Molecular evaluation of *Bacillus thuringiensis* isolates from the soil and production of transgenic tomato plants harboring *Bt* gene for controlling lepidopterous insects in Egypt

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Abstract

This study has two main approaches. Firstly, search for indigenous *Bacillus thuringiensis* strains from Egyptian soils was made and the activity was evaluated against *Spodoptera littoralis*, and *Helicoverpa (=Heliothis) armigera*. Different techniques were adopted for molecular characterization of these isolates. In a second approach, transgenic tomato (cv. *Money maker*) over expressing *Bt* gene was produced using *Agrobacterium*-mediated transformation method. For this, a rapid and efficient regeneration system via direct shoot organogenesis has been developed. The developed tissue culture system was used for the production of transgenic plants. Several parameters affecting *Agrobacterium*-mediated transformation (bacterial densities, type of explants, co-cultivation durations and pre-culture periods) were optimized. Molecular analysis confirmed the expression and integration of the transgene into tomato genome. The potential of feeding larvae of *S. littoralis, H. armigera* and *Phthorimaea operculella* on *Bt* tomato transgenic plants was determined and the effect caused to the predator *Chrysoperla carnea* (Stephens) when fed on lepidopterous insects treated with transformed plants was determined. Histopathological effects caused after feeding on transformed tomato plants compared to those caused after treatment with *Bt* endotoxin were carried out using Transmission Electron Microscopy (TEM).

Key words: Isolation, *Bacillus thuringiensis*, *Agrobacterium*-mediated transformation, transgenic tomato harboring *Cry* 2Ab, lepidopterous insects, *Chrysoperla carnea*, Histopathology, Transmission Electron Microscopy (TEM).

List of Tables

List of Figures

List of abbreviations

g: gram.

Mg: Milligram (10-3 g).

µg: Microgram.

ml: Milliliter.

µl: Microliter.

mM: Milimolar.

pmol: Pico mole.

v/v: volume/volume.

w/v: weight/volume.

Da: Dalton.

Kb: kilobase.

bp: Base pair.

ha: Hectare.

min.: minute.

sec.: Second.

°C: Degree Centigrade.

rpm: Rotation per minute.

Bt: Bacillus thuringiensis.

Cry proteins: Crystal proteins.

GMO: Genetically Modified Organisms.

GM: Genetically Modified.

EPA: Environment Protection Agency.

FAO: Food and Agriculture Organization of the United Nations.

ISAAA: International Service for the Acquisition of Agri-biotech Applications.

LB medium: Luria-Bertani medium.

PCR: Polymerase Chain Reaction.

RAPD: Random amplified polymorphic DNA.

T-DNA: Transferred DNA of *Agrobacterium*.

Ti-plasmid: Tumor-inducing plasmid of *Agrobacterium.*

DNA: Deoxyribonucleic acid.

dNTP: Deoxyribonucleoside triphosphates.

CTAB: Cetyl trimethyl ammonium-bromide.

EDTA: Ethylenediamineetetraacetic acid.

Tris: Tris-(hydroxymethyl)-aminomethan.

TE: Tris EDTA.

SDS: Sodium dodecyl-sulphate.

Acetosyringone: 3`,5`-Dimethoxy-4`-Hydroxyacetophenon.

X-gluc: 5-Bromo-4-Chloro-3-Indolyl-β-D-Glucuronide.

GUS: β-glucuronidase protein from *E. coli.*

35S: 35S promoter of CaMV.

nos: Gene encoding nopaline synthase.

nptII: Gene encoding neomycin phosphotransferase type II protein from *E. coli.*

CaMV: Cauliflower mosaic virus.

uidA: Gene encoding β-glucuronidase protein from *E. coli.*

hpt: Hygromycin phosphotransferase-gene.

MS: Murashige and Skoog salt mixture.

B5 vit: Gamborg's vitamine mixture.

BAP: 6-Benzylaminopurine.

FDA: Food and Drug Administration.

Kan: Kanamycin.

APS: Ammonium Persulphate.

cfu: colony formation unit.