SUMMARY

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STUDY TITLE

Bioinformatics Analysis of Maize Event DAS-40278-9 Insert and Its Flanking Border Sequences

DATA REQUIREMENTS

N/A

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STUDY COMPLETED ON

28 – June – 2010

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101708

Bioinformatics Analysis of Maize Event DAS-40278-9 Insert and Its Flanking Border Sequences

SUMMARY

A plant-optimized aryloxyalkanoate dioxygenase-1 (*aad*-1) gene, originally from common soil bacterium *Sphingobium herbicidovorans,* was integrated into maize (*Zea mays*) to produce event DAS-40278-9 by Whiskers®-mediated transformation using a DNA fragment released by *Fsp* I from plasmid pDAS1740. The AAD-1 protein, encoded by the *aad*-1 gene, provides tolerance to 2,4-dicholorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (*e.g*. haloxyfop, cyhalofop, quizalofop, etc.) based herbicides. Molecular characterization indicated that the event DAS-40278-9 contained a single insert including an intact AAD-1 expression cassette. DNA sequences of the insert, flanking genomic borders, and parental locus of event DAS-40278-9 maize have been previously cloned and characterized.

To update the characterization of event DAS-40278-9 maize, the sequences of the flanking borders and parental genomic locus were analyzed using BLAST search algorithms along with up-to-date GenBank nucleotide collection [Nucleotide collection (nr/nt)], Non-Human and Non-Mouse ESTs (est_others), and protein [Non-redundant protein sequences (nr)] databases. BLASTn and BLASTx analysis of the sequences comprising the insert of DAS-40278-9 and its 5' and 3' flanking border regions revealed identities only to maize derived or the *Fsp* I fragment of pDAS1740 derived sequences. The insert of DAS-40278-9 was most likely integrated into a region containing sequences homologous to a Grande retrotransposon in the maize genome.

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DATA REQUIREMENTS

N/A

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: Maize AAD-1 event DAS-40278-9

Title: Bioinformatics Analysis of Maize Event DAS-40278-9 Insert and Its Flanking Border Sequences

- STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS:

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STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Bioinformatics Analysis of Maize Event DAS-40278-9 Insert and Its Flanking Border Sequences

Study Initiation Date: 26/04/2010

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

Organisation for Economic Co-Operation and Development
ENV/MC/CHEM(98)17, Paris January 26, 1998

At the time this study was conducted, it was not subject to the Good Laboratory Practice Standards and was, therefore, not monitored by the quality assurance unit.

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Bioinformatics Analysis of Maize Event DAS-40278-9 Insert and Its Flanking Border Sequences

ABSTRACT

A plant-optimized aryloxyalkanoate dioxygenase-1 (*aad*-1) gene, originally from common soil bacterium *Sphingobium herbicidovorans,* was integrated into maize (*Zea mays*) to produce event DAS-40278-9 by Whiskers®-mediated transformation using a DNA fragment released by *Fsp* I from plasmid pDAS1740. The AAD-1 protein, encoded by the *aad*-1 gene, provides tolerance to 2,4-dicholorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (*e.g*. haloxyfop, cyhalofop, quizalofop, etc.) based herbicides. Molecular characterization indicated that the event DAS-40278-9 contained a single insert including an intact AAD-1 expression cassette. DNA sequences of the insert, flanking genomic borders, and parental locus of event DAS-40278-9 maize have been previously cloned and characterized.

To update the characterization of event DAS-40278-9 maize, the sequences of the flanking borders and parental genomic locus were analyzed using BLAST search algorithms along with up-to-date GenBank nucleotide collection [Nucleotide collection (nr/nt)], Non-Human and Non-Mouse ESTs (est_others), and protein [Non-redundant protein sequences (nr)] databases. BLASTn and BLASTx analysis of the sequences comprising the insert of DAS-40278-9 and its 5' and 3' flanking border regions revealed identities only to maize derived or the *Fsp* I fragment of pDAS1740 derived sequences. The insert of DAS-40278-9 was most likely integrated into a region containing sequences homologous to a Grande retrotransposon in the maize genome.

INTRODUCTION

A plant-optimized aryloxyalkanoate dioxygenase-1 (*aad*-1) gene, originally from common soil bacterium *Sphingobium herbicidovorans,* was integrated into maize (*Zea mays*) to produce event DAS-40278-9 by Whiskers®-mediated transformation using a DNA fragment released by *Fsp* I from plasmid pDAS1740. The AAD-1 protein, encoded by the *aad*-1 gene, provides tolerance to 2,4-dicholorophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (*e.g*. haloxyfop, cyhalofop, quizalofop, etc.) based herbicides. Molecular characterization indicated that the event DAS-40278-9 contained a single insert including an intact AAD-1 expression cassette (1). DNA sequences of the insert, flanking genomic borders, and parental locus of event DAS-40278-9 maize were also cloned and characterized (2).

