Variability of Bt expression among commercial transgenic cotton varieties in the United States

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ABSTRACT

During 1999-2002, field and laboratory studies were conducted to determine if all commercial transgenic Bt cotton varieties provided the same level of control against various Lepidopteran pests. Studies showed that certain varieties expressed significantly more toxin, which was correlated with insect performance. Segregation analysis indicated that the genetic background shows variability in expression in transgenic Bt cotton. It is anticipated that these studies may be beneficial for transgenic breeders to select the highest expressing varieties in addition to agronomic traits.

Introduction

Variations in overall expression levels of Cry1Ac (i.e. Bt) among Bollgard® cotton (event 531) have been correlated to survival levels in various Lepidoptera indicating that all cultivars of transgenic Bt cotton do not provide the same level of larval control for certain species (Adamczyk *et al.,* 2001; Kranthi *et al.,* this volume). Although genetic transformation events often modify the agronomics of the transgenic variety compared to the corresponding parental variety (e.g. plant maturity and mean height), few studies have been published that examine differential expression of Bt toxin among different plant parts and varieties (Olsen *et al.*, this volume; Fitt *et al*., this volume). Factors that have been proposed to influence the levels of expressed Bt among varieties are still not fully understood, but site-of-gene insertion and cultivar or parental background has been implicated (Sachs *et al.,* 1998). Furthermore, current varieties do not provide a high-dose to control intrinsically tolerant Lepidoptera (i.e. armyworms, loopers, and bollworms). Managing resistance to these insects may be further complicated by differential expression of Bt among plant parts and varieties that could create a spatial source for resistance to develop (Gutierrez *et al.,* 2003). The purpose of this research was to determine if differences in expression among Bollgard® varieties have genetic influences. These studies are much needed to determine if transgenic crops can be selected based on their plant-insect resistance traits (i.e. highest expression varieties) in addition to their agronomic traits.

Experimental procedure

To determine if expression differences among Bollgard® varieties are under genetic control, crosses were made from plants bred from distantly related backgrounds. A Stoneville Pedigree Seed Company variety (ST4691B) and a Delta and Pineland Company variety (PM 1218B/RR) were chosen to cross to two other varieties (NuCOTN 33B and DP 458 BR: Delta and Pineland Co.). Both NuCOTN 33B and DP 458 BR express significantly more Bt in all plant structures compared to other varieties grown in the mid-southern United States including ST4691B and PM 1218 BR (Adamczyk and Sumerford 2001; Figure 1). NuCOTN 33B and DP 458 BR's recurrent conventional variety was DP 5415. Reciprocal crosses were made between DP 458 BR x PM 1218 BR plants and NuCOTN 33B x ST4691B plants. Seeds from the F_1 crosses were then planted in the greenhouse and seeds from the F_2 generation were harvested. Parents, F_1 and F_2 crosses were planted on May 1, 2002 in field plots located in Stoneville, MS. Plots were arranged in a randomized complete block design with six genetic types and four blocks. The F_2 crosses had 3 plots in each block (i.e. an extra level of replication), while the parents and F_1 crosses only had one level of replication per block. All plots were maintained according to local management practices. The amount of Bt was quantified on a per plant basis by using a commercially available quantification kit (Envirologix, Inc., Portland, ME). A detailed Bt quantification procedure can be found at: http://www.insectscience.org/1.13.

Results

In these studies the expression differences among Bt varieties of Bollgard® are under simple genetic control. Significant differences in mean expression (ppm) were found among the crosses and parents (Tables 1 and 2). In addition, the variances for the F_2 crosses were much higher than the F_1 crosses and the parents (Tables 3 and 4). Further analysis indicated that the variance attributed to genetic factors was much higher than the variance attributed to environmental factors (Tables 5 and 6). Tests for dominance and epistasis were not significant for the NuCOTN 33B and ST4691B cross (Table 5); however, the initial test for dominance was significant for the DP 458 BR and PM 1218 BR cross (Table 6). Estimation on the number of genes conferring expression differences was calculated using a modification to the Castle and Wright formula suggested by Cockerham (Cockerham, 1986) . An estimation of a small number of genes was concluded for both crosses (NuCOTN 33B x ST4691B = 0.98; DP 458 BR x PM 1218 BR = 1.08).

Discussion/Conclusion

This initial study provides key information regarding genetic effects of expression of Bt among different varieties. Future work will involve planting progeny rows of additional F_2 crosses to determine if plants can be selected for the highest expressing individuals in a given population. Hopefully, these data will provide valuable insight on selecting the best plants in a transgenic breeding program.

References

- Adamczyk., J.J. Jr. and Sumerford, D.V. (2001). Po-

tential factors impacting season-long expression of Cry1Ac in 13 commerical varieties of Bollgard® cotton. *Journal of Insect Science*, **1**: 13. Available online: insectscience.org/1.13.

- Adamczyk, J.J. Jr., Hardee, D.D., Adams, L.C. and Sumerford, D.V. (2001). Correlating differences in larval survival and development of bollworms (Lepidoptera: Noctuidae) and fall armyworms (Lepidoptera: Noctuidae) to differential expression of Cry1Ac delta-endotoxin in various plant parts among commercial cultivars of transgenic Bacillus thuringiensis cotton. *Journal of Economic Entomology,* **94**: 284-290.
- Cockerham, C.C. (1986). Modification in estimating the number of genes for a quantitative character. *Genetics*, **114**: 659-664.
- Gutierrez, A.P., Ponsard, S. and Adamczyk, J.J. Jr. (2003). A physiologically based model of Bt cotton-pest interactions: I. bollworm-defoliator-natural enemy interactions. *Acta Oecologica*. (In review).
- Sachs, E.S., Benedict, J.H., Stelly, D.M., Taylor, J.F., Altman, D.W., Berberich, S.A. and Davis, S.K. (1998). Expression and segregation of genes encoding CryIA insecticidal proteins in cotton. *Crop Science,* **38**: 1-11.

