SAFETY AND NUTRITIONAL SUMMARY ASSESSMENT FOR INSECT-RESISTANT HERBICIDE-TOLERANT MZIR098 CORN

OECD Unique Identifier: SYN-ØØØ98-3

Applicant

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Table of Contents

List of Tables

List of Figures

Table of Abbreviations, Acronyms, and Symbols

35S-04 promoter	promoter region of the cauliflower mosaic virus
aadA-03	spectinomycin resistance gene
acetyl CoA	acetyl coenzyme A
ADF	acid detergent fiber
ANOVA	analysis of variance
BC	backcross
BLASTP	Basic Local Alignment Search Tool for Proteins
bp	base pair
Bt	*Bacillus thuringiensis*
CFIA	Canadian Food Inspection Agency
CMP-04 promoter	promoter from cestrum yellow leaf curling virus
CoASH	coenzyme A
ColE1-06 ori	Origin of replication that permits replication in *E.coli*
DNA	deoxyribonucleic acid
DTNB	5,5′-dithiobis(2-nitrobenzoic acid)
DW	dry weight
ECCB	Exclusive Capturable Commercial Benefit
ELISA	enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
F_1	first generation of progeny from a breeding cross
F_1,F_2,F_3,F_4,F_5,F_6,F_7	first generation and subsequent generations of progeny from a breeding cross
FSANZ	Food Standards Australia New Zealand
FW	fresh weight
HRP	horseradish peroxidase
ILSI	International Life Sciences Institute
kb	kilobase pairs
kDa	kilodalton
LB-01-01	left border
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
N	sample size
N/A	not applicable
NCBI	National Center for Biotechnology Information
NDF	neutral detergent fiber
ng	nanogram
NOS	nopaline synthase
NOS-02 enhancer	nopaline synthase sequence from *A. tumefaciens* which increases gene expression
NOS-05-01 terminator	nopaline synthase terminator sequence from *A. tumefaciens*
NOS-20 terminator	nopaline synthase terminator sequence from *A. tumefaciens*
OECD	Organisation for Economic Co-operation and Development
ori	origin of replication

P	probability
PAGE	polyacrylamide gel electrophoresis
PAT	phosphinothricin acetyltransferase
pat-08	phosphinothricin acetyltransferase gene
PCR	polymerase chain reaction
pg	picogram
RB-01-01	right border
repA-03	replication gene from *Pseudomonas aeruginosa* plasmid VS1
SEM	standard error of the mean
SDS	sodium dodecyl sulfate
T_0	T_0 is the designation used for the original transformed plant
T-DNA	Transfer- DNA
SDS	sodium dodecyl sulfate
TIU	trypsin inhibitor unit
TNB	2-nitro-5-thiobenzoic acid
TNB^{2-}	2-nitro-5-thiobenzoate anion
Ubi1-18 promoter	corn ubiquitin promoter
US	United States of America
USDA	United States Department of Agriculture
v.	version
virG-01	part of the regulatory system for the virulence regulon in *Agrobacterium tumefaciens*
VS1-02 ori	serves as the origin of replication in the *Agrobacterium tumefaciens* host
×	cross, cross-pollination
⊗	self-pollination
χ^2	chi squared
µg	microgram

Corn Growth Stages (Abendroth *et al.* 2011)

Vegetative:	
V2	first two leaves collared
V3	first three leaves collared
V4	first four leaves collared
V5	first five leaves collared
V6	first six leaves collared
V7	first seven leaves collared
V8	first eight leaves collared
V9	first nine leaves collared
V10	first ten leaves collared
V11	first eleven leaves collared
V12	first twelve leaves collared
V13	first thirteen leaves collared
VT	tassel

Reproductive:

R1	silking
R2	blister
R3	milk
R4	dough
R5	dent
R6	physiological maturity

Amino Acids

Ala, A	alanine
Arg, R	arginine
Arn, N	asparagine
Asp, D	aspartic acid
Cys, C	cysteine
Gln, Q	glutamine
Glu, E	glutamic acid
Gly, G	glycine
His, H	histidine
Ile, I	isoleucine
Leu, L	leucine
Lys, K	lysine
Met, M	methionine
Phe, F	phenylalanine
Pro, P	proline
Ser, S	serine
Thr, T	threonine
Trp, W	tryptophan
Tyr, Y	tyrosine
Val, V	valine

Part I. General Information

I.A. Name, Address, and Contact Information for Applicant

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I.B. Purpose of the Application

This application seeks to vary Food Standards Australia New Zealand (FSANZ) Standard 1.5.2 to allow the use of genetically modified corn (maize; *Zea mays* L.) derived from Event MZIR098 corn (hereafter MZIR098 corn) in Australia and New Zealand food industries.

I.C. Justification for the Application

Crops improved through modern biotechnology have brought significant benefits to agriculture in the form of improved yields, pest management, and crop quality. Continued innovation in this area will benefit growers, consumers, and the environment.

Adoption of genetically engineered crops with insect resistance and herbicide tolerance traits has increased dramatically since the first commercial introductions of transgenic corn, cotton, and soybean in 1996. Net economic benefits at the farm level have been substantial (Hutchison *et al.* 2010). Improved weed and insect control have led to increased crop yields, reductions in conventional pesticide applications, and environmental benefits.

MZIR098 corn plants have been created to offer dual modes of action for corn rootworm control in a single event. The rootworm control traits expressed in MZIR098 corn are the same traits expressed in the previously authorized Syngenta events 5307 corn and MIR604 corn, eCry3.1Ab and mCry3A respectively. In addition, MZIR098 also expresses the PAT protein which offers commercial levels of tolerance to glufosinate-ammonium herbicide products.

As in 5307 corn the eCry3.1Ab produced in MZIR098 corn is highly effective in controlling three of the major rootworm pests of corn in North America: *D. virgifera virgifera* LeConte (western corn rootworm), *D. longicornis barberi* Smith and Lawrence (northern corn

rootworm), and *D. virgifera zeae* Krysan and Smith (Mexican corn rootworm). As in MIR604, the modified protein mCry3A produced by MZIR098 corn has enhanced activity against corn root worm in particular, western corn rootworm and northern corn rootworm (Chen and Stacy 2003).

The combination of eCry3.1Ab and mCry3A in the same corn hybrid offers important advantages for insect resistance management. It has been demonstrated that eCry3.1Ab and mCry3A have unique properties that, when combined, serve to prevent, delay, or mitigate the evolution of target pest resistance to either protein. Although the proteins act by the same general mechanism (i.e., pore formation in the target pest gut), the evidence indicates that they have unique gut binding sites in the target pest, thus effectively representing different modes of action (Walters *et al.* 2010). The concurrent deployment of eCry3.1Ab and mCry3A in the same hybrid corn offerings to growers is expected to help preserve pest susceptibility to both proteins. Furthermore, by reducing the selection pressure on target-pest populations to evolve resistance to any single method of rootworm control, this strategy is expected to help prolong pest susceptibility to other *B. thuringiensis* derived proteins in transgenic corn cultivars used for rootworm control, as well as to other traditional control methods (e.g., insecticides and crop rotation). By combining three traits eCry3.1Ab, mCry3A, and PAT at a single breeding locus, MZIR098 corn will also increase the efficiency of trait conversion into elite genetic lines, thus increasing the speed with which multiple traits can be combined in commercial corn products to meet growers' needs.

I.D. Costs and Benefits and Impact on Trade

The costs and benefits and impact on trade are the same as those described in previous corn applications submitted to FSANZ (A1060; A1001).

I.E. Exclusive Capturable Commercial Benefit (ECCB)

This application is likely to result in an amendment to the FSANZ Food Standards Code. Approval for use of MZIR098 as food in Australia is likely to provide an ECCB for Syngenta, and therefore Syngenta will pay the full cost of processing this application.

I.F. Confidential Commercial Information

Syngenta will request that FSANZ treat parts of the information supplied in this application as confidential information. This information will be clearly marked as Confidential Business Information.

I.G. References Cited in Part I

Chen, E. and Stacy, C. 2003. Modified Cry3A toxins and nucleic acid sequences coding therefore. WO Patent No. 03/018810.

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster LV, Hunt TE, Wright RJ, Pecinovsky K, Rabaey TL, Flood BR, Raun ES. 2010. Areawide suppression of

European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science* 330:222–225. DOI: 10.1126/science.1190242.

Walters, F.S., C.M. deFontes, H.Hart, G.W. Warren, and J.S. Chen. 2010. Lepidopteran-active variable-region sequence imparts coleopteran activity in eCry3.1Ab, an engineered *Bacillus thuringiensis* hybrid insecticidal protein. *Applied and Environmental Microbiology* 76: 3082-3088.

Part II. Submissions

II.A. Submissions for Cultivation Approvals

Syngenta is pursuing regulatory approvals for MZIR098 corn cultivation in the United States and Canada, and may seek cultivation approvals in other countries in the future.

Submissions requesting approvals of MZIR098 corn for cultivation in Canada will soon be made to the Canadian Food Inspection Agency.

Syngenta does not plan to cultivate MZIR098 corn in Australia or New Zealand. Food products derived from MZIR098 corn will therefore enter the Australian and New Zealand food supply as only imported and largely processed food ingredients.

II.B. International Submissions for Food, Feed, and Processing Import Approvals

Submissions requesting approvals of MZIR098 corn for importation will be sought on an as-needed basis.

II.C. International Standards

Syngenta reports and studies included in the information supporting this application have been conducted according to international standards. In the safety assessment of biotechnology products, Syngenta referred primarily to the *Codex Alimentarius* Commission Foods Derived from Modern Biotechnology (CAC 2009), and the relevant Codex Standard is as follows:

Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003.

II.D. References Cited in Part II

CAC. 2009. *Foods Derived from Modern Biotechnology,* 2nd ed. Codex Alimentarius Commission. Rome, Italy: World Health Organization, Food and Agriculture Organization of the United Nations. 85 pp. ftp://ftp.fao.org/docrep/fao/011/a1554e/a1554e00.pdf.

Part III. Synopsis of the Safety and Nutritional Assessment for MZIR098 Corn

III.A. Description of the GM Organism - Identity, Source and Purpose of the Genetic Modification

Syngenta transformed corn (maize; *Zea mays* L.) to produce MZIR098 corn, which offers dual modes of action for corn rootworm control in a single event. In addition, MZIR098 corn also offers commercial levels of tolerance to glufosinate-ammonium herbicide products.

MZIR098 corn plants contain the transgenes *ecry3.1Ab* and *mcry3A*, which encode the insecticidal proteins eCry3.1Ab and mCry3A, and the transgene *pat-08*, which encodes the enzyme phosphinothricin acetyltransferase (PAT). The native Cry3A from the soil bacterium *Bacillus thuringiensis* subsp. *tenebrionis* is active against certain coleopteran pests. The modified protein mCry3A produced by MZIR098 corn has enhanced activity against western corn rootworm (*Diabrotica virgifera virgifera*) and other related coleopteran pests of corn. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab that is also active against *D. virgifera virgifera* and other related pests of corn. The native Cry1Ab from *B. thuringiensis* subsp. *kurstaki* is active against certain lepidopteran pests; however, the portion of Cry1Ab included in eCry3.1Ab has not preserved the activity of Cry1Ab against lepidopterans. Although the proteins act by the same general mechanism (i.e., pore formation in the target pest gut), the evidence indicates that they have unique gut binding sites in the target pest, thus effectively representing different modes of action (Walters *et al.* 2010).

The transgene *pat-08* was derived from the soil bacterium *Streptomyces viridochromogenes*. PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. PAT was used as a selectable marker in the development of MZIR098 corn.

III.B. Designation of Transformation Event

The designation of the transformant is Event MZIR098 corn, which has been assigned the OECD Unique Identifier SYN-ØØØ98-3.

III.C. The Types of Products Likely to Include the Food or Food Ingredient

MZIR098 corn, and the food and feed derived from it, are not materially different from conventional corn. The uses of MZIR098 corn are expected to be the same as conventional corn.

Domestic production of corn in Australia, and New Zealand is supplemented by the import of corn-based products from places such as North America, which is one of largest producers of corn (FAOSTAT 2015). Corn grown in North America is predominantly of the yellow dent type, a commodity crop largely used to feed domestic animals, as either grain or silage. The remainder of the crop is exported for food, feed, or industrial uses or processed by wet milling, dry milling, or alkali treatment to yield products such as high-fructose corn syrup and starch or oil, grits, and flour. These processed products are used extensively in the food industry. For example, corn starch serves as a raw material for an array of processed foods and is also used in industrial manufacturing processes. Since the early 1980s, a significant amount of corn grain

has also been used for fuel ethanol production. The by-products from these distilling processes are often used in animal feeds.

III.D. Description of Host Organism into Which the Genes Were Transferred

III.D.1. Recipient Corn Line

The recipient germplasm for transformation to produce MZIR098 corn was an elite Syngenta inbred corn line, NP2222 (Plant Variety Protection certificate 200200071, issued November 2004; USDA-AMS 2010). This inbred line was used because it is well-suited to *Agrobacterium tumefaciens* mediated transformation and regeneration from tissue culture. NP2222 is a Stiff-Stalk family, yellow dent inbred.

III.D.2. Biology of Corn

The Consensus Document on the Biology of *Zea mays* subsp. *mays* (Maize), published by the Organisation for Economic Co-operation and Development (OECD 2003), provides comprehensive information regarding the biology of corn. This Consensus Document is referenced in support of MZIR098 corn, and includes the following information:

- Uses of corn as a crop plant
- Taxonomic status of the genus *Zea*
- Identification methods among races of *Zea mays* and wild species
- Centers of origin and diversity of corn
- Reproductive biology of corn
- Intra-specific and inter-specific crosses of corn and gene flow
- Agro-ecology of corn, including cultivation, volunteers, weediness, soil ecology, and corn-insect interactions
- Corn biotechnology
- Common diseases and insect pests of corn

III.D.3. Parts and/or Processing of Corn for Food or Feed

Kernels from MZIR098 corn are the most likely tissue to enter the food supply, either as grain or grain by-products. Humans would potentially consume corn at the senescence stage of development, whereas livestock would be more likely to consume the kernels at maturity.

As mentioned previously, corn is typically processed by wet milling, dry milling, or alkali treatment to yield products such as high-fructose corn syrup and starch or oil, grits, and flour. No special processing is required to render the food safe to eat.

III.D.4. Significance of Corn to the Diet in Australia and New Zealand

Corn for grain is the number one produced cereal crop worldwide with just over one billion tonnes produced in 2013, according to the Food and Agricultural Organization estimates (FAOSTAT 2015). In 2013, the top producer continued to be the U.S. with 353.7 million tonnes or 35% of global production. The U.S was followed by China which produced 218.5 million tonnes (22% of global production). Australia produced 0.51 million tonnes and New

Zealand produced 0.20 million tonnes. Domestic production of corn in Australia and New Zealand is supplemented by the import of corn based products.

III.E. Description of the Donor Organisms from Which the Genetic Elements are Derived

MZIR098 corn contains the transgenes *ecry3.1Ab* and *mcry3A* derived from *B. thuringiensis*, a ubiquitous soil bacterium. The engineered protein eCry3.1Ab is a chimera of mCry3A and Cry1Ab. The native Cry3A protein is derived from *B. thuringiensis* subsp. *tenebrionis* whereas the native Cry1Ab protein is derived from *B. thuringiensis* subsp. *kurstaki.* Insecticidal Cry proteins from *B. thuringiensis* have a long history of safe use in food crops.

MZIR098 corn contains the transgene *pat-08*, derived from *S. viridochromogenes*, a common nonpathogenic soil bacterium. Bacteria are not known to be sources of allergenic proteins (Taylor and Hefle 2001).

III.F. References Cited in Part III

FAOSTAT. 2015. Food and Agriculture Organization of the United Nations Statistics Division. http://faostat3.fao.org/home/E (accessed June 29, 2015).

OECD. 2003. *Consensus Document on the Biology of* Zea mays *subsp.* mays *(maize).* Series on Harmonisation of Regulatory Oversight in Biotechnology, No. 27. Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, Environment Directorate, Organisation for Economic Co-operation and Development. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2003)11.

Taylor SL, Hefle SL. 2001. Will genetically modified foods be allergenic? *Journal of Allergy and Clinical Immunology* 107:765–771.

USDA-AMS. 2010. Plant Variety Protection Office, Agricultural Marketing Service, United States Department of Agriculture. http://apps.ams.usda.gov/CMS/ (accessed November 29, 2010).

Walters FS, deFontes CM, Hart H, Warren GW, Chen JS. 2010. Lepidopteran-active variable-region sequence imparts coleopteran activity in eCry3.1Ab, an engineered *Bacillus thuringiensis* hybrid insecticidal protein. *Applied and Environmental Microbiology* 76:3082–3088.

Part IV. The Nature of the Genetic Modification

This Part describes the method by which corn was transformed, the development of MZIR098 corn, and production of MZIR098 corn and nontransgenic, near-isogenic corn seed lots for use in the studies described in this document.

IV.A. Description of the Transformation Method

Transformation of *Z. mays* to produce MZIR098 corn was accomplished through the use of immature embryos of a proprietary corn line via *Agrobacterium tumefaciens*–mediated transformation, as described by Negrotto *et al*. 2000. By this method, genetic elements within the left and right border regions of the transformation plasmid were efficiently transferred and integrated into the genome of the target plant cell, while genetic elements outside these border regions were not transferred.

Immature embryos were excised from corn ears (NP2222) that were harvested 8 to 12 days after pollination. The embryos were rinsed with fresh medium and mixed with a suspension of *A. tumefaciens* strain LBA4404 harboring plasmids pSB1 (Komari *et al.* 1996) and pSYN17629. The embryos in suspension were vortexed for 30 seconds and allowed to incubate for an additional 5 minutes. Excess *A. tumefaciens* suspension was removed by aspiration, and the embryos were moved to plates containing a nonselective culture medium. The embryos were co-cultured with the remaining *A. tumefaciens* at 22°C for 2 to 3 days in the dark. The embryos were then transferred to culture medium supplemented with ticarcillin (200 mg/l) and silver nitrate (1.6 mg/l) and incubated in the dark for 10 days. The *pat-08* gene was used as a selectable marker during the transformation process (Negrotto *et al*. 2000). The embryos producing embryogenic calli were transferred to a cell culture medium containing glufosinate-ammonium as a selection agent. The transformed tissue was transferred to a selective medium containing the broad-spectrum antibiotic cefotaxime at 500 mg/l (a concentration known to kill *A. tumefaciens* [Xing *et al*. 2008]) and grown for four months, ensuring that the *A. tumefaciens* was cleared from the transformed tissue.

The regenerated plantlets were tested for the presence of *ecry3.1Ab*, *mcry3A*, and *pat-08* and for the absence of the spectinomycin resistance gene (*aadA-03*) present on the vector backbone by real-time polymerase chain reaction (PCR) analysis (Ingham *et al*. 2001). This screen allowed for the selection of transgenic events that carried the transferred deoxyribonucleic acid (T-DNA) and were free of plasmid backbone DNA. Plants that tested positive for *ecry3.1Ab*, *mcry3A*, and *pat-08* and negative for *aadA-03* were transferred to the greenhouse for further propagation.

IV.B. Intermediate Host Organisms Used for All Laboratory Manipulations Prior to Plant Transformation

Standard strains of *Escherichia coli* were used for laboratory manipulations prior to plant transformation using disarmed *A. tumefaciens*. Such strains of *E. coli* used in molecular biology are considered non-pathogenic (Muhldorfer and Hacker 1994).

IV.C. Development of MZIR098 Corn

Progeny of the original transformants (T_0 plants) were field tested for resistance to corn rootworm feeding damage, tolerance to glufosinate-ammonium, and agronomic performance in multiple elite lines of corn. MZIR098 corn was selected as the lead commercial candidate among several transformation events and underwent further field testing and development. Figure IV-1 shows the steps in the development of MZIR098 corn.

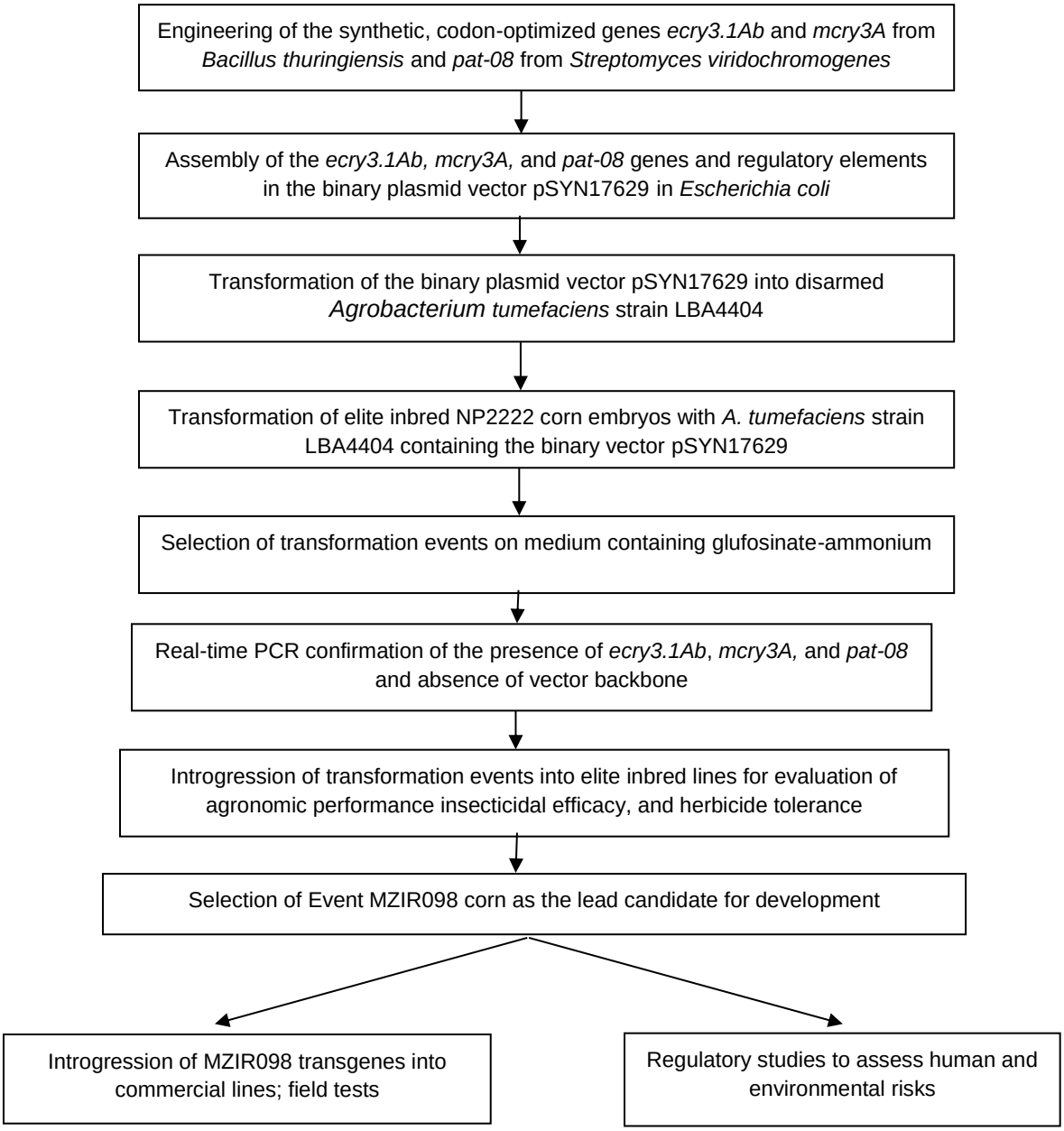


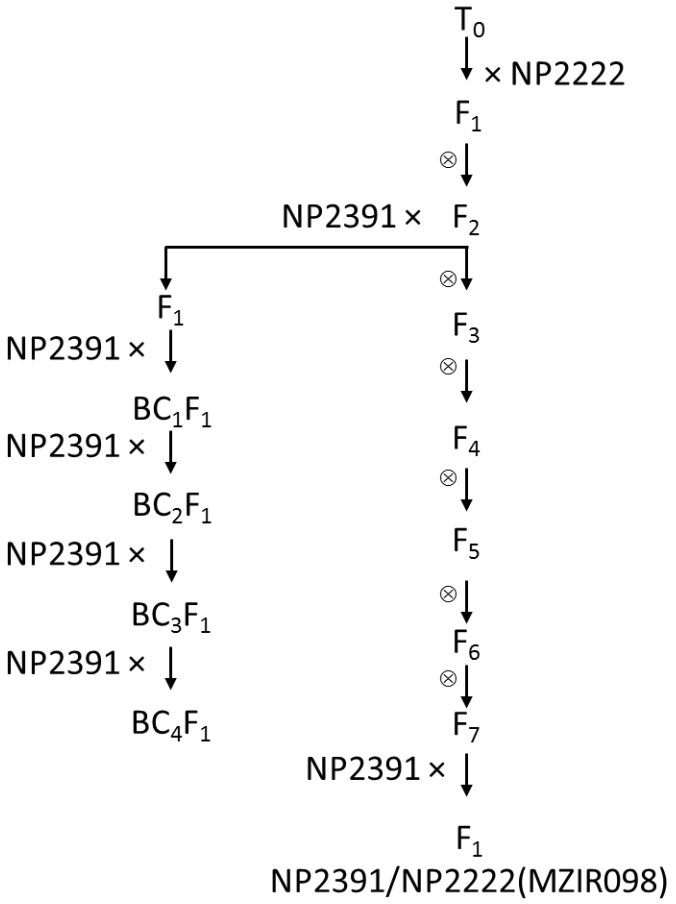
Figure IV-1. Steps in the development of MZIR098 corn

IV.D. Production of Test and Control Seed

Production of all MZIR098 corn and nontransgenic, near-isogenic control corn seed lots used in the studies described in this application were carried out under controlled and isolated conditions under the direction of Syngenta breeders and field researchers. Figure IV-2 shows the pedigree of MZIR098 corn seed materials.

