Application to Amend the Australia New Zealand Food Standards Code
Schedule 26 - *Food Produced Using Gene Technology*

OECD Unique Identifier: COR-ØØ121-4

COR121 Maize

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SUMMARY

Corteva Agriscience Australia Pty Ltd, on behalf of Pioneer Hi-Bred International, Inc. (Pioneer), is submitting this application to FSANZ to vary the Code to approve food uses of insect-resistant maize (*Zea mays* L.) event COR-ØØ121-4 (referred to as COR121 maize), a new food produced using gene technology.

COR121 maize was genetically modified to expresses the IPD083Cb protein for control of certain susceptible lepidopteran pests and the phosphomannose isomerase (PMI) protein that was used as a selectable marker. The IPD083Cb protein has recently been evaluated by FSANZ in the insect-protected soybean line COR23134, which was added to Schedule 26 in August 2025 (A1323 application). The PMI protein is found in several approved events that are currently in commercial use (ISAAA, 2025).

This application presents information supporting the safety and nutritional comparability of COR121 maize to conventional maize. The molecular characterization analyses conducted on COR121 maize demonstrated that the introduced genes are integrated at a single locus, stably inherited across multiple generations, and segregate according to Mendel's law of genetics. The allergenic and toxic potential of the IPD083Cb and PMI proteins were evaluated, and these proteins were found unlikely to be allergenic or toxic to humans or animals. A composition assessment demonstrated that the nutrient composition of COR121 maize forage and grain is comparable to that of conventional maize, represented by non-genetically modified (non-GM), near-isoline maize and non-GM commercial maize.

Overall, data and information contained herein support the conclusion that COR121 maize, containing the IPD083Cb and PMI proteins, is as safe and nutritious as conventional maize for food use.

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STATUTORY DECLARATION

Statutory Declarations Act 1959 [1]

I, [redacted] Regulatory Manager of Corteva Agriscience, Level 9, 67 Albert Ave, Chatswood, NSW 2067 make the following declaration under the *Statutory Declarations Act 1959*:

1. the information provided in this application fully sets out the matters required
2. the information provided in this application is true to the best of my knowledge and belief
3. no information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

[redacted]

[*Signature of person making the declaration*]

Declared at Chatswood on 9th of February 2022

Before me,

[redacted]

Legal Practitioner
Corteva Agriscience, Level 9, 67 Albert Ave, Chatswood, NSW 2067

[1] http://www.comlaw.gov.au/Series/C1959A00052.

GENERAL INFORMATION ON THE APPLICATION

The chapter numbering follows section numbers from the FSANZ Application Handbook (Chapters 3.1 and 3.5.1).

B. Applicant

This application is submitted by:

Corteva Agriscience Australia Pty Ltd, Member of Corteva Agriscience group of companies, on behalf of Pioneer Hi-Bred International, Inc.
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The primary contact is:

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The Managing Director of Corteva Agriscience Australia Pty Ltd is:

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Email: [redacted]

C. Purpose of the application

Pioneer has developed COR121 maize (OECD Unique Identifier COR-ØØ121-4), a new event that has been transformed to express the IPD083Cb protein for protection against certain susceptible lepidopteran pests and the phosphomannose isomerase (PMI) protein that was used as a selectable marker.

As a result of this application, Pioneer seeks an amendment of Standard 1.5.2 *Food produced using gene technology* by inserting the following into table to Schedule 26 3(4) after the last entry: insect-protected corn line COR121.

D. Justification for the application

D (a) Need for the proposed change

Pioneer is a member of Excellence Through Stewardship™ (ETS). Pioneer has developed the new maize event COR121, which will be commercialized in accordance with the ETS Product Launch Stewardship Guidance and in compliance with Pioneer polices regarding stewardship of GM products. In line with these guidelines, Pioneer's process for responsible launches of new products

includes a longstanding process to evaluate export market information, value chain consultations, and regulatory functionality. Pioneer's application to amend Standard 1.5.2 with respect to COR121 maize is aligned with these policies.

D (b) Advantage of the genetically modified food

Maize has multiple downstream uses for feed, fuel, and food that are significant for the global supply of this crop commodity. The introduction of insect-resistant COR121 maize is intended to help growers keep pace with increasing maize demand globally. The United States is one of the world's largest maize producers and a leading exporter of maize. In 2023, more than 15 billion bushels of maize were produced in the United States from approximately 94 million planted acres, valued at nearly $69 billion (NCGA, 2024; USDA-NASS, 2025).

Insect Resistance

Certain lepidopteran insects are serious pests of maize in the United States. Control of lepidopteran maize pests has historically been managed with crop rotation, broad-spectrum insecticides, and transgenic crops expressing crystalline (Cry) proteins. As adoption of Bt maize has increased, the selection pressure on target insects to develop resistance has become greater. Insect resistance to transgenic traits can reduce the efficacy of the traits over time, increasing costs of maize production and/or reducing yield. The IPD083Cb protein expressed in COR121 maize provides an alternative to existing Bt-based insect control traits to help control certain susceptible lepidopteran insects.

Selectable Marker

The PMI protein was incorporated into COR121 maize to enable selection of plants containing the intended genes during the event development process.

D.1 Regulatory impact

Pioneer has developed the new maize event COR121, which will be commercialized in accordance with the ETS Product Launch Stewardship Guidance and in compliance with Pioneer polices regarding stewardship of GM products. In line with these guidelines, Pioneer's process for responsible launches of new products includes a longstanding process to evaluate export market information, value chain consultations, and regulatory functionality. Growers and end-users must take all steps within their control to follow appropriate stewardship requirements and confirm their buyer's acceptance of the grain or other material being purchased.

Refer to the OECD Consensus Document on Compositional Considerations for New Varieties of Maize (Zea mays): Key Food and Feed Nutrients, Anti-nutrients and Secondary Plant Metabolites (OECD, 2002), for the following aspects of the food uses of maize:

- Production of maize for food and feed
- Processing of maize
- Wet Milling
- Dry Milling

- Masa Production
- Feed Processing

The majority of grain and forage derived from maize is used for animal feeds. Less than 10% of maize grain is processed for human food products. Maize grain is also processed into industrial products, such as ethyl alcohol by fermentation and highly refined starch by wet-milling to produce starch and sweetener products. In addition to milling, maize germ can be processed to obtain maize oil.

Domestic production of maize in Australia (ca. 440,000 t) and New Zealand is supplemented by import of a small amount of maize-based products, largely as high-fructose maize syrup, which is not currently manufactured in either Australia or New Zealand. Such products are processed into breakfast cereals, baking products, extruded confectionery and maize chips. Other maize products such as maize starch are also imported. This is used by the food industry for the manufacture of dessert mixes and canned foods (www.grdc.com.au).

D.1.1 Costs and benefits for industry, consumers and government

Pioneer launches new products in accordance with the Pioneer Product Launch Policy and Excellence Through Stewardship Product Launch Guidance. Innovative technologies like COR121 maize are designed to deliver increased value and added performance to the farmers that produce grain from these products, along with helping farmers meet global demand for food and grain products. In line with these guidelines, Pioneer's approach to responsible launches of new products includes a long-standing process to evaluate export market information, value chain consultations, and regulatory functionality. Pioneer continues to advocate for a global synchronous, science-based and predictable regulatory system. We also encourage farmers, industry, and consumer groups to continue to advocate for the acceptance of new, innovative technologies that help to improve farm productivity and profitability and contribute to the global economy and environmental sustainability.

Pioneer does not develop nor import food or feed products into the Australian or New Zealand markets. The proposed amendment to the Standard, however, may result in increasing Australia and New Zealand's access to international grain food markets while supporting Pioneer's sale of seed in markets where COR121 maize is to be cultivated. In this sense, and in an effort to maintain transparency with FSANZ, Pioneer acknowledges that there may be a capturable commercial benefit to Pioneer as defined in Section 8 of the FSANZ Act. Any relevant local costs are made up of Pioneer personnel time both locally and globally as well as of the direct fees associated with the submission.

Most of the sweet corn consumed in Australia is grown domestically. Domestic production of corn in Australia and New Zealand is supplemented by importation of a small amount of corn-based products usually frozen or canned, largely as high-fructose corn syrup, which is not currently manufactured in either Australia or New Zealand (www.grdc.com.au). Although not requiring a FSANZ approval for livestock feed, from time to time, mainly during periods of drought where local supply of feed grain is limited, maize may be imported from the United States or other global grain producing countries for use as stock feed, predominantly in the pig and poultry markets. The requested variation to the Standard permits the import and use of food derived or developed from COR121 maize. This offers benefits to the industry and consumers in Australia and New Zealand,

which result from the advantages of grower access to COR121 maize in cultivation countries (see Section D(b) Advantage of the genetically modified food of the dossier above).

While Pioneer does not possess quantitative data, which would allow it to estimate the benefits in monetary terms, COR121 maize is anticipated to contribute to the maintenance of stable global maize supply, choice, and affordability for consumers. No specific costs associated with the approval of COR121 maize for Australian and New Zealand consumers have been identified.

Similarly, an analysis in monetary terms for the grain and food industry is hard to determine, however, Australian and New Zealand importers are expected to benefit from trade access, which the approval of COR121 maize will support (see Section D.1.2 Impact on international trade below). Compliance with import requirements is also anticipated to be simplified when sourcing from markets in which COR121 maize is commercialized as a seed product. The only identified costs associated with the approval of COR121 maize for Australian and New Zealand industry is meeting their GM labelling requirements for those foods derived from COR121 maize which trigger them, similarly to other existing GM maize varieties.

No dollar value of the costs and benefits for the governments can be assigned with the available information. However, from the government perspective, approval of COR121 maize will support global regulatory harmonization and limit potential instances of non-compliance related to the regulation of GM foods. No costs associated with the approval of COR121 maize for the Australian and New Zealand governments have been identified.

D.1.2 Impact on international trade

The addition of COR121 maize to Schedule 26 is anticipated to help support import access to maize from the applicable cultivation countries. Without such an approval, grain handlers may undertake a scientifically unnecessary and costly activities to segregate COR121 maize and food products derived from it for Australian and New Zealand markets. Therefore, amending the Food Code to include COR121 maize is anticipated to have a positive impact on Australian and New Zealand access to international commodity trade markets.

A. TECHNICAL INFORMATION ON THE FOOD PRODUCED USING GENE TECHNOLOGY

A.1 Nature and identity of the genetically of the genetically modified food

A.1 (a) Description of the GM organisms, nature and purpose of the genetic modification

COR121 maize was genetically modified to express the IPD083Cb protein for control of certain susceptible lepidopteran pests and the PMI protein that was used as a selectable marker.

The IPD083Cb protein is encoded by the insecticidal protein gene, *ipd083Cb.1*, derived from giant maidenhair fern (*Adiantum trapeziforme* var. *braziliense*). The expressed IPD083Cb protein is effective against certain susceptible lepidopteran pests by causing disruption of the midgut epithelial cells, providing an alternative to traditional *Bt*-based insect control traits. This is the same protein that has recently been evaluated by FSANZ in the insect-protected soybean line COR23134, which was added to Schedule 26 in August 2025 (A1323 application).

The phosphomannose isomerase (PMI) protein is encoded by the *pmi* gene, from *Escherichia coli*. The expressed PMI protein serves as a selectable marker during transformation which allows for the growth of tissue using mannose as the carbon source. The PMI protein is found in several approved events that are currently in commercial use (ISAAA, 2025).

A.1 (b) GM Organism Identification

In accordance with OECD's "Guidance for the Designation of a Unique Identifier for Transgenic Plants", this event has an OECD identifier of COR-ØØ121-4, also referred to as COR121 maize.

A.1 (c) Trade name

Maize event COR121 is at a pre-commercialization development stage and has not yet been assigned a commercial product name.

A.2 History of use of the host and donor organisms

A.2 (a) Donor organisms

Adiantum trapeziforme: donor of the ipd083Cb.1 gene

- Class: Polypodiopsida
- Order: Polypodiales
- Family: Pteridaceae
- Genus: *Adiantum L.*
- Species: *A. trapeziforme L.*
- Sub-species: *braziliense*

Adiantum trapeziforme is known as the giant maidenhair fern or diamond maidenhair fern. Ferns are among the oldest living organisms on the planet and, with the exception of Antarctica, are globally distributed (Fernández, 2011). Ferns of the genus *Adiantum L.* are found in temperate and tropical regions worldwide. *A. trapeziforme L.* is native to the tropical rainforests of Central and South America (Kew Science, 2020) and has been introduced in the state of Florida in the United States (USDA-NRCS, 2023).

Humans have used ferns for many applications including occasional sources of food (Simmons and Herman, 2023). Members of the maidenhair fern family and other non-seed plants have been utilized for ethnomedicinal purposes from treating respiratory infections such as cough, colds, to pneumonia with research continuing into the potential benefits of compounds from members of this genus (Rastogi *et al.*, 2018). Many species of genus *Adiantum L.* are used in traditional medicine as infusions, decoctions, or pastes (Rastogi *et al.*, 2018). There are no reports of *A. trapeziforme* being poisonous to humans or livestock.

