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EVALUATION OF A COMMERCIAL ELISA KIT FOR QUANTIFYING CRY1AC PRODUCTION IN GLANDLESS COTTON

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Abstract

Genes from *Bacillus thuringiensis* (Bt) have been genetically engineered into cotton (*Gossypium* spp.) plants to promote insect resistance. For this study, we were interested in quantifying the expression of the Cry1Ac protein encoded by an introduced Bt gene. The amount of Cry1Ac protein produced by plants in four cotton backcross populations—segregating for the glandless gene (Gl_2^e)—and their respective recurrent Bt-containing parents was quantified using the ELISA-based Envirologix QuantiPlate Kit for Cry1Ab/Cry1Ac. Our objective was to use this kit to identify glandless plants with two copies of the Bt gene (homozygous) versus glandless plants with one copy (heterozygous). Our results clearly indicated the lack of Cry1Ac protein in our negative control while showing a continuous range of values for those plants expressing Cry1Ac. There was no definite break among the values that would indicate heterozygotes versus homozygotes having twice the expression level of the heterozygotes. Although we were unable to identify homozygotes with this kit, the kit was easy to use and gave a range of values to select plants with the highest Cry1Ac expression.

Introduction

Several loci and alleles at those loci have been identified that impart the glandless trait in different parts of the cotton plant. Gl_2^e is an allele at the gl_2 locus and was discovered in glandless Egyptian cotton. This allele behaves as a single dominant gene. In the heterozygous state, the Gl_2^e allele results in marginal or reduced glandedness on the cotyledons and reduced or no glands on the hypocotyls (Fig. 1; Kohel and Lee, 1984).

Figure 1. Hypocotyls of cotton seedlings expressing Gl_2^e glanding patterns. From left to right, homozygous glanded, heterozygous, homozygous glandless.

We were interested in showing that this dominant glandless trait could be introduced into commercial transgenic cotton varieties containing a Monsanto Bollgard (BG) trait. Bollgard refers to the Cry1Ac Bt gene in these varieties. We needed a method to evaluate Cry1Ac expression to ensure that the Bt gene was still present following the initial cross to Gl_2^e and the subsequent backcrosses. For our objective to measure Cry1Ac expression, we tested an ELISA kit that reportedly would provide quantitative rather than qualitative measurements in an effort to identify homozygous and heterozygous Bt plants.

Materials and Methods

Germplasm

Four commercial transgenic cotton (*Gossypium hirsutum*) varieties—DPL 33BBG, PM 1569BG, SG 501BGRR, and STV 4691BG—were crossed to a source of the glandless trait (Gl_2^e) to form four populations. The progeny from

this initial cross were backcrossed (BC) to the commercial variety (recurrent parent) three times to form the BC3 generation. Following the BC3 generation, 6-7 plants from each population selected for heterozygous glanded and high Cry1Ac were selfed to form seed for the SBC3 generation. Twenty-five SBC3 progeny from each of the selected BC3 plants were evaluated for glandless and high Cry1Ac expression. Selfed seeds from selected plants were sent to the Cotton Winter Nursery in Tecoman, Mexico for evaluation.

Glandless segregation

The glandless trait was selected for in all generations. All progeny from the initial cross were heterozygous for the glandless trait. The BC generations segregated 1 heterozygous : 1 homozygous glanded. Heterozygous glanded plants were selected in all BC generations. The progeny resulting from selfing BC3 plants segregated 1 homozygous glandless : 2 heterozygous : 1 homozygous glanded. Homozygous glandless plants were also selected in the SBC3 generation.

Cry1Ac testing

Expression of Cry1Ac was measured in the BC3 and SBC3 generation using the Enzyme-Linked ImmunoSorbent Assay (ELISA)-based QuantiPlate kit formerly available from Envirologix**. Two leaf discs were taken from a single terminal leaf of each plant. The discs were weighed and crushed to obtain a sample extract. This extract was added to the wells of a 96-well plate previously coated with Cry protein antibodies. The kit protocol was followed for the ELISA assay and subsequent calculations of Cry1Ac concentration.