⊗ Self-pollinated

X Crossed with



The transformation recipient line was corn inbred NP2222.

Figure IV-2. Pedigree of the MZIR098 plant materials used in regulatory studies

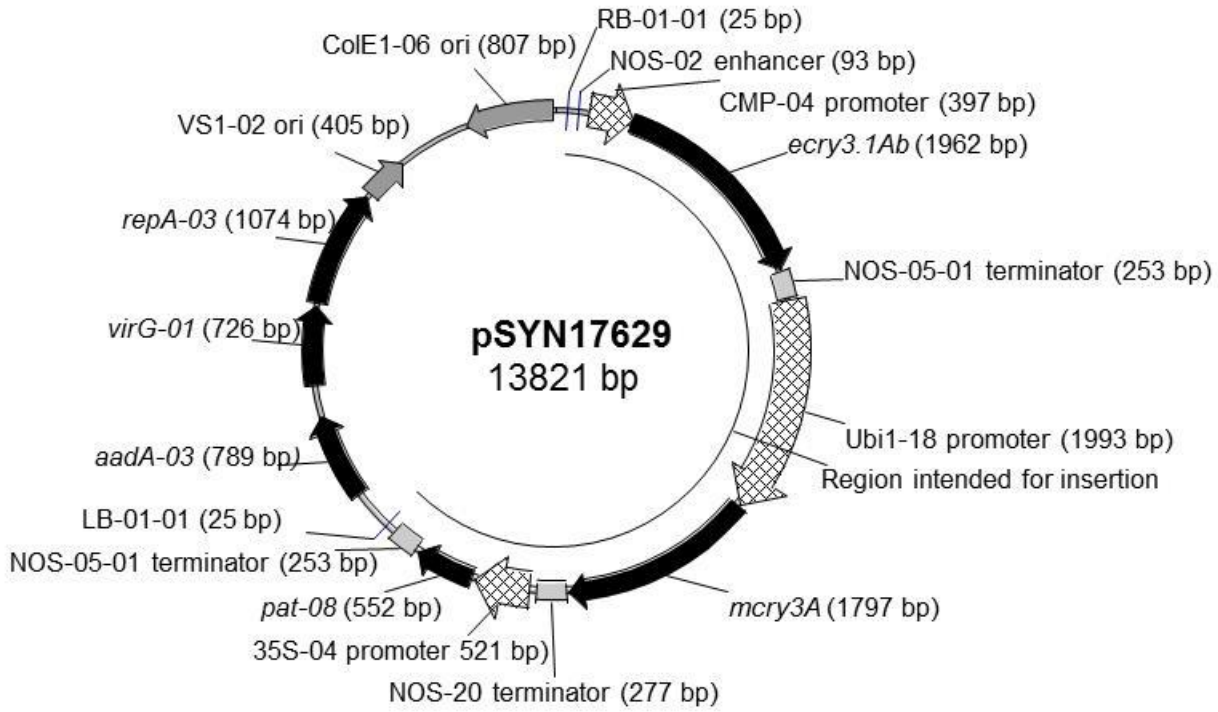
IV.E. Quality Control of Test and Control Materials

All MZIR098 and nontransgenic, near-isogenic control corn seed lots used in regulatory studies were analyzed by real-time PCR for the presence of MZIR098 DNA and the absence of adventitious DNA from other transformation events. All MZIR098 corn seed lots were confirmed to contain Event MZIR098-specific DNA. MZIR098 DNA was not detected in any

nontransgenic, near-isogenic control corn seed lots. None of the MZIR098 or nontransgenic, near-isogenic control corn seed lots contained any detectable sequences indicative of DNA from other regulated transgenic corn products under development at Syngenta or from other transgenic corn products, such as commercial varieties, for which testing methodology is available

IV.F. Description of the Gene Construct and the Transformation Vector

The transformation plasmid pSYN17629 was used to produce MZIR098 corn by *A. tumefaciens*–mediated transformation of immature corn embryos. The DNA region between the left and right borders of the transformation plasmid included gene-expression cassettes for *ecry3.1Ab*, *mcry3A*, and *pat-08*. The *ecry3.1Ab* expression cassette consisted of the *ecry3.1Ab* coding region regulated by a CMP promoter from cestrum yellow leaf curling virus (CMP-04) and the nopaline synthase (NOS) terminator sequence from *A. tumefaciens* (NOS-05-01), as well as the NOS enhancer sequence (NOS-02). The *mcry3A* expression cassette consisted of the *mcry3A* coding region regulated by a corn ubiquitin promoter (Ubi1-18) and NOS terminator (NOS-20). The *pat-08* expression cassette consisted of the *pat-08* coding region regulated by the 35S promoter from cauliflower mosaic virus (35S-04) and the NOS terminator (NOS-05-01). A map of the transformation plasmid is shown in Figure IV-3, and each genetic element in the transformation plasmid is described in Table IV-1



bp = base pairs

Figure IV-3. Plasmid map for the vector pSYN17629

Table IV-1. Description of the genetic elements in vector pSYN17629

Genetic element	Size (bp)	Position	Description
***ecry3.1Ab* cassette**			
Region-1	80	26 to 105	Region used for cloning.
NOS-02 enhancer	93	106 to 198	Enhancer sequence from the NOS gene of *A. tumefaciens* which increases gene expression (NCBI accession number V00087.1) (Bevan *et al.* 1983).
Region-2	5	199 to 203	Region used for cloning.
CMP-04 promoter	397	204 to 600	Cestrum yellow leaf curling virus promoter region (Hohn *et al*. 2007). Provides constitutive expression in corn.
Region-3	9	601 to 609	Region used for cloning.
ecry3.1Ab	1962	610 to 2571	An engineered Cry gene active against certain corn rootworm *(Diabrotica)* species (NCBI accession number GU327680.1). As an engineered chimeric protein, eCry3.1Ab has similarities to other well-characterized Cry proteins. Because Cry proteins share structural similarities, chimeric Cry genes can be engineered via the exchange of domains that are homologous between different Cry genes. The gene *ecry3.1Ab* consists of a fusion between the 5′ end (domain I, domain II, and 15 amino acids of domain III) of a modified Cry3A gene (*mcry3A*) and the 3′ end (domain III and variable region 6 [Höfte and Whiteley 1989]) of a synthetic Cry1Ab gene (see description of *mcry3A* and *cry1Ab* below). Upstream of the *mcry3A* domain, the gene *ecry3.1Ab* carries a 67-bp oligomer extension at its 5′ end, which was introduced during the engineering of the variable regions and is translated into the following 22 amino acid residues: MTSNGRQCAGIRPYDGRQQHRG. The next 459 amino acid residues are identical to those of mCry3A, followed by 172 amino acid residues of Cry1Ab. Description of *cry1Ab*: The gene *cry1Ab* was originally cloned from *B. thuringiensis* subsp. *kurstaki* strain HD-1 (Geiser *et al*. 1986). Its amino acid sequence has been codon-optimized (Koziel *et al*. 1997) to accommodate the preferred codon usage for corn (Murray *et al*. 1989).
Region-4	21	2572 to 2592	Region used for cloning.
NOS-05-01 terminator	253	2593 to 2845	Terminator sequence from the NOS gene of *A. tumefaciens* (NCBI accession number V00087.1). Provides a polyadenylation site (Bevan *et al*. 1983).

Continued

Genetic element	Size (bp)	Position	Description
***mcry3A* cassette**			
Region-5	20	2846 to 2865	Region used for cloning.
Ubi1-18 promoter	1993	2866 to 4858	Promoter region from *Z. mays* polyubiquitin gene which contains the first intron (NCBI accession number S94464.1), in which three bp have been altered to remove restriction sites. Provides constitutive expression in monocots (Christensen *et al*. 1992).
Region-6	9	4859 to 4867	Region used for cloning.
mcry3A	1797	4868 to 6664	A corn-optimized cry3A was synthesized to accommodate the preferred codon usage for corn (Murray et al. 1989). The synthetic sequence was based on the native Cry3A protein sequence from B. thuringiensis subsp. tenebrionis (Sekar *et al.* 1987). The corn-optimized gene was then modified to incorporate a consensus cathepsin G protease recognition site within the expressed protein. The amino acid sequence of the encoded mCry3A corresponds to that of the native Cry3A, except that (1) its N-terminus corresponds to M-48 of the native protein and (2) a cathepsin G protease recognition site has been introduced, beginning at amino acid residue 155 of the native protein. This cathepsin G recognition site has the amino acid sequence AAPF, and has replaced the amino acids V-155, S-156, and S-157 in the native protein (Chen and Stacy 2007).
Region-7	19	6665 to 6683	Region used for cloning.
NOS-20 terminator	277	6684 to 6960	Terminator sequence from the NOS gene of A. tumefaciens (NCBI accession number V00087.1). Variation of the NOS-05-01 terminator with nucleotide changes to eliminate cross-border unintended open reading frames. Provides a polyadenylation site (Bevan *et al*. 1983).
Region-8	59	6961 to 7019	Region used for cloning.
***pat-08* cassette**			
35S-04 promoter	521	7020 to 7540	Promoter region of cauliflower mosaic virus (Odell *et al.* 1985). Provides constitutive expression in plants.
Region-9	24	7541 to 7564	Region used for cloning.
pat-08	552	7565 to 8116	*S. viridochromogenes* strain Tü494 gene encoding the selectable marker PAT. The native coding sequence (Wohlleben *et al*. 1988) was codon-optimized for enhanced expression (NCBI accession number DQ156557.1). The synthetic *pat*

Continued

Genetic element	Size (bp)	Position	Description
			was obtained from AgrEvo, Germany. PAT confers resistance to herbicides containing glufosinate-ammonium (phosphinothricin). The gene *pat*-08 was altered to remove an *Xma*I site.
Region-10	31	8117 to 8147	Region used for cloning.
NOS-05-01 terminator	253	8148 to 8400	Terminator sequence from the NOS gene of *A. tumefaciens* (NCBI accession number V00087.1). Provides a polyadenylation site (Bevan *et al*. 1983).
Region-11	87	8401 to 8487	Region used for cloning.
Border Region			
LB-01-01	25	8488 to 8512	Left border region of T-DNA from *A. tumefaciens* nopaline Ti plasmid (NCBI accession number J01825.1). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Yadav *et al*. 1982).
Plasmid backbone			
Region-12	349	8513 to 8861	Region used for cloning.
aadA-03	789	8862 to 9650	Aminoglycoside adenylyltransferase gene from *Escherichia coli* transposon Tn7 (similar to NCBI accession number X03043.1). Confers resistance to streptomycin and spectinomycin and is used as a bacterial selectable marker (Fling *et al*. 1985).
Region-13	299	9651 to 9949	Region used for cloning.
virG-01	726	9950 to 10675	The VirGN54D gene from pAD1289 (similar to NCBI accession number AF242881.1). The N54D substitution results in a constitutive VirG phenotype. The gene *virG* is part of the two-component regulatory system for the virulence regulon in *A. tumefaciens* (Hansen *et al*. 1994).
Region-14	29	10676 to 10704	Region used for cloning.
repA-03	1074	10705 to 11778	Gene encoding the pVS1 replication protein from *Pseudomonas aeruginosa* (similar to NCBI accession number AF133831.1), which is a part of the minimal pVS1 replicon that is functional in Gram-negative, plant-associated bacteria (Heeb *et al*. 2000).
Region-15	42	11779 to 11820	Region used for cloning.
VS1-02 Ori	405	11821 to 12225	Consensus sequence for the origin of replication and partitioning region from pVS1 of *P. aeruginosa* (NCBI accession number U10487.1). Serves as the origin of replication in the *A. tumefaciens* host (Itoh *et al*. 1984).

Continued

Region-16	677	12226 to 12902	Region used for cloning.
ColE1-06 ori	807	12903 to 13709	Origin of replication (similar to NCBI accession number V00268.1) that permits replication of plasmids in E. coli (Itoh and Tomizawa 1979).
Region-17	112	13710 to 13821	Region used for cloning.
Border region			
RB-01-01	25	1 to 25	Right border region of T-DNA from *A. tumefaciens* nopaline Ti plasmid (NCBI accession number J01826.1). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Wang *et al*. 1984).

IV.G. Molecular Characterization of MZIR098 Corn

An extensive genetic characterization of the T-DNA insert in MZIR098 corn was performed by means of nucleotide sequencing and Southern blot analyses. Sequencing results confirmed the expected copy number of each of the functional elements in the T-DNA. In addition, the corn genomic sequences flanking the MZIR098 insert were identified and characterized. The genetic stability of the insert and absence of plasmid backbone sequence in the MZIR098 corn genome was assessed both by Southern blot analyses over at least three generations of MZIR098 corn. Further stability of the insert was assessed by examining the inheritance patterns of the transgenes over three generations of MZIR098 corn. These data collectively demonstrate that no deleterious changes occurred in the MZIR098 corn genome as a result of the T-DNA insertion.

Parts IV.G.1 through IV.G.4 below, describe the design, results, and conclusions of each genetic characterization study, and the general conclusions of the genetic characterization studies are summarized in Part IV.H.

IV.G.1. Nucleotide Sequence of the T-DNA Insert

Five overlapping DNA fragments that covered the entire MZIR098 insert were amplified via PCR from genomic DNA extracted from MZIR098 F_3 generation corn. Four of the fragments were cloned. A fifth fragment was not cloned but was directly sequenced. A consensus sequence was generated from all of the fragments and compared with the sequence of the T-DNA in plasmid pSYN17629, the transformation plasmid used to create MZIR098 corn.

Comparison of the MZIR098 insert sequence with the transformation plasmid pSYN17629 showed that the 8467-bp MZIR098 insert was intact, with no rearrangements or base-pair changes. Some truncation occurred at the right and left border ends of the T-DNA during the transformation process that resulted in MZIR098 corn. The right border, along with 10 base pairs (bp) of non-coding sequence were truncated, and 10 bp from the left border were truncated. These deletions have no effect on the functionality of the T-DNA insert. Sequence analysis of the MZIR098 insertion site demonstrated that 24 bp from the corn genomic sequence were deleted during the integration of the MZIR098 insert.

The copy number and sequence of each of the functional elements in MZIR098 corn is as expected based on the pSYN17629 T-DNA sequence. The MZIR098 insert contains a single

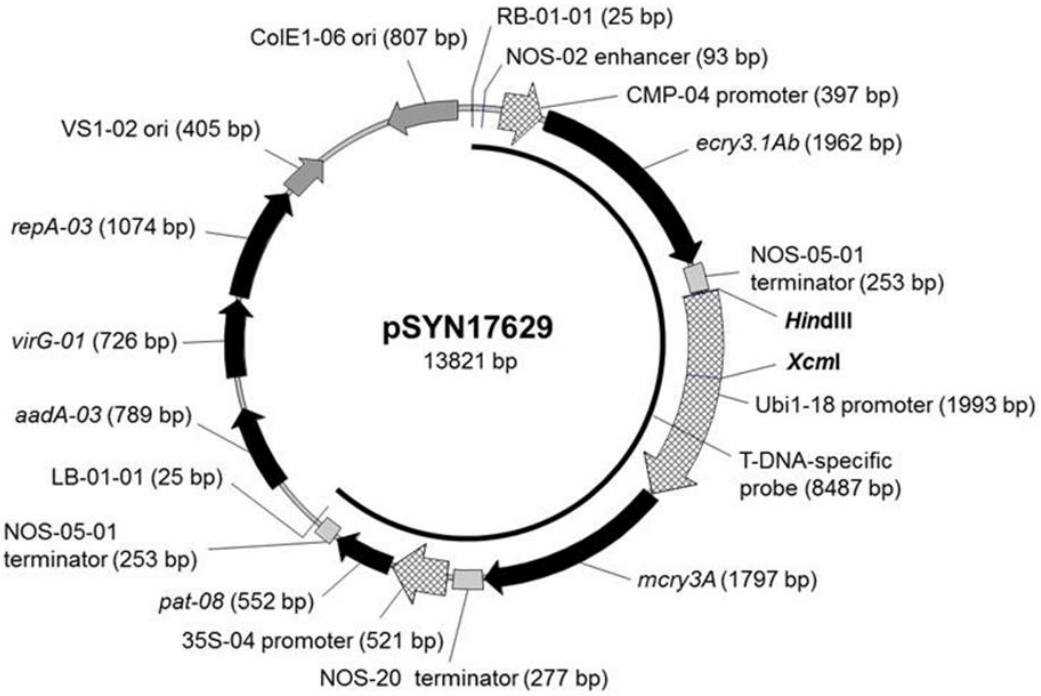
copy of each of the functional elements *ecry3.1Ab*, *mcry3A*, *pat-08*, NOS-02 enhancer, CMP-04 promoter, Ubi1-18 promoter, NOS-20 terminator, 35S-04 promoter and two copies of the NOS-05-01 terminator as expected.

IV.G.2. Characterization of the MZIR098 Corn Insert by Southern Blot Analysis

Southern blot analyses were performed to characterize the transgenic insert of MZIR098 corn by determining the number of plasmid pSYN17629 T-DNA integration sites and the presence or absence of pSYN17629 backbone sequence or additional extraneous fragments of T-DNA.

IV.G.2.a. T-DNA Integrations Sites in MZIR098 Corn and Stability across Multiple Generations

The number of T-DNA integration sites within the MZIR098 corn genome and the number of copies of the T-DNA at each integration site within the MZIR098 corn genome were determined through the use of a single T-DNA-specific probe that covered every base pair of the pSYN17629 T-DNA expected to be transferred and integrated into the corn genome. The template for the probe was a segment of the pSYN17629 T-DNA corresponding with the NOS-02 enhancer through the NOS-05-01 terminator near the left border region (Figure IV-4).



The restriction enzyme *Bmt*I was used during the Southern blot analyses but is not present on this map because pSYN17629 does not contain the recognition sequence for this enzyme.

Figure IV-4. Location of the 8.5-kb full length T-DNA-specific probe and the restriction sites *Hin*dIII and *Xcm*I in the transformation plasmid pSYN17629

The MZIR098 corn generations used in Southern blot analyses were the F_2, F_3, F_4, F_5, and F_1 generations. The F_2 through F_5 generations were in the genetic background NP2222. The F_1

generation was in the background NP2391/NP2222 and was representative of a commercial corn hybrid. Five generations of MZIR098 corn were included to demonstrate stability of the T-DNA insert over multiple generations. The control substances were nontransgenic, near-isogenic NP2222, NP2391, and NP2222/NP2391 corn. DNA from nontransgenic, near-isogenic control corn was used as a negative control to identify any endogenous corn DNA sequences that hybridize to the probe. For all samples, leaf tissue from seven plants were sampled, pooled, and subjected to DNA extraction. Genomic DNA was isolated from leaf tissue by a method modified from that described by Murray and Thompson (1980).

To demonstrate the sensitivity of the analyses, each Southern blot analysis included two positive assay controls representing 1-copy and 1/7-copy per genome of a DNA fragment of known size in the corn genome. The positive assay controls were PCR-amplified fragments that corresponded to the full length T-DNA-specific fragment and were loaded in a well together with 7.5 µg of digested DNA from nontransgenic, near-isogenic NP2222/NP2391 corn, in order to more accurately reflect their migration speeds in the corn genome matrix.

The amount of positive assay control (in picograms for one copy) was calculated by the following formula (Arumuganathan and Earle 1991):

$$\left\{\left(\frac{\text{positive assay control size (bp)}}{\text{genome size (bp)} \times \text{ploidy}}\right) \times \mu\text{g loaded}\right\} \times 1 \times 10^{6} = \text{pg for 1 copy}$$

The following factors were used to calculate the amounts of the positive assay controls:

corn genome size (bp)	2.67×10^{9}
corn ploidy	2
DNA loaded in each lane (µg)	7.5
full length T-DNA-specific DNA fragment (bp)	8487

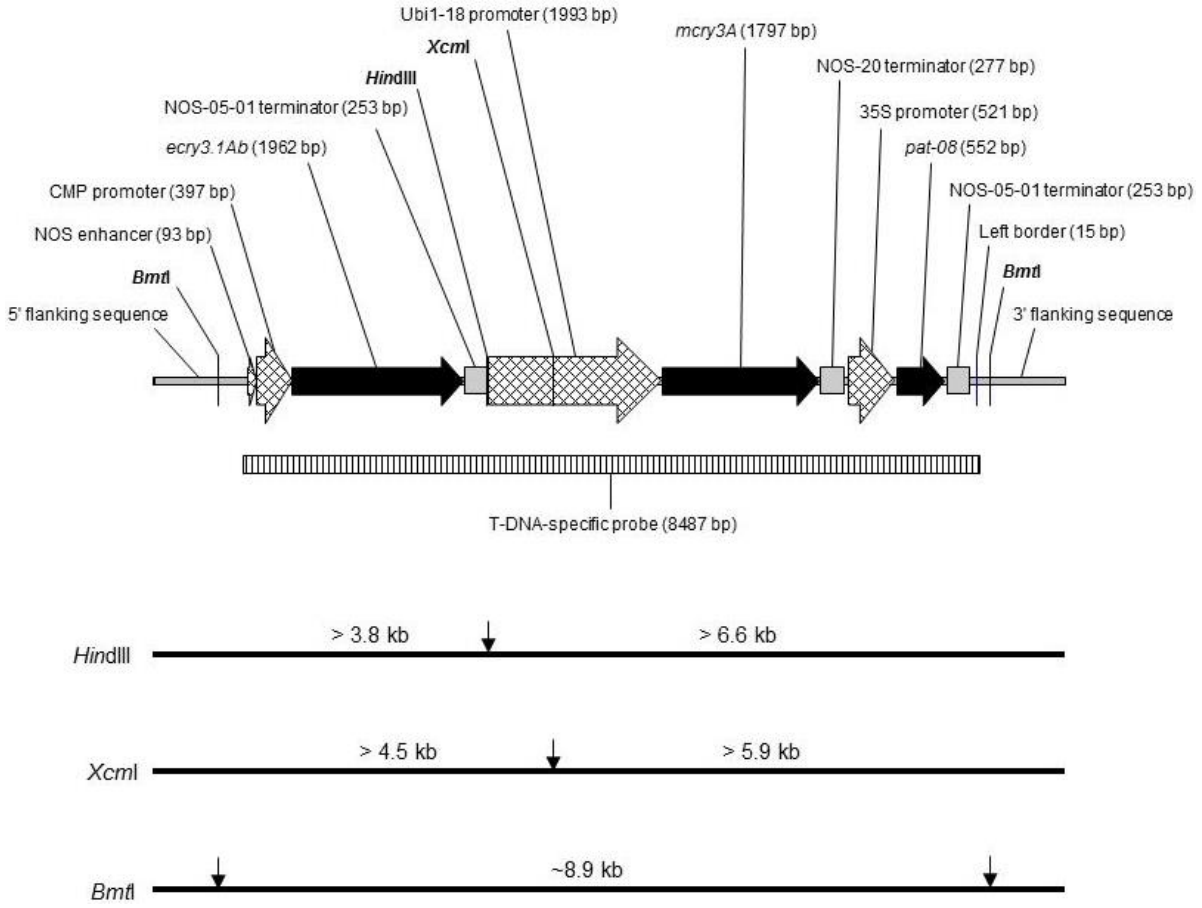
A total of 11.9 pg of the full length T-DNA-specific DNA fragment was added to the 1-copy Southern blot lanes and 1.7 pg was added to the 1/7-copy lanes.

Corn genomic DNA was analyzed via two restriction enzyme digestion strategies. The first strategy was used to determine the number of T-DNA integration sites in the MZIR098 genome, while the second strategy was used to determine the integrity of the insert and the presence of any extraneous DNA fragments of plasmid pSYN17629 T-DNA closely linked to the MZIR098 insert. Data from both strategies were used to infer the number of copies of the insert at the MZIR098 locus.

