Transformation of Eggplant with Synthetic *cryIIIA* Gene Produces a High Level of Resistance to the Colorado Potato Beetle

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ABSTRACT. A population of 300 putative transgenic eggplants (*Solanum melongena* L.) carrying the *syn cryIIIA* gene was produced and tested for resistance to the Colorado potato beetle [CPB; *Leptinotarsa decemlineata* (Say)]. Toxicity tests *in planta* and *in vitro* demonstrated that 69% of the transformed plants were resistant to neonate larvae and adult CPB. Transgenic plants were confirmed by studies of GUS expression and Southern and northern analysis. Primary transformants, having a single insert of the construct, upon selfing, produced progenies cosegregating for the *uidA* and *syn cryIIIA* genes at the expected 3:1 ratios with a few exceptions in which only one of the genes was expressed. The latter was attributed to the gene silencing phenomenon. The segregating resistant R₁ seedlings showed the same level of resistance as the parental genotypes in growth chamber tests and under field conditions. One genotype carrying two copies of the construct, upon selfing, segregated at a 15:1 ratio for GUS expression and resistance to CPB, while Southern analysis revealed a 9:3:3:1 genotypic segregation ratio for individual copies of the construct.

Eggplant (*Solanum melongena* L.) is an important crop species in Europe, Asia, and North America. However, in some parts of the world its commercial production is frequently hampered by devastating attacks by the Colorado potato beetle (CPB; *Leptinotarsa decemlineata*) (Cotty and Lashomb, 1981). In the absence of an effective pest control program, CPB may cause total destruction of eggplant crops. Chemical pesticide application is costly to farmers and damaging to the environment (Dulmage, 1980). The native *cryIIIB* gene encodes a protein in spores of *Bacillus thuringiensis* (*Bt*), which is toxic to certain coleopteran insects, including CPB (Donovan et al., 1988). Spore preparations containing the delta endotoxin of *cryIIIB* have been used for a number of years as a biological pesticide to control CPB on various crops (Aronson et al., 1986). In an attempt to produce eggplant resistant to the CPB, we first used the native *cryIIIB* *Bacillus thuringiensis* gene for transformation (Chen et al., 1995). It was found that this gene was poorly expressed when incorporated into eggplant. Other researchers have reported similar results with different plant species (Adang et al., 1987; Murray et al., 1991). Experimental evidence suggests that the transcription process of the native *Bt* genes in certain plant cells generates, instead of entire transcripts, fragmented mRNA, which is nonfunctional in the translation process (Murray et al., 1991; van Aarssen et al., 1995). In these instances the lack of CPB resistance in transgenic plants is directly related to

the absence of toxic protein synthesis in the cells.

Analysis of the coding sequences of a number of native *Bt* genes has revealed structural features not found in most plant genes (Adang et al., 1993; Perlak et al., 1991; Sutton et al., 1992). For instance, along the coding region of the native *Bt* gene there are frequent stretches of multiple As and Ts. In addition, the GC content of the native *Bt* gene is much lower than in plant genes. The polyadenylation signal sequence AATAAA of plant genes and the mRNA destabilizing ATTTA sequence is often found in the middle of the coding region. Also, the codon use of the native *Bt* gene is different from many plant genes. Modification of sequences in the native *Bt* genes by nucleotide substitution was undertaken with the goal of making the *Bt* coding sequence more similar to plant gene coding sequences. Perlak modified the lepidopteran *cryIA(b)* and *cryIA(c)* *Bt* genes by changing 3% and 20%, respectively, of the nucleotides (Perlak et al., 1990, 1991). Compared to the corresponding native genes, the toxic protein production from the synthetic *Bt* genes in transgenic cotton and tomato was increased. Modified versions of the coleopteran *cryIIIA* gene have been engineered by Sutton et al. (1992) and Adang et al. (1993), who changed the coding sequence by 17% to 11% respectively. The modified genes enhanced production of the toxic protein in tobacco (Sutton et al., 1992) and potato cells (Adang et al., 1993), enhancing their resistance to a representative insect pest. Recently, it has been reported that modified *cryIA(b)* and *cryIC* genes were highly expressed in tomato (van der Salm et al., 1994), *syn cryIA* in corn (Armstrong et al., 1995), and *syn cryIA(b)* in rice (Wunn et al., 1996). These results convincingly demonstrated that recovery of a high level of pest resistance in plants could be achieved by using modified *Bt* genes rather than native ones. The objective of our research was to transform eggplant with the synthetic *cryIIIA* gene from *Bacillus thuringiensis* var. *tenebrionis* (*Btt*) and produce plants resistant to CPB.