Considering the nature of random integration through Whiskers-mediated transformation, insertion of an exogenous gene into maize genome could disrupt an existing gene, open reading frame, or regulatory element. The purpose of this study is to characterize the location of the insert within the maize genome and the nature of the flanking border sequences using BLASTn and BLASTx algorithms and up-to-date nucleotide and protein databases, thus evaluating if the transgenic insert in maize event DAS-40278-9 had been integrated into an endogenous gene or genetic element.

METHODS

Query Sequence Preparation

The whole insert and its flanking border sequences were divided into 4 regions according to the previous analysis (Figure 1). DNA sequence Regions 1 and 4 include the 5' and 3' flanking genomic sequences, respectively. DNA sequence Region 2 includes 21 bp of novel sequence that was identified between the 5' genomic border and the AAD-1 insert. DNA sequence from Region 3 includes the AAD-1 insert of the *Fsp* I fragment derived from pDAS1740. The

parental locus represents the sequence from the maize genomic region prior to insertion of the transgene. The query sequences were prepared in FASTA format for the BLASTn and BLASTx search programs.

Sequence Search and Databases

Query sequences of Regions 1, 4, and the parental locus were analyzed for similarities with known sequences, to better characterize the insertion site of maize event DAS-40278-9. Searches were conducted against the GenBank nucleotide database [Nucleotide collection (nr/nt)] (http://www.ncbi.nlm.nih.gov, update to April 30, 2010) and GenBank Non-mouse and Non-human ESTs (est_others) (update to April 30, 2010) using BLASTn (Basic Local Alignment Search Tool, Version 2.2.21). The 6-frame translations of the DNA sequences were also searched against the non-redundant protein dataset (Non-redundant Protein Sequences "nr") including non-redundant GenBank CDS translation along with protein sequences from SWISS-PROT (http://www.expasy.org/sprot/), PIR (http://pir.georgetown.edu/), PRF (http://www.prf.or.jp/aboutdb-e.html), and PDB (http://www.wwpdb.org/) (update to April 30, 2010) using BLASTx (version 2.2.21). BLASTn and BLASTx searches were performed in an internal UNIX computer using the default setting of algorithm parameters (BLASTn: Expectation = 10, Gap Costs = Existence: 5, Extension: 2; BLASTx and BLASTp: Expectation = 10, Matrix = BLOSUM 62, Gap Costs: Existence: 11, Extension: 1; Word Size =3).

Region 2 was previously analyzed for similarities to sequences in the public databases (2) and was searched here for matches with the sequence of pDAS1740 by scanning. Region 3 was previously analyzed by aligning with the corresponding parts of the sequence in pDAS1740 (2).

RESULTS AND CONCLUSIONS

Sequence similarity search results from BLASTn and BLASTx and descriptions are summarized in Table 1.

Region 1 consists of 1852 bp, representing the 5' border. BLASTn search of Region 1 returned numerous alignments with maize genomic DNA sequences, but none of them are 100% identical (Table 1, APPENDIX 1). The top scoring alignment in complementary orientation (E() = 2×10^{-96}) is with a maize genomic clone AMMBBb-136N21 (Accession #: AC165175.2) , covering nucleotides (nt) 1298 to 1848 with 83% identity. Nucleotides 1523 – 1850 of Region 1 are also complementarily aligned with a maize Grande retrotransposon DNA (Accession #: EF468501) with an E-value of 3×10^{-46} (Table 1). BLASTn search against the EST database returned alignments with various cDNAs of maize and all of those alignments are partial with less than 100% identity (APPENDIX 2). BLASTx search returned no significant alignments (APPENDIX 3).

Region 2 consists of the 21 additional bp at the 5' border of the insertion. It was previously reported that BLASTn search of Region 2 against the GenBank Nucleotide Collection (nt/nr) or the EST database did not return any high scoring alignments with maize DNA sequences (2). Neither did the search of the 21 nucleotides against the maize genome sequence database (http://www.maizesequence.org/index.html). Further analysis indicates the 21 nucleotides match 8 nucleotides from MAR RB7 in a sense orientation and 13 nucleotides from ZmUbi1 promoter in complementary orientation (Table 1). Region 2 is too short to generate biological meaningful hits using BLASTx search.