***Escherichia coli*: donor of the *pmi* gene**

- Class: Gammaproteobacteria
- Order: Enterobacteriales
- Family: Enterobacteriaceae
- Genus: *Escherichia*
- Species: *E. coli*
- Strain: K-12

Escherichia coli (*E. coli*) is a Gram-negative, facultatively anaerobic, rod-shaped bacterium. The strain *E. coli* K-12 is a strain which has been debilitated, does not normally colonize the human intestine, and has a poor survival rate in the environment. *E. coli* K-12 has a history of safe use in human drug and specialty chemical production (US-EPA, 1997).

A.2 (b) Host organism

Information relating to maize, the host organism, was included in many previous safety assessments prepared by FSANZ. Repeating it is not considered necessary in this submission.

A.3 Nature of the genetic modification

A.3 (a) Transformation method

[redacted] The use of SSI for targeted transgene insertion has advantages compared to random transformation by allowing the ability to pre-select the insertion location to avoid endogenous gene disruption and pre-test the genomic location for agronomic neutrality (Gao *et al.*, 2020). Thus, the SSI approach can simplify risk assessment of the event intended for commercialization as it concerns potential for insertional effects.

[redacted]

[redacted]

Table 1. List of Relevant Genetic Elements in Plasmids Used in the First Transformation and Their Presence in COR121 Maize

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

[redacted]

Table 2. List of Relevant Genetic Elements in the T-DNA Region of Plasmid [redacted] Used in the Second Transformation and Their Presence in COR121 Maize

[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]

Figure 1. Map of Plasmid [redacted]

[redacted]

Figure 2. Map of Plasmid [redacted]

[redacted]

Figure 3. Map of Plasmid

Figure 4. Map of Plasmid [redacted]

[redacted]

Figure 5. Map of the T-DNA Region from Plasmid [redacted]

Figure 6. Map of Plasmid [redacted]

[redacted]

Figure 7. Map of the T-DNA Region from Plasmid

Figure 8. Map of Plasmid [redacted]

[redacted]

Figure 9. Map of the COR121 Maize Insertion

[redacted]

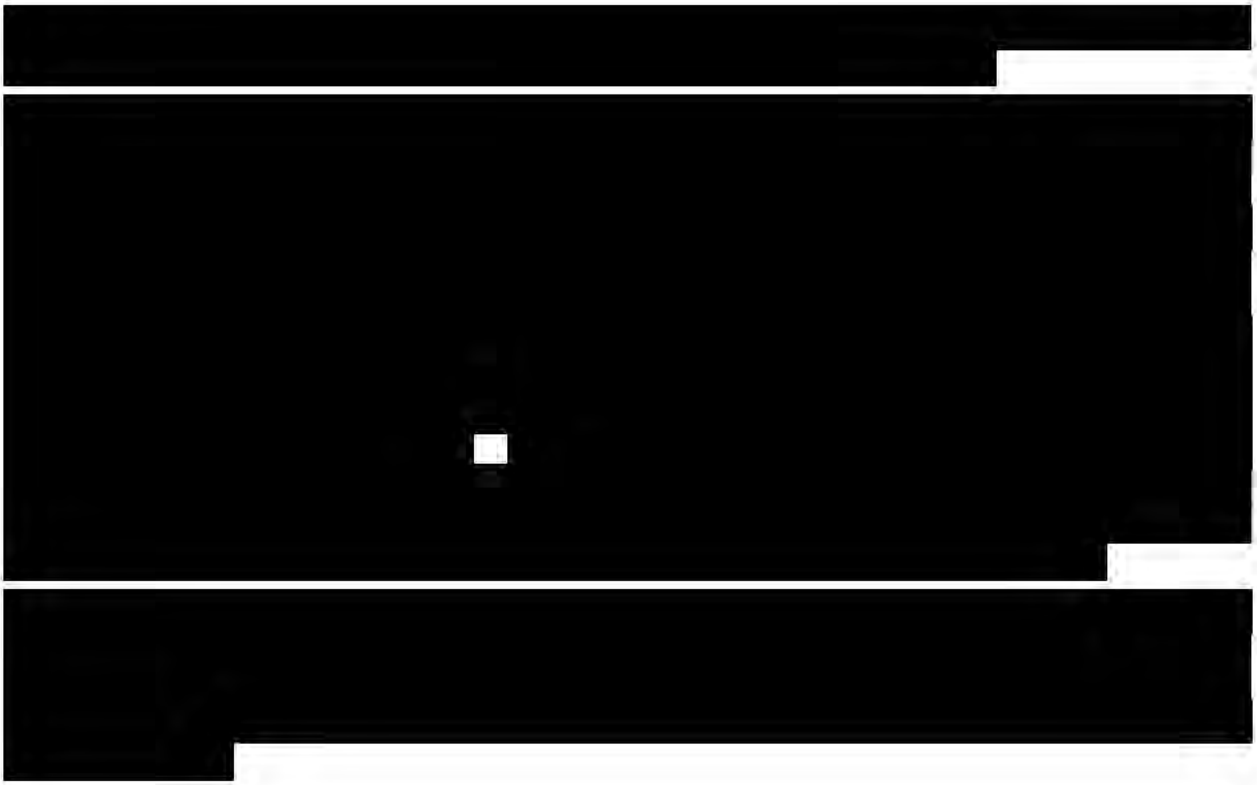


Table 3. Description of Genetic Elements in Plasmid [redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]

Table 4. Description of Genetic Elements in the T-DNA Region from Plasmid

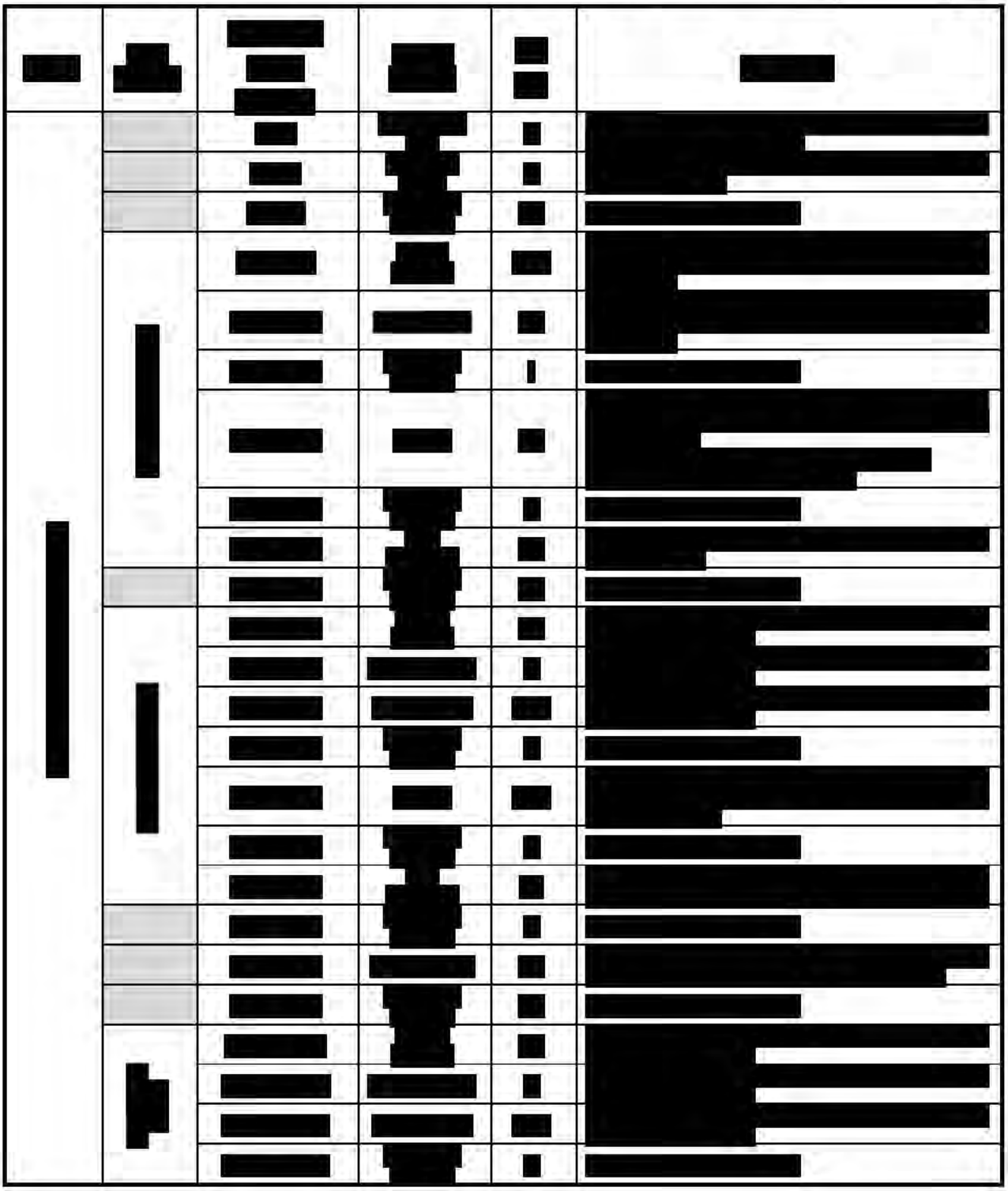


Table 4. Description of Genetic Elements in the T-DNA Region from Plasmid [redacted] (continued)

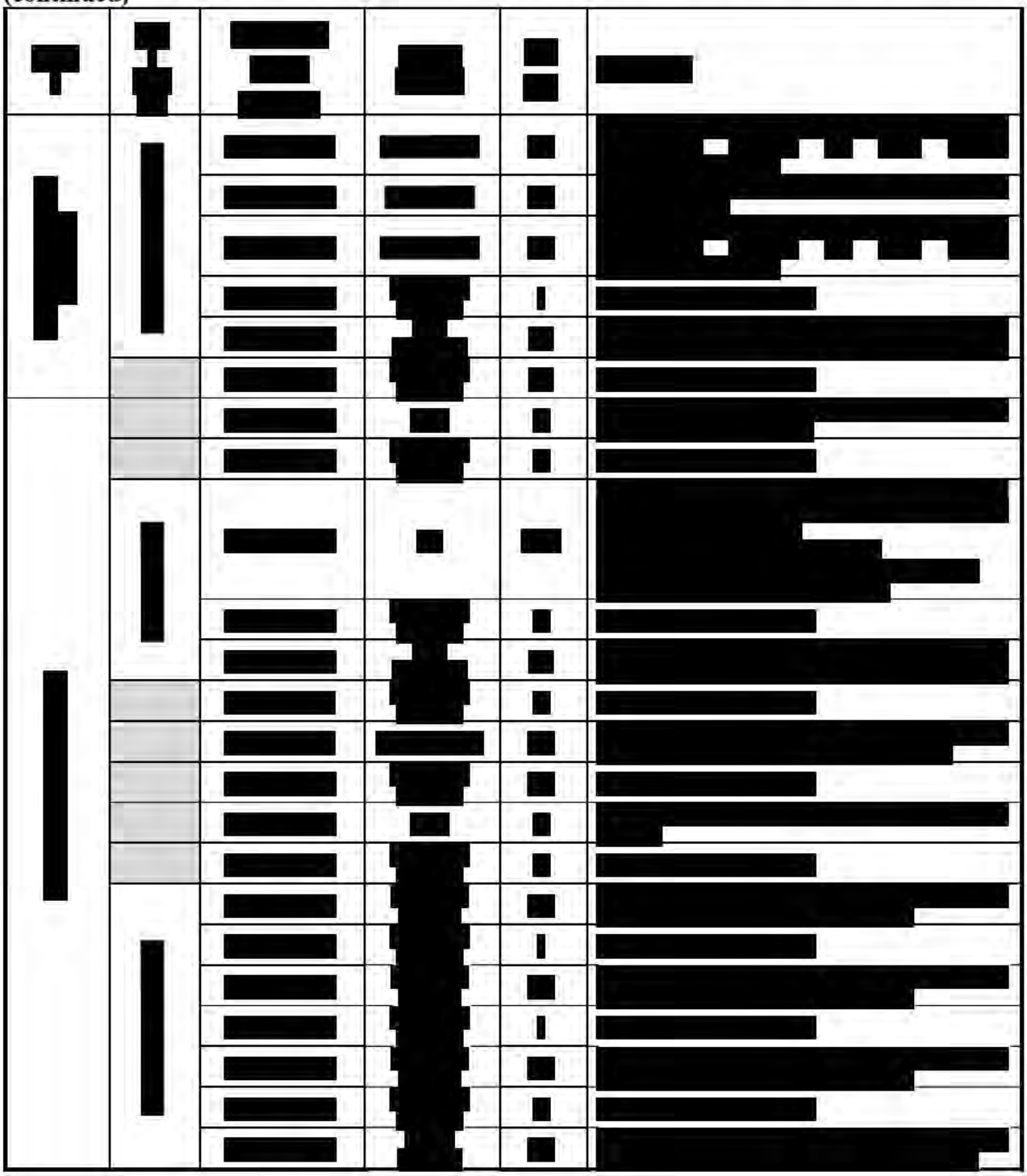


Table 4. Description of Genetic Elements in the T-DNA Region from Plasmid [redacted] (continued)

A.3 (b) Description of the construct and the transformation vectors used

Please refer to Section *A.3 (a) Transformation method* for the description of the construct and the transformation vectors used.