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Materials and Methods

ENGINEERING THE CHIMERIC *syn cryIIIA* CONSTRUCT. The coding sequence of the *syn cryIIIA* gene (Sutton et al., 1992) was excised by restriction with *Bam* HI and *Kpn* I from plasmid pSP73 (Promega, Madison, Wis.) and, by blunt-end ligation, inserted into the *Pst* I site of plasmid pCaMVCN (Pharmacia, Piscataway, N.J.) with a deleted CAT gene. This insertion placed the coding sequence of the *syn cryIIIA* gene between the CaMV 35S promoter at its 5' terminus and the NOS terminator at its 3' terminus. Restriction with *Hind* III excised this chimeric fragment, which was then inserted at the *Hind* III site of pBI121 (Clontech, Palo Alto, Calif.), between the NPTII selectable and *uidA* reporter markers (Fig. 1A). This plasmid (pSB1) was transferred into *Agrobacterium tumefaciens* LBA4404 (Clontech) and used in the transformation of eggplant.

TRANSFORMATION, REGENERATION, AND TRANS-GENICITY ASSAY. Establishment of eggplant stock plants and the transformation and regeneration protocols from leaf segments have been described (Billings et al., 1997). Young leaves of putative transformed plants were assayed for GUS as described by Jefferson (1987).

SOUTHERN AND NORTHERN BLOT ANALYSIS. Isolation of DNA and RNA, their electrophoresis and blotting were carried out as described previously (Chen et al., 1995). Two different probes were used in filter hybridization: a 1.8-kb *syn cryIIIA* fragment excised from pSP73 with *Kpn* I and *Bam* HI and a 1.8-kb coding sequence of the GUS reporter gene restricted from pBI221 (Clontech) with *Sst* I and *Xba* I. The probes were DIG-labeled with the nonradioactive random-primed Genius method (Boehringer Mannheim, Indianapolis). Hybridization and detection was carried out with the Genius method.

WESTERN BLOT ANALYSIS. Protein was extracted by grinding 2 g of leaf tissue in liquid nitrogen and then resuspending in 4 mL extraction buffer as described (Kozziel et al., 1993). After centrifugation, protein concentration in the supernatant was determined, and 100 µg of each sample was electrophoresed on a SDS denaturing gel. Electrophoresis and blotting was carried out according to Maniatis et al. (1989). The *cryIIIA* antibody was supplied to us by Ecogen (Philadelphia) and detection and quantification of the protein was carried out with an Immun-blot assay (AP) kit from Bio Rad (Hercules, Calif.).

TOXICITY TEST IN PLANTA. Vegetative propagules of the transgenic and control plants were planted in 15-cm² pots and grown in a growth chamber with a photoperiod of 16 h light (300 µmol·m⁻²·s⁻¹) at 27 °C and 8 h dark at 22 °C. When the plants reached the four to five leaf stage of growth and development, a CPB egg mass, with ≈20 to 30 eggs, was placed on the basal leaf of each plant and fixed with a small dab of petroleum jelly. Hatching, development, and feeding of the larvae was checked on a daily basis. Resistance was determined by recording the mortality of the larvae and the stage of their development 10 d after hatching. The level of damage to leaves of individual eggplants after a period of 10 d was assessed by visual evaluation. In addition, damage to transgenic plants from mature larvae and adult CPB, raised on control eggplants, was