Results and Discussion

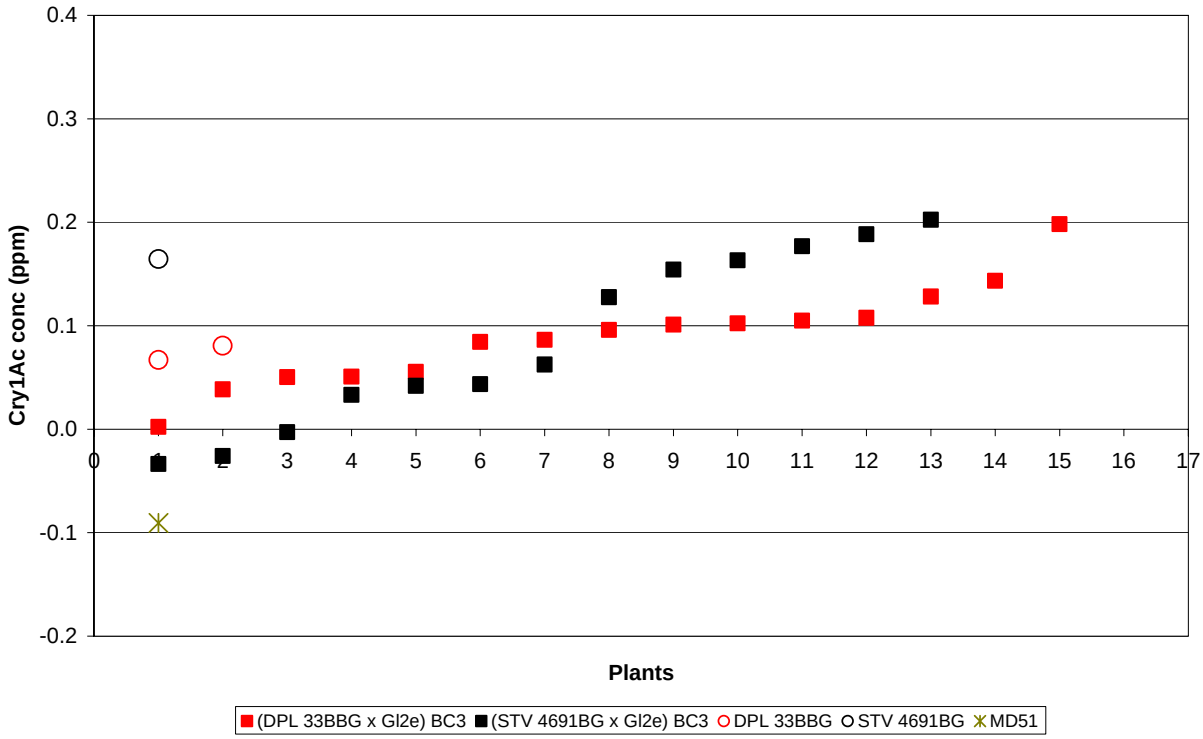
Glandless segregation

BC3 populations met chi-square expectations for a 1:1 segregation ratio (overall $p = 0.753$, $df = 3$). SBC3 progeny of the 25 selected BC3 plants did not meet expectations for a 1:2:1 segregation ratio (overall $p = 2.55 \times 10^{-11}$, $df = 48$). Those progeny exceptions included one selection of (PM 1569BG x Gl_2^e)BC3 with increased heterozygotes, one selection of (DPL 33BBG x Gl_2^e)BC3 with increased glanded plants, and two selections of (STV 4691BG x Gl_2^e)BC3 where all progeny were glandless. These exceptions may be explained by the duplicate recessive gl_2/gl_3 system of glandless alleles (McMichael, 1959) remaining in the genetic background of the parents.

Cry1Ac testing (BC3 generation)

The non-transgenic control, MD51, has a negative Cry1Ac concentration compared with BC3 plants and parents (Fig. 2). There is no definite break in the range of values for the BC3 plants; therefore, the highest 6-7 plants from each population were selected for further study.

(a).



(b).