A.3 (c) Molecular Characterization of the Inserted DNA in COR121 Maize

Molecular Characterization of the inserted DNA in COR121 maize was conducted using Whole Genome Sequencing (WGS) to determine the insertion copy number and organization within the plant genome and to confirm the absence of plasmid backbone and other unintended plasmid sequences. WGS was performed to confirm stable genetic inheritance of the inserted *ipd083Cb.1* and *pmi* gene cassettes across multiple generations during the breeding process. Segregation analysis was conducted for five generations of COR121 maize to confirm stable Mendelian inheritance.

Whole Genome Sequencing Analysis to Determine Insertion Copy Number and Organization, Confirm Absence of Plasmid Backbone, and Demonstrate Genetic Stability Across Generations

WGS analysis utilizes Next Generation Sequencing (NGS) techniques and bioinformatics procedures to sequence and identify inserted DNA within the maize genome. By compiling a large number of unique sequencing reads and mapping them against the sequences of the transformation plasmids, intended insertion (Figure 10), and control maize genome, unique junctions due to the inserted DNA are identified in the bioinformatics analysis and used to determine the insertion copy number and organization within the plant genome and confirm the absence of plasmid backbone sequences.

Genomic DNA is sequenced using a Next Generation Sequencing (NGS) procedure and the results are analyzed using bioinformatics tools. During the analysis, junction reads are identified as those sequencing reads where part of the read aligns to the plasmid DNA sequence while the rest of the read aligns to genome (plasmid-genome junction) or non-contiguous plasmid sequence (plasmid-plasmid junction). Multiple sequencing reads are generated for each junction and are compiled into a consensus sequence for the junction. By compiling a large number of unique sequencing reads and comparing them to the transformation plasmid and control maize genome, unique junctions due to the inserted DNA can be identified. A unique junction is defined as one in which the 20-bp plasmid-derived sequence and the 30-bp adjacent sequence are the same across multiple reads, although the overall length of the multiple reads for that junction may vary due to the sequencing process. The number of unique junctions is related to the number of plasmid insertions present in the maize genome (for example, a single T-DNA insertion is expected to have two unique plasmid-genome junctions). Detection of additional unique junctions beyond the two expected for a single insertion would indicate the presence of rearrangements or deletions within the insertion, or additional insertions derived from plasmid DNA. The absence of any junctions indicates there are no detectable insertions within the maize genome.

The [redacted] generation of COR121 maize was analyzed by WGS to determine the insertion copy number and organization and to confirm the absence of plasmid backbone sequences. WGS analysis was performed on samples from an additional four generations of COR121 maize ([redacted]

[redacted]) to demonstrate genetic stability. WGS was also performed on one PH184C control maize plant, and on positive control samples to confirm that the assay could reliably detect plasmid DNA diluted in control maize DNA. Based on the results obtained for transgenic COR121 maize, a schematic diagram of the COR121 insertion was developed and is provided in Figure 11.

Several genetic elements in the transformation plasmids are derived from maize, and thus these endogenous elements (Table 5; Figures 1-4 and 6) will have sequencing reads in the WGS results due to the homologous elements in the PH184C control maize genome. However, if no junctions are detected, these sequencing reads only indicate the presence of the endogenous elements in their normal context of the maize genome and are not from the inserted DNA.

Sequencing yielded high-quality data, with genome-wide average sequencing depths ranging from 153x to 259x, confirming that the WGS data provided sufficient read depth for robust analysis (Table 6).

Characterization of the [redacted] Generation of COR121 Maize for Determining the Insertion Copy Number and Organization, and Confirming the Absence of Backbone Sequence

WGS analysis of the COR121 maize sample from the [redacted] generation demonstrated the presence of the inserted DNA (Figure 11) by the identification of two unique plasmid-genome junctions (Figure 12, Panel A), one at each end of the insertion. The insertion, derived from plasmid [redacted], starts with the 5' junction at bp [redacted] and ends with the 3' junction at bp [redacted]. These results are consistent with a single insertion of the COR121 Intended Insertion. The number of supporting reads for each plasmid-genome junction is summarized in Table 7.

Furthermore, there were no plasmid-genome junctions between the backbone sequence of the transformation plasmids and the maize genome, demonstrating that no plasmid backbone sequences were incorporated into COR121 maize (Figure 12, Panels B-G).

Confirmation of Genetic Stability of COR121 Maize Across Five Generations

Whole Genome Sequencing (WGS) analysis of the [redacted] generations of COR121 maize showed that each generation contained the same unique plasmid-genome junctions as the [redacted] generation when aligned to the COR121 Intended Insertion (Table 7; Figure 13). No additional plasmid-genome or plasmid-plasmid junctions that would result from rearrangements, deletions, or duplications were detected. This confirms that the insertion, copy number and organization remained stable during the breeding process.

WGS Specificity and Sensitivity Assessment via Controls

Sequencing reads of the non-GM PH184C control maize were aligned to the COR121 Intended Insertion and transformation plasmids (Figure 14, Panel A); however, coverage was only obtained for the endogenous genetic elements derived from the maize genome. No plasmid-genome junctions were detected between the sequencing reads and the maize genome, indicating that the reads were solely due to the endogenous genetic elements present in the maize genome (Figure 14, Panels B-G). WGS analysis of the positive control samples analyzed at 1 copy per genome resulted in sequence coverage across the entire length of plasmid of each plasmid (Appendix A, Figure

A.1). As expected for non-integrated DNA, no plasmid-genome junctions were detected. These control results demonstrate that the WGS methodology can reliably detect single-copy plasmid sequences while distinguishing between integrated and non-integrated DNA.

WGS analysis of the [redacted] generation of COR121 maize demonstrated that COR121 maize contained a single copy of the inserted DNA, derived from plasmids [redacted], with the expected organization and that no additional insertions, deletions, or plasmid backbone sequences are present in its genome as shown with the two plasmid-genome junctions identified.

Additional details regarding analytical methods for WGS analysis are provided in Appendix A.

Table 5. Maize Endogenous Elements in the Plasmids and COR121 Intended Insertion

Number[a]	Maize Endogenous Genetic Element[b]	Plasmid or Intended Insertion[b]
1	[redacted]	[redacted]
2		[redacted] COR121 Intended Insertion
3		[redacted]
4		[redacted]
5		[redacted]
6		[redacted]
7		[redacted], COR121 Intended Insertion
8		[redacted], COR121 Intended Insertion
9		[redacted]
10		[redacted]
11		[redacted]

Note: Untranslated region (UTR).

[a] The numbers indicating the maize endogenous genetic elements in the plasmid or COR121 Intended Insertion shown as circled numbers in the linear maps in Figures 12-14 and Appendix A (Figure A.1)

[b] As shown in the plasmid [redacted] and COR121 Intended Insertion maps in Figures 1-4, 6, and 10, respectively.

[c] As [redacted] are found in their native context in the maize genome, they are considered part of the flanking regions and not part of the COR121 Intended Insertion (Figure 10).

Table 6. WGS Sequencing Overview

Sample Description	Total Number of Bases Sequenced (Gb)[a]	Ave Seq Depth of the Genome[b]
COR121 Maize ([redacted] Generation)	354.1	153
COR121 Maize ([redacted] Generation)	427.7	185
COR121 Maize ([redacted] Generation)	513.1	223
COR121 Maize ([redacted] Generation)	423.6	184
COR121 Maize ([redacted] Generation)	385.9	167
Non-GM PH184C Control Maize	572.7	249
[redacted] Positive Control	595.8	259
[redacted] Positive Control	472.3	205
[redacted] Positive Control	443.1	192
[redacted] Positive Control	563.0	244
[redacted] Positive Control	390.8	169
[redacted] Positive Control	555.2	241

[a] Total number of bases sequenced per sample, expressed in Gigabases (Gb).

[b] Genome-wide average read depth was estimated using the Lander–Waterman formula (*D = N × L / G, where D is average read depth, N is the number of reads, L is the average read length, and G is the effective genome size of maize (2.5Gb)* Lander and Waterman, 1988).

Table 7. Junction Analysis for COR121 Maize and Controls

Sample Description	# Unique Plasmid-Genome Junctions Detected[a]	# Unique Plasmid-Plasmid Junctions Detected[b]	# Reads Alighed to 5' Plasmid-Genome Junction[c]	# Reads Aligned to 3' Plasmid-Genome Junction[d]
COR121 Maize ([redacted] Generation)	2	0	29	25
COR121 Maize ([redacted] Generation)	2	0	40	42
COR121 Maize ([redacted] Generation)	2	0	84	78
COR121 Maize ([redacted] Generation)	2	0	73	78
COR121 Maize ([redacted] Generation)	2	0	74	89
non-GM PH184C Control Maize	0	0	N/A	N/A
[redacted] Positive Control	0	0	N/A	N/A
[redacted] Positive Control	0	0	N/A	N/A
[redacted] Positive Control	0	0	N/A	N/A
[redacted] Positive Control	0	0	N/A	N/A
[redacted] Positive Control	0	2[e]	N/A	N/A
[redacted] Positive Control	0	2[e]	N/A	N/A

N/A = Not Applicable

[a] Total number of unique plasmid-genome junctions detected (Figures 12-14; Appendix A (Figure A.1).

[b] Total number of unique plasmid-plasmid junctions detected (Figures 12-14; Appendix A (Figure A.1).

[c] Total number of sequencing reads mapping to the 5' plasmid-genome junction of the COR121 insertion (Figures 12-14; Appendix A (Figure A.1).

[d] Total number of sequencing reads mapping to the 3' plasmid-genome junction of the COR121 insertion (Figures 12-14; Appendix A (Figure A.1).

[e] The two plasmid-plasmid junctions detected are the result of aligning sequencing reads from a circular plasmid to a linear map.

Figure 10. Map of the COR121 Intended Insertion

Schematic diagram of the COR121 Intended Insertion (indicated by dashed vertical lines) in COR121 maize following SSI at the [redacted] and [redacted] sites. The size of the COR121 Intended Insertion is [redacted] bp and it includes sequences from [redacted] (Figure 1) and [redacted] (Figure 6). The flanking maize genomic regions are represented by horizontal black bars. Although [redacted] are present in [redacted], they are derived from the maize genome and appear in their natural context in the chromosome and are therefore considered to be parts of the flanking maize genome and are not included in the COR121 Intended Insertion.

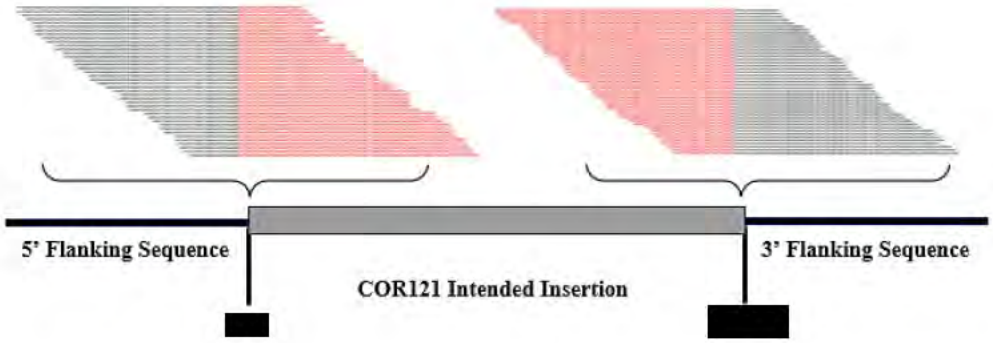


Figure 11. Map of the COR121 Maize Insertion

Schematic diagram of the DNA insertion in COR121 maize based on the WGS analysis described. The flanking maize genomic regions are indicated in the map by black bars. A single copy of the COR121 Intended Insertion derived from [redacted] as shown by the gray box, was integrated into the COR121 maize genome. Vertical lines show the locations of the two unique plasmid-genome junctions. The numbers below the vertical lines indicate the bp location of the junction position in reference to the sequence of the COR121 Intended Insertion (Figure 10). Individual sequencing reads for the junction are shown as horizontally stacked lines above each junction (not to scale); black indicates flanking genomic sequence and red indicates plasmid sequence within each sequencing read.

