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GENETIC BACKGROUND SHOWS VARIABILITY IN EXPRESSION OF TRANSGENIC BT COTTON

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Abstract

Research Summary

Variation 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. 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. Factors that have been proposed to influence the level of expressed Bt among varieties are still not fully understood, but site-of-gene insertion and cultivar or parental background has been implicated. Furthermore, by not providing a high-dose strategy to control the 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 temporal source for resistance to develop. The purpose of this research was to determine if differences in expression among Bollgard® varieties are under genetic control. 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. As with any foliar insecticide and herbicide, the efficacy of each variety must be determined to ensure the best recommendation to growers.

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 Co. variety (ST4691B) and a Delta and Pineland Co. variety (PM 1218B/RR) were chosen to cross to two other varieties (NuCOTN 33B and DP 458BR: Delta and Pineland Co.). Both NuCOTN 33B and DP 458BR express significantly more Bt in all plant structures compared to other common mid-south varieties, while both ST4691B and PM 1218BR have been shown to express significantly less Bt than NuCOTN 33B or DP 458BR (Figure 1). NuCOTN 33B and DP 458BR's recurrent conventional variety was DP 5415. Reciprocal crosses were made between DP 458BR X PM 1218BR plants and NuCOTN 33B X ST4691B plants. Seeds from the F1 crosses were then planted in the greenhouse and seeds from the F2 generation were harvested. Parents, F1 and F2 crosses were planted on May 1, 2002 in field plots located in Stoneville, MS. Plots were arranged in a randomized complete block design with 6 genetic types and 4 blocks. The F2 crosses had 3 plots in each block (i.e. an extra level of replication), while the parents and F1 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).

In these studies the expression differences in Bt among varieties of Bollgard® are under simple genetic control. Significant differences among expression means (ppm) were found among the crosses and parents (Tables 1 and 2.). In addition, the variances for the F2 crosses were much higher than the F1 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 epistatsis were not significant for the NuCOTN 33B and ST4691B cross (Table 5); however, the initial test for dominance was significant for the DP 458BR and PM 1218BR 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. An estimation of a small number of genes was concluded for both crosses (NuCOTN 33B X ST4691B = 0.98; DP 458BR X PM 1218BR = 1.08). There was no difference in reciprocal F1 and F2 populations for Bt expression.

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 F2 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.

Disclaimer

Mention of a commercial or propriety product does not constitute an endorsement by the U.S. Department of Agriculture for its use.

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 (Covariance Parameter Estimates)		blk*genetic type=0 plot (blk*genetic type)=0.46 residual=4.39	

Table 2. Expression Differences for Bt in DP 458BR X PM 1218BR.

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 (Covariance Parameter Estimates)		blk*genetic type=0 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 458BR and PM 1218BR.

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 ST4691B 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 458BR and PM 1218BR 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

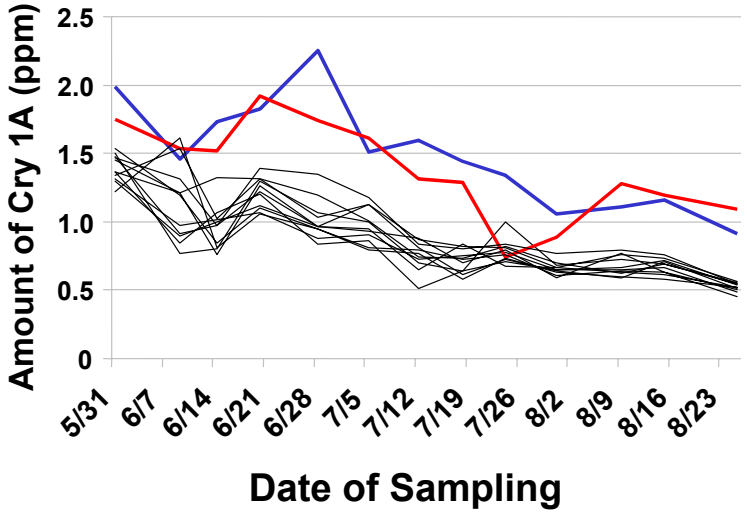

Figure 1. Expression of Cry1A in terminal leaves throughout the growing season for 13 transgenic varieties. Blue line, NuCOTN 33B; red line, DP 458BR; black lines, 11 additional Bt varieties including PM 1218BR and ST4691B. Reprinted from the Journal of Insect Science, 1.13. Available online: http://insectscience.org/1.13.