Fig. 1. Structure of pSB1 construct and Southern blot analysis of primary transformants. (A) The *syn cryIIIA* gene under control of 35S promoter and NOS polyadenylation sequence inserted at the *Hind* III site in pBI121. (B) *Hind* III restriction of genomic DNA from 13 transgenic plants and one control. Each phenotypic group is represented: Lanes 1–4, G+R+; lane 5, G–R–; lanes 6–9, G+R+; lanes 10–11, G+R–; lanes 12–13, G–R+; lane 14, control. Plant DNA was probed with a DNA DIG-labeled 1.8-kb *syn cryIIIA* fragment. C. *Hind* III restriction of plant DNA, same order as above, but probed with a DNA DIG-labeled 1.8-kb *uidA* fragment.

assessed. Feeding habits and viability of the insects on both transgenic and control eggplants was observed daily.

TOXICITY TEST IN VITRO. One or two vigorously growing leaves of each plant were excised and the petiole was inserted into a piece of moist root cube and arranged in an insect rearing box. Ten first and/or second instar larvae of CPB were preweighed together and placed on a leaf in each of the insect rearing boxes. There were five replications for each plant genotype. After 6 d the larvae were weighed again. Mortality of the larvae was also recorded. Data was analyzed via ANOVA and means separation using the Ryan-Ernst-Gabriel-Welsch multiple F test (SAS Institute, Cary, N.C.).

PROGENY TESTS. The primary transgenic plants (R_0) were selfed and seed of these plants were germinated in 2 sphagnum peat : 1 vermiculite (by volume) mix in plastic grow cells under mist. After development of two primary leaves, the seedlings (R_1) were transplanted into 7-cm² pots and grown in the growth chamber. In a similar way, seedlings from nontransgenic plants were produced. The individual seedlings were tested for GUS activity and resistance to the CPB as described above (toxicity test in planta).

Heterozygous S_1 progeny from six primary transformants (Btt5, Btt16, Btt38, Btt55, Btt90, Btt204) were compared in the field with

Table 1. Southern analysis of the four classes of transgenic eggplants.

Phenotype	<i>Btt</i> Probe			<i>Uida</i> Probe							
	Plants tested (no.)	Results		Plants tested (no.)	Results		Inserts (no.)				
		+	-		+	-	0	1	2	3	≥4
G+R+	23	23	-	22	22	-	-	12	9	1	-
G+R-	7	7	-	6	6	-	-	1	3	1	1
G-R+	7	7	-	7	6	1	1	4	1	-	1
G-R-	6	3	3	5	2	3	3	-	1	-	1

nontransgenic plants at the Rutgers Research and Development Center, Bridgeton, N.J., in Summer 1996. Nontransgenic controls were either a) untreated or b) treated with the insecticide imidacloprid (Admire 2F, Provado 1.6F; Bayer, Kansas City, Mo.) applied at 34.4 g·ha⁻¹ (a.i.) as a pot drench in the greenhouse 2 weeks before planting and again on 12 June 1996 at 0.09 g·L⁻¹ (a.i.) using a hand sprayer. All plants (240 plants/treatment) used in the study were seeded into 15-cm pots on 9 Apr. 1996, grown under standard light, temperature, water, and fertilization conditions in the greenhouse, and then hand transplanted into the field on 30 May 1996.

A randomized complete-block design was used in the study. Each block consisted of 8 rows (one row per treatment) of 12 plants using a row spacing of 0.91 m between plants and 4.55 m between rows (total row length, 10.8 m) and was replicated 20 times. To encourage the development of CPB populations in the study plot, eight rows of 'Superior' potatoes (two rows on each exterior border of the plot; four additional rows mid plot) were seeded 5 weeks before planting (25 Apr. 1996) the eggplant transplants. Adult beetles, obtained from a colony maintained by the New Jersey Dept. of Agriculture's Phillip Alampi Biological Control Laboratory, were also released weekly into the potatoes between 20 June to 24 July.

Sampling of the different treatments was conducted weekly between 27 June and 31 July 1996. On each sample date, the total number of beetles (larvae and adults) and harvestable fruit was determined for each of the 10 interior plants within a row and recorded. All data were transformed using $\sqrt{x+1}$ (Snedecor and Cochran, 1978) and analyzed using analysis of variance (SAS). Means for the total number of beetles and number of fruit per plant were separated by a least significant difference test (LSD).