Figure 12. WGS Results for the [redacted] Generation of COR121 Maize: Insertion Copy Number, Organization, and Backbone Integration

The blue coverage graph shows the number of NGS reads for the [redacted] generation of COR121 maize aligned at each position on the COR121 Intended Insertion, plasmids [redacted] using a logarithmic scale at the middle of the graph for the [redacted] generation of COR121 maize. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources.

A. Alignment of NGS Reads to COR121 Intended Insertion

[redacted]

Alignment of sequencing reads against the COR121 Intended Insertion ([redacted] bp; Figure 10) indicating that this sample contains the expected insertion. Arrows in the Junctions panel indicate the two plasmid-genome junctions (black arrows); the numbers below the arrows refer to the bp location of the junction relative to the COR121 Intended Insertion. The presence of two plasmid-genome junctions (Junctions at bp [redacted] and bp [redacted]) demonstrates the presence of a single insertion in the [redacted] generation of COR121 maize genome.

B. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 1). Coverage was obtained for the elements found in COR121 maize (between [redacted] to [redacted] site and between [redacted]). Coverage was obtained for the [redacted] (*) due to an alignment of reads derived from the identical element in the COR121 Intended Insertion. Coverage was also obtained at regions bp [redacted] and bp [redacted] as these regions contain repetitive sequences that aligned to similar sequences in the genome and within the COR121 Intended Insertion. For clarity, the COR121 plasmid-genome junctions shown in panel A are not shown in panel B. The absence of any additional plasmid-genome or plasmid-plasmid junctions to the [redacted] sequence shows that there are no additional insertions, rearrangements, or backbone sequences present in the [redacted] generation of COR121 maize.

Figure 12. WGS Results for the [redacted] Generation of COR121 Maize: Insertion Copy Number, Organization, and Backbone Integration (continued)

The blue coverage graph shows the number of NGS reads for the [redacted] generation of COR121 maize aligned at each position on the COR121 Intended Insertion, plasmids [redacted] using a logarithmic scale at the middle of the graph for the [redacted] generation of COR121 maize. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources.

C. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 2). Coverage was obtained for the endogenous elements and was also obtained for the [redacted] (*) due to alignment of reads derived from the identical element in the COR121 Intended Insertion. Variation in coverage of the endogenous elements is due to sequence variations between the COR121 maize genome and the source of the corresponding genetic elements. The absence of any plasmid-genome or plasmid-plasmid junctions to the [redacted] sequence shows that there are no additional insertions, rearrangements, or backbone sequences present in the [redacted] generation of COR121 maize.

D. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 3). Coverage was obtained for the endogenous elements only. Variation in coverage of the endogenous elements is due to sequence variations between the COR121 maize genome and the source of the corresponding genetic elements. The absence of any plasmid-genome or plasmid-plasmid junctions to the [redacted] sequence shows that there are no additional insertions, rearrangements, or backbone sequences present in the [redacted] generation of COR121 maize.

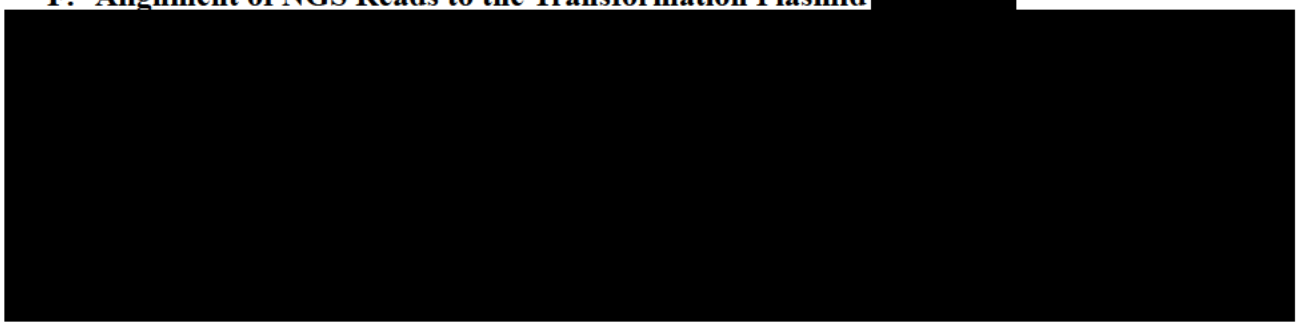
Figure 12. WGS Results for the T1 Generation of COR121 Maize: Insertion Copy Number, Organization, and Backbone Integration (continued)

The blue coverage graph shows the number of NGS reads for the [redacted] generation of COR121 maize aligned at each position on the COR121 Intended Insertion, plasmids [redacted] using a logarithmic scale at the middle of the graph for the [redacted] generation of COR121 maize. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources.

E. Alignment of NGS Reads to the Transformation Plasmid [redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 4). Coverage was obtained for the endogenous elements and was also obtained for the [redacted] (*) due to alignment of reads derived from the identical element in the COR121 Intended Insertion. Variation in coverage of the endogenous elements is due to sequence variations between the COR121 maize genome and the source of the corresponding genetic elements. The absence of any plasmid-genome or plasmid-plasmid junctions to the [redacted] sequence shows that there are no additional insertions, rearrangements, or backbone sequences present in the [redacted] generation of COR121 maize.

F. Alignment of NGS Reads to the Transformation Plasmid [redacted]



Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 6). Coverage was obtained for the recombination fragment between [redacted] that was transferred into COR121 maize (Figure 7). Coverage was also obtained for the endogenous elements in the region between the RB and [redacted] that were not transferred into the COR121 maize genome, and two of the [redacted] (*) elements outside of the [redacted] sites, due to alignment of reads derived from the [redacted] in the intended insertion to all copies of this element in [redacted]. Variation in coverage of the endogenous elements is due to sequence variations between the COR121 maize genome and the source of the corresponding genetic elements. Coverage was also obtained at regions bp [redacted], bp [redacted], and bp [redacted] as these regions contain repetitive sequences that aligned to similar sequences in the genome. The absence of any plasmid-genome or plasmid-plasmid junctions to the [redacted] sequence shows that there are no additional insertions, rearrangements, or backbone sequences present in the [redacted] generation of COR121 maize.

Figure 12. WGS Results for the [redacted] Generation of COR121 Maize: Insertion Copy Number, Organization, and Backbone Integration (continued)

The blue coverage graph shows the number of NGS reads for the [redacted] generation of COR121 maize aligned at each position on the COR121 Intended Insertion, plasmids [redacted] using a logarithmic scale at the middle of the graph for the [redacted] generation of COR121 maize. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources.

G. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 8). The absence of sequencing reads and plasmid-genome junctions for [redacted] indicates that no plasmid sequences were incorporated into the [redacted] generation of COR121 maize.

Figure 13. WGS Results for [redacted] Generation of COR121 Maize: Genetic Stability

WGS results for the [redacted] generations (panels A-E, respectively) of COR121 maize aligned against the COR121 Intended Insertion [redacted] bp; Figure 10). The blue coverage graph shows the number of NGS reads aligned at each point on the COR121 Intended Insertion using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Arrows in the Junctions panel indicate the two plasmid-genome junctions (black arrows); the numbers below the arrows refer to the bp location of the junction relative to the COR121 Intended Insertion. The presence of the same 5' and 3' plasmid-genome junctions in each generation as compared to the [redacted] generation indicates that the insertion in COR121 maize is genetically stable during the breeding process.

A. [redacted] COR121 Maize: Alignment of NGS Reads to COR121 Intended Insertion

B. [redacted] COR121 Maize: Alignment of NGS Reads to COR121 Intended Insertion

C. [redacted] COR121 Maize: Alignment of NGS Reads to COR121 Intended Insertion

Figure 13. WGS Results for [redacted] Generation of COR121 Maize: Genetic Stability (continued)

WGS results for the [redacted] generations (panels A-E, respectively) of COR121 maize aligned against the COR121 Intended Insertion ([redacted] bp; Figure 10). The blue coverage graph shows the number of NGS reads aligned at each point on the COR121 Intended Insertion using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Arrows in the Junctions panel indicate the two plasmid-genome junctions (black arrows); the numbers below the arrows refer to the bp location of the junction relative to the COR121 Intended Insertion. The presence of the same 5' and 3' plasmid-genome junctions in each generation as compared to the [redacted] generation indicates that the insertion in COR121 maize is genetically stable during the breeding process.

D. [redacted] COR121 Maize: Alignment of NGS Reads to COR121 Intended Insertion

[redacted]

E. [redacted] COR121 Maize: Alignment of NGS Reads to COR121 Intended Insertion

[redacted]

Figure 14. WGS Results for non-GM PH184C Control Maize

The blue coverage graph shows the number of individual NGS reads for the non-GM PH184C control maize sample aligned at each point on the COR121 Intended Insertion, [redacted] plasmid using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements are derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Variation in coverage of the endogenous elements is due to sequence variations between the non-GM PH184C control maize genome and the source of the corresponding genetic elements.

A. Alignment of NGS Reads to COR121 Intended Insertion

Alignment of sequencing reads against the COR121 Intended Insertion ([redacted] bp; Figure 10). Coverage was obtained for regions derived from maize endogenous elements only. The absence of plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

B. Alignment of NGS Reads to the Transformation Plasmid [redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 1). Coverage was obtained for regions derived from maize endogenous elements. Coverage was also obtained at the region bp [redacted] as this region contains repetitive sequences that aligned to similar sequences in the genome. The absence of plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

Figure 14. WGS Results for non-GM PH184C Control Maize (continued)

The blue coverage graph shows the number of individual NGS reads for the non-GM PH184C control maize sample aligned at each point on the COR121 Intended Insertion, [redacted] plasmid using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements are derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Variation in coverage of the endogenous elements is due to sequence variations between the non-GM PH184C control maize genome and the source of the corresponding genetic elements.

C. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 2). Coverage was obtained for regions derived from maize endogenous elements only. The absence of plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

D. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 3). Coverage was obtained for regions derived from maize endogenous elements. The absence of plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

Figure 14. WGS Results for non-GM PH184C Control Maize (continued)

The blue coverage graph shows the number of individual NGS reads for the non-GM PH184C control maize sample aligned at each point on the COR121 Intended Insertion, [redacted] plasmid using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements are derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Variation in coverage of the endogenous elements is due to sequence variations between the non-GM PH184C control maize genome and the source of the corresponding genetic elements.

E. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 4). Coverage was obtained for regions derived from maize endogenous elements. The absence of plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

F. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Alignment of sequencing reads against the plasmid [redacted] ([redacted] bp; Figure 6). Coverage was obtained for regions derived from maize endogenous elements. Coverage was also obtained at regions bp [redacted], bp [redacted], and bp [redacted] as these regions contain repetitive sequences that aligned to similar sequences in the genome. The absence of plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

Figure 14. WGS Results for non-GM PH184C Control Maize (continued)

The blue coverage graph shows the number of individual NGS reads for the non-GM PH184C control maize sample aligned at each point on the COR121 Intended Insertion, [redacted] plasmid using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements are derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Variation in coverage of the endogenous elements is due to sequence variations between the non-GM PH184C control maize genome and the source of the corresponding genetic elements.

G. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

G) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 8). The absence of sequencing reads and plasmid-genome junctions indicates that no plasmid sequences were incorporated into the non-GM PH184C control maize genome.

Multi-Generation Segregation Analysis

Segregation analysis was performed on five generations of COR121 maize ([redacted]) to confirm the Mendelian inheritance pattern of the inserted DNA during the breeding process. The observed inheritance pattern predicts the segregation of these genes within COR121 maize as a single genetic locus throughout the commercial breeding process.

The genotypic analysis was performed by quantitative digital PCR (referred to as dPCR) on individual seed chip samples to determine the copy number of event COR-ØØ121-4. For each of the [redacted] generations of COR121 maize, 96 seed chips were tested prior to planting. The observed segregation ratio was determined by genotypic results. Samples were identified as positive if confirmed to contain the COR121 maize event and the associated gene and genetic elements (one or two copy), and samples were identified as negative if not containing the gene and genetic elements (NULL). A chi-square test was performed separately for each generation to compare the observed segregation ratio to the expected segregation ratio (3:1). A chi-square test was not performed for the [redacted] generations of COR121 maize as all samples were identified as positive as expected for a homozygous generation.

A summary of segregation results for COR121 maize ([redacted] generations) is provided in Table 8. No statistically significant deviation from the expected segregation ratio was found in the [redacted] generations of COR121 maize, and the [redacted] generations were confirmed to be non-segregating. The genotypic analyses based on dPCR results demonstrated that the observed segregation ratios matched the expected segregation ratios for all six generations.

The results of the multi-generation segregation analysis demonstrated that the inserted DNA in COR121 maize segregated as a single locus in accordance with Mendelian rules of inheritance for a single genetic locus, indicating stable integration of the insert into the maize genome and a stable genetic inheritance pattern across generations during the breeding process.