In the first strategy, the genomic DNA was digested with an enzyme that cut within the MZIR098 insert and in the corn genome flanking the MZIR098 insert. This first strategy was used twice, with two different enzymes. The enzymes used were *Hind*III and *Xcm*I. The locations of the restriction sites are shown in Figure IV-4, above.

In the second strategy, the genomic DNA was digested with a restriction enzyme that cut within the insertion site to release DNA fragments of predictable size. This strategy was used to

determine the number of copies of the T-DNA at each location within the MZIR098 corn genome, the intactness of the insert, and the presence or absence of any closely linked extraneous T-DNA fragments. The enzyme used was *Bmt*I. The restriction enzyme *Bmt*I was used for Southern blot analysis but is not present on the plasmid map because pSYN17629 does not contain the recognition sequence for this enzyme. The *Bmt*I recognition sequences were verified from the known flanking sequences of the MZIR098 insert. Figure IV-5 shows the digestion strategy used with the full length T-DNA-specific probe.



The vertical arrows indicate the site of restriction digestion.
Sizes of the expected restriction fragments are indicated.
The restriction enzyme *Bmt*I was used during the Southern blot analysis but is not present on pSYN17629. The *Bmt*I recognition sequences were verified from the known flanking sequences of the MZIR098 insert.

Figure IV-5. Locations of the 8.5-kb T-DNA-specific probe and the restriction sites *Hind*III, *Xcm*I, and *Bmt*I in the MZIR098 corn insert

Table IV-2 shows the insert-specific hybridization bands expected and observed in Southern blot analyses of MZIR098 corn with the full length T-DNA-specific probe. Additional, unexpected bands in any of these analyses would indicate the presence of more than one copy of

the T-DNA at more than one location within the MZIR098 corn genome. No hybridization bands were expected in the analyses of genomic DNA from nontransgenic, near-isogenic corn (the negative control). In the analyses of NP2222, NP2391, or NP2391/NP2222 corn genomic DNA, the observation of bands that were also present in genomic DNA from MZIR098 corn were the result of cross-hybridization of the T-DNA-specific probe sequence with the endogenous corn sequence. Figures IV-6 through IV-8 show the results of the Southern blot analyses with the full length T-DNA-specific probe.

In the analysis of genomic DNA digested with *Hin*dIII, two bands of approximately 5.9 kb and 12.0 kb were observed in the lanes containing DNA from MZIR098 corn F_2, F_3, F_4, F_5, and F_1 generations (Figure IV-6, Lanes 2 through 6). These bands were absent from the lanes containing DNA from the nontransgenic NP2222, NP2391, and NP2222/NP2391 corn (Figure IV-6, Lanes 7 through 9) and were, therefore, specific to the MZIR098 insert. As expected, one band of approximately 8.5 kb was observed in the lanes containing the positive controls (Figure IV-6, Lanes 10 and 11).

In the analysis of genomic DNA digested with *Xcm*I, two bands of approximately 8.0 kb and 11.5 kb were observed in the lanes containing DNA from MZIR098 corn F_2, F_3, F_4, F_5, and F_1 generations (Figure IV-7, Lanes 2 through 6). These bands were absent from the lanes containing DNA from nontransgenic NP2222, NP2391, and NP2222/NP2391 corn (Figure IV-7, Lanes 7 through 9) and were, therefore, specific to the MZIR098 insert. As expected, one band of approximately 8.5 kb was observed in the lanes containing the positive controls (Figure IV-7, Lanes 10 and 11).

In the analysis of genomic DNA digested with *Bmt*I, one band of approximately 8.9 kb was observed in the lanes containing DNA from MZIR098 corn F_2, F_3, F_4, F_5, and F_1 generations (Figure IV-8, Lanes 2 through 6). This band was absent in the lanes containing DNA from nontransgenic NP2222, NP2391, and NP2222/NP2391 corn (Figure IV-8, Lanes 7 through 9) and was, therefore, specific to the MZIR098 insert. As expected, one band of approximately 8.5 kb was observed in the lanes containing the positive controls (Figure IV-8, Lanes 10 and 11).

Table IV-2. Expected and observed insert-specific hybridization bands in Southern blot analyses of multiple generations of MZIR098 corn DNA with a full length T-DNA-specific probe and restriction enzymes *HindIII*, *XcmI*, and *BmtI*

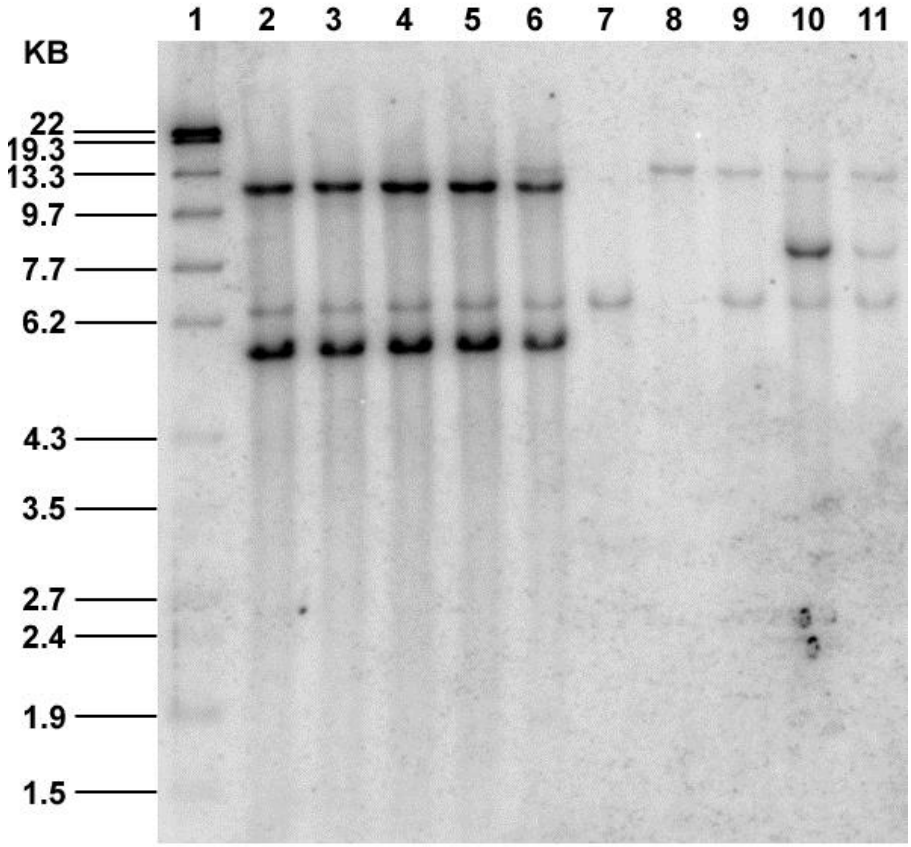
Figure & lane	Source of DNA	Restriction Enzymes	Expected no. of bands[a]	Approximate band size (kb)	
				Expected	Observed[a]
IV-6, 2	MZIR098 F_2 corn	*Hin*dIII	2	>3.8 >6.6	5.9 12.0
IV-6, 3	MZIR098 F_3 corn	*Hin*dIII	2	>3.8 >6.6	5.9 12.0
IV-6, 4	MZIR098 F_4 corn	*Hin*dIII	2	>3.8 >6.6	5.9 12.0
IV-6, 5	MZIR098 F_5 corn	*Hin*dIII	2	>3.8 >6.6	5.9 12.0
IV-6, 6	MZIR098 F_1 corn	*Hin*dIII	2	>3.8 >6.6	5.9 12.0
IV-6, 7	NP2222 corn (negative control)	*Hin*dIII	0	N/A	N/A
IV-6, 8	NP2391 corn (negative control)	*Hin*dIII	0	N/A	N/A
IV-6, 9	NP2222/NP2391 corn (negative control)	*Hin*dIII	0	N/A	N/A
IV-6, 10	1-copy positive control	*Hin*dIII	1	8.5	8.5
IV-6, 11	1/7-copy positive control	*Hin*dIII	1	8.5	8.5
IV-7, 2	MZIR098 F_2 corn	*Xcm*I	2	>4.5 >5.9	8.0 11.5
IV-7, 3	MZIR098 F_3 corn	*Xcm*I	2	>4.5 >5.9	8.0 11.5
IV-7, 4	MZIR098 F_4 corn	*Xcm*I	2	>4.5 >5.9	8.0 11.5
IV-7, 5	MZIR098 F_5 corn	*Xcm*I	2	>4.5 >5.9	8.0 11.5
IV-7, 6	MZIR098 F_1 corn	*Xcm*I	2	>4.5 >5.9	8.0 11.5
IV-7, 7	NP2222 corn (negative control)	*Xcm*I	0	N/A	N/A
IV-7, 8	NP2391 corn (negative control)	*Xcm*I	0	N/A	N/A
IV-7, 9	NP2222/NP2391 corn (negative control)	*Xcm*I	0	N/A	N/A
IV-7, 10	1-copy positive control	*Xcm*I	1	8.5	8.5
IV-7, 11	1/7-copy positive control	*Xcm*I	1	8.5	8.5
IV-8, 2	MZIR098 F_2 corn	*Bmt*I	1	8.9	8.9
IV-8, 3	MZIR098 F_3 corn	*Bmt*I	1	8.9	8.9
IV-8, 4	MZIR098 F_4 corn	*Bmt*I	1	8.9	8.9

Continued

Figure & lane	Source of DNA	Restriction Enzymes	Expected no. of bands[a]	Approximate band size (kb)	
				Expected	Observed[a]
IV-8, 5	MZIR098 F_5 corn	*Bmt*I	1	8.9	8.9
IV-8, 6	MZIR098 F_1 corn	*Bmt*I	1	8.9	8.9
IV-8, 7	NP2222 corn (negative control)	*Bmt*I	0	N/A	N/A
IV-8, 8	NP2391 corn (negative control)	*Bmt*I	0	N/A	N/A
IV-8, 9	NP2222/NP2391 corn (negative control)	*Bmt*I	0	N/A	N/A
IV-8, 10	1-copy positive control	*Bmt*I	1	8.5	8.5
IV-8, 11	1/7-copy positive control	*Bmt*I	1	8.5	8.5

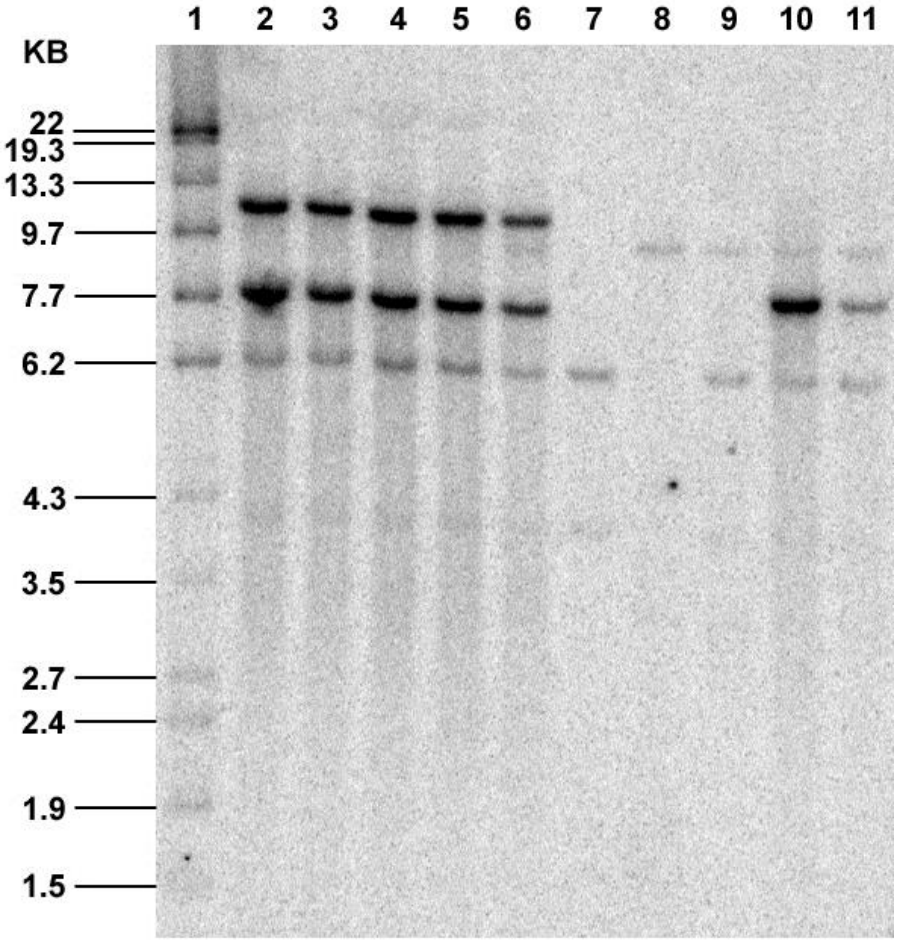
N/A = not applicable.

[a]Bands resulting from cross-hybridization to endogenous corn elements that are not specific to the MZIR098 insert are not included.



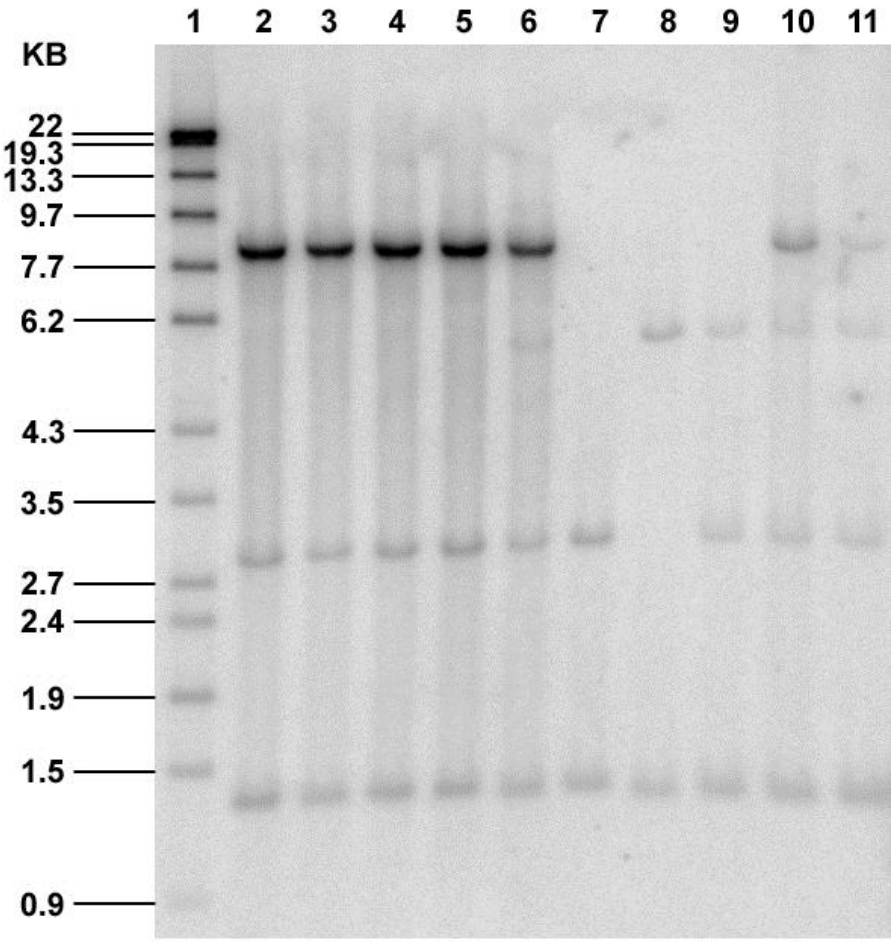
Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_2 corn
Lane 3 = MZIR098 F_3 corn
Lane 4 = MZIR098 F_4 corn
Lane 5 = MZIR098 F_5 corn
Lane 6 = MZIR098 F_1 corn
Lane 7 = NP2222 corn
Lane 8 = NP2391 corn
Lane 9 = NP2222/NP2391 corn
Lane 10 = 1-copy positive control (NP2222/NP2391 corn + 11.9 pg of T-DNA-specific fragment)
Lane 11 = 1/7-copy positive control (NP2222/NP2391 corn + 1.7 pg of T-DNA-specific fragment)

Figure IV-6. Southern blot analysis of MZIR098 corn with the a 8.5-kb full length T-DNA-specific probe and restriction enzyme *Hind*III



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_2 corn
Lane 3 = MZIR098 F_3 corn
Lane 4 = MZIR098 F_4 corn
Lane 5 = MZIR098 F_5 corn
Lane 6 = MZIR098 F_1 corn
Lane 7 = NP2222 corn
Lane 8 = NP2391 corn
Lane 9 = NP2222/NP2391 corn
Lane 10 = 1-copy positive control (NP2222/NP2391 corn + 11.9 pg of T-DNA-specific fragment)
Lane 11 = 1/7-copy positive control (NP2222/NP2391 corn + 1.7 pg of T-DNA-specific fragment)

Figure IV-7. Southern blot analysis of MZIR098 corn with the a 8.5-kb full length T-DNA-specific probe and restriction enzyme *Xcm*I



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_2 corn
Lane 3 = MZIR098 F_3 corn
Lane 4 = MZIR098 F_4 corn
Lane 5 = MZIR098 F_5 corn
Lane 6 = MZIR098 F_1 corn
Lane 7 = NP2222 corn
Lane 8 = NP2391 corn
Lane 9 = NP2222/NP2391 corn
Lane 10 = 1-copy positive control (NP2222/NP2391 corn + 11.9 pg of T-DNA-specific fragment)
Lane 11 = 1/7-copy positive control (NP2222/NP2391 corn + 1.7 pg of T-DNA-specific fragment)

Figure IV-8 Southern blot analysis of MZIR098 corn with the a 8.5-kb full length T-DNA-specific probe and restriction enzyme *Bmt*I

IV.G.2.b. Confirmation of Absence of Plasmid Backbone through Southern Analyses

The elements of the plasmid necessary for its replication and selection in different bacterial hosts are categorized as "plasmid backbone" (the region outside of the T-DNA). In the Southern blot analyses, the presence or absence of plasmid backbone was determined through the use of two backbone-specific probes that together covered every base pair of pSYN17629 outside of the T-DNA. These elements were not expected to be transferred to the plant cell or integrated into the plant genome during T-DNA transfer.

The MZIR098 corn generations used in Southern blot analyses were the F_1 and F_2 generations in the genetic background NP2222. These generations represent the earliest generations of MZIR098 corn. The control substance was nontransgenic, near-isogenic NP2222 corn. DNA from nontransgenic, near-isogenic control corn was used as a negative control to identify any endogenous corn DNA sequences that hybridize to the probe. For the MZIR098 F_1 generation, leaf tissue from 14 plants were sampled and pooled in groups of seven and used for DNA extraction. For the MZIR098 F_2 generation and the nontransgenic, near-isogenic NP2222 corn, leaf tissue from seven plants were sampled, pooled, and used for DNA extraction. Genomic DNA was isolated from leaf tissue by a method modified from that described by Murray and Thompson (1980).

To demonstrate the sensitivity of the analyses, each Southern blot analysis included two positive assay controls representing 1-copy and 1/7-copy per genome of a DNA fragment of known size in the corn genome. The positive assay controls were PCR-amplified fragments that corresponded to the two backbone-specific probes and were loaded in a well together with 7.5 µg of digested DNA from nontransgenic, near-isogenic NP2222 corn, in order to more accurately reflect their migration speeds in the corn genome matrix.

The amount of positive assay control (in picograms for one copy) was calculated by the following formula (Arumuganathan and Earle 1991):

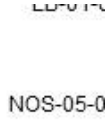
$$\left\{\left(\frac{\text{positive assay control size (bp)}}{\text{genome size (bp)} \times \text{ploidy}}\right) \times \mu\text{g loaded}\right\} \times 1 \times 10^{6} = \text{pg for 1 copy}$$

The following factors were used to calculate the amounts of the positive assay controls:

corn genome size (bp)	2.67×10^9
corn ploidy	2
DNA loaded in each lane (µg)	7.5
backbone-specific DNA fragment 1 (bp)	3311
backbone-specific DNA fragment 2 (bp)	2065

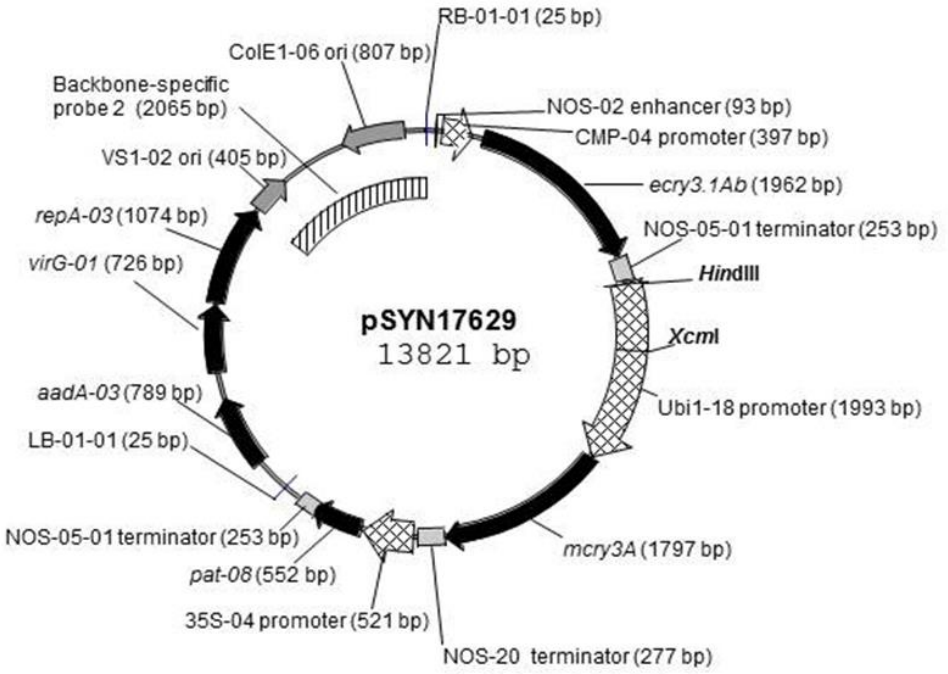
A total of 4.65 and 0.66 pg of backbone-specific DNA fragment 1 were added to the Southern blot for the 1-copy and 1/7-copy lanes, respectively. A total of 2.90 and 0.41 pg of backbone-specific DNA fragment 2 were added to the Southern blot for the 1-copy and 1/7-copy lanes, respectively.

Corn genomic DNA was analyzed via two restriction enzyme digestion strategies. In the first strategy, the genomic DNA was digested with an enzyme that cut within the MZIR098 insert and in the corn genome flanking the MZIR098 insert. This first strategy was used twice, with two different enzymes. The enzymes used were *Hind*III and *Xcm*I (Figures IV-9 and IV-10). In the second strategy the genomic DNA was digested with restriction enzymes that cut within the insert to release DNA fragments of predictable size. The enzyme used was *Bmt*I.



The restriction enzyme *Bmt*I was used during the Southern blot analysis but is not present on this map because pSYN17629 does not contain the recognition sequence for this enzyme.

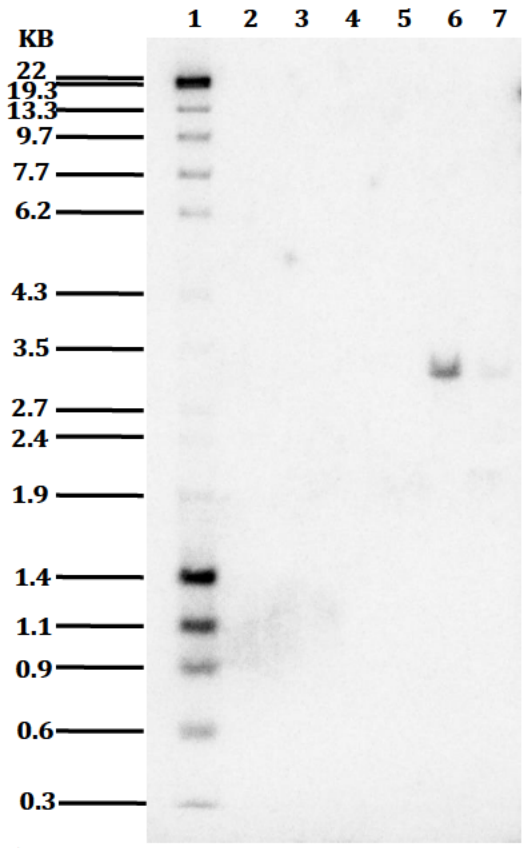
Figure IV-9. Locations of the 3.3-kb backbone-specific probe 1 and the restriction sites *Hind*III and *Xcm*I in the transformation plasmid pSYN17629



The restriction enzyme *Bmt*I was used during the Southern blot analysis but is not present on this map because pSYN17629 does not contain the recognition sequence for this enzyme.