Results

CATEGORIZATION OF PRIMARY TRANSFORMANTS.

A total of 300 putative transformants was regenerated from the eggplant explants through tissue culture. When subjected to the GUS test, 185 tested positive (62%) and 115, negative (38%). Toxicity tests *in planta* with CPB were conducted on 146 GUS-positive, and 23 GUS-negative plants with the expectation that the latter could serve as negative controls. Results from these two tests revealed four phenotypically different categories of primary transformants: most (110% or 65%) were GUS-positive and CPB-resistant (G+R+); 36 (21%) were GUS-positive and CPB-susceptible (G+R-); 7 (4%) were GUS-negative and CPB-resistant (G-R+); and 16 (10%) were GUS-negative and CPB-susceptible (G-R-). Initially, the last two groups were considered to represent "escapees" that occurred during the transformation process but the CPB

bioassays and Southern analysis revealed that most of the plants from this latter group were transformants.

SOUTHERN ANALYSIS. A *Hind* III digest of DNA from transgenic plants, probed with *syn cryIIIA*, was expected to produce a single band at the 2.5-kb position, while the same digest, probed with *uidA*, was expected to produce different sizes and numbers of bands depending on the numbers of insertions (Fig. 1A). To verify transgenic status of the putative transformants, 43 plants representing the 4 phenotypic categories described above were subjected to Southern analysis (Table 1). The *cryIIIA* gene was present in 40 out of 43 and the *uidA* in 36 out of 40 tested plants, proving transgenic status of most of these plants. A sample of the analyzed plants, displaying the Southern profiles for *syn cryIIIA* and *uidA*, is shown in Fig. 1 B and C. In the first category (G+R+) the phenotypic expression of *syn cryIIIA* and *uidA* genes was directly correlated with their presence in the Southern blots (genotypes Btt5, Btt10, Btt65, Btt25a, Btt13, Btt3, Btt77, Btt38). In the next two categories there was correlation of gene expression with either *uidA* (G+R-, genotypes Btt70 and Btt57) or *cryIIIA* (G-R+, genotype Btt53), while the corresponding linked gene, which had not been expressed, was also detected by Southern blot. An obvious exception was plant Btt88 in which the *uidA* gene had been deleted. In the fourth category (G-R-), three plants were found to harbor both genes, which were not expressed (i.e., Genotype Btt162) and three

Fig. 2. Northern analysis of total RNA from primary transformants. (A) RNA of 13 transformants and 1 control (Lanes 1-4, G+R+; lane 5, G-R-; lanes 6-9, G+R+; lanes 10-11, G+R-, lanes 12-13, G-R+; lane 14, control), probed with a DNA DIG-labeled *syn cryIIIA* fragment. (B) RNA of the same 14 genotypes probed with a DNA DIG-labeled *uidA* fragment.

Table 2. Segregation analysis of GUS expression and Colorado potato beetle (CPB) resistance (R = resistant, NR = nonresistant) in R_1 progenies of transgenic eggplants.

Primary transformant ²		Plants tested (no.)	GUS expression		Calculated χ^2	CPB assay		Calculated χ^2
Designation	Phenotype		+	-		R	NR	
10	G+R+	32	24	8	0	NT ³	NT	
16	G+R+	34	26	8	0	26	8	0
38	G+R+	36	31	5	1.81	31	5	1.81
55	G+R+	29	22	7	0.01	26	3	2.59
90	G+R+	36	23	13	1.81	24	12	0.93
204	G+R+	30	24	6	0.18	NT	NT	
53	G-R+	30	12	18	17.78 ^x	5	25	51.37 ^x
25a	G+R+	55	31	2	0.09	31	2	0.09

²The first seven progenies were tested for 3:1 and the last one for 15:1 segregation ratio.

³NT = not tested.