Additional details regarding analytical methods for the multi-generation segregation analysis are provided in Appendix B.

Table 8. Summary of Genotypic Segregation Analyses for Five Generations of COR121 Maize

Entry	Generation	Expected Segregation Ratio	Observed Segregation[a]			Statistical Analysis	
		(Positive:Negative)	Positive	Negative	Total	Chi-Square[b]	P-Value
2	[redacted]	3:1	76	20	96	0.89	0.3458
3		Homozygous	95	0	95[c]	--	--
4		Homozygous	96	0	96	--	--
5		Homozygous	96	0	96	--	--
6		3:1	71	25	96	0.06	0.8137

[a] The observed segregation ratio was determined by genotype results.

[b] Degrees of freedom = 1. A chi-square value greater than 3.84 (P-value less than 0.05) would indicate a significant difference.

[c] One plant was excluded from the analysis due to inconclusive results obtained from polymerase chain reaction (PCR) analysis.

Sequence of Insert and Genomic Border Regions

Sequence characterization analysis was performed to determine the DNA sequence of the COR121 insert and flanking genomic regions. It should be noted that while DNA sequencing provides certain molecular information, the exact nucleotide sequence should not be viewed as static. Spontaneous mutations are a very common phenomenon in plants, presenting a biological mechanism of adaptation to constantly changing environment (Weber *et al.*, 2012). Spontaneous mutations can occur in any part of the plant genome and in both non-GM and GM plants (Waigmann *et al.*, 2013). In GM plants, there is no scientific basis to expect that the frequency of spontaneous mutations in transgenic insert or flanking genomic regions would be greater than in the rest of the plant genome, or that they would have a differential impact on safety (La Paz *et al.*, 2010; Waigmann *et al.*, 2013).

Sequence of the Insert and Flanking Genomic Regions of COR121 Maize is provided as a separate study, 2025-1014.

Additional details regarding analytical methods for event sequencing analysis are provided in Appendix C.

Figure 15. Map of the Insert and Flanking Genomic Regions in COR121 Maize

Genomic Border Region Analysis

[redacted]

Reading Frame Analysis of the Insert/Border Junctions and Bioinformatic Assessment for Allergens and Toxins

A bioinformatics assessment of potentially expressed peptides (i.e., translated stop codon-bracketed frames) was conducted following established international criteria (Codex Alimentarius Commission, 2009; EFSA, 2010; EFSA Panel on Genetically Modified Organisms (GMO), 2011; FAO/WHO, 2001). All translated stop codon-bracketed frames of length ≥ eight amino acids (aa) in COR121 maize that are within the insertion or that cross the boundary between the insertion and its flanking genomic regions were identified and evaluated for sequence similarity of known or putative allergens and protein toxins. [redacted]

Allergenicity Analysis

[redacted]

Toxicity Analysis

[redacted]

[redacted]

Event-Specific Detection Methodology

[redacted] The *hmg-A* taxon-specific PCR assay is a validated maize-specific PCR assay for the High Mobility Protein Group A gene (GenBank Accession ID_AJ131373) (QT-TAX-ZM-002) with the replacement of the TAMRA™ (6carboxytetramethylrhodamine, succinimidyl- ester) quencher with a Black Hole Quencher 1 (BHQ1™) quencher (EU-RL-GMFF, 2012). The assay amplifies a 79-bp product.

Conclusion on the Molecular Characterization of COR121 Maize

WGS, multi-generation segregation, sequencing of the insert and its flanking genomic regions, bioinformatics assessments of the flanking genomic sequences for chromosomal location of the insert and for potential endogenous gene disruption, and bioinformatics assessments of translated stop codon-bracketed frames for allergenicity and toxicity, were conducted to characterize the inserted DNA in COR121 maize.

WGS analysis confirmed that COR121 maize contains a single copy of the inserted DNA with the expected organization and that no additional insertions or plasmid backbone sequences are present in the COR121 maize genome. WGS analysis of five generations of COR121 maize confirmed that the inserted DNA in COR121 maize is consistent and stable across multiple generations during the breeding process. Segregation analysis of five generations of COR121 maize confirmed that the inserted DNA segregated as a single locus in accordance with Mendelian rules of inheritance and stably integrated into the maize genome across generations during the breeding process. Sequencing of the insert and its flanking genomic regions confirmed the integrity of the inserted DNA derived from plasmids [redacted] in COR121 maize. Bioinformatic assessments of the genomic sequences flanking the COR121 maize insert confirmed the chromosomal location of the insert and no disruption of endogenous genes. Bioinformatics

assessments of translated stop codon-bracketed frames that are within the insertion or that cross the boundary between the insertion and its flanking genomic regions confirmed that the COR121 maize insert did not generate biologically relevant amino acid sequence similarities to allergens and protein toxins that are harmful to humans.

Together, these analyses confirmed that a single copy of the inserted DNA, with no plasmid backbone sequences, is present in the COR121 maize genome. The introduced genes are stably inherited across multiple generations and segregated according to Mendel's law of inheritance during the breeding process. Sequencing analysis determined the sequences of the inserted DNA and flanking genomic regions in COR121 maize, and bioinformatic assessments of the genomic sequences flanking the COR121 maize insert confirmed the chromosomal location of the insert and no disruption of endogenous genes. Bioinformatic assessments for allergenicity or toxicity support the conclusion that COR121 maize was found unlikely to be toxic or allergenic to humans. Additionally, an event-specific quantitative real-time PCR detection method was developed and validated for COR121 maize.

A.3 (d) Breeding process

Plants that were regenerated from transformation and tissue culture (designated [redacted] plants) were selected for further characterization. A schematic overview of the transformation and event development process for COR121 maize is provided in Figure 16.

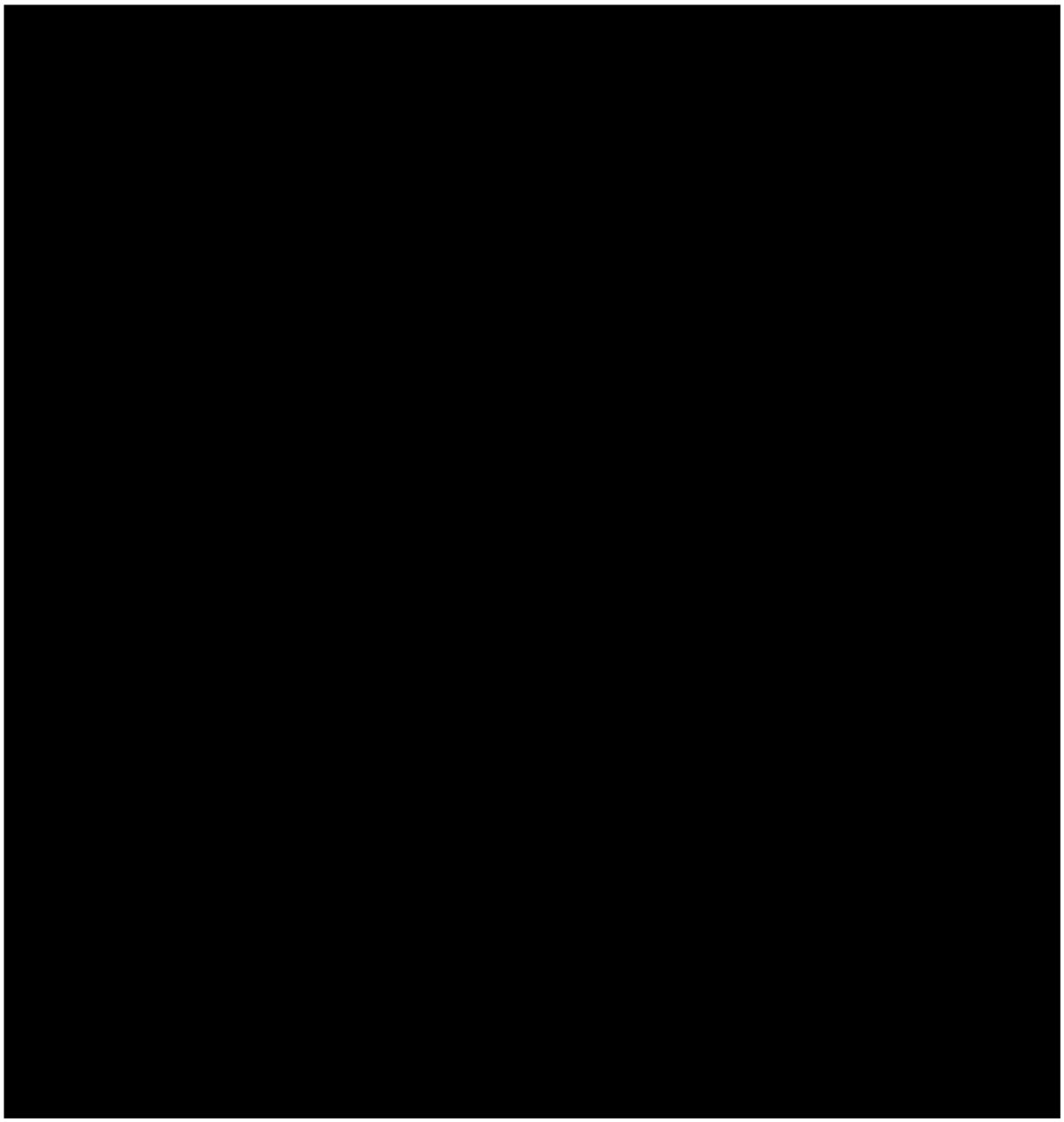


Figure 16. Event Development Process of COR121 Maize

The subsequent breeding of COR121 maize proceeded as indicated in Figure 17 to produce specific generations for the characterization and assessments conducted, as well as for the development of commercial maize lines. Table 9 provides the generations used for each characterization study.

Figure 17. Breeding Diagram for COR121 Maize and Generations Used for Analysis

The breeding steps to produce the generations used for characterization and safety assessments are shown schematically.

Selection of Comparators

For the characterization of COR121 maize, proprietary maize inbred (PH184C) and F1 hybrid (PH47K2xPH184C) were used as experimental controls (Table 9). The control lines selected are non-genetically modified (non-GM) near-isoline maize lines and represent the same genetic background as the maize lines used to produce the COR121 maize generations used in analysis (Figure 17).

In addition, non-GM commercial maize hybrid lines (i.e., reference lines), were used to obtain tolerance intervals for compositional analyses. These maize hybrids were chosen to represent a wide range of conventional varieties. These tolerance intervals represent a portion of the biological variation of the maize crop for compositional analytes and further helped to determine the comparability of COR121 maize to conventional maize.

Table 9. Generations and Comparators Used for Analysis of COR121 Maize

Analysis	Seed Generation(s) Used	Comparators
Insertion copy number, insertion organization, and the absence of plasmid backbone sequences by WGS	[redacted]	PH184C
Insertion organization and stability by WGS		PH184C
Mendelian inheritance by multi-generation segregation analysis		PH184C
Sequence determination of Insert and its flanking genomic regions by Sanger sequencing		PH184C
Nutrient composition and expressed trait protein analysis	F1 (PH47K2xPH184C)	PH47K2xPH184C

A.3 (e) Stability of the genetic changes

Stability of the genetic changes is covered in Section *A.3 (c) Molecular Characterization of the Inserted DNA in COR121 Maize* under subsections *Whole Genome Sequencing Analysis to Determine Insertion Copy Number and Organization, Confirm Absence of Plasmid Backbone, and Demonstrate Genetic Stability Across Generations* and *Multi-Generation Segregation Analysis*.

B. CHARACTERISATION AND SAFETY ASSESSMENT OF NEW SUBSTANCES

B.1 Characterisation and safety assessment of new substances

There are no new substances associated with COR121 maize other than the proteins encoded by the introduced genes (see Section B.2 *New proteins* below).

B.2 New proteins

IPD083Cb Protein

Function and Activity of the IPD083Cb Protein

The IPD083Cb protein expressed in COR121 maize is encoded by the insecticidal protein gene, *ipd083Cb.1*, from giant maidenhair fern (*Adiantum trapeziforme* var. *braziliense*). The expressed IPD083Cb protein confers control of certain susceptible lepidopteran pests (Liu *et al.*, 2019), by causing disruption of the midgut epithelium. The site of action of the IPD083Cb protein appears to be similar to that of Cry proteins derived from *Bacillus thuringiensis* (*Bt*). The IPD083Cb protein binds to target sites located on the midgut of susceptible insects, leading to insect death. The competitive binding assays using brush border membrane vesicles from insect midguts demonstrated that IPD083Cb does not bind to the same target sites as *Bt*-derived insecticidal proteins including Cry2A.127 (a variant of Cry2Ab), Vip3Aa, or Cry1A.88 (a variant of Cry1Ab), indicating that IPD083Cb is unlikely to exhibit cross-resistance with insects that are resistant to these proteins.