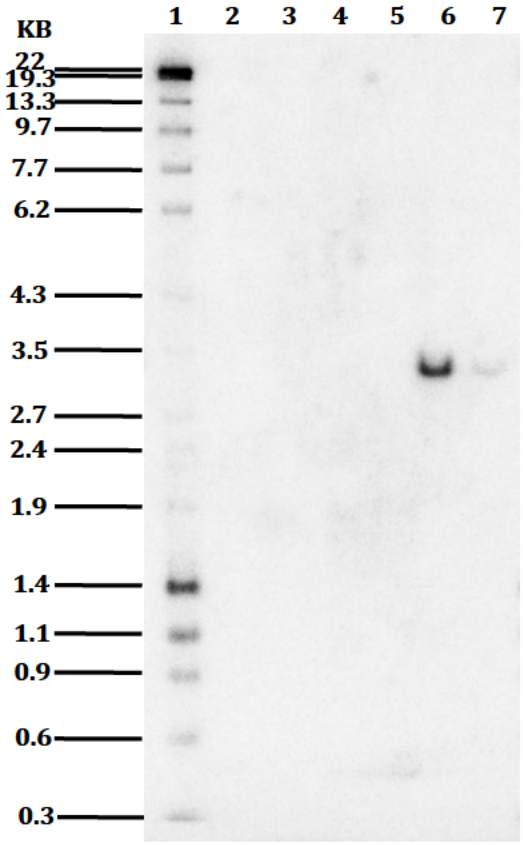
Figure IV-10. Locations of the 2.1-kb backbone-specific probe 2 and the restriction sites *Hind*III and *Xcm*I in the transformation plasmid pSYN17629

Figure IV-11 through IV-13 show the results of the Southern blot analyses with backbone-specific probe 1. In the analyses of genomic DNA digested with *Hin*dIII, *Xcm*I, and *Bmt*I, no bands were observed in any of the lanes containing DNA from MZIR098 corn F_1 and F_2 generations (Figures IV-11 through IV-13, Lanes 2 through 4) or in the lanes containing DNA from nontransgenic NP2222 corn (Figures IV-11 through IV-13, Lanes 5 through 7). One band of approximately 3.3 kb was observed in the lanes containing the 1-copy and 1/7-copy positive controls (Figures IV-11 through IV-13, Lanes 6 and 7), as expected.



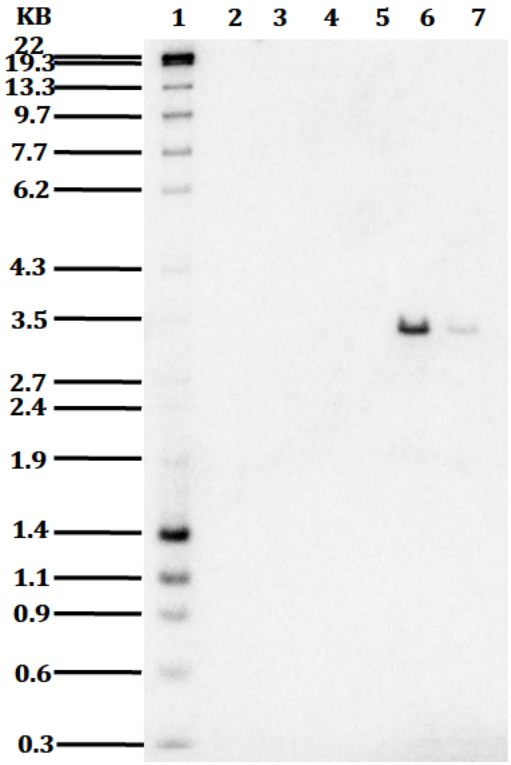
Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_1 (pool 1) corn
Lane 3 = MZIR098 F_1 (pool 2) corn
Lane 4 = MZIR098 F_2 corn
Lane 5 = NP2222 corn
Lane 6 = 1-copy positive control (NP2222 corn + 4.65 pg of backbone-specific fragment 1)
Lane 7 = 1/7-copy positive control (NP2222 corn + 0.66 pg of backbone-specific fragment 1)[a]
[a]Because of limitations in printer resolution, the faint band visible at approximately 3.3 kb in lane 7 may not be visible on the printed copy.

Figure IV-11. Southern blot analysis of MZIR098 corn with the 3.3-kb plasmid pSYN17629 backbone-specific probe 1 and restriction enzyme *Hind*III



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_1 (pool 1) corn
Lane 3 = MZIR098 F_1 (pool 2) corn
Lane 4 = MZIR098 F_2 corn
Lane 5 = NP2222 corn
Lane 6 = 1-copy positive control (NP2222 corn + 4.65 pg of backbone-specific fragment 1)
Lane 7 = 1/7-copy positive control (NP2222 corn + 0.66 pg of backbone-specific fragment 1)[a]
[a]Because of limitations in printer resolution, the faint band visible at approximately 3.3 kb in lane 7 may not be visible on the printed copy.

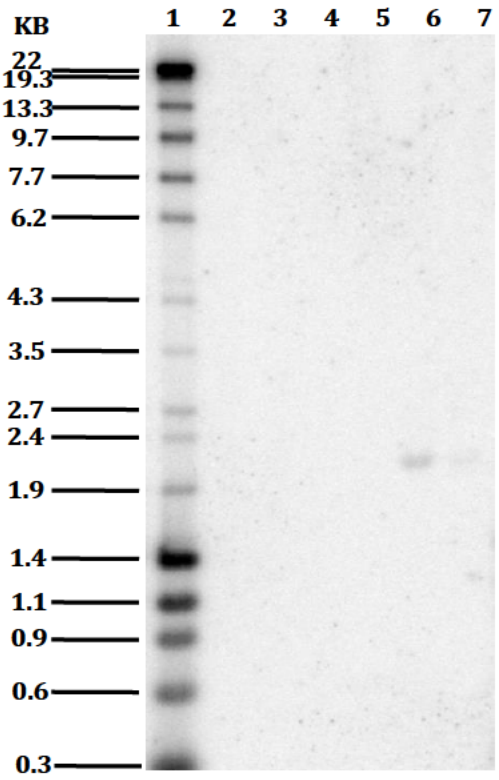
Figure IV-12. Southern blot analysis of MZIR098 corn with the 3.3-kb plasmid pSYN17629 backbone-specific probe 1 and restriction enzyme *Xcm*I



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_1 (pool 1) corn
Lane 3 = MZIR098 F_1 (pool 2) corn
Lane 4 = MZIR098 F_2 corn
Lane 5 = NP2222 corn
Lane 6 = 1-copy positive control (NP2222 corn + 4.65 pg of backbone-specific fragment 1)
Lane 7 = 1/7-copy positive control (NP2222 corn + 0.66 pg of backbone-specific fragment 1)[a]
[a]Because of limitations in printer resolution, the faint band visible at approximately 3.3 kb in lane 7 may not be visible on the printed copy.

Figure IV-13. Southern blot analysis of MZIR098 corn with the 3.3-kb plasmid pSYN17629 backbone-specific probe 1 and restriction enzyme *Bmt*I

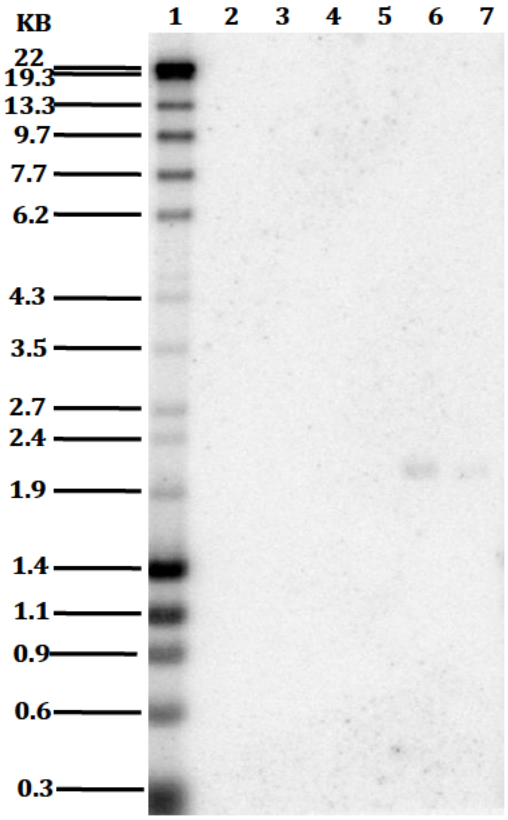
Figures IV-14 through IV-16 show the results of the Southern blot analyses with backbone-specific probe 2. In the analyses of genomic DNA digested with *Hin*dIII, *Xcm*I, and *Bmt*I, no bands were observed in any of the lanes containing DNA from MZIR098 corn F_1 and F_2 generations (Figures IV-14 through IV-16, Lanes 2 through 4) or in the lanes containing DNA from nontransgenic NP2222 corn (Figures IV-14 through IV-16, Lanes 5 through 7). One band of approximately 2.1 kb was observed in the lanes containing the 1-copy and 1/7-copy positive controls (Figures IV-14 through IV-16, Lanes 6 and 7), as expected.



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_1 (pool 1) corn
Lane 3 = MZIR098 F_1 (pool 2) corn
Lane 4 = MZIR098 F_2 corn
Lane 5 = NP2222 corn
Lane 6 = 1-copy positive control (NP2222 corn + 2.90 pg of backbone-specific fragment 2)
Lane 7 = 1/7-copy positive control (NP2222 corn + 0.41 pg of backbone-specific fragment 2)[a]

[a]Because of limitations in printer resolution, the faint band visible at approximately 2.1 kb in lane 7 may not be visible on the printed copy.

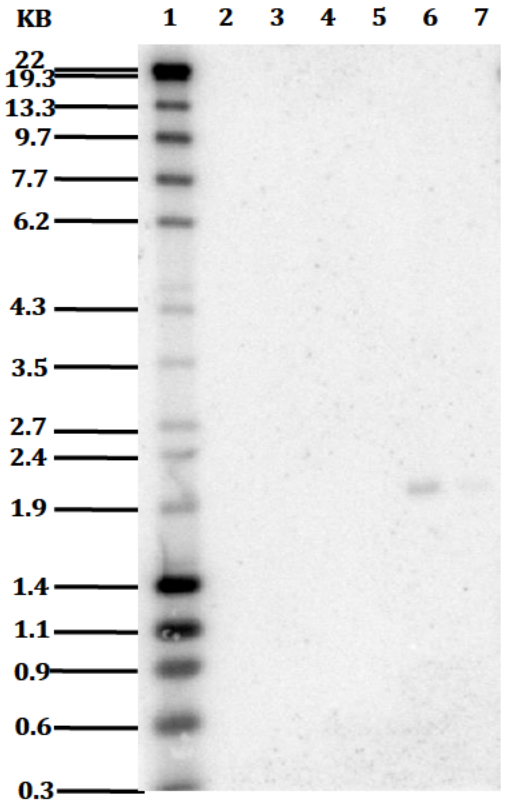
Figure IV-14. Southern blot analysis of MZIR098 corn with the 2.1-kb plasmid pSYN17629 backbone-specific probe 2 and restriction enzyme *Hind*III



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_1 (pool 1) corn
Lane 3 = MZIR098 F_1 (pool 2) corn
Lane 4 = MZIR098 F_2 corn
Lane 5 = NP2222 corn
Lane 6 = 1-copy positive control (NP2222 corn + 2.90 pg of backbone-specific fragment 2)
Lane 7 = 1/7-copy positive control (NP2222 corn + 0.41 pg of backbone-specific fragment 2)[a]

[a]Because of limitations in printer resolution, the faint band visible at approximately 2.1 kb in lane 7 may not be visible on the printed copy.

Figure IV-15. Southern blot analysis of MZIR098 corn with the 2.1-kb plasmid pSYN17629 backbone-specific probe 2 and restriction enzyme *Xcm*I



Lane 1 = molecular weight markers
Lane 2 = MZIR098 F_1 (pool 1) corn
Lane 3 = MZIR098 F_1 (pool 2) corn
Lane 4 = MZIR098 F_2 corn
Lane 5 = NP2222 corn
Lane 6 = 1-copy positive control (NP2222 corn + 2.90 pg of backbone-specific fragment 2)
Lane 7 = 1/7-copy positive control (NP2222 corn + 0.41 pg of backbone-specific -specific fragment 2)[a]
[a]Because of limitations in printer resolution, the faint band visible at approximately 2.1 kb in lane 7 may not be visible on the printed copy.

Figure IV-16. Southern blot analysis of MZIR098 corn with the 2.1-kb plasmid pSYN17629 backbone-specific probe 2 and restriction enzyme *Bmt*I

IV.G.3. Conclusions from the Results of the Southern Blot Analyses

The Southern blot analyses demonstrated that the hybridization bands specific to the MZIR098 insert were identical in all lanes containing genomic DNA extracted from MZIR098 corn plants of the generations tested. These results support the conclusion that the MZIR098 insert is stably inherited from one generation to the next and that MZIR098 corn contains a single T-DNA insert. No unexpected bands were detected, indicating that the MZIR098 corn genome contains no extraneous DNA fragments of the insert. The Southern blot analyses also demonstrated that MZIR098 corn does not contain any backbone sequence from the transformation plasmid pSYN17629.

IV.G.4. Mendelian Inheritance of the T-DNA Insert

Three generations of MZIR098 corn were individually analyzed for the presence of *ecry3.1Ab*, *mcry3A*, and *pat-08* by real-time PCR analysis (Ingham *et al.* 2001). The results from real-time PCR analysis were used to determine the segregation ratios of *ecry3.1Ab*, *mcry3A*, and *pat-08*. Hemizygous MZIR098 corn plants of the F_2 generation were crossed with nontransgenic corn line NP2391. The resulting F_1 generation was backcrossed with the nontransgenic recurrent parent (NP2391) to yield the BC_1F_1 generation. MZIR098 corn plants from the BC_1F_1 generation were backcrossed three more times with the nontransgenic recurrent parent (NP2391) to yield the BC_2F_1, BC_3F_1, and BC_4F_1 generations analyzed in this study. The expected segregation ratio for each gene was 1:1 in each generation (i.e., 50% of the plants in each generation were expected to carry the genes). Chi-square analysis of the segregation data was performed to test the hypothesis that the MZIR098 insert is inherited in a predictable manner according to Mendelian principles and consistent with insertion into a chromosome within the corn nuclear genome. The goodness-of-fit of the observed to the expected segregation ratios was tested by chi-square analysis

$$\chi^2 = \text{sum (observed} - \text{expected)}^2 \div \text{expected}$$

The expected and observed segregation ratios are shown in Table IV-3. The genes *ecry3.1Ab*, *mcry3A*, and *pat-08* co-segregated (i.e., when one gene was present, the other genes were also present). The critical value for rejection of the hypothesis of segregation according to Mendelian inheritance at $\alpha = 0.05$ was 3.84) All of the chi-square values were less than 3.84 for each generation tested, indicating that *ecry3.1Ab*, *mcry3A*, and *pat-08* were inherited in a predictable manner, according to Mendelian principles. These results support the conclusion that the MZIR098 corn insert integrated into a chromosome within the corn nuclear genome.

Table IV-3. Observed and expected frequencies of *ecry3.1Ab*, *mcry3A*, and *pat-08* in three generations of MZIR098 corn

	BC_2F_1		BC_3F_1		BC_4F_1	
Trait[a]	**Observed**	**Expected**	**Observed**	**Expected**	**Observed**	**Expected**
Positive	85	93	69	70	75	74.5
Negative	101	93	71	70	74	74.5
Total	186	186	140	140	149	149
χ^2	1.38[b]		0.03[b]		0.01[b]	

[a]The observed frequencies of *ecry3.1Ab*, *mcry3A*, and *pat-08* were identical; the two genes segregated as one locus.
[b]$P < 0.05$ ($\chi^2 < 3.84$).

IV.H. Summary of the Genetic Characterization of MZIR098 Corn

Genetic characterization studies demonstrated that MZIR098 corn contains, at a single locus within the corn genome, a single copy of each of the following functional elements: *ecry3.1Ab*, *mcry3A*, *pat-08*, NOS-02 enhancer, CMP-04 promoter, Ubi1-18 promoter, NOS-20 terminator, 35S-04 promoter, and two copies of the NOS-05-01 terminator as expected. It does not contain any extraneous DNA fragments of these functional elements elsewhere in the MZIR098 corn

genome, and it does not contain the plasmid backbone sequence from transformation plasmid pSYN17629.

Nucleotide sequence analysis determined that the MZIR098 insert consists of the intact T-DNA region of the pSYN17629. The results of the Southern blot analyses are consistent with the results of the nucleotide sequence analysis. Sequence analysis of the MZIR098 insertion site demonstrated that 24-bp from the corn genomic sequence was deleted during the integration of the MZIR098 insert.

The observed segregation ratios for *ecry3.1Ab*, *mcry3A*, and *pat-08* in three generations of MZIR098 corn plants were as expected for a gene inherited according to Mendelian principles. The data indicate that the insert is inherited as a single locus in the corn nuclear genome. These data and the results of Southern blot analyses of five generations of Event MZIR098 corn indicate that the transgenic locus is stably inherited during conventional breeding.

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Part V. Absence of Genes that Encode Antibiotic Resistance

V.A. Absence of Genes that Encode Antibiotic Resistance in MZIR098 Corn

The aminoglycoside adenyltransferase gene (*aadA-03*) from *E. coli* transposon Tn7 (similar to Accession No. X03043.1 [NCBI 2012]) was a component in the plasmid backbone used to generate MZIR098 corn. Its presence conferred resistance to streptomycin and spectinomycin, and it was used as a bacterial selectable marker. This gene is located outside of the right and left borders of the T-DNA and therefore is not incorporated into the transformed corn genome. Part IV.G.2.b. above, describes the analyses used to confirm the absence of any plasmid backbone sequence in MZIR098 corn.

V.B. References Cited in Part V

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Part VI. Substances in the Food

The safety of the proteins conferring insect resistance and herbicide tolerance in MZIR098 corn have been previously evaluated in the context of Syngenta Events 5307, MIR604, and Bt11 corn and, to date, have proven to be safe. This part characterizes the proteins in MZIR098 corn; demonstrates their equivalence to the proteins expressed by 5307, MIR604, and Bt11; and describes their history of safe use.

VI.A. Identity and Characterization of eCry3.1Ab and mCry3A Produced in MZIR098 corn

The spectrum of insecticidal activity of any individual Cry protein is quite narrow; each Cry protein typically is active against only a few species within a phylogenetic order. The specificity of each *B. thuringiensis* Cry protein is the result of the efficiency of the steps needed to produce an active protein toxin and its subsequent interaction with the epithelial cells in the insect midgut. To exert their insecticidal activity, most known *B. thuringiensis* Cry proteins must be ingested by the insect and solubilized in the insect gut, be activated via specific proteolytic cleavage by midgut enzymes, bind to specific receptors on the surface of the insect midgut, and form ion channels in the gut membrane. The completion of all four of these processes results in disruption of the normal function of the midgut, leading to the death of the insect.

The eCry3.1Ab produced by MZIR098 corn is a chimeric protein engineered by combining portions of two Cry genes modified *cry3A (mcry3A)* and *cry1Ab* (Walters *et al*. 2010), each of which is derived from a native gene of *B. thuringiensis,* a ubiquitous soil bacterium. In addition, 22 amino acids are present at the N-terminus of eCry3.1Ab that are not derived from a Cry protein *per se*, but resulted from a PCR-induced frame shift mutation in a portion of the gene *mcry3A*. Following the 22 N-terminal amino acids are 459 consecutive amino acids from mCry3A, followed by 172 consecutive amino acids from Cry1Ab at the C-terminus, resulting in a 653-amino-acid eCry3.1Ab polypeptide with a molecular weight of 73.7 kDa.

The engineered chimeric protein eCry3.1Ab has similarities to other well-characterized Cry proteins. The eCry3.1Ab protein exhibits the same behavior as other coleopteran-active *B. thuringiensis* Cry proteins, including alkaline solubility, cleavage by chymotrypsin, specificity of brush-border membrane binding, and ion-channel formation (Walters *et al.* 2010). The mode of action of eCry3.1Ab, like that of most other Cry proteins, is highly specific to insects and is not operable in mammalian or other vertebrate species.

The novel gene *mcry3A* is a modified version of a native *cry3A* gene from *Bacillus thuringiensis* subsp. *tenebrionis* (Sekar *et al.* 1987). The native *cry3A* gene was recreated synthetically to optimize for expression in corn and has enhanced activity against the western corn rootworm and northern corn rootworm. The amino acid sequence of the mCry3A protein corresponds to that of the native Cry3A protein, except that its N-terminus corresponds to methionine-48 of the native protein and a cathepsin G protease recognition site has been introduced, beginning at amino acid residue 155 of the native protein. Cathepsin G is a chymotrypisin-like serine protease.

The characterization and equivalency of the proteins produced in MZIR098 to proteins produced in previously approved products is supported by several investigative methods as

follows: comparison of deduced amino acid sequence alignment, peptide mass coverage, western blot analysis to determine molecular weight, and glycosylation analysis. The results of these analyses are reported below.

VI.A.1. Deduced Amino Acid Sequence Alignment of eCry3.1Ab and mCry3A

The eCry3.1Ab protein produced in MZIR098 corn is identical to the eCry3.1Ab protein produced in 5307 corn (OECD unique identifier SYN-Ø53Ø7-1). The nucleotide sequence of *ecry3.1Ab* in MZIR098 corn encoding the eCry3.1Ab protein was confirmed by nucleotide sequencing of the insert. The *ecry3.1Ab* in 5307 corn encoding the eCry3.1Ab protein was confirmed by nucleotide sequencing of the insert. Deduced amino acid sequences of the eCry3.1Ab protein in both MZIR098 corn and 5307 corn are identical (as shown in Figure VI-1).