^xSignificant at <0.005 probability range.

plants that were actual escapees. The frequency of occurrence of transgenic plants containing various copies of the construct is shown in the right side of Table 1. As expected, most transgenic plants (72%) harbor one or two insertions of the construct. Out of 17 plants with one insertion, 70% expressed both genes; out of 14 plants with 2 insertions, 64% expressed both genes. Examples of the latter were genotypes Btt13 and Btt25a. Plants containing more than three insertions, such as genotype Btt162, did not express either of the genes. It was with Southern blot analysis, using the *uidA* probe, that clones which had originated from the same transformation event could be identified. One example is seen with genotypes Btt5 and Btt10, which were derived from the same leaf segment and had the same banding pattern. However, genotypes Btt25a and Btt162, which had also been derived from the same leaf segment, were determined to be individual transformants.

RNA AND PROTEIN ANALYSIS. To ascertain the expression of *uidA* and *syn cryIIIA* at the mRNA level, northern analysis of total RNA was performed (Fig. 2). As expected, a positive correlation was found between the phenotypic expression of the two genes (CPB resistance and GUS activity) and their expression at the mRNA level. Two genotypes were unusual: in Btt25a the *Btt* mRNA was not detected and yet the plant displayed resistance to CPB; in Btt162 the *uidA* mRNA was detected even though the GUS test had been negative. The *cryIIIA* protein was detected in variable amounts in all transgenic plants having a *syn cryIIIA* mRNA band (data not presented).

PROGENY TESTS. The stability of incorporation of the *syn cryIIIA* and *uidA* genes in the primary transformants (R_0) was further assessed by studying their transmission and segregation pattern in R_1 generations. For this purpose 23 primary transformants, which were randomly selected, were self-pollinated and all except two produced seed. The two sterile transgenic plants were later found to have multiple copy numbers of the construct (insert) in their genomes. Eight primary transformants, consistently resistant to CPB, were selected for progeny analysis. Seven of these contained one insert and one contained two inserts (Btt25a). The results of this study are summarized in Table 2. Upon selfing, the first six plants in the table transmitted both genes to $\approx 3/4$ of their progeny, an ex-

pected proportion for a dominant, single copy insert in the hemizygous transgenic plants. Regularity of the segregation for each gene in the R_1 generation is supported by low values of the calculated χ^2 . The cosegregational pattern of the *uidA* and *syn cryIIIA* genes reflect the contiguous position of these genes in the construct. In general, the seedlings showing GUS activity also displayed resistance to CPB. Discrepancy in cosegregation of the two genes in progenies of Btt55 and Btt90 is most likely due to seedling phenotype misclassification. The Btt53 genotype is unusual: although *syn cryIIIA* is expressed in the primary transformant (R_0), only a small proportion of the seedlings (R_1) displayed CPB resistance. In contrast, the *uidA* gene is not expressed in the primary transformant but $\approx 1/2$ of the progeny showed GUS activity. In progenies of the Btt25a genotype high numbers of CPB-resistant and GUS-positive seedlings were obtained. Since this genotype has two incorporated constructs, the ratio of 15:1, obtained from GUS analysis, for segregating duplicate hemizygous loci, indicates that the two inserts are on different homologous chromosomes. Progeny of genotype Btt25a were also analyzed for insert segregation at the DNA level (Fig. 3). Their pattern, when probed with the *uidA* fragment, followed a 9:3:3:1 ratio as indi-

Fig. 3. Southern blot analysis of 33 R_1 seedling offspring of transgenic genotype Btt25a. (A) *Hind* III restriction of genomic DNA probed with a DNA DIG-labeled 1.8-kb *syn cryIIIA* fragment. The segregation of *syn cryIIIA* follows a duplicate gene ratio of 15:1. (B) *Hind* III restriction of genomic DNA probed with a DNA DIG-labeled 1.8-kb *uidA* fragment. Segregation of the *uidA* demonstrates a dihybrid segregation ratio of 9:3:3:1. Lane 1 is DIG-labeled DNA marker; the next 33 lanes are individual seedling offspring of Btt25a. Lane sequence is the same for A and B.