Region 3 encompasses the transgene insert from the *Fsp* I fragment of pDAS1740. As described previously (2), the promoter, gene, and terminator sequences of the transgene are intact but both of the MAR elements, flanking the AAD-1 expression cassette in an opposite orientation, were truncated at their 5'ends (Table 1).

Region 4 consists of 1868 bp of the 3' border region. Similar to Region 1, BLASTn search of Region 4 returned numerous alignments with maize genomic sequences. Nucleotides 2 -1387 are complementarily aligned with a maize Grande retrotransposon DNA (Accession #: EF468501) (E() =0) (Table 1, APPENDIX 4). Grande retrotransposon was also one of the top hits returned in the search of Region 1 (5' end border). Top scoring alignments returned in the

search against EST database are with cDNA sequences from maize, but those alignments are all partial and none of them are 100% identical (APPENDIX 5). BLASTx search of Region 4 retuned a top scoring hit (E()= 1×10^{-155}) with a putative *Zea mays* gag-pol precursor (Accession #: AAP94585.1) (APPENDIX 6), but only covering the nucleotides 107 – 1662 in Region 4.

BLASTn search of the parental locus returned a top scoring alignment (E()= 0) with a maize Grande retrotransposon DNA (Accession #: EF468501), which matches the output from the search of Region 1 and 4 (Table 1, APPENDIX 7). Similar to the Region 1 and Region 4, BLASTn search against EST database returned numerous alignments with maize cDNAs, but they are all partial without 100 % identity (APPENDIX 8). In BLASTx search, the 3' end region down stream the integration site in the parental locus displays a top scoring alignment (E()=1×10^{-108}) with a putative *Zea mays* gag-pol precursor (Accession #: AAP94585.1; APPENDIX 9), which is also observed in the BLASTx search of Region 4.

Taking account of all the results from sequence similarity searches using BLAST tools, the insert of DAS-40278-9 is most likely located in a maize genomic region containing sequences homologous to a Grande retrotransposon. No evidence demonstrated that any known existing gene or genetic element is disrupted due the integration of the insert in DAS-40278-9.

REFERENCES

1. Zhuang, M., Mo, J., Poorbaugh, J. D., Richey, K.A., Cruse, J., Thomas, A., 2009. Molecular Characterization of AAD-1 Corn Event DAS-40278-9. Dow AgroSciences Study Report 081052.
2. Mo, J., Zhou, N., Poorbaugh, J. 2009. Cloning and Characterization of DNA Sequence in the Insert and the Flanking Border Regions of AAD-1 Corn Event DAS-40278-9. Dow AgroSciences Study Report 091023.

Table 1 Description of DNA Sequences from the Insert, Flanking Borders in DAS-. 40278-9, and the Parental Locus

Region	Location	Size (bp)	% (identity)	Homologue Accession #	Location in homologous sequences	Description
1	1-1852	1852	83 (1298-1848 in Region 1)	AC16175.2	46811- 46266 (complementary)	Maize genomic clone (E() = 2×10^{-96})
			87 (1523-1850 in Region 1)	EF468501	5721-5393 (complementary)	Maize Grande retrotransposon DNA (E()=3×10^{-46})
2	1853−1873	21	100	*Fsp*I fragment of pDAS1740	2382-2394 (complementary)	Sequences from ZmUbi1 promoter
				*Fsp*I fragment of pDAS1740	1068-1075	Sequences from MAR RB7
3	1874−6689	4816	100	*Fsp*I fragment of pDAS1740	1038 -5853	Transgene insert in pDAS1740
4	6690−8557	1868	83 (2- 1387 in Region 4)	EF468501	5388-4004 (complementary)	Maize Grande retrotransposon DNA (E()=0)
			55 (107 – 1662 in Region 4, Frame +2)	AAP94585.1 (1833 aa)	1-387 aa	Putative gag-pol precursor (*Zea mays*) (E()=1×10^{-155}
			77 (1315-1662 in Region 4, Frame +1	AAP94585.1 (1833 aa)	388-503 aa	Putative gag-pol precursor (*Zea mays*) (E()=1×10^{-155}
Parental Locus (integration site 1118)		2212	84 (788-2195 in the Locus)	EF468501	5721-4315 (complementary)	Maize Grande retrotransposon DNA (E()=0)
			54 (1225-2211 in the Locus, Frame +1)	AAP94585.1 (1833 aa)	1-313 aa	Putative gag-pol precursor (*Zea mays*) (E()=4×10^{-80}