The IPD083Cb protein has recently been evaluated by FSANZ in the insect-protected soybean line COR23134, which was added to Schedule 26 in August 2025 (A1323 application).

Amino Acid Sequence of the IPD083Cb Protein

The deduced amino acid sequence from the translation of the *ipd083Cb.1* gene encodes the IPD083Cb protein that is 853 amino acids in length and has a molecular weight of approximately 95 kDa (Figure 18). The deduced amino acid sequence from the translation of the *ipd083Cb.1* gene expressed in COR121 maize is identical to that found in the recent application (A1323) for soybean event COR23134.

```
  1 MADYSTLYRD LNQISMPLDR VEFSEVMVIH RMYLRLSDLN VGELPGAERV
 51 KRLYVLADVV ELATFAHPQL LNTRMPGSVT VIILCRLLQF PTDGSFAAWL
101 ELPFMELHTL IEQYRSEIKA ADDAKWGTYV HAEEVQLSPL FNGWPYLVVE
151 AQRCIITAAM HNTFNRPGWV RSITQFTTDQ SGRVDTTLLA RTEFGHIDLP
201 LETDSPTAFS VSHRQSTNLP VEYTGIPVEV VTDPNILMGM QTSVHIAELV
251 KACYPSPELV SAVGVHVNWL NEVLLRVVQK ESQLQGTEAY NECLALLGRI
301 QCVMKMGPFV SVVPQLQYRM YGSLIRQMAQ VAQNYDQDFR QLKLFIAQNQ
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351 ILGSYLLQQN KAFADREVQM ESFHSAVISQ RRQELDDAIA KMDRLSLQME
401 EEDRAMEQAR KEMEEGLKQF QNEQVARAVF AVLKSVAMIA LAFVTAGATA
451 PGAAASAAQA VNIAGQAAQA LRRVVEILEG LEAVMEVVAA IKHLVDALDQ
501 VSQIVDAPPM PDMPSEADWS IFVNEIEAVA EGMPTEVSEV PAWKAKCKNV
551 AALGREMCIT AEQISQLQYD IWVQGLLRDI AQSHADRLAA IQPANLTNYL
601 EMAIQMDMRT TRILIGLLNI MRIQNAALMY EYLLTPTQLT AWPLRMDTVA
651 NLLITHESAA LSGLAQLGPP SDFTSRHVVK GIPVSLLLDG GDWEFEIPVQ
701 GGMSSFPSSW TRVRIRHLEM HFVQEASGGG EIIHQPATQT GTIYILLQGS
751 TVFHDRRREE VMTFQAAVPL NYHYAYRLDT GEATLTNEPS EQFANTFMQM
801 TPFTHWRLRL SASAAENKGL AFPTATAPDS TTEIAITFHV TAIRQIDWRQ
851 EEE*
```

Figure 18. Deduced Amino Acid Sequence of the IPD083Cb Protein

The deduced amino acid sequence from the translation of the *ipd083Cb.1* gene from plasmid [redacted]. The asterisk (*) indicates the translational stop codon. The IPD083Cb protein is 853 amino acids in length and has an approximate molecular weight of 95 kDa.

Characterization of the IPD083Cb Protein Derived from COR121 Maize

The IPD083Cb protein expressed in COR121 maize was purified from the leaf tissue using immunoaffinity chromatography.

In order to have sufficient amounts of the purified IPD083Cb protein for the multiple studies required to assess its safety, the IPD083Cb protein was expressed in a *Nicotiana benthamiana* (tobacco)-based protein expression system. The tobacco-derived protein was purified using immobilized metal affinity chromatography. Please note, the tobacco-derived data is the same as the data presented for COR23134 soybean within the A1323 application.

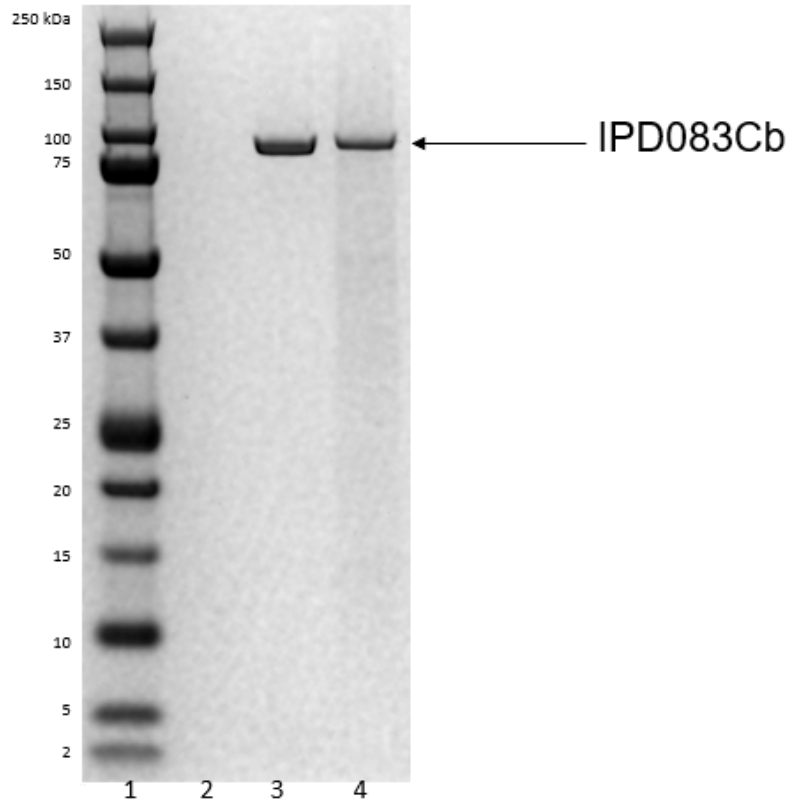
The biochemical characteristics of the COR121 maize-derived and tobacco-derived IPD083Cb proteins were characterized using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot, glycosylation analysis, peptide mapping by liquid chromatography mass spectrometry (LC-MS), and N-terminal amino acid sequencing. For the tobacco-derived IPD083Cb protein, the bioactivity was verified by a sensitive insect bioassay. The results demonstrated that the COR121 maize-derived and tobacco-derived IPD083Cb proteins have the expected molecular weight, immunoreactivity, amino acid sequence, and lack of glycosylation. The tobacco-derived IPD083Cb protein was demonstrated to be an appropriate test substance for use in safety studies.

SDS-PAGE Analysis

Samples of the COR121 maize-derived IPD083Cb protein and the tobacco-derived IPD083Cb protein were analyzed by SDS-PAGE. As expected, the IPD083Cb proteins, derived from both

COR121 maize and the tobacco system, migrated as a predominant band consistent with the expected molecular weight (Figure 19).

Additional details regarding SDS-PAGE analytical methods are provided in Appendix D.



Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	COR121 Maize-Derived IPD083Cb Protein (diluted 1:50)
4	Tobacco-Derived IPD083Cb Protein (1.0 µg)

Note: kilodalton (kDa), lithium dodecyl sulfate and dithiothreitol (LDS/DTT), and microgram (µg).
[a] Molecular weight markers were included to provide a visual estimate of protein migration.

Figure 19. SDS-PAGE Analysis of the IPD083Cb Protein

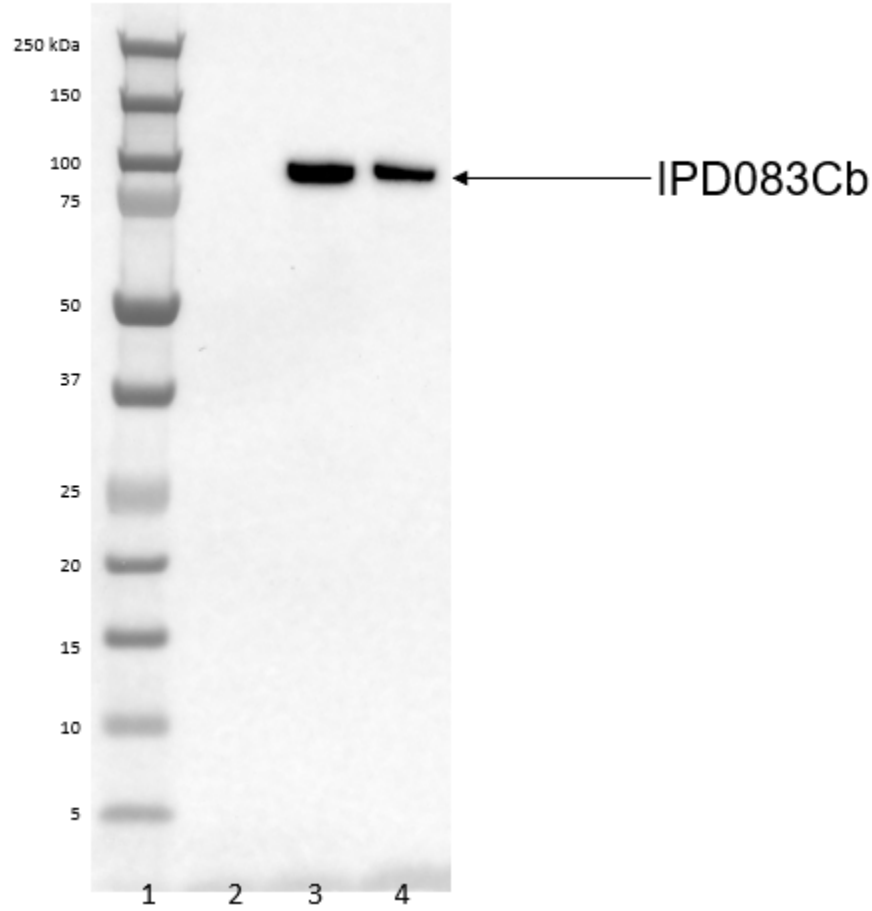
Coomassie blue staining of the SDS-PAGE gel demonstrated the protein migrated as a predominant band consistent with the expected molecular weight for the COR121 maize-derived IPD083Cb protein (Lane 3) and the tobacco-derived IPD083Cb protein (Lane 4).

Western Blot Analysis

Samples of the COR121 maize-derived IPD083Cb protein and the tobacco-derived IPD083Cb protein were analyzed by western blot. As expected, the IPD083Cb proteins derived from both

COR121 maize and the tobacco system are immunoreactive and have the expected molecular weight (Figure 20).

Additional details regarding western blot analytical methods are provided in Appendix D.



Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	COR121 Maize-Derived IPD083Cb Protein (diluted 1:5000)
4	Tobacco-Derived IPD083Cb Protein (10 ng)

Note: kilodalton (kDa), lithium dodecyl sulfate and dithiothreitol (LDS/DTT), and nanogram (ng).
[a] Molecular weight markers were included to provide a visual estimate of protein migration.

Figure 20. Western Blot Analysis of the IPD083Cb Protein

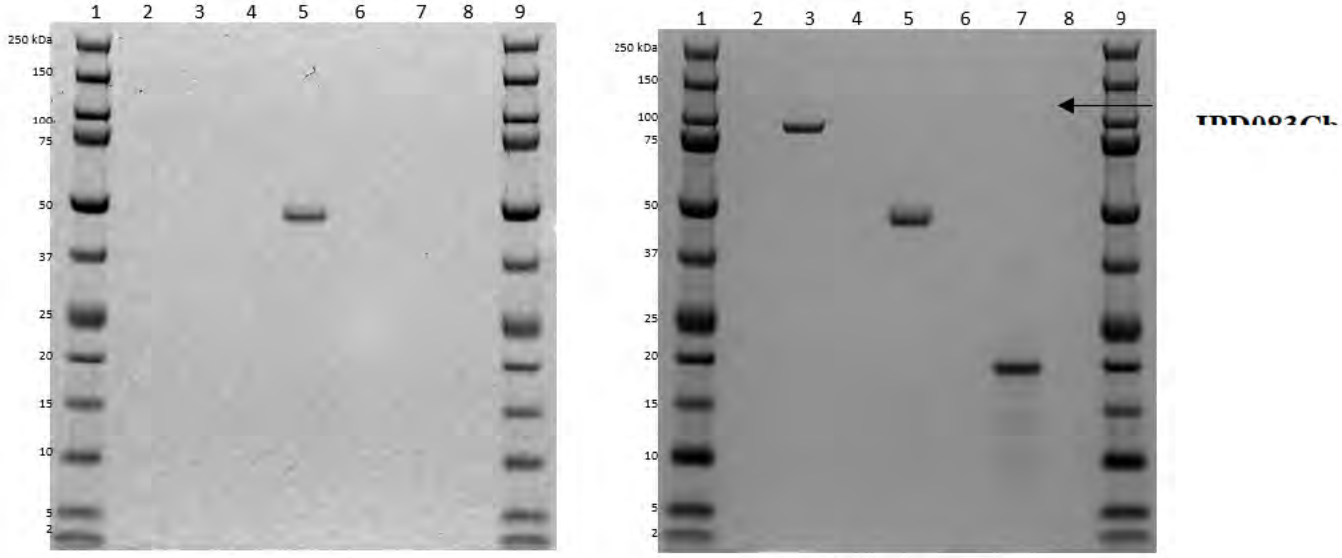
Western blot analysis demonstrated that the IPD083Cb protein was immunoreactive to polyclonal antibody and visible as a predominant band consistent with the expected molecular weight for the COR121 maize-derived IPD083Cb protein (Lane 3) and the tobacco-derived IPD083Cb protein (Lane 4).