The mCry3A protein produced in MZIR098 corn is identical to the mCry3A protein produced in MIR604 corn (OECD Unique Identifier SYN-IR6Ø4-5). The nucleotide sequence of *mcry3A* in MZIR098 corn encoding the mCry3A protein was confirmed by nucleotide sequencing of the insert. The *mcry3A* in MIR604 corn encoding the mCry3A protein was confirmed by nucleotide sequencing of the insert. The deduced amino acid sequences of the mCry3A protein in both MZIR098 corn and MIR604 corn are identical (Figure VI-2).

```
Translation of Event 5307 ecry3.1Ab       (1) MTSNGRQCAGIRPYDGRQQHRGLDS
Translation of Event MZIR098 ecry3.1Ab    (1) MTSNGRQCAGIRPYDGRQQHRGLDS

Translation of Event 5307 ecry3.1Ab      (26) STTKDVIQKGISVVGDLLGVVGFPF
Translation of Event MZIR098 ecry3.1Ab   (26) STTKDVIQKGISVVGDLLGVVGFPF

Translation of Event 5307 ecry3.1Ab      (51) GGALVSFYTNFLNTIWPSEDPWKAF
Translation of Event MZIR098 ecry3.1Ab   (51) GGALVSFYTNFLNTIWPSEDPWKAF

Translation of Event 5307 ecry3.1Ab      (76) MEQVEALMDQKIADYAKNKALAELQ
Translation of Event MZIR098 ecry3.1Ab   (76) MEQVEALMDQKIADYAKNKALAELQ

Translation of Event 5307 ecry3.1Ab     (101) GLQNNVEDYVSALSSWQKNPAAPFR
Translation of Event MZIR098 ecry3.1Ab  (101) GLQNNVEDYVSALSSWQKNPAAPFR

Translation of Event 5307 ecry3.1Ab     (126) NPHSQGRIRELFSQAESHFRNSMPS
Translation of Event MZIR098 ecry3.1Ab  (126) NPHSQGRIRELFSQAESHFRNSMPS

Translation of Event 5307 ecry3.1Ab     (151) FAISGYEVLFLTTYAQAANTHLFLL
Translation of Event MZIR098 ecry3.1Ab  (151) FAISGYEVLFLTTYAQAANTHLFLL

Translation of Event 5307 ecry3.1Ab     (176) KDAQIYGEEWGYEKEDIAEFYKRQL
Translation of Event MZIR098 ecry3.1Ab  (176) KDAQIYGEEWGYEKEDIAEFYKRQL

Translation of Event 5307 ecry3.1Ab     (201) KLTQEYTDHCVKWYNVGLDKLRGSS
Translation of Event MZIR098 ecry3.1Ab  (201) KLTQEYTDHCVKWYNVGLDKLRGSS

Translation of Event 5307 ecry3.1Ab     (226) YESWVNFNRYRREMTLTVLDLIALF
Translation of Event MZIR098 ecry3.1Ab  (226) YESWVNFNRYRREMTLTVLDLIALF

Translation of Event 5307 ecry3.1Ab     (251) PLYDVRLYPKEVKTELTRDVLTDPI
Translation of Event MZIR098 ecry3.1Ab  (251) PLYDVRLYPKEVKTELTRDVLTDPI

Translation of Event 5307 ecry3.1Ab     (276) VGVNNLRGYGTTFSNIENYIRKPHL
Translation of Event MZIR098 ecry3.1Ab  (276) VGVNNLRGYGTTFSNIENYIRKPHL

Translation of Event 5307 ecry3.1Ab     (301) FDYLHRIQFHTRFQPGYYGNDSFNY
Translation of Event MZIR098 ecry3.1Ab  (301) FDYLHRIQFHTRFQPGYYGNDSFNY

Translation of Event 5307 ecry3.1Ab     (326) WSGNYVSTRPSIGSNDIITSPFYGN
Translation of Event MZIR098 ecry3.1Ab  (326) WSGNYVSTRPSIGSNDIITSPFYGN

Translation of Event 5307 ecry3.1Ab     (351) KSSEPVQNLEFNGEKVYRAVANTNL
Translation of Event MZIR098 ecry3.1Ab  (351) KSSEPVQNLEFNGEKVYRAVANTNL

Translation of Event 5307 ecry3.1Ab     (376) AVWPSAVYSGVTKVEFSQYNDQTDE
Translation of Event MZIR098 ecry3.1Ab  (376) AVWPSAVYSGVTKVEFSQYNDQTDE

Translation of Event 5307 ecry3.1Ab     (401) ASTQTYDSKRNVGAVSWDSIDQLPP
Translation of Event MZIR098 ecry3.1Ab  (401) ASTQTYDSKRNVGAVSWDSIDQLPP

Translation of Event 5307 ecry3.1Ab     (426) ETTDEPLEKGYSHQLNYVMCFLMQG
Translation of Event MZIR098 ecry3.1Ab  (426) ETTDEPLEKGYSHQLNYVMCFLMQG

Translation of Event 5307 ecry3.1Ab     (451) SRGTIPVLTWTHKSVDFFNMIDSKK
Translation of Event MZIR098 ecry3.1Ab  (451) SRGTIPVLTWTHKSVDFFNMIDSKK

Translation of Event 5307 ecry3.1Ab     (476) ITQLPLTKSTNLGSGTSVVKGPGFT
Translation of Event MZIR098 ecry3.1Ab  (476) ITQLPLTKSTNLGSGTSVVKGPGFT

Translation of Event 5307 ecry3.1Ab     (501) GGDILRRTSPGQISTLRVNITAPLS
Translation of Event MZIR098 ecry3.1Ab  (501) GGDILRRTSPGQISTLRVNITAPLS

Translation of Event 5307 ecry3.1Ab     (526) QRYRVRIRYASTTNLQFHTSIDGRP
Translation of Event MZIR098 ecry3.1Ab  (526) QRYRVRIRYASTTNLQFHTSIDGRP

Translation of Event 5307 ecry3.1Ab     (551) INQGNFSATMSSGSNLQSGSFRTVG
Translation of Event MZIR098 ecry3.1Ab  (551) INQGNFSATMSSGSNLQSGSFRTVG

Translation of Event 5307 ecry3.1Ab     (576) FTTPFNFSNGSSVFTLSAHVFNSGN
Translation of Event MZIR098 ecry3.1Ab  (576) FTTPFNFSNGSSVFTLSAHVFNSGN

Translation of Event 5307 ecry3.1Ab     (601) EVYIDRIEFVPAEVTFEAEYDLERA
Translation of Event MZIR098 ecry3.1Ab  (601) EVYIDRIEFVPAEVTFEAEYDLERA

Translation of Event 5307 ecry3.1Ab     (626) QKAVNELFTSSNQIGLKTDVTDYHI
Translation of Event MZIR098 ecry3.1Ab  (626) QKAVNELFTSSNQIGLKTDVTDYHI

Translation of Event 5307 ecry3.1Ab     (651) DQV-
Translation of Event MZIR098 ecry3.1Ab  (651) DQV-
```

Figure VI-1. Alignment of the deduced amino acid sequences from *ecry3.1Ab* in 5307 corn and *ecry3.1Ab* in MZIR098 corn

```
Translation of Event MIR604 mcry3A        (1) MTADNNTEALDSSTTKDVIQKGISV
Translation of Event MZIR098 mcry3A       (1) MTADNNTEALDSSTTKDVIQKGISV

Translation of Event MIR604 mcry3A       (26) VGDLLGVVGFPFGGALVSFYTNFLN
Translation of Event MZIR098 mcry3A      (26) VGDLLGVVGFPFGGALVSFYTNFLN

Translation of Event MIR604  mcry3A      (51) TIWPSEDPWKAFMEQVEALMDQKIA
Translation of Event MZIR098 mcry3A      (51) TIWPSEDPWKAFMEQVEALMDQKIA

Translation of Event MIR604  mcry3A      (76) DYAKNKALAELQGLQNNVEDYVSAL
Translation of Event MZIR098 mcry3A      (76) DYAKNKALAELQGLQNNVEDYVSAL

Translation of Event MIR604 mcry3A      (101) SSWQKNPAAPFRNPHSQGRIRELFS
Translation of Event MZIR098 mcry3A     (101) SSWQKNPAAPFRNPHSQGRIRELFS

Translation of Event MIR604 mcry3A      (126) QAESHFRNSMPSFAISGYEVLFLTT
Translation of Event MZIR098 mcry3A     (126) QAESHFRNSMPSFAISGYEVLFLTT

Translation of Event MIR604 mcry3A      (151) YAQAANTHLFLLKDAQIYGEEWGYE
Translation of Event MZIR098 mcry3A     (151) YAQAANTHLFLLKDAQIYGEEWGYE

Translation of Event MIR604 mcry3A      (176) KEDIAEFYKRQLKLTQEYTDHCVKW
Translation of Event MZIR098 mcry3A     (176) KEDIAEFYKRQLKLTQEYTDHCVKW

Translation of Event MIR604 mcry3A      (201) YNVGLDKLRGSSYESWVNFNRYRRE
Translation of Event MZIR098 mcry3A     (201) YNVGLDKLRGSSYESWVNFNRYRRE

Translation of Event MIR604 mcry3A      (226) MTLTVLDLIALFPLYDVRLYPKEVK
Translation of Event MZIR098 mcry3A     (226) MTLTVLDLIALFPLYDVRLYPKEVK

Translation of Event MIR604 mcry3A      (251) TELTRDVLTDPIVGVNNLRGYGTTF
Translation of Event MZIR098 mcry3A     (251) TELTRDVLTDPIVGVNNLRGYGTTF

Translation of Event MIR604 mcry3A      (276) SNIENYIRKPHLFDYLHRIQFHTRF
Translation of Event MZIR098 mcry3A     (276) SNIENYIRKPHLFDYLHRIQFHTRF

Translation of Event MIR604 mcry3A      (301) QPGYYGNDSFNYWSGNYVSTRPSIG
Translation of Event MZIR098 mcry3A     (301) QPGYYGNDSFNYWSGNYVSTRPSIG

Translation of Event MIR604 mcry3A      (326) SNDIITSPFYGNKSSEPVQNLEFNG
Translation of Event MZIR098 mcry3A     (326) SNDIITSPFYGNKSSEPVQNLEFNG

Translation of Event MIR604 mcry3A      (351) EKVYRAVANTNLAVWPSAVYSGVTK
Translation of Event MZIR098 mcry3A     (351) EKVYRAVANTNLAVWPSAVYSGVTK

Translation of Event MIR604 mcry3A      (376) VEFSQYNDQTDEASTQTYDSKRNVG
Translation of Event MZIR098 mcry3A     (376) VEFSQYNDQTDEASTQTYDSKRNVG

Translation of Event MIR604 mcry3A      (401) AVSWDSIDQLPPETTDEPLEKGYSH
Translation of Event MZIR098 mcry3A     (401) AVSWDSIDQLPPETTDEPLEKGYSH

Translation of Event MIR604 mcry3A      (426) QLNYVMCFLMQGSRGTIPVLTWTHK
Translation of Event MZIR098 mcry3A     (426) QLNYVMCFLMQGSRGTIPVLTWTHK

Translation of Event MIR604 mcry3A      (451) SVDFFNMIDSKKITQLPLVKAYKLQ
Translation of Event MZIR098 mcry3A     (451) SVDFFNMIDSKKITQLPLVKAYKLQ

Translation of Event MIR604 mcry3A      (476) SGASVVAGPRFTGGDIIQCTENGSA
Translation of Event MZIR098 mcry3A     (476) SGASVVAGPRFTGGDIIQCTENGSA

Translation of Event MIR604 mcry3A      (501) ATIYVTPDVSYSQKYRARIHYASTS
Translation of Event MZIR098 mcry3A     (501) ATIYVTPDVSYSQKYRARIHYASTS

Translation of Event MIR604 mcry3A      (526) QITFTLSLDGAPFNQYYFDKTINKG
Translation of Event MZIR098 mcry3A     (526) QITFTLSLDGAPFNQYYFDKTINKG

Translation of Event MIR604 mcry3A      (551) DTLTYNSFNLASFSTPFELSGNNLQ
Translation of Event MZIR098 mcry3A     (551) DTLTYNSFNLASFSTPFELSGNNLQ

Translation of Event MIR604 mcry3A      (576) IGVTGLSAGDKVYIDKIEFIPVN-
Translation of Event MZIR098 mcry3A     (576) IGVTGLSAGDKVYIDKIEFIPVN-
```

Figure VI-2. Alignment of the deduced amino acid sequence from *mcry3A* in MIR604 corn and *mcry3A* in MZIR098 corn

VI.A.2. Peptide Mass Coverage Analysis of eCry3.1Ab and mCry3A Produced in MZIR098 Corn

Peptide mass coverage analysis was used to determine the identity of purified eCry3.1Ab and of purified mCry3A from MZIR098 corn extract by liquid chromatography–tandem mass spectrometry (LC-MS/MS) using an Ultra-Performance Liquid Chromatography system.

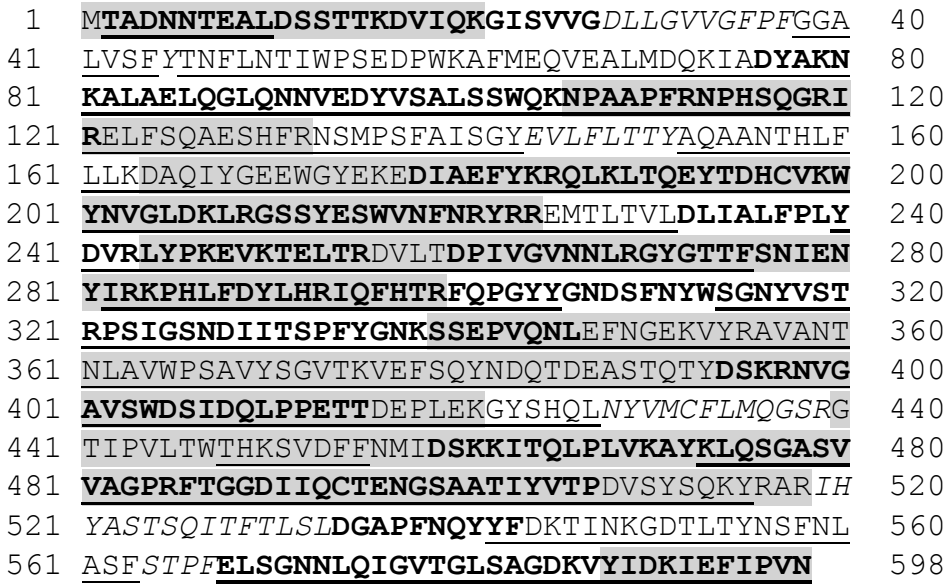
The collective analysis of the three proteolytic digests of the purified eCry3.1Ab preparation from MZIR098 corn extract yielded coverage of 85.9% of the total predicted eCry3.1Ab amino acid sequence. Evidence for 64.3%, 75.2%, and 46.4% of eCry3.1Ab protein amino acid sequence was obtained for trypsin, chymotrypsin, and endoproteinase Asp-N, respectively. The sequence coverage map is shown in Figure VI-3.

1 *M***TSNGRQCAGIRPYDGRQQHRGLDSSTTKDVIQKGISVVG** 40
41 *DLLGVVGFPFGGALVSFY*TNFLNTIWPSEDPWKAF*MEQVE* 80
81 *ALMDQKIA***DYAKNKALAELQGLQNNVE**DYVSALSSWQKNP 120
121 AAPFRNPHSQGRIRELFSQAESHFRNSMPSFAISGYEVL*F* 160
161 *LTTY*AQAANTHLFLLKDAQIYGEEWGYEKE**DIAEFYKRQL** 200
201 **KLTQEYTDHCVKWYNVGL**DKLRGSSYESWVNFNRYRR*EMT* 240
241 *LTVL***DLIALFPLYDVRLYPKEVKTELTR**DVLT**DPIVGVNN** 280
281 **LRGYGTTFSNIENYIRKPHLFDYLHRIQFHTRFQPGYYGN** 320
321 **DSFNYWSGNYVSTRPSIGSNDIITSPFYGNKSSEPVQNL**E 360
361 FNGEKVYRAVANTNLAVWPSAVYSGVTKVEFSQYNDQT**DE** 400
401 **ASTQTYDSKRNVGAVSWDSIDQLPPETT**DEPLEKGY*SHQL* 440
441 *NYVMCF*LMQGSRGTIPVLTWTHKSVDFFNMI**DSKKITQLP** 480
481 **LTKSTNLGSGTSVVKGPGFTGG**DILRRTSPGQISTLRVNI 520
521 TAPLSQRYR*VRIRY*ASTTNLQFHTSIDGRPINQGNF*SATM* 560
561 *SSGSNLQSGSFRTVGFTTPFNFSNGSSVF*TLSAHVFNSGN 600
601 EVYI**DRIEFVPAEVTF**EAEY**DLERAQKAVNELFTSSNQIG** 640
641 **LKTDVTDYHIDQV** 653

Legend:
Trypsin-detected
Chymotrypsin-detected
Endoproteinase Asp-N-detected
Italic indicates amino acids not identified

Figure VI-3. Amino acid sequence identified for eCry3.1Ab from MZIR098 corn by peptide mass coverage analysis

The collective analysis of the three proteolytic digests of the purified mCry3A preparation from MZIR098 corn extract yielded coverage of 91.6% of the total predicted mCry3A amino acid sequence. Evidence for 56.7%, 78.3%, and 57.2% of mCry3A protein amino acid sequence coverage was obtained for trypsin, chymotrypsin, and endoproteinase Asp-N, respectively. The sequence coverage map is shown in Figure VI-4.

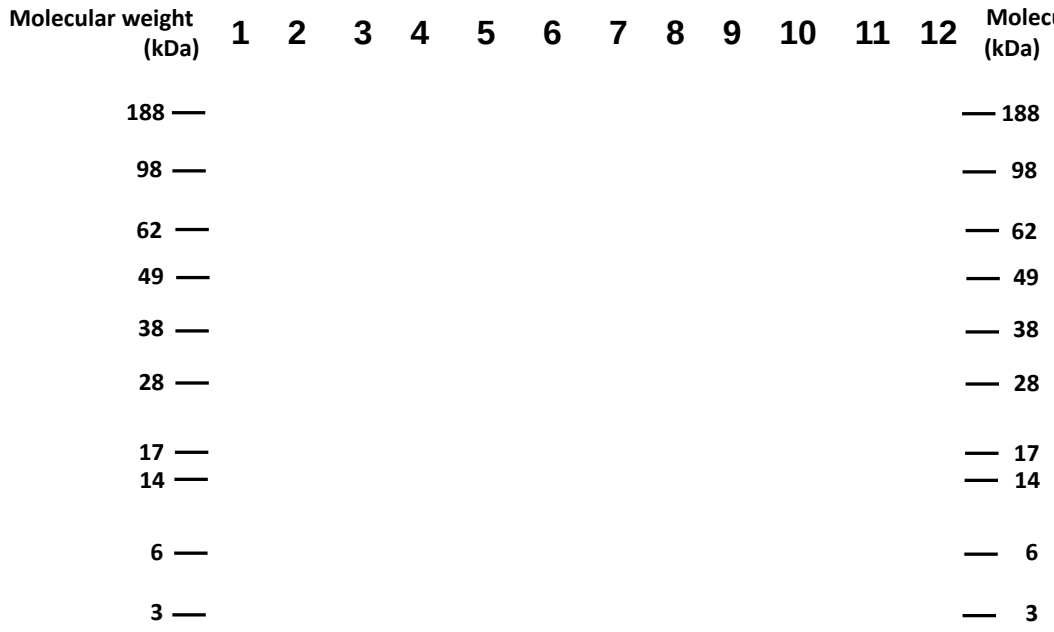


Legend: Trypsin-detected
Chymotrypsin-detected
Endoproteinase Asp-N-detected
Italic indicates amino acids not identified

Figure VI-4. Amino acid sequence identified for mCry3A from MZIR098 corn by peptide mass coverage analysis

VI.A.3. Immunoreactivity and Molecular Weight of eCry3.1Ab and mCry3A Produced in MZIR098 Corn

Western blot analysis demonstrated that the apparent molecular weight of eCry3.1Ab produced in MZIR098 corn was consistent with the predicted molecular weight of 73.7 kDa (Figure VI-5, lanes 5 and 6). Likewise, western blot analysis of mCry3A in MZIR098 was consistent with the predicted molecular weight of 67.7 kDa (Figure VI-5, lanes 6 and 7). Two bands of lower molecular weight (ca. 55 kDa and ca. 35 kDa) were present only in the plant-produced sample of MZIR098 corn crude extract (Figure VI-5, lane 6). These bands most likely corresponded to degradation products from *in planta* degradation of mCry3A, as was also seen in the western blot analysis with MIR604 corn extract when probed with the mCry3A antibody.



Lane 1: Molecular weight standard
Lane 2: Microbially produced eCry3.1Ab reference substance (7.44 ng of eCry3.1Ab)[a]
Lane 3: Microbially produced eCry3.1Ab batch (7.44 ng of eCry3.1Ab)[b]
Lane 4: Nontransgenic corn extract, (12.2 µg of total protein) fortified with microbially produced eCry3.1Ab batch (7.44 ng of eCry3.1Ab)
Lane 5: eCry3.1Ab purified preparation from MZIR098 corn extract (7.44 ng of eCry3.1Ab)
Lane 6: MZIR098 corn extract (12.2 µg of total protein, 7.44 ng of eCry3.1Ab, 3.94 ng of mCry3A)
Lane 7: mCry3A purified preparation from MZIR098 corn extract (3.94 ng of mCry3A)
Lane 8: Nontransgenic corn extract, (12.2 µg of total protein) fortified with microbially produced mCry3A batch (3.94 ng of mCry3A)
Lane 9: Microbially produced mCry3A batch (3.94 ng of mCry3A)[b]
Lane 10: Microbially produced mCry3A reference substance (3.94 ng of mCry3A)[a]
Lane 11: Nontransgenic corn extract, (12.2 µg of total protein)
Lane 12: Molecular weight standard

[a] Microbially produced protein was used as a positive assay control. The reference substance is the original lot of microbial protein that was used to establish the safety of the proteins eCry3.1Ab and mCry3A in the safety data packages submitted for 5307 corn and MIR604 corn, respectively.

[b] A new microbially produced protein batch was required for additional studies. The microbial protein reference substance and the microbial protein batch were made using the identical procedure in the same facility.

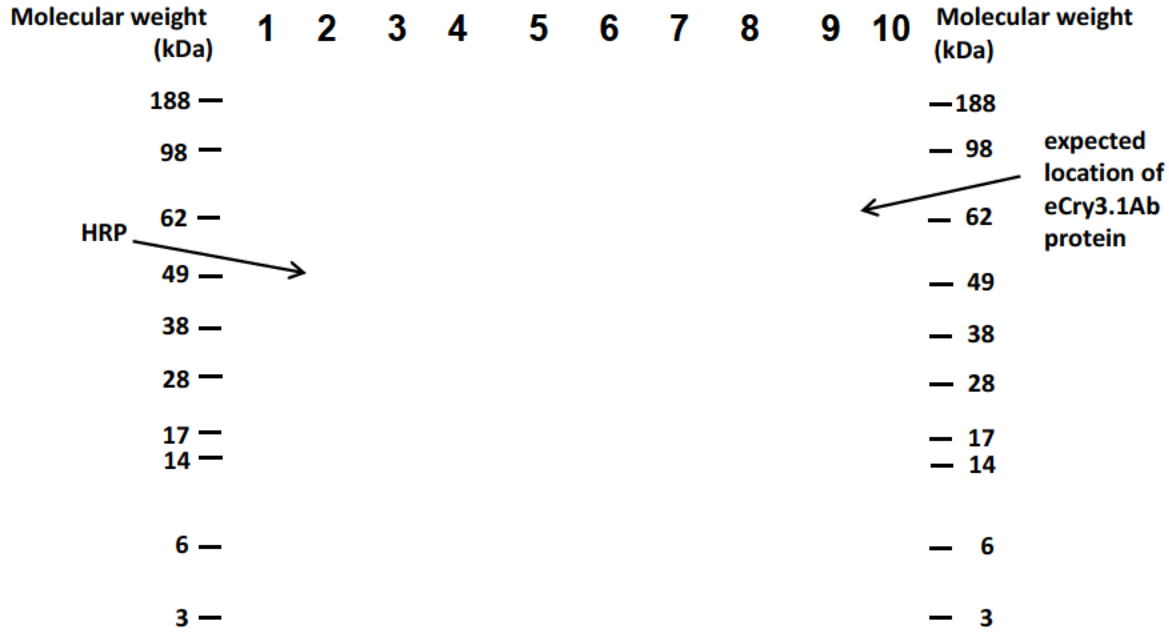
Figure VI-5. Western blot analysis of plant-produced eCry3.1Ab and mCry3A produced in MZIR098 corn

VI.A.4. Glycosylation Analysis of eCry3.1Ab and mCry3A Produced in MZIR098 corn

The eCry3.1Ab and mCry3A produced in MZIR098 corn was analyzed to ensure that no post-translational glycosylation of the protein(s) had occurred *in planta*.

As shown in Figure VI-6, glycosylation analysis demonstrated the absence of post-translational glycosylation of eCry3.1Ab produced in MZIR098 corn. No bands corresponding to the presence of glycosylated eCry3.1Ab were visible in samples derived from MZIR098 corn (Figure VI-6, Lane 8). The positive control, horseradish peroxidase (HRP), generated a visible band when applied to the gel at 25, 10, 5, 2.5, and 1 pmol (Figure VI-6, Lanes 2 through 6). The negative control, soybean trypsin inhibitor was not detected (Figure VI-6, Lane 7).

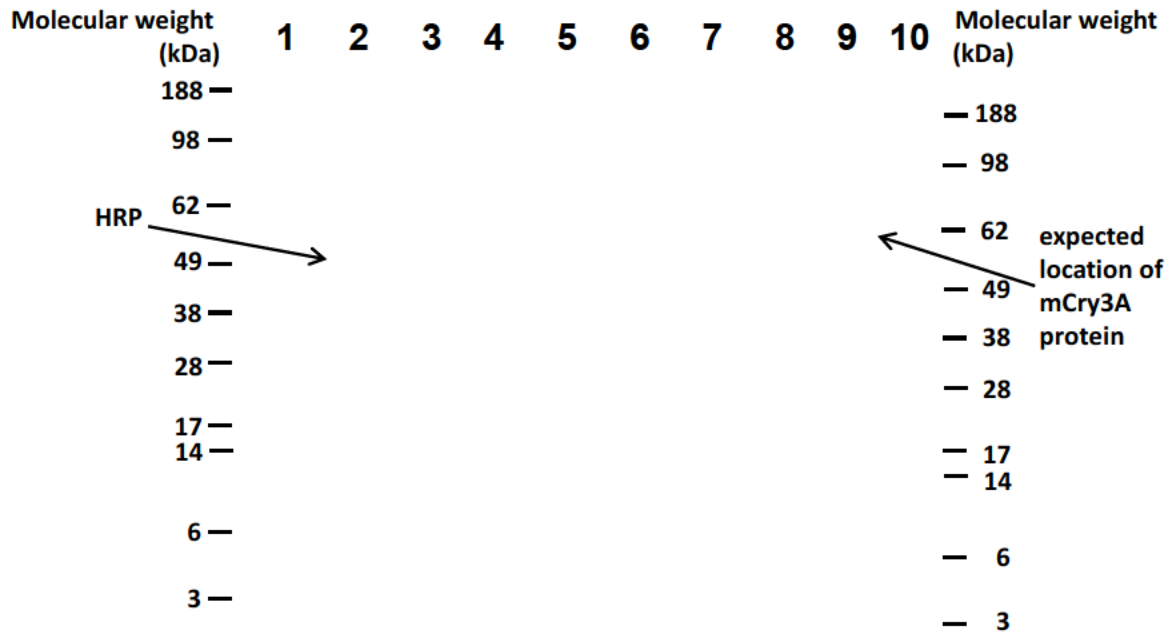
Therefore, these results support the conclusion that eCry3.1Ab produced in MZIR098 corn is not glycosylated.