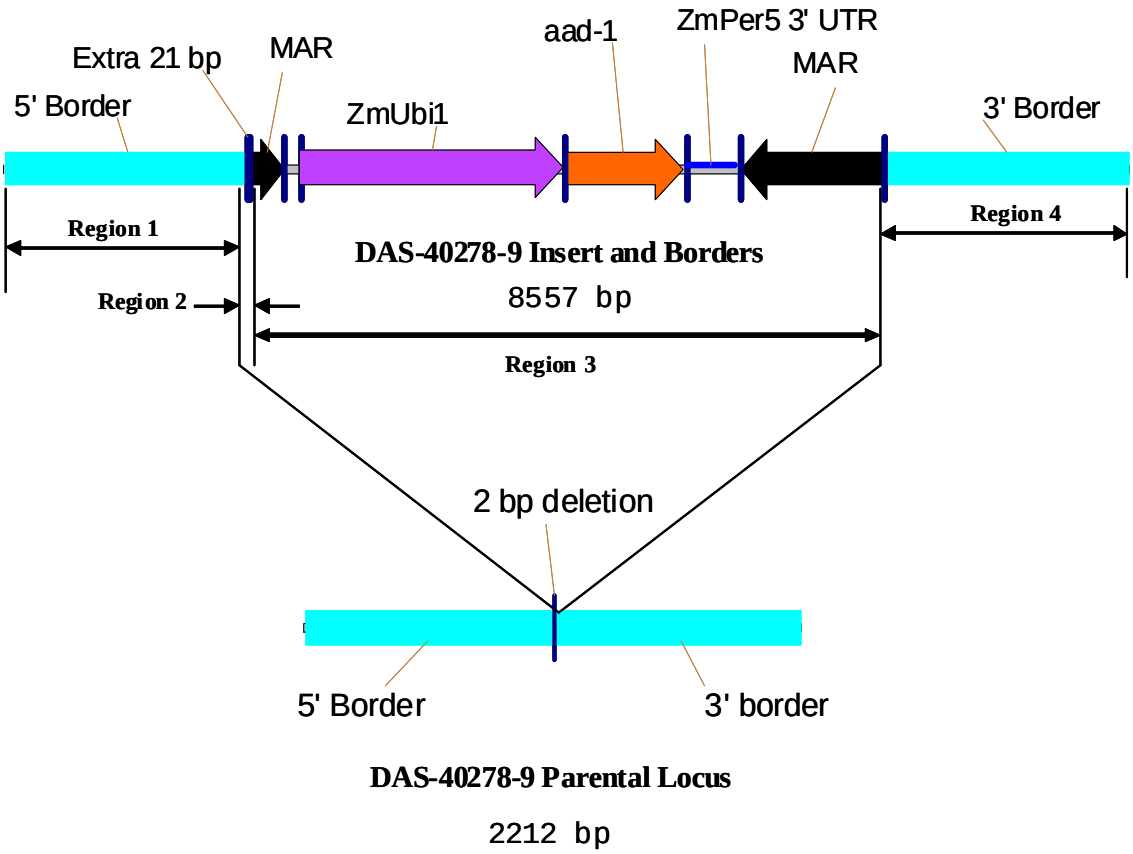

Figure 1. Diagram of the Insert, Flanking Borders, and the Parental Locus in Maize Event DAS-40278-9

APPENDIX

1. BLASTn Search Outputs of the 5' Border Sequence in Maize Event DAS-40278-9 against GenBank Nucleotide Collection (nr/nt)

2. BLASTn Search Outputs of the 5' Border Sequence in Maize Event DAS-40278-9 against GenBank No-mouse and No-human ESTs (est_others)

3. BLASTx Search Outputs of the 5' Border Sequence in Maize Event DAS-40278-9 against GenBank Non-redundant Proteins Sequences "nr"

4. BLASTn Search Outputs of the 3' Border Sequence in Maize Event DAS-40278-9 against GenBank Nucleotide Collection (nr/nt)

5. BLASTn Search Outputs of the 3' Border Sequence in Maize Event DAS-40278-9 against GenBank No-mouse and No-human ESTs (est_others)

6. BLASTx Search Outputs of the 3' Border Sequence in Maize Event DAS-40278-9 against GenBank Non-redundant Protein Sequences "nr"

7. BLASTn Search Outputs of the Parental Locus in Maize Event DAS-40278-9 against GenBank Nucleotide Collection (nr/nt)

8. BLASTn Search Outputs of the Parental Locus in Maize Event DAS-40278-9 against GenBank No-mouse and No-human ESTs (est_others)

9. BLASTx Search Outputs of the Parental Locus in Maize Event DAS-40278-9 against GenBank Non-redundant Protein Sequences "nr"

NOTE: All The BLAST search output files are electronically stored in a secured computer in Dow AgroSciences and available for view in PDF format.