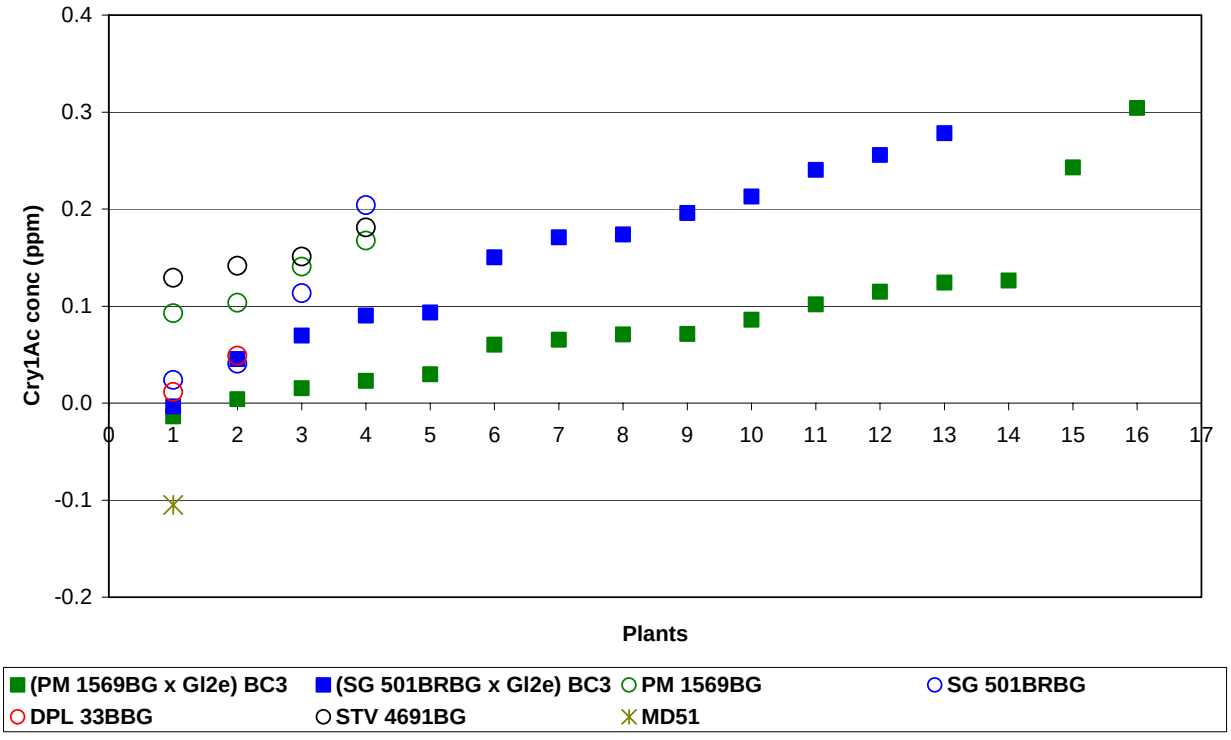
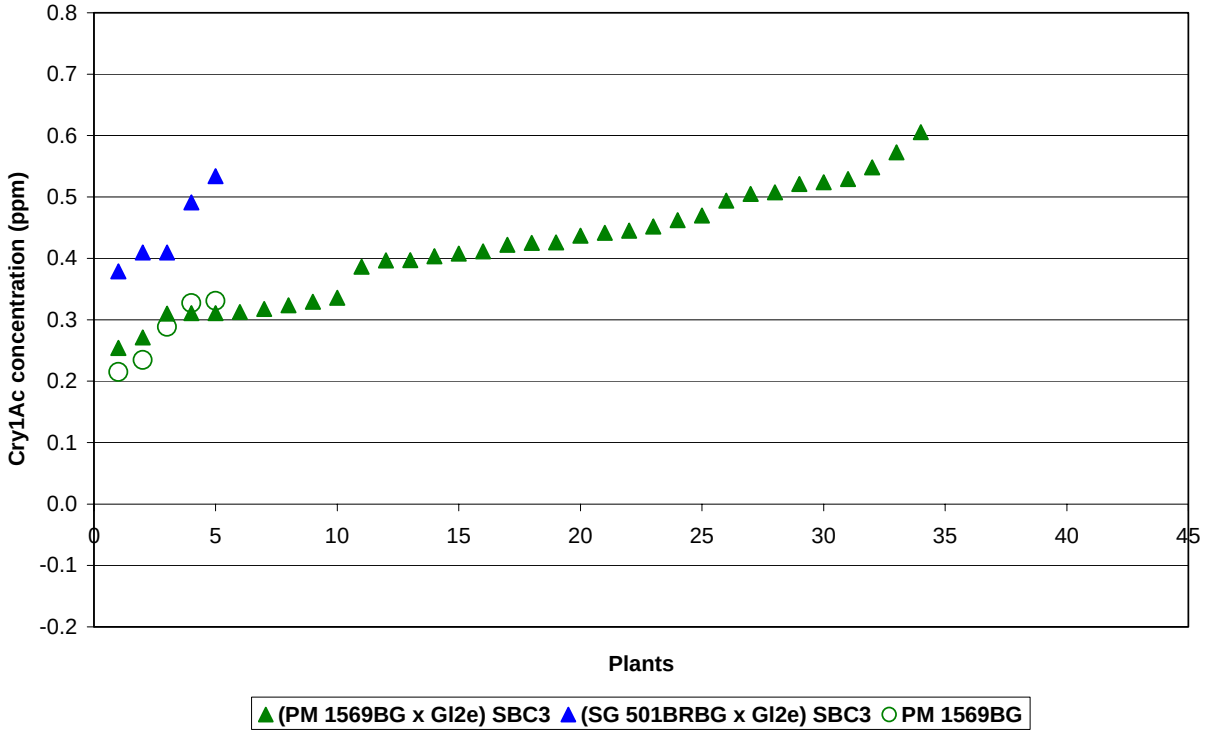


