OVERPRODUCTION OF THE CRY2A BT PROTEIN IN TOBACCO CHLOROPLASTS CONFERS PLANT RESISTANCE TO SUSCEPTIBLE AND BT-RESISTANT TOBACCO BUDWORM, COTTON BOLLWORM AND BEET ARMYWORM

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Abstract

Evolving levels of resistance in insects to the bioinsecticide *Bacillus thuringiensis* (Bt) can be dramatically reduced through the genetic engineering of chloroplasts in plants. When transgenic tobacco leaves expressing Cry2A protoxin in chloroplasts were fed to susceptible, Cry1A resistant (20,000-40,000 fold), and Cry2A resistant (330-393 fold) tobacco budworm, *Heliothis virescens*, cotton bollworm, *Helicoverpa zea*, and the beet armyworm, *Spodoptera exigua*, 100% mortality was observed against all insect species and strains. Cry2A was chosen for this study because of its toxicity to many economically important insect pests, relatively low levels of cross resistance against Cry1A-resistant insects, and can be expressed as a protoxin instead of a toxin because of its relatively small size (65 kDa). Southern blot analysis confirmed stable integration of *cry*2A into all of the chloroplast genomes (5,000-10,000 copies per cell) of transgenic plants. Transformed tobacco leaves expressed Cry2A protoxin at levels between 2 and 3% of total soluble protein, 20-30 fold higher levels than current commercial nuclear transgenic plants. These results suggest that plants expressing high levels of a non-homologous Bt protein should be able to overcome or at the very least, significantly delay, broad spectrum Bt resistance development in the field.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 2:926-926 (1999)
National Cotton Council, Memphis TN