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Study Title:

Sequencing of the Bt10 insert and comparison with the previously reported Bt11 sequence

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Study Completed On: January 28, 2005

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Report Number: SSB-104-05

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STATEMENT OF DATA CONFIDENTIALITY CLAIMS

Information claimed confidential on the basis of its falling within the scope of FIFRA sec. 10(d)(1)(A), (B), or (C) has been removed to a confidential appendix, and is cited by cross-reference number in the body of the study.

Company: Syngenta Seeds, Inc. – Field Crops - NAFTA

Company Agent: Jeff Stein Date: Jan 28, 2005

Title: Head of Regulatory Affairs-NAFTA

Signature: 

These data are the property of Syngenta Seeds, Inc. – Field Crops – NAFTA and, as such, are considered to be confidential for all purposes other than compliance with FIFRA §10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other statute in any other country.

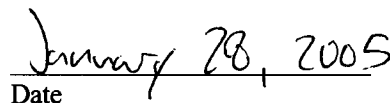
STATEMENT OF GOOD LABORATORY PRACTICES

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

PRINCIPAL INVESTIGATOR:



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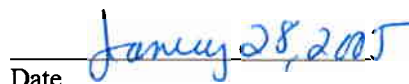


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Syngenta Biotechnology, Inc.
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Research Triangle Park, NC, USA

Final Report

Sequencing of the Bt10 insert and comparison with the previously reported Bt11 sequence

A. Summary

The DNA sequence of the transgene insert in the corn event Bt10 was deduced and the sequence was compared to the previously reported DNA sequence of the transgene insert present in Syngenta Seeds event Bt11 (EPA Reg No 67979-1). A total of three nucleotide changes were identified. Two of these changes were located in the intervening sequences contained within the Bt10 insert and one was located in the NOS terminator associated with the *cry1Ab* gene. No nucleotide changes were identified in any of the coding sequences and promoters within the Bt10 insert.

B. Chromosomal mapping

The transgene insert present in Event Bt10 is located on Chromosome 1, Bin 1.07. This was determined using a backcross population comprised of approximately 400 individual plants resulting from the cross of the Event Bt10 donor line to an elite corn inbred, followed by an additional cross of the resulting progeny to the elite inbred (i.e. plants represented Backcross Generation 1). Plants were analyzed using approximately 60 molecular markers distributed throughout the genome. Plants were also subjected to an over-the-top spray application of Liberty herbicide [glufosinate] at label rate to evaluate resistance to the herbicide. Given that the construct used to create Event Bt10 (and Bt11) contains the phosphinothricin acetyltransferase (*pat*) gene, which confers tolerance to glufosinate, the herbicide response provides an easily observable trait that can be used as an indicator of events expressing the *pat* gene product (e.g., Event Bt10, Bt11, or other events transformed with the *pat* gene). Association analysis was used to assess the relationships between each of the molecular markers and the expression of the *pat* gene. Because the chromosomal locations of the markers are known, strong relationships between banding patterns obtained for a given marker and glufosinate response (present or absent) serve to 'map' the site of insertion. Whereas the insertion for Bt11 is known to be situated on Chromosome 8 (Mettler et al., 2001. US Patent 6,222,04), mapping results indicated that the insertion site for Event Bt10 is located on Chromosome 1, in the region of Bin 1.06 to Bin 1.08. Further saturation of this chromosomal region with additional markers more accurately pinpointed the location to Chromosome 1, Bin 1.07.

The population which provided the Bt donor used to generate the mapping population described above also served as the parent population to generate the 5 plants used for sequence determination.