Fig. 4. Transgenic eggplant (left) which is resistant and control eggplant (right) which is susceptible to Colorado potato beetle (CPB). Photo taken 10 d after hatching and feeding of CPB larvae.

cated by the calculated χ^2 value of 0.892. From the R_1 seedlings of genotypes Btt16 and Btt55 (G+R+), several homozygous genotypes have been isolated. All of their R_2 progenies were GUS-positive and will be used for future seed production and CPB-resistance studies in the field.

TOXICITY ASSAYS. A single egg mass placed on the adaxial side of a leaf lamina produced 20 to 30 first instar larvae. On resistant transgenic plants the larvae spread over the lamina, eating small amounts of tissue, causing the formation of barely visible lesions. In some cases they even produced a few minute holes. In four to six hours after hatching, they became immobilized, ceased feeding, and in 15 to 20 h appeared dead. All larvae died, which was evidence of a high level of resistance. When adults or mature larvae were raised on the control plants and then transferred onto resistant transgenic plants, they initially consumed a noticeable amount of leaf tissue. However, after a few hours, their feeding became sporadic; they appeared less mobile and would languish for days in the same place on the leaf lamina. They died on the plants within 5 to 7 d. In contrast, the control plants were defoliated completely in <10 d by developing larvae (Fig. 4). In a few primary transformants the resistance to CPB was assessed by following the growth rate and survival of the second-instar larvae on detached leaves. The weight of larvae raised on control plants was significantly higher than that of larvae raised on transgenic leaves (Fig. 5). As in the *in planta* tests, larval growth was arrested very early in development, and none survived after feeding on the *Btt* transgenic leaves.

The primary transformants, characterized as resistant to CPB by either test above, were field-planted in Spring 1995, to assess their resistance under conditions of natural infestation with CPB. Individual plants were rated for their general vigor and the severity of damage by CPB. Those plants with the best ratings were selected as parents for S_1 progenies which were evaluated for their horticultural values and particularly their resistance to CPB in a replicated trial, under field conditions, in Summer 1996. This showed that field populations of CPB (adults and larvae) were affected by the progenies tested (Table 3). Throughout the study significantly

fewer beetles ($P \leq 0.05$) were observed on the transgenic progenies when compared to untreated non-transgenic plants. In addition, on most sample dates, significantly fewer beetles ($P \leq 0.05$) were found on the transgenic plants than on control plants treated with imidacloprid. Overall, there were small differences amongst tested transgenic progenies in resistance to CPB. This resistance was manifested in higher numbers of fruit per plant in the heterozygous transgenic population in comparison to non treated controls (Fig. 6). These results indicate that a commercial eggplant variety, developed from the homozygous derivatives of the resistant seedlings, would provide a viable alternative to the control of CPB.

Discussion

A population of primary transgenic eggplants carrying the *syn cryIIIA* gene displayed resistance to CPB in 69% of the primary transformants. The biotoxicity assays of the resistant plants have demonstrated conclusively that the *syn cryIIIA* gene is expressed at a level that is toxic to the first or second instar larvae of CPB. The effect of the toxic protein is swift and consistent: 1) on the leaves of transgenic plants, all the neonate larvae died within a few hours after hatching and 2) the adult insects, raised on nontransgenic genotypes and transferred onto transgenic leaves languished within 8 to 10 d, causing minor damage to various plant parts on different transgenic plants before they die. The segregating R_1 resistant seedlings, tested in the growth chamber, showed similar levels and patterns of resistance under field conditions. This would strongly suggest that the observed resistance in the growth chamber tests, with laboratory-derived strains of CPB, reflected the sensitivity of the general, natural CPB population to the *syn cryIIIA* gene. The level of resistance in the studied progeny is adequate for controlling CPB under commercial conditions. It should be noted that from the field-tested progeny, $\approx 1/4$ were nonresistant segregants. However, the average survival of larvae and adult insects was still lower than in control seedlings. Homozygous nonsegregating genotypes isolated from the S_1 generation are presently being tested under field conditions, with the expectation that the level of resistance in these transgenic seedlings will be increased. A logical

Fig. 5. Comparison of mean weight gain (g) of 10 larvae, 10 d after feeding on transgenic (T101, T25a, T65) or control plant leaves kept in insect rearing boxes.