Figure 2. Cry1Ac concentration (ppm) for four populations of BC3 generation heterozygous glanded plants and their recurrent parents. Figure (a) and (b) represent results from two separate 96-well ELISA plates. MD51 is a non-transgenic negative control.

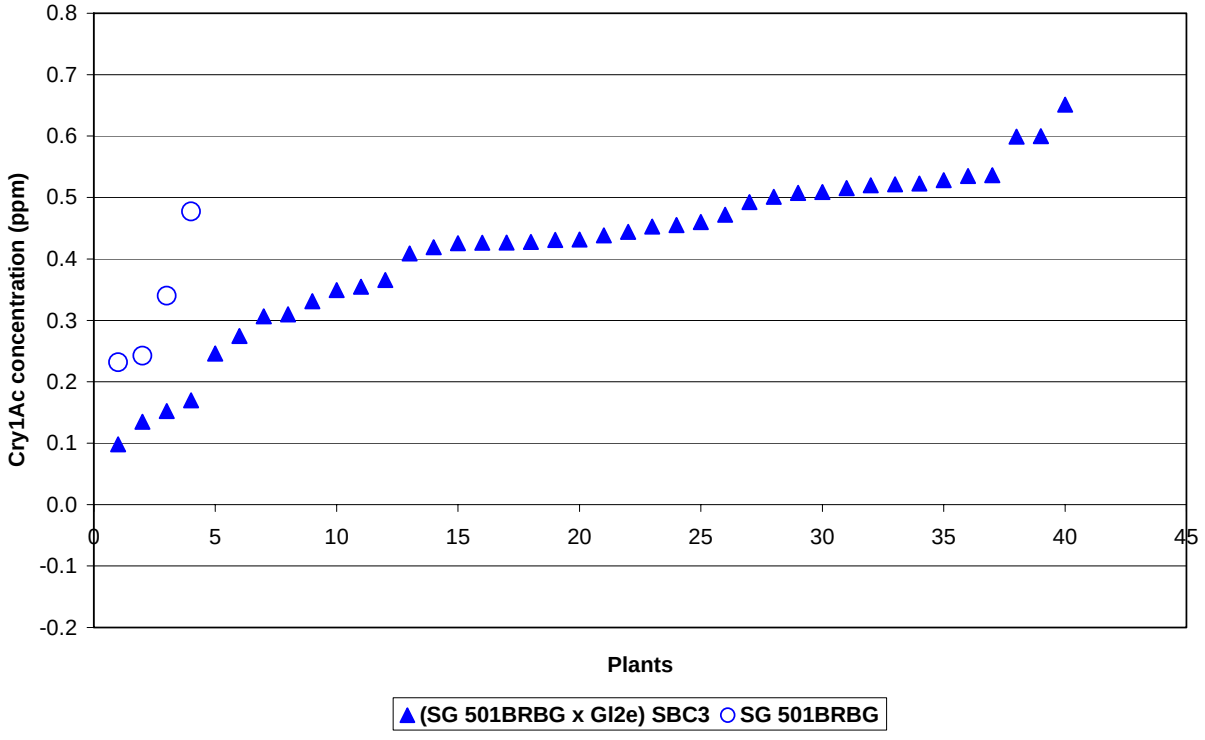
Cry1Ac testing (SBC3 generation)