C. Insert sequencing

The nucleotide sequence of the entire Bt10 insert was determined, enabling a direct comparison to the previously reported Bt11 sequence (Chalk and Rabe, 2004). The Bt10 insert was amplified by PCR as eight overlapping fragments (Figure 1) based upon the sequence of the *NotI* fragment from plasmid pZO1502 that was used to generate Event Bt10 (and Event Bt11). PCR was performed on maize genomic DNA extracted from five individual plants from JHID829BB seed lot 2613E10F17 using the Wizard® Magnetic 96 DNA Plant System (Promega Cat. No. FF3760) following the protocol from Promega (Technical Bulletin 289). Each individual PCR product was amplified using the pair of oligonucleotides indicated in Table 1 and was carried out using the JumpStart Red AccuTaq® DNA polymerase (Sigma, Cat. No. D1313) following the protocol from Sigma (Technical Bulletin MB-805). PCR products were sequenced directly following clean up using the Qiaquick® PCR purification kit from Qiagen (Cat. No. 28106) following the manufacturer's protocol. Sequencing was carried out using the ABI3730XL analyzer using ABI BigDye® 1.1 or Big Dye 3.1 dGTP (for GC rich templates) chemistry. Figure 2 represents regions of the Bt10 insert for which high quality sequence data were obtained. For all coding regions contained within the Bt10 insert the sequence was verified in at least four of the individual plants analyzed. For all remaining regions of the Bt10 insert, sequence was verified in one plant and confirmed in one to four additional plants. Alignments were performed using the ClustalW program with the following parameters: scoring matrix blosum55, gap opening penalty 15, gap extension penalty 6.66 (Thompson *et al.*, 1994).

D. Results

No nucleotide differences were found in any of the coding sequences contained within the Bt10 insert when compared to the previously reported Bt11 sequence.

A total of three nucleotide changes were identified. The locations of these changes are represented graphically in Figure 2 as Chg 1 and 2, and within the alignment comparing the Bt10 insert sequence to the previously reported Bt11 sequence (Figure 3). Two of the changes are adjacent nucleotides and are labeled as a single change (Chg 1). In Change 1, the nucleotide sequence in Bt10 is "TA" whereas the nucleotide sequence for those positions in Event Bt11 is "AT". In Change 2, the nucleotide sequence in Bt10 is "A" whereas the nucleotide sequence for that position in Event Bt11 is "C"(Figure 3).

Change1 is located within intervening sequences contained within the Bt10 insert, and does not impact any functional elements within the insert. Change 2 is located within the NOS terminator associated with the *cry1Ab* gene. This change is not located within the poly-adenylation signal contained within the NOS terminator and it is highly unlikely to have an effect on the functionality of the terminator (Depicker, *et al.*, 1982).

E. Conclusion

A total of three nucleotide changes were identified when the Bt10 insert sequence was compared to the previously reported Bt11 sequence. None of these changes would be anticipated to impact the functionality of the elements contained within the Bt10 insert or the characteristics of the expressed proteins when compared to Bt11. Two of the changes were located in intervening sequence within the Bt10 insert. The remaining change is in the NOS terminator associated with the *cry1Ab* gene, would not be anticipated to impact its poly-adenylation function.

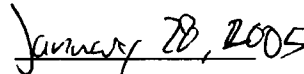
F. Records Retention

Raw data, the original copy of this report, and other relevant records are archived at Syngenta Biotechnology, Inc., 3054 East Cornwallis Rd., Research Triangle Park, NC, USA 27709

G. Report Signatures



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Date

Approved by:



Jeff Stein
Head of Regulatory Affairs-NAFTA



Date

H. References

Unpublished Syngenta Seeds Report

Chalk, C. and Rabe, S. (2004) Further sequencing of the Bt11 insert and comparison with the previously reported Bt11 sequence. Unpublished Syngenta data volume SSB-110-04.

Literature References

Depicker, A., Stachel, S., Dhaese, P., Zambryski, P., & Goodman, H. M. (1982). Nopaline synthase: transcript mapping and DNA sequence. *Journal of Molecular Applied Genetics*, 1, 561-573.

Mettler; I. J., Krier; M., Mies, D. (2001) US Patent 6,222,04 Inbred maize line NP948.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673-4680.