Lane 1: Molecular weight standard
Lane 2: HRP (positive control), 25 pmol
Lane 3: HRP (positive control), 10 pmol
Lane 4: HRP (positive control), 5 pmol
Lane 5: HRP (positive control), 2.5 pmol
Lane 6: HRP (positive control), 1 pmol
Lane 7: Soybean trypsin inhibitor (negative control), 25 pmol
Lane 8: Purified eCry3.1Ab preparation from MZIR098 corn leaf extract, 25 pmol
Lane 9: Microbially produced eCry3.1Ab 25 pmol
Lane 10: Molecular weight standard

Figure VI-6. Glycosylation analysis of eCry3.1Ab produced in MZIR098 corn

As shown in Figure VI-7, glycosylation analysis demonstrated the absence of post-translational glycosylation of mCry3A produced in MZIR098 corn. No bands corresponding to the presence of glycosylated mCry3A were visible in samples derived from MZIR098 corn (Figure VI-7, Lane 8). The positive control, horseradish peroxidase (HRP), generated a visible band when applied to the gel at 25, 10, 5, 2.5, and 1 pmol (Figure VI-7, Lanes 2 through 6). The negative control, soybean trypsin inhibitor was not detected (Figure VI-7, Lane 7). Therefore, these results support the conclusion that mCry3A produced in MZIR098 corn is not glycosylated.



Lane 1: Molecular weight standard
Lane 2: HRP (positive control), 25 pmol
Lane 3: HRP (positive control), 10 pmol
Lane 4: HRP (positive control), 5 pmol
Lane 5: HRP (positive control), 2.5 pmol
Lane 6: HRP (positive control), 1 pmol
Lane 7: Soybean trypsin inhibitor (negative control), 25 pmol
Lane 8: Purified mCry3A preparation from MZIR098 corn leaf extract, 25 pmol
Lane 9: Microbially produced mCry3A, 25 pmol
Lane 10: Molecular weight standard

Figure VI-7. Glycosylation analysis of mCry3A produced in MZIR098 corn

VI.B. Identity and Characterization of the PAT Produced in MZIR098 Corn

MZIR098 corn contains the transgene *pat-08*, derived from the soil bacterium *Streptomyces viridochromogenes,* which encodes the enzyme PAT. PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. Specifically, PAT catalyzes the transfer of the acetyl group from acetyl coenzyme A to phosphinothricin. The released free thiol reacts with 5,5′-dithiobis(2-nitrobenzoic acid) to form 2-nitro-5-thiobenzoate anion under mild alkaline conditions (pH 7 to 8) (Habeeb 1972) (as shown in Figure VI-8).

Glufosinate-ammonium (L-phosphinothricin) inhibits glutamine synthetase, an enzyme in the nitrogen assimilation pathway. PAT is a highly specific enzyme for acetylation of glufosinate-ammonium. It does not acetylate glutamate (the closest structural analog to glufosinate-ammonium) or other L-amino acids and only poorly recognizes analogues such as methionine sulfoximine and hydroxylysine (Wehrmann *et al.* 1996, Hérouet *et al.* 2005).

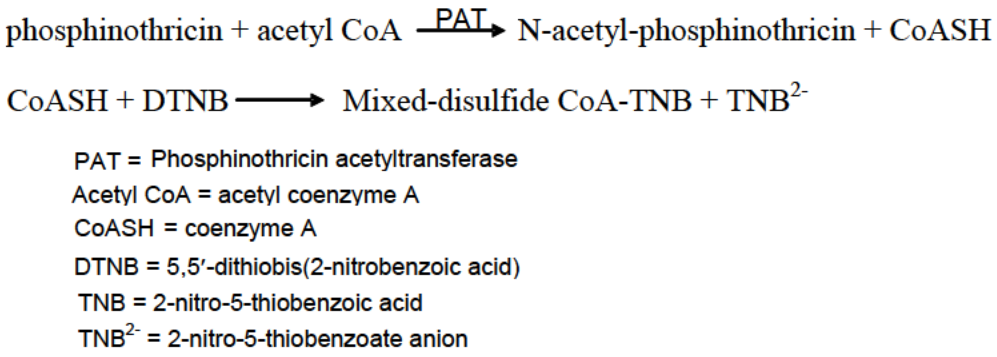


Figure VI-8. The reaction catalyzed by PAT

VI.B.1. Deduced Amino Acid Sequence Alignment for PAT

The PAT produced in MZIR098 corn (SYN-ØØØ98-3) is identical to the PAT produced in Bt11 corn (OECD identifier SYN-BTØ11-1). The nucleotide sequence of *pat-08* encoding the PAT in MZIR098 corn and the nucleotide sequence of *pat* encoding the PAT in Bt11 corn were confirmed by nucleotide sequencing of the inserts. The deduced amino acid sequence of the PAT protein in MZIR098 corn and Bt11 corn is identical (Figure VI-9).

```
Translation of Event Bt11 pat            (1) MSPERRPVEIRPATAADMAAVCDIV
Translation of Event MZIR098 pat-08      (1) MSPERRPVEIRPATAADMAAVCDIV

Translation of Event Bt11 pat           (26) NHYIETSTVNFRTEPQTPQEWIDDL
Translation of Event MZIR098 pat-08     (26) NHYIETSTVNFRTEPQTPQEWIDDL

Translation of Event Bt11 pat           (51) ERLQDRYPWLVAEVEGVVAGIAYAG
Translation of Event MZIR098 pat-08     (51) ERLQDRYPWLVAEVEGVVAGIAYAG

Translation of Event Bt11 pat           (76) PWKARNAYDWTVESTVYVSHRHQRL
Translation of Event MZIR098 pat-08     (76) PWKARNAYDWTVESTVYVSHRHQRL

Translation of Event Bt11 pat          (101) GLGSTLYTHLLKSMEAQGFKSVVAV
Translation of Event MZIR098 pat-08    (101) GLGSTLYTHLLKSMEAQGFKSVVAV

Translation of Event Bt11 pat          (126) IGLPNDPSVRLHEALGYTARGTLRA
Translation of Event MZIR098 pat-08    (126) IGLPNDPSVRLHEALGYTARGTLRA

Translation of Event Bt11 pat          (151) AGYKHGGWHDVGFWQRDFELPAPPR
Translation of Event MZIR098 pat-08    (151) AGYKHGGWHDVGFWQRDFELPAPPR

Translation of Event Bt11 pat          (176) PVRPVTQI-
Translation of Event MZIR098 pat-08    (176) PVRPVTQI-
```

Figure VI-9. Alignment of the deduced amino acid sequence for PAT encoded by *pat* in Bt11 corn and *pat-08* in MZIR098 corn

VI.B.2. Peptide Mass Coverage Analysis of PAT Produced in MZIR098 Corn

Peptide mass coverage analysis was used to determine the identity of the purified PAT preparation from MZIR098 corn extract by LC-MS/MS using an Ultra-Performance Liquid Chromatography system.

The collective analysis of the three proteolytic digests of the purified PAT preparation from MZIR098 corn extract resulted in coverage of 96% of the total predicted PAT amino acid sequence. Evidence for 50%, 74%, and 66% of the PAT protein amino acid sequence was

obtained for trypsin, chymotrypsin, and endoproteinase Asp-N, respectively. The sequence coverage map is shown in Figure VI-10.

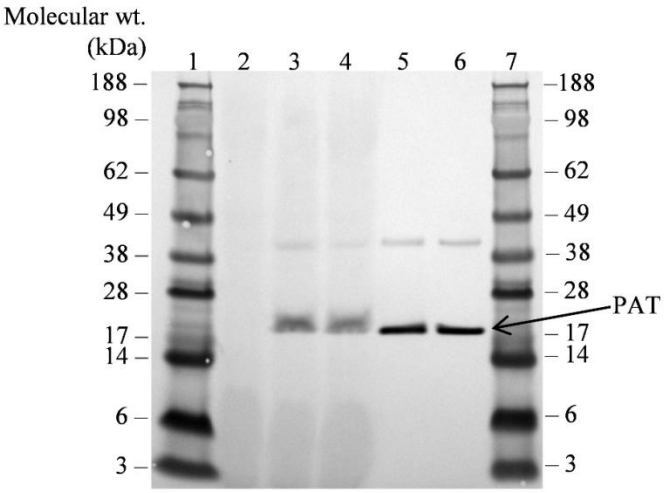
1 *M***SPERRPVEIRPATAA***DMAAVC***DIVNHYIETSTVNFRTEP** 40
41 **QTPQEWIDDLERLQDRYPWLVAEVEGVVAGIAYAGPWKAR** 80
81 **NAY**DWTVESTVYVSHRHQRLGLGSTLYTHLLKSM**EAQGFK** 120
121 **SVVAVIGLPNDPSVRLHEALGYTARGTLRAAGYKHGGWHD** 160
161 **VGFWQRDFELPAPPRPVRPVTQI** 183

Legend: Trypsin-detected
Chymotrypsin-detected
Endoproteinase Asp-N-detected
Italic indicates amino acids not identified

Figure VI-10. Amino acid sequence identified for PAT from MZIR098 corn by peptide mass coverage analysis

VI.B.3. Immunoreactivity and Molecular Weight of PAT Produced in MZIR098 Corn

Western blot analysis demonstrated that the apparent molecular weight of PAT in MZIR098 corn was consistent with the predicted molecular weight of 20.5 kDa, and the protein cross-reacted with the PAT-specific antibody (as shown in Figure VI-11, lanes 4 and 5).



Lane 1: Molecular weight standard
Lane 2: Nontransgenic corn extract (165 µg of total protein)
Lane 3: Microbially produced PAT-fortified nontransgenic corn extract (10 ng of PAT, 165 µg of total protein)
Lane 4: MZIR098 corn extract (10 ng of PAT, 165 µg of total protein)
Lane 5: Purified PAT preparation from MZIR098 corn extract (10 ng of PAT)
Lane 6: Microbially produced PAT (10 ng of PAT)
Lane 7: Molecular weight standard

Figure VI-11. Western blot analysis of PAT produced in MZIR098 corn

VI.B.4. Glycosylation Analysis of PAT Produced in MZIR098 Corn

The PAT produced in MZIR098 corn was analyzed to ensure that no post-translational glycosylation of the protein had occurred *in planta*. As shown in Figure VI-12, this analysis

demonstrated the absence of post-translational glycosylation of PAT produced in MZIR098 corn. No bands corresponding to the presence of glycosylated PAT were visible in PAT samples derived from MZIR098 corn (Figure VI-12, Lane 8). The positive control, HRP, generated a visible band when applied to the gel at 25, 10, 5, 2.5, and 1 pmol (Figure VI-12, Lanes 2 through 6). The negative control, soybean trypsin inhibitor, was not detected (Figure V-12, Lane 7). Therefore, these results support the conclusion that PAT produced in MZIR098 corn is not glycosylated.

Lane 1: Molecular weight standard
Lane 2: HRP (positive control), 25 pmol
Lane 3: HRP (positive control), 10 pmol
Lane 4: HRP (positive control), 5 pmol
Lane 5: HRP (positive control), 2.5 pmol
Lane 6: HRP (positive control), 1 pmol
Lane 7: Soybean trypsin inhibitor (negative control), 25 pmol
Lane 8: PAT purified preparation from MZIR098 corn leaf extract, 25 pmol
Lane 9: Microbially produced PAT, 25 pmol
Lane 10: Molecular weight standard

Figure VI-12. Glycosylation of PAT produced in MZIR098 corn

VI.C. Levels of eCry3.1Ab, mCry3A and PAT Produced in MZIR098 Corn Tissue

The concentrations of eCry3.1Ab, mCry3A and PAT in various MZIR098 corn tissues were quantified by enzyme-linked immunosorbent assay (ELISA) to establish an expression profile for these proteins as produced in MZIR098 corn. The tissues analyzed were leaves and roots at four growth stages (V6, R1, R6, and senescence), whole plants at three stages (V6, R1, and R6), kernels at two stages (R6 and senescence), and pollen at one stage (R1). The tissues were collected from MZIR098 corn and a nontransgenic, near-isogenic control corn grown concurrently according to local agronomic practices at four U.S. locations in 2013. The

genotypes of the plants used in these studies were NP2391/NP2222(MZIR098) (Figure IV-2) and NP2391/ NP2222.

At each location, one plot was planted with MZIR098 corn, and one plot was planted with nontransgenic corn. Five replicate samples of each tissue type except pollen were collected from each plot. For pollen, a pooled sample was collected from 10 to 15 tassels per plot. All tissue samples except pollen were ground to a powder, and all samples were then lyophilized. The percent dry weight (DW) of each sample was determined from the sample weight before and after lyophilization.

Protein was extracted from representative aliquots of the lyophilized tissue samples. The sample extracts were analyzed by ELISA in duplicate or triplicate, and a standard curve was generated for each ELISA plate with known amounts of the corresponding reference protein. Concurrent analysis of tissues from the nontransgenic corn confirmed the absence of plant-matrix effects on the analysis methods. All protein concentrations were adjusted for extraction efficiency.

Table VI-1 shows the ranges of protein concentrations observed in each tissue type across all growth stages and locations on a dry-weight (DW) and fresh-weight (FW) basis for MZIR098 corn.

Table VI-1. Ranges of concentrations of eCry3.1Ab, mCry3A, and PAT in tissues of MZIR098 corn across multiple growth stages and across four locations

Tissue	No. of stages	Dry-weight concentration (µg/g)			Fresh-weight concentration (µg/g)		
		eCry3.1Ab	mCry3A	PAT	eCry3.1Ab	mCry3A	PAT
Leaves	4	2.09–333.92	3.50–114.42	<LOD–13.05	1.68–46.68	2.81–26.90	<LOD-1.85
Roots	4	1.84–96.44	5.07–110.86	<LOD–3.27	0.34–13.84	0.53-18.23	<LOD-0.50
Whole plants	3	2.70–221.05	9.20–95.09	<LOD–6.82	1.57–29.39	3.48-12.62	<LOD-0.91
Pollen	1	<LOD	293.87–308.71	<LOD	<LOD	152.64-246.96	<LOD
Kernels	2	0.82–5.90	6.69–22.83	<LOD-<LOQ	0.67–3.44	5.38-15.38	<LOD-<LOQ

Limit of Detection (LOD) for eCry3.1Ab in pollen = 0.08 µg/g DW.
LOD for PAT in leaves, whole plants, pollen, and kernels = 0.025 µg/g DW
Limit of Quantitation (LOQ) for PAT in kernels = 0.031 µg/g DW.

Kernels from MZIR098 corn are the most likely tissue to enter the food supply, as either grain or grain by-products. Humans would potentially consume corn at the senescence stage of development, whereas livestock would be more likely to consume the kernels at maturity. The average eCry3.1Ab concentration measured in kernels from MZIR098 corn was 2.08 µg/g dry weight at senescence and 2.42 µg/g dry weight at maturity (R6). The average mCry3A concentration measured in kernels from MZIR098 corn was 11.21 µg/g dry weight at senescence and 14.59 µg/g dry weight at maturity (R6).

The PAT concentration measured in kernels from MZIR098 corn was below the LOD for the assay (0.025 μg/g dry weight) at senescence and ranged from the LOD to LOQ (0.31 μg/g) dry weight at maturity.

VI.D. History of Safe Exposure

Insecticidal Cry proteins from *B. thuringiensis* have a long history of safe use in food crops. Their modes of action are highly specific within narrow ranges of related insect species and are not relevant to mammals or other vertebrates. PAT belongs to the class of acetyltransferase enzymes common in plants and animals, and it shares similar three-dimensional structure, molecular weight, and functional properties with other acetyltransferase enzymes, which are present as natural components of human and animal diets. It is likely that small amounts of acetyltransferase enzymes from various sources have always been present in the food and feed supply, because of the ubiquitous occurrence of PAT proteins in nature.

Breeding stacks containing 5307 corn were first introduced to the U.S. market in 2014. Syngenta has combined 5307 corn with other approved biotechnology-derived traits in two combinations that have been reviewed and approved globally for cultivation and food/feed uses, as shown in Appendix A, Table A1. These combinations both include MIR604 corn.

MIR604 corn, containing mCry3A has been cultivated in the U.S. and Canada since 2008. Syngenta has combined MIR604 corn with other approved biotechnology-derived traits in multiple combinations that have been reviewed and approved globally for cultivation and food/feed uses, as shown in Appendix A, Table A2.

Bt11 corn containing PAT, either as a stand-alone or stacked corn product, has been available to farmers and in the food and feed supply for almost two decades. A list of approved products containing PAT proteins derived from *Streptomyces* spp. and encoded by either *pat* or *bar,* a similar gene, are listed in Appendix A, Table A-3.

Approximately 91 million acres of corn were planted in the United States in 2014, of which 93% was biotechnology-derived (USDA 2015). Most of the biotechnology-derived corn currently grown in the United States and Canada consists of transgenic varieties that are herbicide tolerant and/or resistant to insects.

VI.E. Existing Safety Data

MZIR098 corn produces the proteins eCry3.1Ab, mCry3A, and PAT; the safety of these proteins has been previously established by numerous regulatory agencies, including FSANZ.

Evidence presented earlier in Part VI demonstrate the proteins eCry3.1Ab, mCry3A, and PAT in MZIR098 corn are identical to the eCry3.1Ab, mCry3A, and PAT proteins assessed in safety assessments conducted by FSANZ, as listed below:

(1) The eCry3.1Ab protein produced by MZIR098 corn is identical to the eCry3.1Ab protein produced by Syngenta's Event 5307 corn, which was first approved by FSANZ in 2012 (Approval no. 1060).

(2) The mCry3A protein produced by MZIR098 corn is identical to the mCry3A produced by Syngenta's Event MIR604 corn, which was first approved by FSANZ in 2006 (Approval no. 564).
(3) The PAT protein produced by MZIR098 corn is identical to the PAT produced by Syngenta's Event Bt11 corn, which was first approved by FSANZ in 2001(Approval no. 386).

In these assessments conducted by FSANZ, it was found that eCry3.1Ab and mCry3A did not present any safety concerns, based on the following evidence:

- The proteins lack sequence similarity to known mammalian toxins or allergens.
- No adverse effects were observed when the proteins were ingested by mice.
- *In vitro* digestive fate studies showed that the protein(s) are rapidly degraded in simulated gastric fluid.
- The proteins are not glycosylated.
- The proteins did not retain bioactivity activity after incubation at 95 °C for 30 minutes

Collectively, these data indicate that the proteins are unlikely to be toxic or allergenic to mammals.

Syngenta data for the PAT protein was recently submitted to FSANZ as part of the safety assessment for MZHG0JG corn A1112-Appendix B, pages 109-112. The information submitted supports the same conclusions described above for eCry3.1Ab and mCry3A, however the thermoliability of PAT was determined using immunoreactivity rather than bioactivity, where it was shown that after incubation at 95°C, the immunoreactivity of PAT was below the LOD. In addition, a comprehensive characterization and safety assessment of the PAT protein is available in a 2005 article published in *Regulatory Toxicology and Pharmacology* (Hérouet *et al.* 2005).

VI.F. Recent Bioinformatics Searches

The amino acid sequences of the eCry3.1Ab and mCry3A proteins were compared with known and putative protein allergens and toxins as part of a weight-of-evidence approach to assessing potential mammalian toxicity and allergenicity. The recent (2015) bioinformatics assessments for PAT can be found in FSANZ A1112-Food derived from Herbicide-Tolerant Corn Line MZHG0JG, pages 111-112, and therefore will not be provided here.

VI.F.1. Amino Acid Similarity to Known or Putative Allergens of eCry3.1Ab and mCry3A

To determine whether eCry3.1Ab and mCry3A had biologically relevant amino acid sequence similarity to known or putative allergens, two different searches were performed against the Food Allergy Research and Resource Program Protein Allergen Protein Database (FARRP 2015), which contained 1897 nonredundant sequences of known and putative allergens. A full-length sequence search with the FASTA algorithm (Pearson and Lipman 1988) and a separate search for exact matches of eight or more contiguous amino acids were used to compare the

eCry3.1Ab and mCry3A amino acid sequences with each of the known or putative allergen sequences.

In the FASTA search, no sequence similarity greater than 35% shared identity over 80 or more amino acids was observed between the eCry3.1Ab or mCry3A amino acid sequence and any sequence in the FARRP database. In the exact match search, no alignments of eight or more contiguous amino acids were found between the eCry3.1Ab or mCry3A amino acid sequence and any sequence in the FARRP database. Together, these results support the conclusion that eCry3.1Ab and mCry3A share no biologically relevant amino acid sequence similarity to known or putative protein allergens.

VI.F.2. Amino Acid Similarity to Known or Putative Toxins of eCry3.1Ab and mCry3A

The amino acid sequences of eCry3.1Ab and mCry3A were systematically compared with the latest posting of the NCBI Entrez Protein Database (NCBI 2015). The Basic Local Alignment Search Tool for Proteins (BLASTP) program (Altschul *et al.* 1997) was used to compare the eCry3.1Ab and mCry3A amino acid sequences with all entries in the NCBI Entrez Protein Database sequences. This analysis addressed two questions: (1) whether any protein(s) in the database had a high degree of sequence similarity to the eCry3.1Ab or mCry3A amino acid sequence and (2) whether any proteins demonstrating a high degree of sequence similarity to the eCry3.1Ab or mCry3A amino acid sequence were known or putative toxins.

The BLASTP searches were performed with the default parameters, and the threshold for statistical significance of *E*-values (a measure of the probability that matches between sequences occurred by chance) was established by analysis of searches using randomly shuffled versions of the eCry3.1Ab or mCry3A amino acid sequences. The threshold *E*-values were $<1 \times 10^{-5}$ for eCry3.1Ab and mCry3A.

For eCry3.1Ab, all 982 of the most similar alignments from NCBI were below the screening threshold E-value of 1×10^{-5}. Of these alignments, 825 were categorized as "Cry proteins". Similarly, for mCry3A, 975 of the most similar alignments were also below the threshold E-value, and 814 of these alignments were categorized as "Cry proteins," the same class of protein to which both eCry3.1Ab and mCry3A belong.

BLASTP analyses were also conducted with the Syngenta Toxin Database, a separate database of known toxin sequences. No significant alignments (to indicate the potential to act as a toxin) were observed when eCry3.1Ab or mCry3A were compared with any of the entries in the Syngenta Toxin Database.

The results of both database comparisons confirm that eCry3.1Ab and mCry3A are not toxic proteins and they do not share significant sequence similarity with other known or putative protein toxins.

V.I.F.3. Conclusions on the Amino Acid Similarity to Known or Putative Allergens and Toxins of eCry3.1Ab and mCry3A

These results support the conclusion that eCry3.1Ab and mCry3A proteins are unlikely to be toxic or allergenic proteins, since they share no biologically relevant amino acid sequence similarity to known or putative protein allergens or toxins.

VI.G. Conclusions on the Characterization and Safety of eCry3.1Ab, mCry3A and PAT Produced in MZIR098 Corn

The safety of eCry3.1Ab, mCry3A, and PAT have been thoroughly assessed by Syngenta. Numerous regulatory agencies globally, including Food Standards Australia and New Zealand have assessed the safety of these proteins in the context of 5307, MIR604, and Bt11 corn. The conclusions of safety of eCry3.1Ab, mCry3A and PAT are supported by the following:

- Characterization demonstrates that eCry3.1Ab, mCry3A and PAT produced in MZIR098 corn are identical to eCry3.1Ab, mCry3A, and PAT produced in 5307MIR604, and Bt11 corn, respectively.
    - Amino acid sequences of the proteins are identical based on deduced amino acid sequence comparison.
    - The peptide mass maps were as expected.
    - The molecular weights of the proteins, based on western blot analyses, were as expected and the same as what has been previously submitted.
    - The proteins were not glycosylated.

- The proteins eCry3.1Ab, mCry3A, and PAT have very specific and well-characterized modes of action.

- The proteins eCry3.1Ab, mCry3A, and PAT are present in other previously approved and marketed corn varieties and no adverse effects associated with intake have been reported

- Given the low levels of eCry3.1Ab, mCry3A, and PAT in MZIR098 kernels, dietary exposure can be considered minimal.

- Recently updated bioinformatics searches support previous assessments that the proteins eCry3.1Ab, mCry3A, and PAT share no biologically relevant amino acid sequence similarity to known or putative protein allergens or toxins.

The weight of evidence from the data presented in this application, a history of safe use either through their presence in transgenic crops or abundance in nature, the weight of evidence in the publicly available literature, previous conducted safety assessment reports by regulatory agencies around the world, and recently updated bioinformatics data demonstrate that the eCry3.1Ab, mCry3A, and PAT proteins do not present a risk to the health of humans or livestock through consumption of MZIR098 corn.

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Part VII. Absence of Other Novel Substances Produced as a Result of the Genetic Modification.