Protein Glycosylation Analysis

Samples of the COR121 maize-derived IPD083Cb protein and the tobacco-derived IPD083Cb protein were analyzed by SDS-PAGE followed by the glycoprotein staining for glycosylation analysis. Each gel also included a positive control (horseradish peroxidase) and a negative control (soybean trypsin inhibitor). The gel was first stained using a Pierce Glycoprotein Staining Kit to visualize any glycoproteins, imaged, and then stained with the Coomassie blue reagent to visualize all protein bands.

Glycosylation was determined to be negative for both the COR121 maize-derived and tobacco-derived IPD083Cb proteins (Figures 21 and 22, respectively). The horseradish peroxidase positive control was clearly visible as a stained band. The soybean trypsin inhibitor negative control was not stained by the glycoprotein stain.

Additional details regarding glycosylation analytical methods are provided in Appendix D.



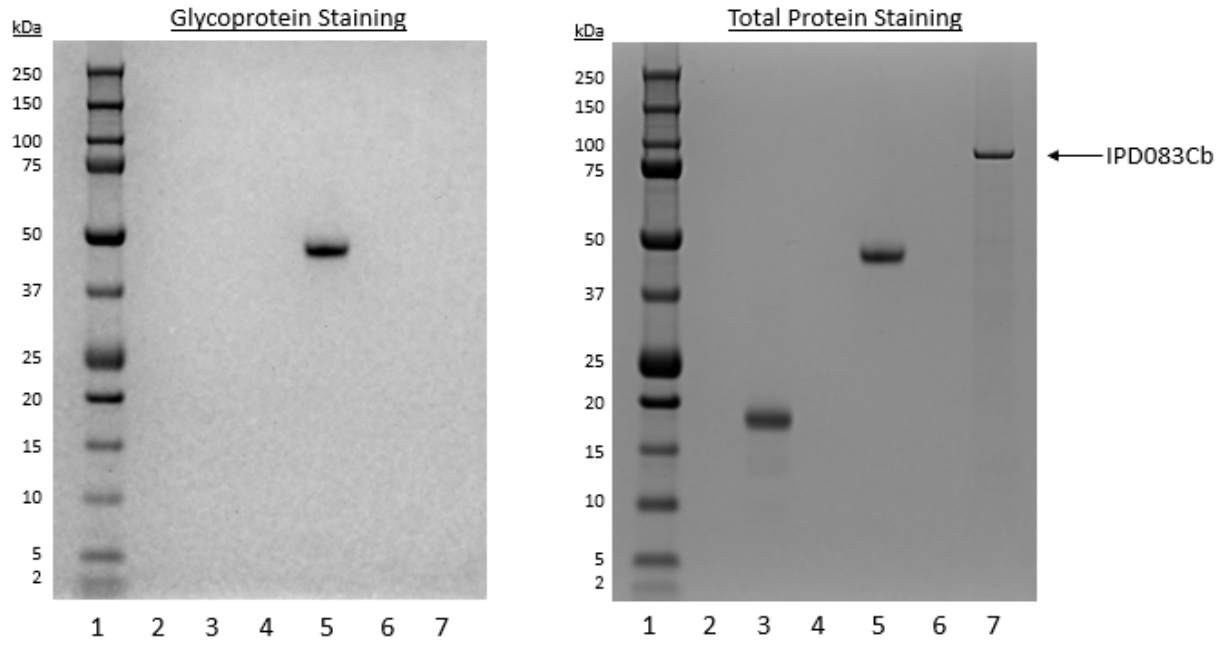
Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	COR121 Maize-Derived IPD083Cb Protein
4	1X LDS/DTT Sample Buffer Blank
5	Positive Control Horseradish Peroxidase (1.0 µg)
6	1X LDS/DTT Sample Buffer Blank
7	Negative Control Soybean Trypsin Inhibitor (1.0 µg)
8	1X LDS/DTT Sample Buffer Blank
9	Pre-stained Protein Molecular Weight Marker[a]

Note: kilodalton (kDa), lithium dodecyl sulfate and dithiothreitol (LDS/DTT), and microgram (µg). The gel was stained with glycoprotein staining reagent; following glycoprotein detection, the same gel was then stained with Coomassie for total proteins.

[a] Molecular weight markers were included to provide a visual estimate of protein migration.

Figure 21. Glycosylation Analysis of the COR121 Maize-Derived IPD083Cb Protein

A) Glycoprotein staining: Glycosylation was not detected for the COR121 maize-derived IPD083Cb protein (Lane 3). The horseradish peroxidase positive control was stained (Lane 5), and the soybean trypsin inhibitor negative control was not stained (Lane 7). **B)** Total protein staining: Subsequent Coomassie blue staining of the same gel for total proteins detected the COR121 maize-derived IPD083Cb protein (Lane 3) and both the positive (Lane 5) and negative (Lane 7) control proteins.



Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	Soybean Trypsin Inhibitor Negative Control (1 µg)
4	1X LDS/DTT Sample Buffer Blank
5	Horseradish Peroxidase Positive Control (1 µg)
6	1X LDS/DTT Sample Buffer Blank
7	Tobacco-Derived IPD083Cb Protein (1 µg)

Note: kilodalton (kDa) and microgram (µg). The glycoprotein staining gel was stained with glycoprotein staining reagent. The total protein staining gel was stained with glycoprotein staining reagent followed by staining with Coomassie for total proteins.

[a] Molecular weight markers were included to provide a visual estimate that migration was within the expected range of the predicted molecular weight.

Figure 22. Glycosylation Analysis of the Tobacco-Derived IPD083Cb Protein

A) Glycoprotein staining: Glycosylation was not detected for the tobacco-derived IPD083Cb protein (Lane 7). The horseradish peroxidase positive control was stained (Lane 5), and the soybean trypsin inhibitor negative control was not stained (Lane 3). **B)** Total protein staining: Subsequent Coomassie blue staining of the same gel for total proteins detected the tobacco-derived IPD083Cb protein (Lane 7) and both the positive (Lane 5) and negative (Lane 3) control proteins.

Mass Spectrometry Peptide Mapping Analysis

Samples of the COR121 maize-derived IPD083Cb protein and the tobacco-derived IPD083Cb protein were analyzed by SDS-PAGE. The gel was stained with Coomassie blue reagent, and the bands containing the IPD083Cb protein were excised for each sample. The excised IPD083Cb protein bands were digested with trypsin or chymotrypsin. Digested samples were analyzed using liquid chromatography-mass spectrometry (LC-MS). The resulting MS data were used to search and match the peptides from the IPD083Cb protein sequence, and the combined sequence coverage was calculated.

The combined sequence coverage of the identified tryptic and chymotryptic peptides for the COR121 maize-derived IPD083Cb protein accounts for 98% (835/852) of the amino acid sequence (Figure 23). The combined sequence coverage of the identified tryptic and chymotryptic peptides for the tobacco-derived IPD083Cb protein accounts for 87.5% (751/858) of the amino acid sequence (Figure 24).

Additional details regarding peptide mapping analytical methods are provided in Appendix D.

```
  1 ADYSTLYRDL NQISMPLDRV EFSEVMVIHR MYLRLSDLNV GELPGAERVK
 51 RLYVLADVVE LATFAHPQLL NTRMPGSVTV IILCRLLQFP TDGSFAAWLE
101 LPFMELHTLI EQYRSEIKAA DDAKWGTYVH AEEVQLSPLF NGWPYLVVEA
151 QRCIITAAMH NTFNRPGWVR SITQFTTDQS GRVDTTLLAR TEFGHIDLPL
201 ETDSPTAFSV SHRQSTNLPV EYTGIPVEVV TDPNILMGMQ TSVHIAELVK
251 ACYPSPELVS AVGVHVNWLN EVLLRVVQKE SQLQGTEAYN ECLALLGRIQ
301 CVMKMGPFVS VVPQLQYRMY GSLIRQMAQV AQNYDQDFRQ LKLFIAQNQI
351 LGSYLLQQNK AFADREVQME SFHSAVISQR RQELDDAIAK MDRLSLQMEE
401 EDRAMEQARK EMEEGLKQFQ NEQVARAVFA VLKSVAMIAL AFVTAGATAP
451 GAAASAAQAV NIAGQAAQAL RRVVEILEGL EAVMEVVAAI KHLVDALDQV
501 SQIVDAPPMP DMPSEADWSI FVNEIEAVAE GMPTEVSEVP AWKAKCKNVA
551 ALGREMCITA EQISQLQYDI WVQGLLRDIA QSHADRLAAI QPANLTNYLE
601 MAIQMDMRTT RILIGLLNIM RIQNAALMYE YLLTPTQLTA WPLRMDTVAN
651 LLITHESAAL SGLAQLGPPS DFTSRHVVKG IPVSLLLDGG DWEFEIPVQG
701 GMSSFPSSWT RVRIRHLEMH FVQEASGGGE IIHQPATQTG TIYILLQGST
751 VFHDRREEEV MTFQAAVPLN YHYAYRLDTG EATLTNEPSE QFANTFMQMT
801 PFTHWRLRLS ASAAENKGLA FPTATAPDST TEIAITFHVT AIRQIDWRQE
851 EE
```

Red bold type	Red bold type indicates the sequence of the COR121 maize-derived IPD083Cb peptides identified using LC-MS analysis against the expected IPD083Cb protein sequence.
Amino acid residue abbreviations	Alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), tryptophan (W), tyrosine (Y), and valine (V).

Note: The expected IPD083Cb sequence does not include the N-terminal methionine as it is anticipated to be absent. The N-terminal peptide was acetylated.

Figure 23. Identified Tryptic and Chymotryptic Peptide Amino Acid Sequence of the COR121 Maize-Derived IPD083Cb Protein Using LC-MS Analysis

```
  1 ADYSTLYRDL NQISMPLDRV EFSEVMVIHR MYLRLSDLNV GELPGAERVK
 51 RLYVLADVVE LATFAHPQLL NTRMPGSVTV IILCRLLQFP TDGSFAAWLE
101 LPFMELHTLI EQYRSEIKAA DDAKWGTYVH AEEVQLSPLF NGWPYLVVEA
151 QRCIITAAMH NTFNRPGWVR SITQFTTDQS GRVDTTLLAR TEFGHIDLPL
201 ETDSPTAFSV SHRQSTNLPV EYTGIPVEVV TDPNILMGMQ TSVHIAELVK
251 ACYPSPELVS AVGVHVNWLN EVLLRVVQKE SQLQGTEAYN ECLALLGRIQ
301 CVMKMGPFVS VVPQLQYRMY GSLIRQMAQV AQNYDQDFRQ LKLFIAQNQI
351 LGSYLLQQNK AFADREVQME SFHSAVISQR RQELDDAIAK MDRLSLQMEE
401 EDRAMEQARK EMEEGLKQFQ NEQVARAVFA VLKSVAMIAL AFVTAGATAP
451 GAAASAAQAV NIAGQAAQAL RRVVEILEGL EAVMEVVAAI KHLVDALDQV
501 SQIVDAPPMP DMPSEADWSI FVNEIEAVAE GMPTEVSEVP AWKAKCKNVA
551 ALGREMCITA EQISQLQYDI WVQGLLRDIA QSHADRLAAI QPANLTNYLE
601 MAIQMDMRTT RILIGLLNIM RIQNAALMYE YLLTPTQLTA WPLRMDTVAN
651 LLITHESAAL SGLAQLGPPS DFTSRHVVKG IPVSLLLDGG DWEFEIPVQG
701 GMSSFPSSWT RVRIRHLEMH FVQEASGGGE IIHQPATQTG TIYILLQGST
751 VFHDRREEV MTFQAAVPLN YHYAYRLDTG EATLTNEPSE QFANTFMQMT
801 PFTHWRLRLS ASAAENKGLA FPTATAPDST TEIAITFHVT AIRQIDWRQE
851 EEHHHHHH
```

Red bold type	Red bold type indicates IPD083Cb peptides identified using LC-MS analysis against the expected IPD083Cb protein sequence.
Amino acid residue abbreviations	Alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), tryptophan (W), tyrosine (Y), and valine (V).

Note: The expected IPD083Cb protein sequence does not include the N-terminal methionine as it is anticipated to be absent and the N-terminal alanine residue of the protein was acetylated.