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Table 1. *Expression differences for Bt in NuCOTN 33B x ST4691B.*

Genetic Type	No. Plants Tested	Mean (ppm) ± SE
NuCOTN 33B	20	9.15 ± 0.373
ST4691B	19	2.47 ± 0.259
NuCOTN 33B X ST4691B F1	18	5.67 ± 0.248
ST4691B X NuCOTN 33B F1	20	5.40 ± 0.299
NuCOTN 33B X ST4691B F2	78	6.00 ± 0.278
ST4691B X NuCOTN 33B F2	67	6.21 ± 0.325

Fixed Effects	blk
	genetic type (F=13.20; P<0.0001)
Random Effects	blk*genetic type=0
(Covariance Parameter Estimates)	plot (blk*genetic type)=0.46
	residual=4.39

Table 2. *Expression differences for Bt in DP 458 BR x PM 1218 BR.*

Genetic Type	No. Plants Tested	Mean (ppm) ± SE
DP 458BR	18	7.80 ± 0.444
PM 1218BR	19	3.14 ± 0.349
DP 458BR X PM 1218BR F1	20	4.08 ± 0.362
PM 1218BR X DP 458BR F1	20	4.20 ± 0.385
DP 458BR X PM 1218BR F2	74	4.02 ± 0.254
PM 1218BR X DP 458BR F2	78	4.77 ± 0.246
Fixed Effects	blk	
	genetic type (F=12.06; P<0.0001)	
Random Effects	blk*genetic type=0	
(Covariance Parameter Estimates)	plot (blk*genetic type)=0.32	
	residual=3.06	

Table 3. *Variance for genetic types involving NuCOTN 33B and ST4691B.*

Genetic Type	Variance
NuCOTN 33B	1.7798
ST4691B	0.9635
NuCOTN 33B X ST4691B F1	0.3479
ST4691B X NuCOTN 33B F1	1.2154
NuCOTN 33B X ST4691B F2	6.1238
ST4691B X NuCOTN 33B F2	7.2813
F-Value	64.53
P-Value	<0.0001

Table 4. *Variance for genetic types involving DP 458 BR and PM 1218 BR.*

Genetic Type	Variance
DP 458BR	2.0927
PM 1218BR	1.5939
DP 458BR X PM 1218BR F1	1.3818
PM 1218BR X DP 458BR F1	1.3401
DP 458BR X PM 1218BR F2	4.5795
PM 1218BR X DP 458BR F2	3.8257
F-Value	25.45
P-Value	<0.0001

Table 5. *Environmental and genetic influences, and dominance and epistasis for NuCOTN 33B and ST469 LB test.*

Covariance Parameter	Estimate	
Variance (environment)	1.112	
Variance (environment) + variance (genetic)	6.526	
Contrasts	F-value	P-value
Mean Parents vs Mean F1	1.02	0.3165
Mean Parents vs Mean F2	0.23	0.6508
Epistasis	0.53	0.5058

Table 6. *Environmental and genetic influences and dominance and epistasis for DP 458 BR and PM 1218 BR test.*

Covariance Parameter	Estimate	
Variance (environment)	1.513	
Variance (environment) + variance (genetic)	4.052	
Contrasts	F-value	P-value
Mean Parents vs Mean F1	22.58	<0.001
Mean Parents vs Mean F2	1.70	0.2401
Epistasis	0.29	0.6100

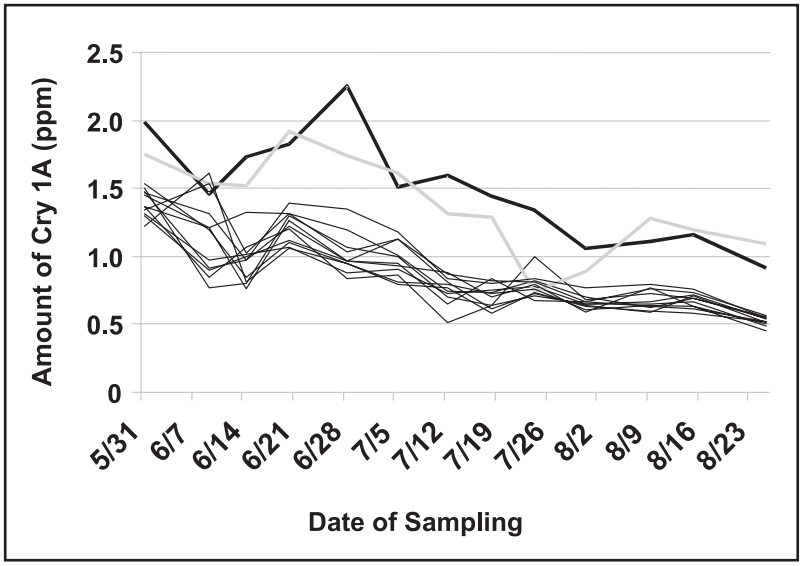

Figure 1.
Expression of Cry1A in terminal leaves throughout the growing season for 13 transgenic varieties. Thick black line, NuCOTN 33B; thick gray line, DP 458 BR; thin black lines, 11 additional Bt varieties including PM 1218 BR and ST4691B. (Reprinted from the Journal of Insect Science, 1.13. Available online: http://insectscience.org/1.13.)