciará el intercambio de conocimientos y experiencias entre los investigadores dedicados a esta rama, fundamentalmente en los países de América Latina.

Los temas del curso comprenden, entre otros: microbiología clínica e inmunología; epidemiología y diagnóstico; biología molecular de los principales antígenos de la membrana externa; vacunas; modelos animales para la enfermedad meningocócica.

El curso tendrá una matrícula máxima de 30 estudiantes. La cuota de inscripción es de 620 USD, que asegura alojamiento por 12 noches, desayuno, almuerzo y comida, actividades sociales de bienvenida y clausura, así como el material didáctico del curso.

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