A continuous range of values were also observed for the SBC3 progeny equivalent to or exceeding the value of the commercial parent (Fig. 3). Since there is no definite break in values, plants expressing the highest concentration of Cry1Ac were selfed and are currently under evaluation in the Cotton Winter Nursery.

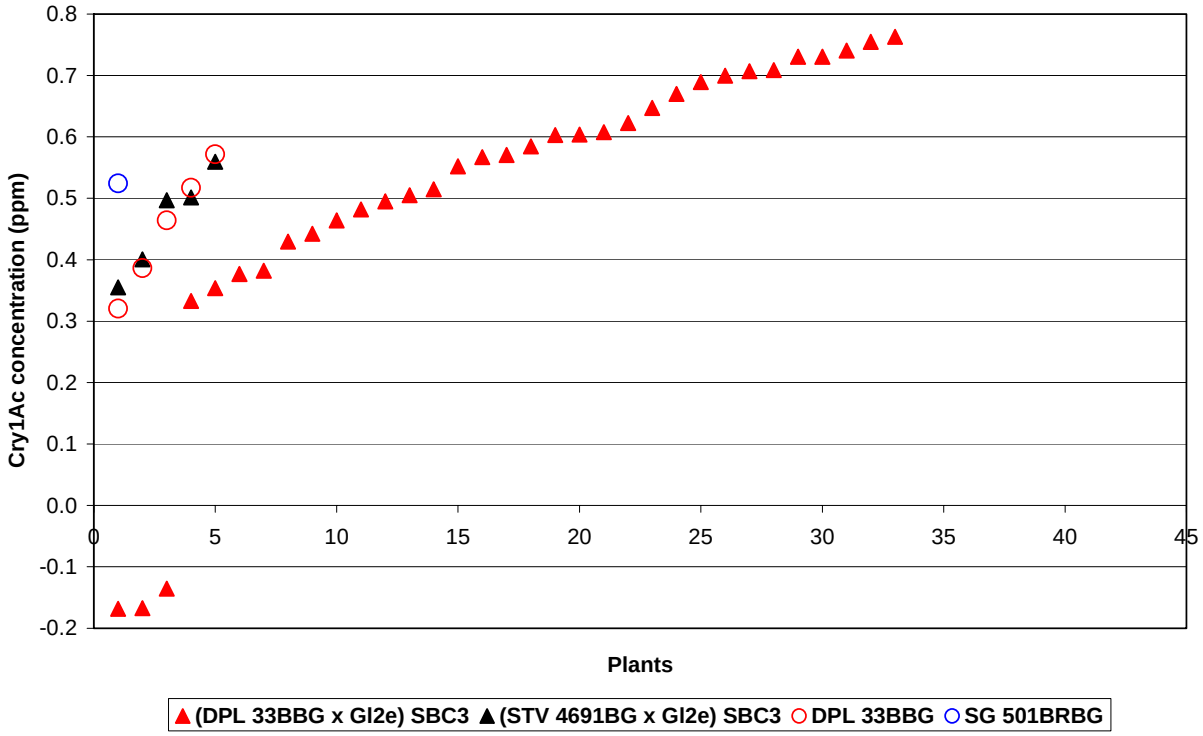
(a).