Figure 1. Location of amplified PCR products in the *NotI* fragment of pZO1502.

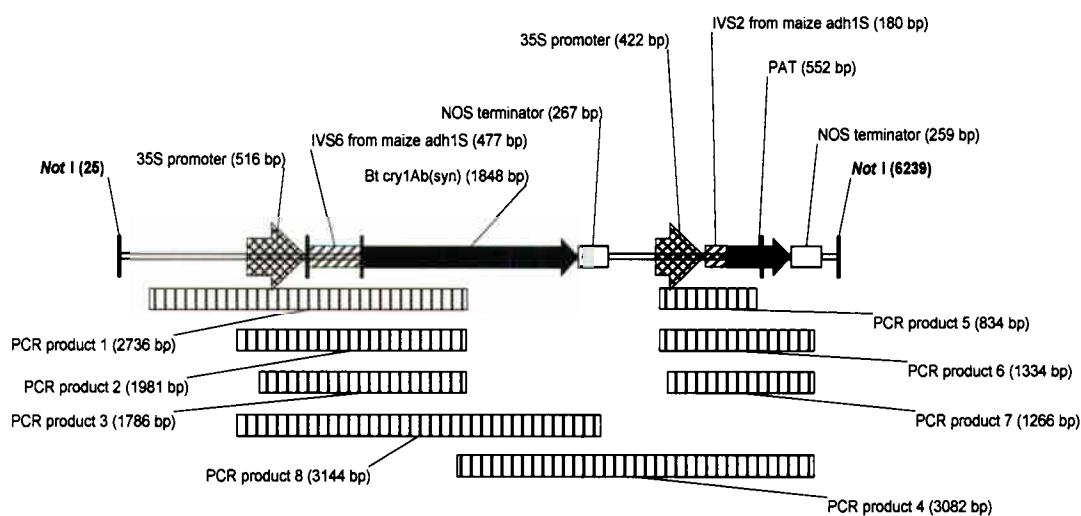
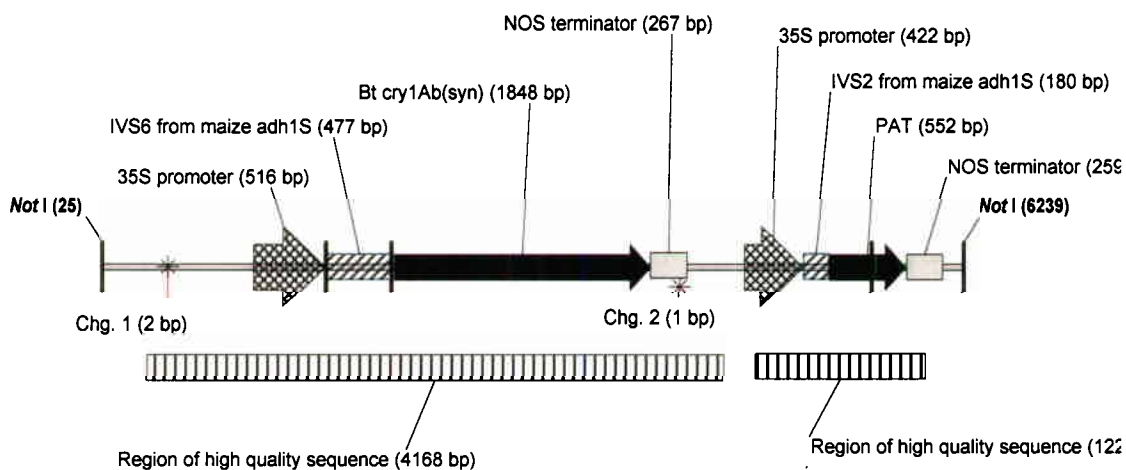


Figure 2. Location of changes identified in Bt10 when compared to Bt11.



CROSS REFERENCE 1

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