MZIR098 corn produces the intended proteins eCry3.1Ab, mCry3A and PAT. The three genes expressed in MZIR098 corn, *eCry3.1Ab, mCry3A* and *pat-08*, are functional as intended, suggesting there is no gene silencing due to the expression of either transgene. These proteins are identical to the transgenic proteins produced in other approved biotechnology-derived products, which have a history of safe use.

No other novel metabolites were identified or expected as a result of the genetic modification. Specifically, no new herbicide metabolites are expected from spraying MZIR098 corn with glufosinate-ammonium herbicides. The herbicide metabolic profiles resulting from the transgenic protein–herbicide interaction in corn have been established for PAT through a significant history of use. Corn grown in the United States and Canada (and exported to many countries, including Australia, New Zealand,) has included transgenic varieties that are glufosinate-ammonium tolerant for a number of years.

Part VIII. Compositional Analysis of the GM Food

Corn grown in the U.S. is predominantly of the yellow dent type, a commodity crop. Roughly 60% of the crop is fed to livestock either as grain or silage. Livestock that feed on corn include cattle, pigs, poultry, sheep, and goats. The remainder of the crop is exported or processed by wet milling, dry milling, or alkali treatment to yield products such as high-fructose corn syrup, starch, oil, grits, and flour. These processed products are used extensively in the food industry. For example, corn starch serves as a raw material for an array of processed foods and is also used in industrial manufacturing processes. Since the early 1980s, a significant amount of grain has also been used for fuel ethanol production. The by-products from these processes are often used in animal feeds. Described below is a study conducted to measure and compare key nutrients and anti-nutrients in forage and grain from MZIR098 and conventional corn.

VIII.A. Composition Study Design and Methods

Compositional analyses of MZIR098 corn, the corresponding nontransgenic, near-isogenic control corn, and six nontransgenic corn reference varieties were performed to assess nutritional equivalence. This assessment consisted of quantitative analyses of 73 nutritional components of grain and nine nutritional components in forage, including key food and feed nutrients, secondary plant metabolites, and anti-nutrients.

Compositional analyses were conducted on corn forage and grain samples harvested from replicated field trials planted at eight locations in the United States in 2013. The test substance was MZIR098 corn, and the control substance was nontransgenic, near-isogenic corn. Six nontransgenic commercial corn varieties were included in the study design as reference entries to establish a range of natural variation in germplasm with a history of production in the area of cultivation. The test, control, and reference entries are listed in Table VIII-1.

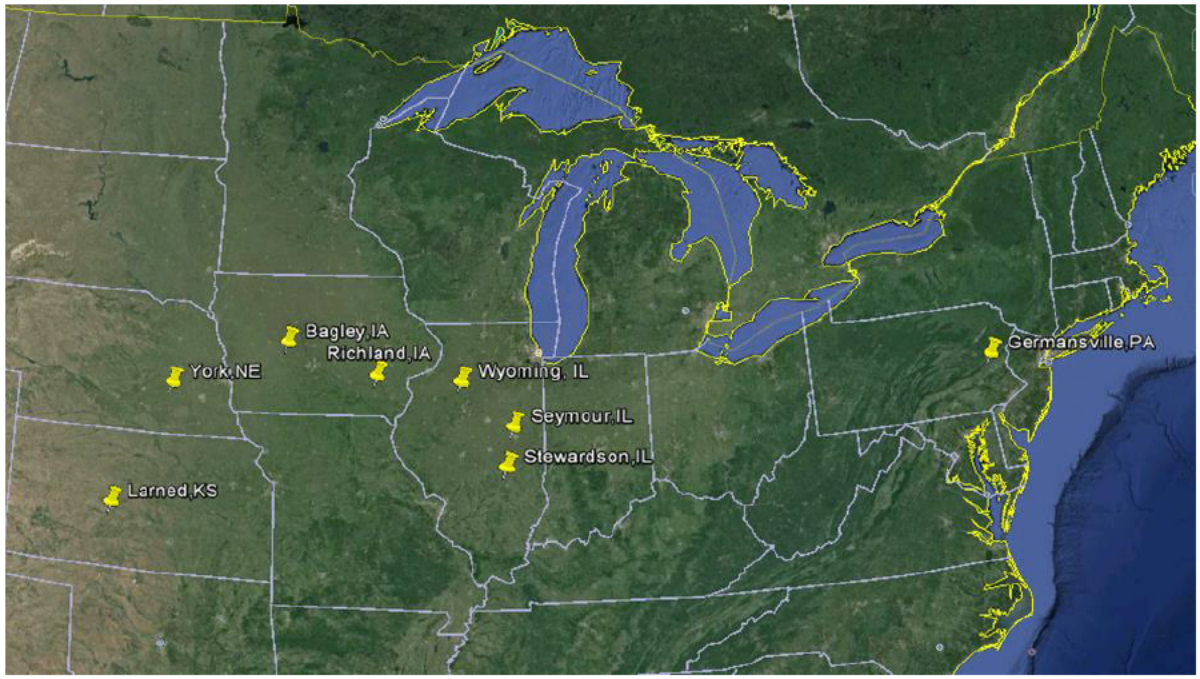
Table VIII-1. Identification of test, control, and reference corn varieties

Entry Identification	Seed description	Hybrid genotype	Material Identification
E06	Nontransgenic, near-isogenic corn (control)	NP2391/NP2222	12MG027028
E07	MZIR098 corn (test)	NP2391/NP2222 (MZIR098)	12MG027027
E09	Corn reference line 1*	H-7191	09BIC000006
E10	Corn reference line 2*	H-7540	09BIC000007
E11	Corn reference line 3*	SY Sincero	12PS804310
E12	Corn reference line 4*	NK Lucius	12PS804303
E13	Corn reference line 5*	NK Cisko	12PS804304
E14	Corn reference line 6*	SY Provial	12PS804307

*Corn reference "line" is also described as reference "variety"

The locations selected were representative of agricultural regions suitable for the cultivation of the hybrid corn varieties. At each location, the entries were grown in a randomized complete block design with four replicate plots. The plots were six rows spaced 76 cm apart and 6m long planted with approximately 40 seeds per row.

The plots were managed according to local agricultural practices, and all plots at a given location were managed identically with regard to irrigation, fertilization, and pest control. A satellite view of the composition trial locations is shown in Figure VIII-1. The locations, with soil type, previous year's crop, and planting date for each location, are listed in Table VIII-2.



The location designated is the city nearest to the field plots.

Figure VIII-1. Satellite view of composition trial locations in the United States

Table VIII-2. Field-trial locations

Location		Soil type	Previous crop	Planting date (2013)
L01	Richland, Iowa	silty clay loam	soybean	June 4
L02	York, Nebraska	silty clay loam	soybean	June 3
L03	Seymour, Illinois	silty clay loam	corn	June 20
L04	Bagley, Iowa	clay loam	field corn	June 13
L05	Larned, Kansas	loam	sorghum	June 12
L06	Stewardson, Illinois	silt loam	corn	June 10
L09	Wyoming, Illinois	silt loam	corn	June 8
L10	Germansville, Pennsylvania	clay loam	general vegetable	June 20

Forage samples collected from each plot consisted of the entire above-ground portions of five plants harvested at dough stage (R4 growth stage, as defined by Abendroth *et al.* 2011). The plants were chopped and pooled to create a composite sample for each plot. After the plants reached physiological maturity (R6 growth stage), 15 ears were collected from each plot for

grain samples. The ears were dried mechanically or in the field until the grain contained not more than 17% moisture.

The nutritional components measured in corn forage and grain were chosen based on recommendations of the OECD (2002) for comparative assessment of the composition of new varieties of corn. The components analyzed in forage and grain are listed in Tables VIII-3 and VIII-4.

Table VIII-3. Nutritional components analyzed in corn forage

Proximates		Minerals
moisture	carbohydrates	calcium
protein	ADF[a]	phosphorus
fat	NDF[b]	
ash		

[a]Acid detergent fiber.
[b]Neutral detergent fiber.

Table VIII-4. Nutritional components analyzed in corn grain

Proximates and starch	Minerals	Vitamins	Amino acids	
moisture	calcium	A (β-carotene)	alanine	lysine
protein	copper	B_1 (thiamine)	arginine	methionine
fat	Iron	B_2 (riboflavin)	aspartic acid	phenylalanine
ash	magnesium	B_3 (niacin)	cystine	proline
carbohydrates	manganese	B_6 (pyridoxine)	glutamic acid	serine
ADF	phosphorus	B_9 (folic acid)	glycine	threonine
NDF	potassium	E (α-tocopherol)	histidine	tryptophan
TDF[a]	selenium		isoleucine	tyrosine
starch	sodium		leucine	valine
	zinc			

Fatty acids		Secondary metabolites	Anti-nutrients
8:0 caprylic	18:0 stearic	*p*-coumaric acid	phytic acid
10:0 capric	18:1 oleic	ferulic acid	raffinose
12:0 lauric	18:2 linoleic	furfural	trypsin inhibitor
14:0 myristic	18:3 gamma linolenic	inositol	
14:1 myristoleic	18:3 linolenic		
15:0 pentadecanoic	20:0 arachidic		
15:1 pentadecenoic	20:1 eicosenoic		
16:0 palmitic	20:2 eicosadienoic		
16:1 palmitoleic	20:3 eicosatrienoic		
17:0 heptadecanoic	20:4 arachidonic		
17:1 heptadecenoic	22:0 behenic		

[a]Total detergent fiber.

The component levels were converted to equivalent units of dry weight based on the moisture content of each sample. All compositional analyses were conducted according to methods published and approved by AOAC International, or were other industry-standard methods, or were based on literature references and developed and validated by the analytical laboratory.

VIII.B. Data Analysis

The mean levels of each component across locations were computed. The data for each quantifiable component were subjected to analysis of variance (ANOVA) using the following mixed model:

$$Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk}$$

In this model, Y_{ijk} is the observed response for entry i at location j block k, U is the overall mean, T_i is the entry effect, L_j is the location effect, $B(L)_{jk}$ is the effect of block within location, LT_{ij} is the location-by-entry interaction effect, and e_{ijk} is the residual error. Entry was regarded as a fixed effect, while the effects of location, block within location, and location-by-entry interaction were regarded as random. In the across-location ANOVA, only the control and test entries were included, to avoid inflation of the residual error by any interaction that may have been present between location and the reference varieties.

For each component, *t*-tests were used to assess the statistical significance of the comparison of interest (MZIR098 vs. control). Significance was based on an alpha level of 0.05, and denominator degrees of freedom were determined by the Kenward-Roger method (Kenward and Roger 1997). The standard error of the mean (SEM) was also determined for each component.

In cases where some or all values for a component were below the limit of quantitation and substitution of the LOQ was not appropriate because of the number or distribution of substitutions required, calculation of the mean and ANOVA could not be performed, and only the range is reported.

The across-location means for the components of MZIR098 corn were also compared nonstatistically with the ranges of component levels from the nontransgenic corn reference varieties and with the ranges for conventional corn published in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI 2014).

VIII.C. Compositional Analysis Results

Parts VIII.C.1 and VIII.C.2 describe the compositional analysis results for MZIR098 corn forage and grain and compare them with the results for the nontransgenic, near-isogenic control corn, as well as the reference-variety and ILSI database ranges. The conclusions from the compositional analysis are presented in Section VIII.C.3.

VIII.C.1. Forage

Across-location statistics for proximate and mineral composition of corn forage are shown in Table VII-5. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant differences were observed in the levels of moisture, protein, fat, ash, carbohydrates, ADF, NDF, calcium, or phosphorus.

In both MZIR098 corn and the nontransgenic control corn, the mean levels of all proximates and minerals were within the ranges for the reference varieties and the ranges reported in the ILSI database.

Table VIII-5. Proximate and mineral composition of forage from MZIR098 corn and nontransgenic corn

Data source	Statistic	Moisture	Protein	Fat	Ash	Carbohydrates	ADF	NDF	Calcium	Phosphorus
MZIR098	mean	68.7	7.17	1.90	4.00	86.9	25.7	43.8	1971	1731
	range	62.2-75.9	5.24-9.50	1.05-2.84	2.73-5.70	84.0-90.2	17.2-32.2	35.0-52.9	1240-2800	1290-2570
Control	mean	68.4	7.36	1.95	3.94	86.8	24.9	42.5	1861	1832
	range	65.5-74.8	5.61-9.10	1.14-2.81	3.03-5.26	84.1-89.8	20.0-32.2	33.9-49-.8	1300-2570	1200-2450
ANOVA (*t*-test) entry effect and SEM										
	P	0.649	0.206	0.626	0.608	0.386	0.264	0.311	0.176	0.052
	SEM	0.99	0.211	0.099	0.239	0.37	0.72	0.98	76	101
Reference varieties	mean	69.8	7.19	2.18	3.79	86.8	24.1	40.3	1920	1817
	range	61.2–80.0	4.54–9.54	0.587–3.79	2.41–5.98	82.0–90.7	15.8–32.8	28.8–57.0	1010–3300	1090–2800
ILSI (2014)	mean	69.9	7.68	2.063	4.30	86.0	25.80	41.88	1902.87	1938.01
	range	48.8–82.0	3.14–15.20	<LOQ–6.755	0.66–13.20	74.3–92.9	9.90–47.39	20.29–67.80	582.00–5767.90	689.78–4385.20
	N	4316	3897	3873	4316	3897	4116	4116	3650	3650

MZIR098: $N = 32$.
Control: $N = 32$.
Reference varieties: $N = 192$.
ILSI: *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ.
Proximate levels shown as percent dry weight, except moisture which is shown as percent fresh weight.
Calcium and phosphorus levels shown as milligrams per kilogram dry weight.
Results significantly different ($p < 0.05$) are shown in bold italic type.

VIII.C.2. Grain

VIII.C.2.a. Proximates, Starch, Minerals, and Vitamins

Across-location statistics for proximate and starch components of corn grain are shown in Table VIII-6. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant differences were observed in in the levels of any proximates and starch

Across-location statistics for mineral components of corn grain are shown in Table VIII-7. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant differences were observed in the levels of iron, magnesium, manganese, phosphorus, or zinc. The levels of calcium, copper and potassium were significantly higher in MZIR098 corn than in the control corn. For selenium and sodium, levels below the LOQ precluded calculation of the means and statistical comparisons across locations.

Across-location statistics for vitamin components of corn grain are shown in Table VII-8. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant differences were observed in the levels of vitamins B_1, B_2, B_3, B_6, B_9 or vitamin E (α-tocopherol). The level of vitamin A (β-carotene) was significantly higher in MZIR098 corn than in the control corn.

In both MZIR098 corn and the nontransgenic control corn, the mean levels of all proximates, starch, minerals, and vitamins were within the ranges for the reference varieties and the ranges reported in the ILSI database.

Table VIII-6. Proximate and starch composition of grain from MZIR098 corn and nontransgenic corn

Data source	Statistic	Moisture[a]	Protein	Fat	Ash	Carbohydrates	ADF	NDF	TDF	Starch
MZIR098	mean	12.9	10.3	3.96	1.43	84.3	3.98	11.3	16.4	65.5
	range	8.59-18.8	8.58-13.1	3.33-4.69	1.16-1.73	81.8-86.2	3.20-4.72	9.91-12.4	14.3-19.9	56.0-76.6
Control	mean	12.8	10.4	3.93	1.42	84.3	4.06	11.1	16.3	65.8
	range	9.22-17.6	8.46-13.9	3.22-4.76	1.25-1.69	81.3-86.6	3.12–4.88	9.58-12.8	14.0-20.1	58.1-75.0
ANOVA (*t*-test) entry effect and SEM										
	P	–	0.416	0.654	0.974	0.618	0.294	0.223	0.726	0.657
	SEM	–	0.32	0.098	0.035	0.34	0.100	0.17	0.36	0.75
Reference varieties	mean	12.2	10.3	3.40	1.48	84.8	3.40	9.54	13.6	66.4
	range	7.99–17.4	7.68–13.9	2.39–4.41	1.18–1.87	81.3–88.0	2.43–4.48	7.42–12.2	11.2–20.0	53.3–79.6
ILSI (2014)	mean	14.5	10.31	3.829	1.415	84.5	3.72	10.31	13.90	66.6
	range	5.1–40.5	5.72–17.26	1.363–7.830	0.616–6.282	77.4–89.7	1.41–11.34	4.28–22.64	8.73–35.31	26.5–83.7
	N	6616	5790	5790	6190	5765	5942	5941	3763	1931

MZIR098: $N = 32$.
Control: $N = 32$.
Reference varieties: $N = 192$.
Proximate and starch levels shown as percent dry weight, except moisture which is shown as percent fresh weight.
Results significantly different ($p < 0.05$) are shown in bold italic type.
[a] Grain was dried in the field, or mechanically after harvest, so moisture levels were not subjected to analysis of variance (ANOVA).

Table VIII-7. Mineral composition of grain from MZIR098 corn and nontransgenic corn

Data source	Statistic	Ca	Cu	Fe	Mg	Mn	P	K	Se[a]	Na[b]	Zn
MZIR098	Mean	37.8	2.02	19.9	1178	5.98	3030	3680	–	–	22.2
	Range	26.1-51.3	1.47-3.49	16.2-23.2	1030-1350	3.86-9.29	2450-3600	3190-4040	<LOQ–0.667	<LOQ	17.3-53.2
Control	Mean	35.5	1.90	19.7	1176	5.93	2989	3549	–	–	20.4
	Range	23.6-50.1	1.32-2.57	16.9-28.0	994–1360	3.34-10.4	2540-3620	3220-3930	<LOQ–0.582	<LOQ	15.7-24.4
ANOVA (*t*-test) entry effect and SEM											
	P	***0.023***	***0.013***	0.655	0.926	0.717	0.314	***<0.001***	–	–	0.105
	SEM	2.28	0.131	0.35	25	0.586	82	59	–	–	0.92
Reference varieties	Mean	41.2	2.09	20.3	1168	5.80	3053	3807	–	–	21.3
	Range	27.4–59.1	1.33–3.20	13.4–28.8	867–1400	3.15–9.10	2410–3750	3170–4640	<LOQ–0.802	<LOQ–185	12.7–29.3
ILSI (2014)	Mean	44.2	1.71	20.56	1217.0	6.45	3142.0	3690.6	0.28	24.94	22.8
	Range	<LOQ–1010.0	<LOQ–21.20	9.51–191.00	594.0–1940.0	1.69–14.30	1300.0–5520.0	1810.0–6030.0	<LOQ–1.51	<LOQ–731.54	6.5–42.6
	N	5932	5650	5819	5823	5822	5938	5823	973	1110	5823

MZIR098: $N = 32$.
Control: $N = 32$.
Reference varieties: $N = 192$.
ILSI: *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ.
Mineral levels shown as milligrams per kilogram (mg/kg) dry weight.
Results significantly different ($p < 0.05$) are shown in bold italic type. When some or all values were < LOQ, and substitution with the LOQ was not appropriate due to the number or distribution of substitutions required, calculation of the mean and analysis of variance (ANOVA) could not be performed and only the range is shown.
[a] Original units of parts per billion (ppb) were converted to mg/kg. The LOQ for selenium was 0.033–0.036 mg/kg dry weight.
[b] The LOQ for sodium was 109–121 mg/kg dry weight.

Table VIII-8. Vitamin composition of grain from MZIR098 corn and nontransgenic corn

Data source	Statistic	Vitamin A[a] (β-carotene)	Vitamin B_1 (thiamine)	Vitamin B_2 (riboflavin)	Vitamin B_3 (niacin)	Vitamin B_6 (pyridoxine)	Vitamin B_9 (folic acid)	Vitamin E[b] (α-tocopherol)
MZIR098	Mean	0.154	0.380	0.206	2.11	0.557	0.0440	0.0124
	Range	0.125-0.175	0.275-0.504	0.131-0.336	1.74-2.53	0.372-0.642	0.0322-0.0584	0.00887-0.0156
Control	Mean	0.145	0.374	0.217	2.09	0.563	0.0441	0.0121
	Range	0.105-0.190	0.305-0.458	0.132-0.346	1.76-2.42	0.410-0.698	0.0355-0.0602	0.00814-0.0155
ANOVA (*t*-test) entry effect and SEM								
	P	***0.037***	0.359	0.437	0.453	0.580	0.940	0.286
	SEM	0.0045	0.0130	0.0115	0.044	0.0161	0.00233	0.00066
Reference varieties	Mean	0.134	0.368	0.214	2.45	0.632	0.0414	0.0132
	Range	0.064–0.318	0.249–0.506	0.114–0.375	1.55–4.17	0.365–0.910	0.0232–0.0640	0.00762–0.0221
ILSI (2014)	Mean	0.481	0.383	0.190	2.094	0.601	0.0575	0.0106
	Range	<LOQ–4.990	<LOQ–4.000	<LOQ–0.735	<LOQ–4.694	<LOQ–1.214	<LOQ–0.3500	<LOQ–0.0687
	N[b]	4373	4981	4061	4999	4998	5460	4480

MZIR098: *N* = 32.
Control: *N* = 32.
Reference varieties: *N* = 192.
ILSI: *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ.
Vitamin levels shown as milligrams per 100 grams (mg/100 g) dry weight, except vitamin E which is shown as milligrams per gram (mg/g).
Results significantly different ($p < 0.05$) are shown in bold italic type.
[a] β-carotene is measured in this study vitamin A is not produced in plants.
[b] Original units of mg/100 g were converted to mg/g.

VIII.C.2.b. Amino Acids, Fatty Acids, Secondary Metabolites, and Anti-nutrients

Across-location statistics for amino acid components of corn grain are shown in Table VIII-9. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant differences were observed in the levels of 17 amino acids. The levels of lysine was significantly lower in MZIR098 corn than in the control corn

The across-location statistics for ten quantifiable fatty acids in corn grain are shown in Table VIII-10. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant differences were observed in the proportions of 16:0 palmitic, 16:1 palmitoleic, 18:3 linolenic, 20:1 eicosenoic, or 22:0 behenic acid. The proportions of 17:0 heptadecanoic and 18:2 linoleic acid were significantly higher in MZIR098 corn than in the control corn, and the proportions of 18:0 stearic, 18:1 oleic, and 20:0 arachidic were significantly lower.

Twelve fatty acids analyzed had levels below the LOQ in all replicates at all locations (8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 17:1 heptadecenoic, 18:3 gamma linolenic, 20:2 eicosadienoic, 20:3 eicosatrienoic, and 20:4 arachidonic acids).

Across-location statistics for secondary metabolite and anti-nutrient components of corn grain are shown in Table VIII-11. In statistical comparisons between MZIR098 corn and the nontransgenic control corn, no significant were observed in the levels of ferulic acid, *p*-coumaric acid, inositol, phytic acid, trypsin inhibitor or raffinose. For furfural, levels below the LOQ precluded calculation of the means and statistical comparisons across locations.

In both MZIR098 corn and the nontransgenic control corn, the mean levels of all amino acids, quantifiable fatty acids, quantifiable secondary metabolites, and anti-nutrients, except ferulic acid, were within the ranges for the reference varieties and the ranges reported in the ILSI database. In both MZIR098 corn and the nontransgenic control corn, the mean levels of ferulic acid were within the ranges reported in the ILSI database.

Table VIII-9. Amino acid composition of grain from MZIR098 corn and nontransgenic corn

Data source	Statistic	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
MZIR098	mean	6.57	3.54	4.65	19.0	8.91	3.76	7.85	2.03	4.61
	range	5.70-8.30	3.02-4.37	3.87-6.16	15.8-25.7	7.75-11.2	3.17-4.35	6.50-10.2	1.71–2.44	3.84-5.81
Control	mean	6.66	3.57	4.70	19.1	8.97	3.78	7.91	2.03	4.63
	range	5.52-9.19	2.99-4.93	3.72-6.58	15.1-29.4	7.16-12.6	3.14-4.55	6.17-11.8	1.59-2.46	3.67-6.42
ANOVA (*t*-test) entry effect and SEM										
	P	0.277	0.370	0.470	0.689	0.615	0.637	0.619	0.981	0.857
	SEM	0.195	0.107	0.155	0.75	0.277	0.085	0.297	0.048	0.140
Reference varieties	mean	6.79	3.58	4.74	19.1	9.10	3.83	7.81	2.06	4.67
	range	4.87–8.94	2.56–4.74	3.33–7.04	12.8–28.9	5.97–12.6	2.70–4.82	5.42–11.4	1.52–2.59	3.27–6.23
ILSI (2014)	mean	6.82	3.68	4.97	19.70	9.19	3.88	7.89	2.14	4.83
	range	3.35–12.08	2.19–6.66	1.82–7.69	9.65–35.40	4.62–17.50	1.84–6.85	4.39–14.80	1.16– 5.14	2.66–8.55
	N	5918	5918	5918	5918	5918	5918	5918	5917	5918
Data source	**Statistic**	**Met**	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	**Trp**
MZIR098	mean	2.14	3.55	12.8	4.01	5.13	2.89	2.58	4.90	0.843
	range	1.45-2.55	3.08–4.66	10.6-17.4	3.518-5.02	4.38-6.73	2.19-3.26	2.04-3.08	3.84-5.87	0.716-0.930
Control	mean	2.16	3.55	12.9	4.02	5.17	2.97	2.56	4.91	0.839
	range	1.73–2.84	2.81-5.31	10.1–20.9	3.29-5.90	4.09-8.01	2.43-3.41	2.16-3.27	3.83-6.09	0.690-.965
ANOVA (*t*-test) entry effect and SEM										
	P	0.602	0.982	0.670	0.829	0.595	***0.044***	0.596	0.767	0.704
	SEM	0.066	0.131	0.56	0.148	0.206	0.051	0.067	0.138	0.0202
Reference varieties	mean	2.02	3.55	12.8	4.03	5.19	2.92	2.68	5.02	0.859
	range	1.51–2.49	2.38–5.18	8.30–20.7	2.69–6.09	3.52–7.92	1.88–3.85	1.95–3.58	3.47–6.54	0.639–1.02
ILSI (2014)	mean	2.10	3.68	13.03	3.54	5.30	2.94	2.87	4.65	0.712
	range	1.05–4.68	1.79–6.92	6.42–24.92	1.03–7.34	2.44–9.30	1.29–6.68	1.37–4.56	1.19–7.08	0.271–2.150
	N	5915	5918	5918	5918	5918	5909	5918	5918	5916

MZIR098: *N* = 32.
Control: *N* = 32.
Reference lines: *N* = 192; ILSI: *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ.
Amino acid levels shown as milligrams per gram dry weight. ; Results significantly different ($p < 0.05$) are shown in bold italic type.