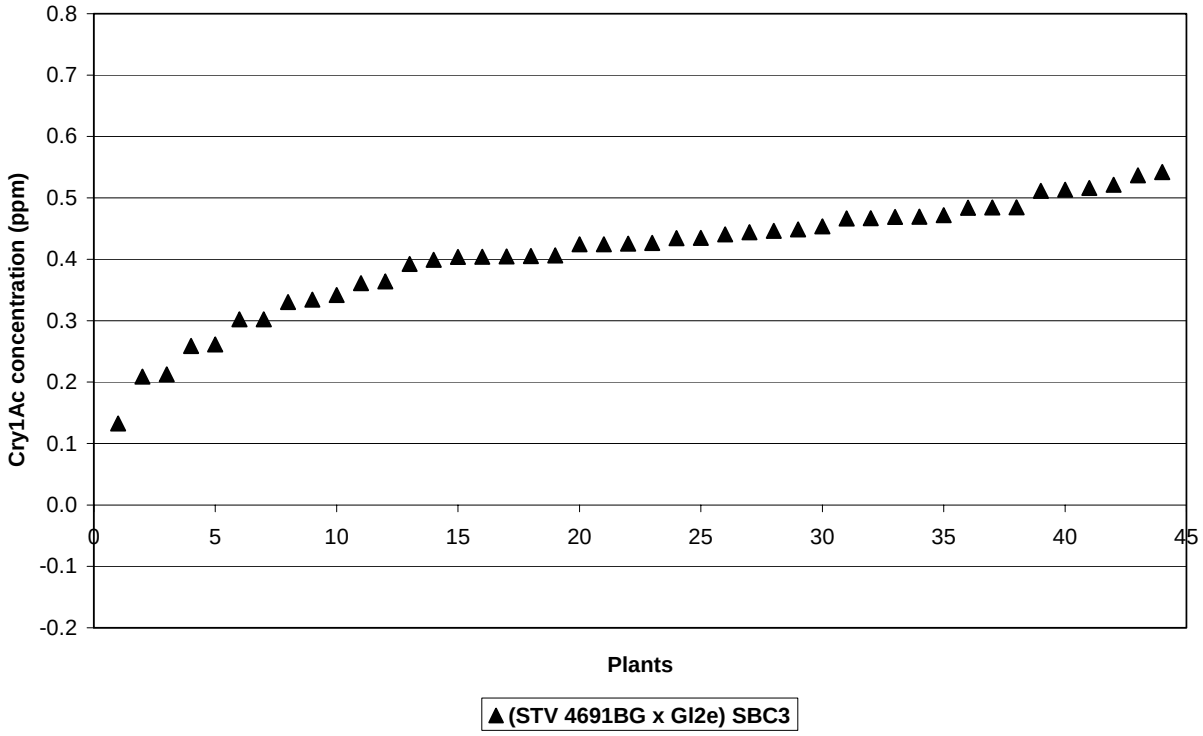
(b).



(c).


Cry1Ac concentration (ppm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
5
10
15
20
25
30
35
40
45
Plants
(DPL 33BBG x Gl2e) SBC3
(STV 4691BG x Gl2e) SBC3
DPL 33BBG
SG 501BRBG

(d).


Cry1Ac concentration (ppm)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
0
5
10
15
20
25
30
35
40
45
Plants
(STV 4691BG x Gl2e) SBC3

(e).