Table 3. Effect of transgenic and nontransgenic eggplant on Colorado potato beetle (CPB) densities.

Treatment	CPBs (no.)/plant ^z					
	27 June	3 July	10 July	17 July	24 July	31 July
Btt5	9.75 b	6.02 cd	1.45 c	0.82 c	0.48 bcd	0.24 bc
Btt16	6.15 bc	5.01 d	1.98 b	1.00 bcd	0.30 cd	0.31 b
Btt38	4.50 bc	11.94 a	2.18 b	0.88 bc	0.90 a	0.34 b
Btt55	7.70 bc	5.19 d	1.98 b	1.08 bc	0.33 bcd	0.56 a
Btt90	6.15 c	8.96 ab	1.94 b	0.70 d	0.17 d	0.15 c
Btt204	3.80 c	7.44 bc	2.08 b	1.08 bcd	0.48 bc	0.28 bc
Control-imi ^y	1.65 d	6.26 cd	3.24 a	1.17 b	0.47 abc	0.65 a
Control-nt ^x	14.75 a	10.03 a	4.25 a	1.75 a	0.66 ab	0.58 a

^zMeans within a column followed by the same letter are not significantly different at $P \leq 0.05$ (LSD); $n = 200$.

^yControl-imi = nontransgenic plants, treated with insecticide imidacloprid.

^xControl-nt = nontransgenic plants, not treated with insecticide.

followup to these studies is to select the most suitable (resistant) genotype for development of a management strategy, under commercial conditions, that would deter development of insect tolerance or resistance to the *syn cryIIIA* gene product (Mallet and Porter, 1992). Most importantly, it is clear that the expression of *syn cryIIIA* gene is higher than the previously tested native *cryIIB* gene in transgenic eggplant (Chen et al., 1995). Those results are in agreement with the expression of the same gene in tobacco and potato (Kemp, personal communication) and other modified *cryI* genes incorporated into various plant species (Armstrong et al., 1995; Van der Salm et al., 1994; Wunn et al., 1996).

The chimeric construct was found to be incorporated stably in all the transgenic plants studied and was transmitted through the sexual process in a regular manner, leading to Mendelian segregation ratios in all but one genotype (Btt53). The cosegregational pattern of the *uidA* and *syn cryIIIA* genes in the R_1 progenies indicates structural integrity of the construct in the derived plants. The genotypes having two constructs segregated for each construct according to an independent assortment pattern (Fig. 3), and

expression of the *syn cryIIIA* and *uidA* gene in genotype Btt25a demonstrated the expected ratio (Table 1). The failure to detect *syn cryIIIA* RNA of transformant Btt25a is most likely due to a low level of transcript in the cells. In some of the transgenic eggplants with a single copy of the construct, either the *uidA* or *syn cryIIIA* gene was not expressed in spite of the physical presence in the chromosomes. Currently, there is an abundant body of evidence that incorporated genes may be epigenetically silenced and then sometimes again reactivated (Fujiwara et al., 1993; Hobbs, 1990; Matzke and Matzke, 1993). Although various mechanisms have been suggested to account for this phenomenon, methylation of cytosine is most often considered to be the causal factor (Finnegan and McElroy, 1994; Ronemus et al., 1996). In one of our primary transformants (Btt53) the *uidA* gene was silenced in all stages of its development, while the progeny segregation ratio for this gene was $\approx 1:1$. Presently we are in the process of studying the nature of gene silencing in this plant's incorporated construct. Attempts are underway to identify transgenic eggplants in which the NPTII and *uidA* are permanently silenced and only the *syn cryIIIA* gene is expressed. This might ease public concern over the use of transgenic products containing products of alien genes other than *Bt*, since *Bt* products have been used in biological control of CPB for the past three decades and have not shown detrimental effects on humans or wildlife.

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Fig. 6. Effect of Colorado potato beetles on the yield of transgenic (Btt5, Btt16, Btt38, Btt55, Btt90, Btt204) and nontransgenic [control-imi (imidacloprid-treated); control-nt (not insecticide treated)] eggplant.

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