Table VIII-10. Fatty acid composition of grain from MZIR098 corn and nontransgenic corn

Data source	Statistic	16:0 Palmitic	16:1 Palmitoleic	17:0 Heptadecanoic	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenoic	22:0 Behenic
MZIR098	mean	14.2	0.131	0.0842	2.10	26.3	54.6	1.78	0.421	0.227	0.174
	range	13.7-15.0	0.104-0.149	0.0745-0.0947	1.75–2.39	22.6-28.7	52.3-58.6	1.67-1.92	0.358-0.468	0.201–0.244	0.134–0.202
Control	mean	14.2	0.131	0.0821	2.13	27.1	53.8	1.76	0.427	0.228	0.174
	range	13.8-14.7	0.106-0.157	0.0730-0.0913	1.73-2.44	23.5-29.2	51.7-58.0	1.65-1.85	0.353-0.477	0.203–0.253	0.134–0.206
ANOVA (*t*-test) entry effect and SEM											
	P	0.493	0.686	***0.010***	***0.038***	***<0.001***	***<0.001***	0.219	***0.029***	0.626	0.921
	SEM	0.08	0.0034	0.00199	0.058	0.63	0.63	0.017	0.0107	0.0039	0.0058
Reference varieties	mean	15.1	0.127	0.0871	2.06	24.9	55.1	1.73	0.415	0.256	0.187
	range	13.2–17.0	0.0876–0.200	0.0698–0.121	1.59–2.48	16.5–31.1	47.5–64.1	1.39–2.12	0.329–0.485	0.178–0.348	0.0977–0.247
ILSI (2014)	mean	12.55	0.147	0.089	1.90	26.52	56.72	1.38	0.419	0.270	0.185
	range	6.81– 26.55	<LOQ–0.453	<LOQ–0.203	1.02– 3.83	17.40–42.81	34.27–67.68	0.55–2.33	0.267–0.993	<LOQ–1.952	<LOQ–0.417
	N	4682	2119	265	4682	4682	4682	4682	4344	4322	3858

MZIR098: *N* = 32.
Control: *N* = 32.
Reference varieties: *N* = 192.
ILSI: *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ.
Fatty acids shown as percent of total fatty acids.
Results significantly different ($p < 0.05$) are shown in bold italic type.
When some or all values were < LOQ, and substitution with the LOQ was not appropriate due to the number or distribution of substitutions required, calculation of the mean and analysis of variance (ANOVA) could not be performed and only the range is shown. Levels < LOQ were observed for all replicates at all locations for 8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic acid, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 17:1 heptadecenoic, 18:3 gamma linolenic, 20:2 eicosadienoic, 20:3 eicosatrienoic, and 20:4 arachidonic fatty acids

Table VIII-11. Secondary metabolite and anti-nutrient composition of grain from MZIR098 corn and nontransgenic corn

Data source	Statistic	*p*-Coumaric acid (mg/kg)	Ferulic acid (mg/kg)	Furfural[a] (mg/kg)	Inositol (ppm)	Phytic acid (%)	Raffinose[b] (%)	Trypsin inhibitor (TIU/mg)
MZIR098	mean	304	3357	–	2505	0.859	0.111	3.94
	range	220-346	2930-3820	<LOQ	1850-3540	0.643-1.13	<LOQ–0.196	2.49-5.38
Control	mean	300	3357	–	2520	0.885	0.105	4.03
	range	220-364	3030-3970	<LOQ	1900-3470	0.707-1.12	<LOQ–0.169	2.90-4.90
ANOVA (*t*-test) entry effect and SEM								
	P	0.445	0.987	–	0.852	0.282	0.076	0.580
	SEM	9.2	62	–	74	0.0358	0.0130	0.123
Reference varieties	mean	222	2249	–	2606	0.893	0.172	4.04
	range	113–435	1700–2920	<LOQ	1720–3890	0.503–1.34	<LOQ–0.386	1.67–6.09
ILSI (2014)	mean	224.2	2254.93	3.697	1737.1	0.861	0.174	3.51
	range	<LOQ–820.0	291.93–4397.30	<LOQ–6.340	<LOQ–4750.0	<LOQ–1.570	<LOQ–0.443	<LOQ–8.42
	N	5371	5378	14	4003	5762	4585	4089

MZIR098: *N* = 32.
Control: *N* = 32.
Reference varieties: *N* = 192.
ILSI: *N* is the number of ILSI values used to calculate the mean and excludes values < LOQ.
Units for anti-nutrients are shown in column headings: milligrams per kilogram (mg/kg), parts per million (ppm), percent (%), trypsin inhibitor unit (TIU). All are expressed on a dry weight basis.
Results significantly different ($p < 0.05$) are shown in bold italic type.
When some or all values were < LOQ, and substitution with the LOQ was not appropriate due to the number or distribution of substitutions required, calculation of the mean and analysis of variance (ANOVA) could not be performed and only the range is shown.
[a] The LOQ for furfural was 0.543–0.605 mg/kg DW.
[b] The LOQ for raffinose was 0.057–0.060 mg/kg DW. Levels for one test sample and two control samples were replaced with the LOQ to perform ANOVA.

VIII.C.3. Conclusions from Compositional Analysis

In the compositional assessment of MZIR098 corn forage and grain, the levels of the majority of nutritional components did not differ significantly between MZIR098 corn and nontransgenic, near-isogenic control corn.

Across-location mean levels of all quantifiable components except ferulic acid were within the ranges observed in the nontransgenic commercial corn reference varieties grown in the same field trials. The levels of ferulic acid did not differ significantly between the MZIR098 and nontransgenic control corn. The across-location mean levels of all components of MZIR098 corn were within the ranges published in the ILSI Crop Composition Database.

These results indicate that the levels of the majority of nutritional components did not differ between MZIR098 corn and near-isogenic, nontransgenic control corn, and that those levels that did differ fell within ranges considered to be normal for conventional corn. Therefore, the results of this assessment of key nutritional components of forage and grain from MZIR098 corn support the following conclusions:

- No biologically relevant impact on the nutritional status of forage and grain originating from Event MZIR098 corn resulted from the transformation process or the expression of the transgenes in MZIR098 corn.
- Forage and grain from MZIR098 corn are not materially different in nutrient composition from forage and grain of the nontransgenic, near-isogenic comparator or of conventional field corn

Based on the conclusions from the compositional analysis, there is no reason to perform any additional nutritional impact studies.

VIII.D. References Cited in Part VIII

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Appendix A. Transgenic Crops Approved for Food and Feed Use Globally that Contain eCry3.1Ab, mCry3A, and PAT

This appendix contains a list of biotechnology-derived traits containing transgenic eCry3.1Ab, mCry3A, and PAT proteins with the potential to currently be in commerce. Companies listed are only those that contribute to biotradestatus.com. BCS = Bayer CropScience, Dow = Dow AgroSciences LLC. Product commercial names may vary by region, the list of products and approving countries may be incomplete, and therefore, this list may not be comprehensive.

Table A-1. Transgenic crops approved for food and feed use globally that contain eCry3.1Ab proteins

Company	Product name	Event/Stacked event	OECD unique identifier	Countries with approvals
Syngenta	Agrisure Duracade™	5307	SYN-Ø53Ø7-1	Australia, Canada, Colombia, Japan, Korea, Mexico, New Zealand, Russia, Taiwan, United States
Syngenta	Agrisure Duracade™ E-Z Refuge™ 5122	Bt11 × MIR604 × TC1507 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × SYN-Ø53Ø7-1 × MON-ØØØ21-9	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, South Africa, Taiwan, United States
Syngenta	Agrisure Duracade™ E-Z Refuge™ 5222	Bt11 × MIR162 × MIR604 × TC1507 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × SYN-Ø53Ø7-1 × MON-ØØØ21-9	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, South Africa, Taiwan, United States

Sources: CropLife International (2015)

Table A-2. Transgenic crops approved for food and feed use globally that contain mCry3A proteins

Company	Product name	Event/stacked event	OECD unique identifier	Countries with approvals
Syngenta	Agrisure® 3000GT	Bt11 × MIR604 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4-5 × MON-ØØØ21-9	Argentina, Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Syngenta	Agrisure® 3122	Bt11 × DAS-59122-7 × MIR604 × TC1507 × GA21	SYN-BTØ11-1 × DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, Philippines, South Africa, Taiwan, United States
Syngenta	Agrisure® CB/LL/RW	Bt11 × MIR604	SYN-BTØ11-1 × SYN-IR6Ø4-5	Argentina, Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Syngenta	Agrisure Duracade™ E-Z Refuge™ 5122	Bt11 × MIR604 × TC1507 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × SYN-Ø53Ø7-1 × MON-ØØØ21-9	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, South Africa, Taiwan, United States
Syngenta	Agrisure Duracade™ E-Z Refuge™ 5222	Bt11 × MIR162 × MIR604 × TC1507 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × SYN-Ø53Ø7-1 × MON-ØØØ21-9	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, South Africa, Taiwan, United States

Continued

Company	Product name	Event/stacked event	OECD unique identifier	Countries with approvals
Syngenta	Agrisure® GT/RW	MIR604 × GA21	SYN-IR6Ø4-5 × MON-ØØØ21-9	Argentina, Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Syngenta	Agrisure® RW	MIR604	SYN-IR6Ø4-5	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Indonesia, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Syngenta	Agrisure Viptera® 3111	Bt11 × MIR162 × MIR604 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × MON-ØØØ21-9	Argentina, Australia, Brazil, Canada, Colombia, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Syngenta	Enogen®/ Agrisure® 3000GT	3272 × Bt11 × MIR604 × GA21	SYN-E3272-5 × SYN-BTØ11-1 × SYN-IR6Ø4-5 × MON-ØØØ21-9	Australia, Canada, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, Taiwan, United States
DuPont Pioneer	Optimum® Intrasect® Xtreme	TC1507 × DAS-59122-7 × MON810 × MIR604 × NK603	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ81Ø-6 × SYN-IR6Ø4-5 × MON-ØØ6Ø3-6	Australia, Canada, China, Japan, Korea, Mexico, New Zealand, Taiwan, United States
DuPont Pioneer	Optimum® TRIsect®	TC1507 × MIR604 × NK603	DAS-Ø15Ø7-1 × SYN-IR6Ø4-5 × MON-ØØ6Ø3-6	Australia, Canada, Japan, Korea, Mexico, New Zealand, Taiwan, United States

Sources: CropLife International (2015)

Table A-3. Transgenic crops approved for food and feed use globally that contain PAT proteins

Crop	Company	Product name	Event/ stacked event	OECD unique identifier	Source of PAT gene	Countries with approvals
Corn	BCS	LibertyLink® Corn	T25	ACS-ZMØØ3 -2	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Malaysia, Mexico, New Zealand, Philippines, Singapore, South Africa, Taiwan, United States
Corn	Dow	HERCULEX® I	TC1507	DAS-Ø15Ø7 -1	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Paraguay, Philippines, Singapore, South Africa, Taiwan, United States, Uruguay

Continued

Crop	Company	Product name	Event/ stacked event	OECD unique identifier	Source of PAT gene	Countries with approvals
Corn	Dow	HERCULEX® I × Roundup Ready® Corn 2	TC1507 × NK603	DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States, Uruguay
Corn	Dow	HERCULEX® RW Rootworm Protection	DAS-59122-7	DAS-59122-7		Australia, Canada, China, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Singapore, Taiwan, United States
Corn	Dow	HERCULEX® XTRA Insect Protection	TC1507 × DAS-59122-7	DAS-Ø15Ø7-1 × DAS-59122-7	*S. viridochromogenes*	Australia, Canada, China, European Union, Japan, Korea, Mexico, New Zealand, Philippines, South Africa, Taiwan, United States
Corn	Dow	HERCULEX® XTRA × Roundup Ready® Corn 2	TC1507 × DAS-59122-7 × NK603	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, China, European Union, Japan, Korea, Mexico, New Zealand, Philippines, South Africa, Taiwan, United States
Corn	Dow	POWERCORE™	MON 89034-3 × TC1507 × NK603	MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Paraguay, Philippines, South Africa, Taiwan, United States, Uruguay
Corn	Dow	SmartStax® Corn	MON 89034 × TC1507 × MON 88017 × DAS-59122-7	MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-88Ø17-3 × DAS-59122-7	*S. viridochromogenes*	Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, South Africa, Taiwan, United States
Corn	DuPont Pioneer	HERCULEX® I Insect Protection	TC1507	DAS-Ø15Ø7 -1	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Honduras, Japan, Korea, Malaysia, Mexico, New Zealand, Paraguay, Philippines, Singapore, south Africa, Taiwan, United States, Uruguay
Corn	DuPont Pioneer	HERCULEX® I Insect Protection × Roundup Ready® Corn 2	TC1507 × NK603	DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Honduras, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Corn	DuPont Pioneer	HERCULEX® RW Rootworm Protection	DAS-59122-7	DAS-59122-7	*S. viridochromogenes*	Australia, Canada, China, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Corn	DuPont Pioneer	HERCULEX® RW Rootworm Protection × Roundup Ready® Corn 2	DAS-59122-7 × NK603	DAS-59122-7 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, China, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Corn	DuPont Pioneer	HERCULEX® XTRA Insect Protection	TC1507 × DAS-59122-7	DAS-Ø15Ø7-1 × DAS-59122-7	*S. viridochromogenes*	Australia, Canada, China, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States

Continued

Crop	Company	Product name	Event/ stacked event	OECD unique identifier	Source of PAT gene	Countries with approvals
Corn	DuPont Pioneer	HERCULEX® XTRA × Roundup Ready® Corn 2	TC1507 × DAS-59122-7 × NK603	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, China, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Corn	DuPont Pioneer	Optimum® Intrasect®	TC1507 × MON810 × NK603	DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Brazil, Canada, China, Colombia, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Corn	DuPont Pioneer	Optimum® Intrasect® Xtra	TC1507 × DAS-59122-7 × MON810 × NK603	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ81Ø-6 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, China, Japan, Korea, Mexico, New Zealand, Taiwan, United States
Corn	DuPont Pioneer	Optimum® Intrasect® Xtra × Xtreme	TC1507 × DAS-59122-7 × MON810 × MIR604 × NK603	DAS-Ø15Ø7-1 × DAS-59122-7 × MON-ØØ81Ø-6 × SYN-IR6Ø4-5 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, China, Japan, Korea, Mexico, New Zealand, Taiwan, United States
Corn	DuPont Pioneer	Optimum® Leptra®	TC1507 × MON810 × MIR162 × NK603	DAS-Ø15Ø7-1 × MON-ØØ81Ø-6 × SYN-IR162-4 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, Japan, Korea, Mexico, New Zealand, Taiwan, United States
Corn	DuPont Pioneer	Optimum® Trisect®	TC1507 × MIR604 × NK603	DAS-Ø15Ø7-1 × SYN-IR6Ø4-5 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Australia, Canada, China, Japan, Korea, Mexico, New Zealand, Taiwan, United States
Corn	Monsanto	Genuity® SmartStax®	MON 89034 × TC1507 × MON 88017 × DAS-59122-7	MON-89Ø34-3 × DAS-Ø15Ø7 × MON-88Ø17-3 × DAS-59122-7	*S. viridochromogenes*	Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Corn	Monsanto	POWERCORE™	MON89034 × TC1507 × NK603	MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6	*S. viridochromogenes*	Argentina, Brazil, Canada, Japan, Korea, Philippines, South Africa
Corn	Monsanto	Roundup Ready® Corn 2/Glufosinate Tolerant Corn	NK603 × T25	MON-ØØ6Ø3-6 × ACS-ZMØØ3-2	*S. viridochromogenes*	Canada, Colombia, Japan, Korea, Mexico, Philippines, Taiwan, United States

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Crop	Company	Product name	Event/ stacked event	OECD unique identifier	Source of PAT gene	Countries with approvals
Corn	Syngenta	KnockOut®	176	SYN-EV176-9	*S. hygroscopicus*	Argentina, Australia, Canada, China, European Union, Japan, Korea, New Zealand, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure® 3000GT	Bt11 × MIR604 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4-5 X MON-ØØØ21-9	*S. viridochromogenes*	Argentina, Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure® 3122	Bt11 × DAS-59122-7 × MIR604 × TC1507 × GA21	SYN-BTØ11-1 × DAS-59122-7 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × MON-ØØØ21-9	*S. viridochromogenes*	Canada, Japan, Korea, Mexico, Philippines, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure® CB/LL	Bt11	SYN-BTØ11-1	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, Colombia, European Union, Indonesia, Japan, Korea, Malaysia, Mexico, New Zealand, Paraguay, Philippines, Russia, South Africa, Switzerland, Taiwan, Turkey, United States, Uruguay, Vietnam
Corn	Syngenta	Agrisure® CB/LL/RW	Bt11 × MIR604	SYN-BTØ11-1 × SYN-IR6Ø4-5	*S. viridochromogenes*	Argentina, Australia, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure Duracade™ E-Z Refuge™ 5122	Bt11 × MIR604 × TC1507 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × SYN-Ø53Ø7-1 × MON-ØØØ21-9	*S. viridochromogenes*	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure Duracade™ E-Z Refuge™ 5222	Bt11 × MIR162 × MIR604 × TC1507 × 5307 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × DAS-Ø15Ø7-1 × SYN-Ø53Ø7-1 × MON-ØØØ21-9	*S. viridochromogenes*	Australia, Canada, Japan, Korea, Mexico, New Zealand, Russian Federation, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure® GT/CB/LL	Bt11 × GA21	SYN-BTØ11-1 × MON-ØØØ21-9	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, Colombia, European Union, Japan, Korea, Mexico, New Zealand, Philippines, South Africa, Taiwan, Turkey, United States, Uruguay
Corn	Syngenta	Agrisure Viptera® 3110	Bt11 × MIR162 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × MON-ØØ×21-9	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, Colombia, Japan, Korea, Mexico, New Zealand, Philippines, South Africa, Taiwan, United States, Uruguay

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Crop	Company	Product name	Event/ stacked event	OECD unique identifier	Source of PAT gene	Countries with approvals
Corn	Syngenta	Agrisure Viptera® 3111	Bt11 × MIR162 × MIR604 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × SYN-IR6Ø4-5 × MON-ØØØ21-9	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, Colombia, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Corn	Syngenta	Agrisure Viptera® 3220	Bt11 × MIR162 × TC1507 × GA21	SYN-BTØ11-1 × SYN-IR162-4 × DAS-Ø15Ø7-1 × MON-ØØØ21-9	*S. viridochromogenes*	Argentina, Australia, Canada, Japan, Korea, Mexico, New Zealand, Philippines, Russian Federation, South Africa, Taiwan, United States
Corn	Syngenta	Enogen® Agrisure® 3000GT	3272 × Bt11 × MIR604 × GA21	SYN-E3272-5 × SYN-BTØ11-1 × SYN-IR6Ø4-5 × MON-ØØØ21-9	*S. viridochromogenes*	Australia, Canada, Japan, Korea, Mexico, New Zealand, Philippines, Taiwan, United States
Canola	BCS	LibertyLink® Canola	T45	ACS-BNØØ8-2	*S. viridochromogenes*	Australia, Canada, China, European Union, Japan, Korea, Mexico, Russian Federation, South Africa, Taiwan, United States
Canola	BCS	SeedLink®	MS1/RF1	ACS-BNØØ4-7 × ACS-BNØØ1-4	*S. hygroscopicus*	Australia, Canada, China, Japan, Korea, Mexico, New Zealand, South Africa, United States
Canola	BCS	SeedLink®	MS1/RF2	ACS-BNØØ4-7 × ACS-BNØØ2-5	*S. hygroscopicus*	Australia, Canada, China, Japan, Korea, Mexico, New Zealand, South Africa, United States
Canola	BCS	SeedLink®/ InVigor®	MS8/RF3	ACS-BNØØ5-8 × ACS-BNØØ3-6	*S. hygroscopicus*	Australia, Canada, China, European Union, Japan, Korea, Mexico, New Zealand, South Africa, United States
Cotton	BCS	GlyTol® × LibertyLink® Cotton	GHB614 × LL25Cotton	BCS-GHØØ2-5 × ACS-GHØØ1-3	*S. hygroscopicus*	Australia, Brazil, Canada, Colombia, Japan, Korea, Mexico, New Zealand, United States
Cotton	BCS	GlyTol® × TwinLink®	GHB614 × T304-40 × GHB119	BCS-GHØØ2-5 × BCS- GHØØ4-7 × BCS- GHØØ5-8	*S. hygroscopicus*	Australia, Brazil, Canada, Japan, Korea, Mexico, New Zealand, United States
Cotton	BCS	LibertyLink® Cotton	LLCotton25	ACS-GHØØ1-3	*S. hygroscopicus*	Australia, Brazil, Canada, China, Colombia, European Union, Japan, Korea, Mexico, South Africa, New Zealand, United States
Cotton	BCS	LibertyLink® × Bollgard II® Cotton	LLCotton25 × MON15985	ACS-GHØØ1-3 × MON 15985-7	*S. hygroscopicus*	Australia, Canada, Japan, Korea, Mexico, New Zealand, United States
Cotton	BCS	TwinLink®	T304-40 × GHB119	BCS- GHØØ4-7 × BCS- GHØØ5-8	*S. hygroscopicus*	Australia, Brazil, Canada, Korea, Mexico, New Zealand, United States

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Crop	Company	Product name	Event/ stacked event	OECD unique identifier	Source of PAT gene	Countries with approvals
Cotton	Dow	WideStrike®	281-24-236 × 3006-210-23	DAS-21Ø23-5 × DAS-24236-5	*S. viridochromogenes*	Australia, Brazil, Canada, European Union, Japan, Korea, Mexico, New Zealand, United States
Cotton	Dow	WideStrike® × Roundup Ready® Flex Cotton	281-24-236 × 3006-210-23 × Mon 88913	DAS-24236-5 × DAS-21Ø23-5 × MON-88913-8	*S. viridochromogenes*	Australia, Canada, Japan, Korea, Mexico, New Zealand, United States
Cotton	Dow	WideStrike® × RR1445 Cotton	281-24-236 × 3006-210-23 × Mon 1445	DAS-24236-5 × DAS-21Ø23-5 × MON-Ø1445-2	*S. viridochromogenes*	Australia, Canada, Japan, Korea, Mexico, New Zealand, United States
Cotton	Monsanto	Bollgard II® XtendFlex™	MON 88701 × MON 88913 × MON 15985	MON-887Ø1-3 × MON-88913-8 × MON-15985-7	*S. hygroscopicus*	Australia, Canada, Japan, Mexico, New Zealand, United States
Rice	BCS	LibertyLink® Rice	LLRICE06,LLRICE 62, LLRICE601	ACS-OSØØ2-5	*S. hygroscopicus*	Australia, Canada, Colombia, Honduras, Mexico, New Zealand, Philippines, Russian Federation, South Africa, United States
Soybean	BCS	LibertyLink® Soybean	A2704-12	ACS-GMØØ5-3	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, European Union, Japan, Korea, Malaysia, Mexico, New Zealand, Philippines, Russian Federation, Singapore, South Africa, Taiwan, United States, Uruguay
Soybean	BCS	LibertyLink® Soybean	A5547-127	ACS-GMØØ6-4	*S. viridochromogenes*	Argentina, Australia, Brazil, Canada, China, European Union, Japan, Korea, Malaysia, Mexico, New Zealand, Philippines, Russian Federation, Singapore, Taiwan, United States, Uruguay

Sources: CropLife International (2015), OECD (2015)

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