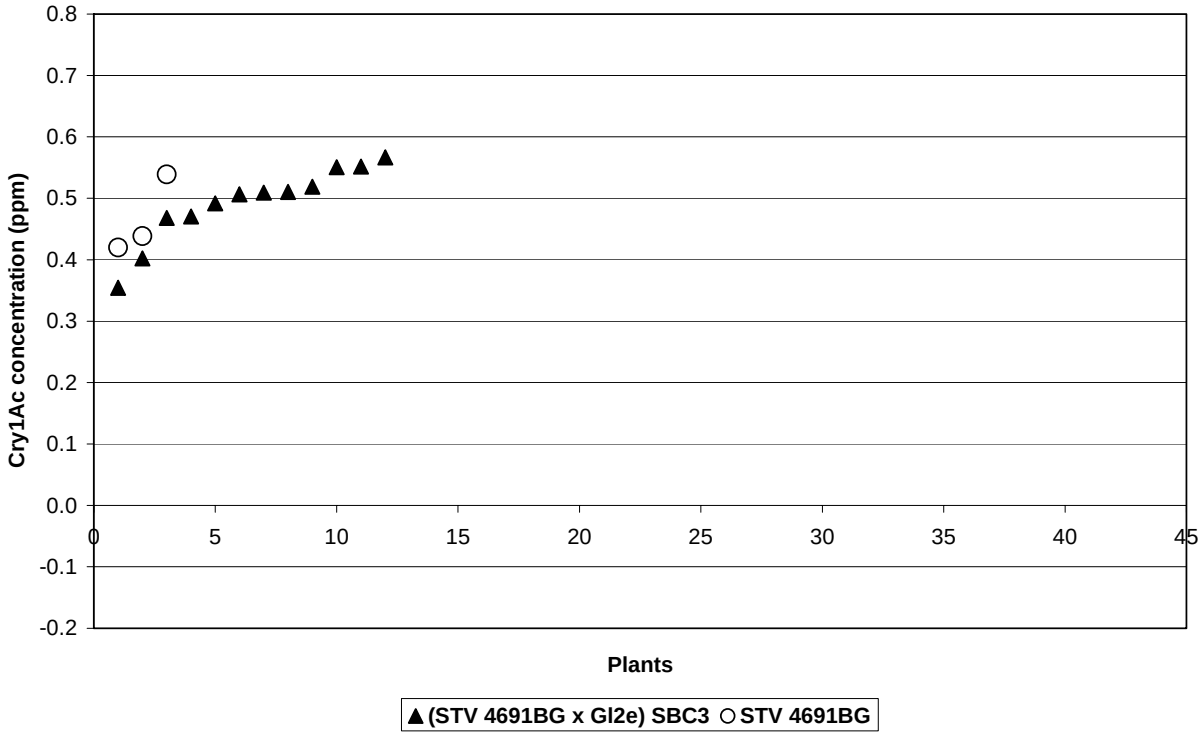


Figure 3. Cry1Ac concentration (ppm) for four populations of SBC3 generation homozygous glandless plants and their recurrent parents. Figures (a) through (e) represent results from five separate 96-well ELISA plates.

Gl_2^e is a single dominant allele that is genetically easier to work with than the gl_2/gl_3 system of duplicate recessives for imparting the glandless trait in cotton (Kohel and Lee, 1984; McMichael, 1959). The glandless character is of interest to those working in cottonseed quality research to maintain low gossypol content in glandless cotton; incorporating Gl_2^e into the glandless system produces glandless hybrid seeds if the glandless plant outcrosses with a normal glanded plant (Kohel and Lee, 1984).

We were unable to distinguish heterozygotes and homozygotes quantitatively using this ELISA kit. We later discovered this kit was discontinued by the manufacturer due to variation in the ELISA standards. Therefore, we interpreted the results within each plate separately to obtain a relative ranking of plants in each population assayed within each plate.

****NOTE:** Envirologix no longer offers a QuantiPlate kit for Cry1Ab/Cry1Ac. A company representative noted that the calibrating standards fluctuated to the extent that this test was not up to their standards for quality. Therefore, results within a single plate are comparable relative to each other, but results cannot reliably be compared from one plate to another.

DISCLAIMER: Mention of trade names or commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

References

Kohel, R.J. and J.A. Lee. 1984. Genetic analysis of Egyptian glandless cotton. Crop Sci. 24:1119-1121.

McMichael, S.C. 1959. Hopi cotton, a source of cottonseed free of gossypol pigments. Agron. J. 51:630.