Figure 24. Identified Tryptic and Chymotryptic Peptide Amino Acid Sequence of Tobacco-Derived IPD083Cb Protein Using LC-MS Analysis

N-Terminal Amino Acid Sequence Analysis

The Edman N-terminal amino acid sequence analysis of the COR121 maize-derived IPD083Cb protein and the tobacco-derived protein did not obtain sequence data, suggesting the N-terminus of the protein was blocked. The N-terminal peptide was identified as ADYSTLYR from the LC-MS analysis of the trypsin-digested protein. The results confirmed the N-terminal methionine was absent as expected (Dummitt *et al.*, 2003; Sherman *et al.*, 1985) and the N-terminal alanine residue of the protein was acetylated.

Additional details regarding N-terminal amino acid sequencing analytical methods are provided in Appendix D.

Bioactivity Assay

The biological activity of the tobacco-derived IPD083Cb protein was evaluated by conducting a 7-day bioassay using *Chrysodeixis includens* (soybean looper; Lepidoptera: Noctuidae), a species sensitive to the IPD083Cb protein.

Bioactivity analysis demonstrated that the tobacco-expressed IPD083Cb protein had insecticidal activity toward a target insect, *C. includens* (Table 10). The biological activity of the test diet containing 50 µg of the IPD083Cb protein per cm^2 agar-based diet was demonstrated increased mortality and decreased weight in *C. includens* compared to *C. includens* fed the buffer control diet.

Additional details regarding bioactivity assay methods are provided in Appendix D.

Table 10. Summary of the Tobacco-Derived IPD083Cb Protein Bioactivity Assay Using *Chrysodeixis includens*

Treatment	Treatment Description	Concentration (µg IPD083Cb Protein/cm^2)	Total Number of Observations	Mortality (%)	Number of Surviving Organisms	Weight of Surviving Organisms (mg)	
						Mean ± Standard Deviation	Range
1	Buffer Control Diet	0	24	16.7	20	15.2 ± 4.6	0.9-22.6
2	Test Diet	50.0	24	45.8	13	0.7 ± 0.5	0.1-1.6

Note: The summary of *Chrysodeixis includens* mortality data consisted of the calculation of dead larvae divided by the total number of observed larvae at the end of the study and multiplied by 100.

Allergenicity and Toxicity of the IPD083Cb Protein

The IPD083Cb protein has recently been evaluated by FSANZ in the insect-protected soybean line COR23134, which was added to Schedule 26 in August 2025 (A1323 application). In accordance with the FSANZ Application Handbook, only the updated bioinformatics analysis is required for safety assessment of proteins evaluated previously.

Bioinformatic Analysis of IPD083Cb Sequence Similarity to Known and Putative Allergens

Assessing newly expressed proteins for potential cross-reactivity with known and putative allergens is an important part of the weight-of-evidence approach used to evaluate the safety of these proteins in genetically modified plant products (Codex Alimentarius Commission, 2009). A bioinformatic assessment of the IPD083Cb protein sequence (853 amino acids [aa]) for its sequence similarity with known or putative allergens was conducted by following established international criteria (Codex Alimentarius Commission, 2009; FAO/WHO, 2001).

Two separate searches for the IPD083Cb protein sequence were performed using the Comprehensive Protein Allergen Resource (COMPARE) 2025 database (January 27, 2025 (van Ree *et al.*, 2021)). This peer-reviewed database is compiled by the Health and Environmental Sciences Institute (HESI) and contains 2,836 sequences.

The first search was the sliding 80-mer window search, accomplished with an internally developed Perl script running FASTA v36.3.8 (Pearson and Lipman, 1988) with an *E*-score cutoff set to 100. In a sliding window search, each sequentially overlapping 80 aa sub-sequence of the overall IPD083Cb protein sequence is used as a query against the COMPARE allergen database sequences. The script examined all alignments generated from the query and reported any possessing > 35% identity over an alignment length of ≥ 80 aa. Additionally, the script rescaled the percent identity to an 80-mer window for any alignments possessing an alignment length shorter than 80 aa; the number of identities in these alignments would be divided by 80, then multiplied by 100, and would report any alignment possessing an adjusted percent identity > 35%.

The second search used EMBOSS fuzzpro v6.6.0 (Rice *et al.*, 2000) to identify any eight or greater contiguous identical amino acid matches between the IPD083Cb protein sequence and the COMPARE allergen sequences.

Results of the search of the IPD083Cb protein sequence against the COMPARE allergen database sequences found no alignments that were a length of 80 aa or greater with a sequence identity of > 35% and no alignments shorter than 80 aa with a sequence identity > 35% when normalized to an 80-mer window. No contiguous 8-residue exact matches between the IPD083Cb protein sequence and the known or putative allergen sequences were identified in the second search. Collectively, these data indicate that no allergenicity concern arose from the bioinformatics assessment of the IPD083Cb protein.

Bioinformatics evaluation of the IPD083Cb protein sequence did not generate biologically relevant amino acid sequence similarities to known or putative allergens that are harmful to humans.

Bioinformatic Analysis of IPD083Cb Sequence Similarity to Known and Putative Protein Toxins

Assessing newly expressed proteins for potential sequence similarity with known or putative protein toxins is an important part of the weight-of-evidence approach used to evaluate the safety of these proteins in genetically modified plant products (Codex Alimentarius Commission, 2009). The potential toxicity of the IPD083Cb protein was assessed by comparison of its sequence to the National Center for Biotechnology Information (NCBI) non-redundant (nr) protein database (January 2025) with an *E*-value set to 10^{-4} for searches.

The BLASTP search of the IPD083Cb protein against the NCBI nr protein database returned the maximum number of alignments (96) with an *E*-value range from 0 to 1.07×10^{-5} to various closely related IPD083 proteins or hypothetical proteins. None of the accessions returned by the BLASTP search are proteins known to be toxic to humans.

Bioinformatics evaluation of the IPD083Cb protein sequence did not generate biologically relevant amino acid sequence similarities to known or putative protein toxins that are harmful to humans.

Conclusion on the Safety of the IPD083Cb protein in COR121 Maize

In conclusion, protein characterization results via SDS-PAGE, western blot, glycosylation analysis, mass spectrometry peptide mapping analysis, and N-terminal amino acid sequence analysis have demonstrated that the IPD083Cb protein derived from COR121 maize and the tobacco system has the expected molecular weight, immunoreactivity, and amino acid sequence, and is not glycosylated as well as that it is identical to the IPD083Cb protein, which has recently been evaluated by FSANZ in the insect-protected soybean line COR23134. Characterization of the tobacco derived IPD083Cb protein demonstrated that it is an appropriate test substance for use in safety studies.

In accordance with the FSANZ Application Handbook, the allergenic potential of the IPD083Cb protein was evaluated by assessing a bioinformatic comparison of the amino acid sequence of the IPD083Cb protein with known and putative allergen sequences.

The bioinformatic comparison of the IPD083Cb protein sequence to known and putative allergen and protein toxin sequences showed that the IPD083Cb protein is unlikely to be allergenic or toxic for humans. This data support the conclusion that COR121 maize expressing the IPD083Cb protein, is as safe as conventional maize for the food and feed supply.

Based on this weight of evidence, consumption of the IPD083Cb protein from COR121 maize is unlikely to cause an adverse effect on humans.

PMI Protein

Function and Activity of the PMI Protein

The PMI protein expressed in COR121 maize is encoded by the *pmi* gene from *Escherichia coli*. The mode of action of PMI has been previously characterized and described (Negrotto et al., 2000; Privalle, 2002; Reed et al., 2001; Weisser et al., 1996). PMI is widely present in nature and is expressed in fungi, insects, plants, and mammals (Slein, 1950; US-EPA, 2004). The United States EPA has granted an exemption from the requirement of a tolerance for the PMI protein as an inert ingredient in plants (US-EPA, 2004). The PMI protein catalyzes the reversible interconversion between mannose-6-phosphate and fructose-6-phosphate. Mannose is phosphorylated by hexokinase to mannose-6-phosphate and in the presence of PMI enters the glycolytic pathway after isomerization to fructose 6-phosphate. In the absence of PMI, mannose-6-phosphate accumulates in the plant cells and inhibits glycolysis; additionally, high levels of mannose can lead to other impacts on photosynthesis and ATP production (Negrotto et al., 2000; Privalle, 2002). However, in the presence of PMI, plant cells may survive on media containing mannose as a carbon source, thus allowing PMI to be utilized as a selectable marker (Negrotto et al., 2000; Reed et al., 2001).

Amino Acid Sequence of the PMI Protein

The deduced amino acid sequence from the translation of the *pmi* gene encodes the PMI protein that is 391 amino acids in length and has a molecular weight of approximately 43 kDa (Figure 25).

```
  1 MQKLINSVQN YAWGSKTALT ELYGMENPSS QPMAELWMGA HPKSSSRVQN
 51 AAGDIVSLRD VIESDKSTLL GEAVAKRFGE LPFLFKVLCA AQPLSIQVHP
101 NKHNSEIGFA KENAAGIPMD AAERNYKDPN HKPELVFALT PFLAMNAFRE
151 FSEIVSLLQP VAGAHPAIAH FLQQPDAERL SELFASLLNM QGEEKSRALA
201 ILKSALDSQQ GEPWQTIRLI SEFYPEDSGL FSPLLLNVVK LNPGEAMFLF
251 AETPHAYLQG VALEVMANSD NVLRAGLTPK YIDIPELVAN VKFEAKPANQ
301 LLTQPVKQGA ELDFPIPVDD FAFSLHDLSD KETTISQQSA AILFCVEGDA
351 TLWKGSQQLQ LKPGESAFIA ANESPVTVKG HGRLARVYNK L*
```

Figure 25. Deduced Amino Acid Sequence of the PMI Protein

The deduced amino acid sequence from the translation of the *pmi* gene from plasmid [redacted] The asterisk (*) indicates the translational stop codon.

Characterization of the PMI Protein Derived from COR121 Maize

The PMI protein expressed in COR121 maize was purified from maize stalk tissue using ammonium sulfate precipitation and immunoaffinity chromatography.

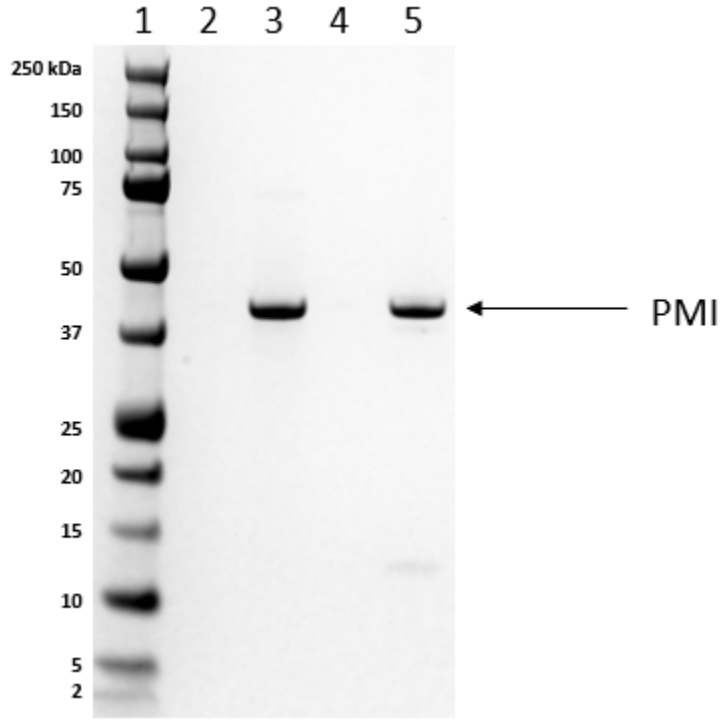
The biochemical characteristics of the COR121 maize-derived PMI protein was characterized using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot,

glycosylation analysis, peptide mapping by liquid chromatography mass spectrometry (LC-MS), and N-terminal amino acid sequencing. The results demonstrated that the COR121 maize-derived PMI protein has the expected molecular weight, immunoreactivity, amino acid sequence, and lack of glycosylation.

SDS-PAGE Analysis

Samples of the COR121 maize-derived PMI protein and the microbially derived PMI protein were analyzed by SDS-PAGE. As expected, the PMI proteins, derived from both COR121 maize and the microbial system, migrated as a predominant band consistent with the expected molecular weight (Figure 26).

Additional details regarding SDS-PAGE analytical methods are provided in Appendix E.



Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	COR121 Maize-Derived PMI Protein (diluted 1:1.75)
4	1X LDS/DTT Sample Buffer Blank
5	PMI Protein Reference Substance (1.0 µg)

Note: kilodalton (kDa), lithium dodecyl sulfate and dithiothreitol (LDS/DTT), and microgram (µg).
[a] Molecular weight markers were included to provide a visual estimate of protein migration.

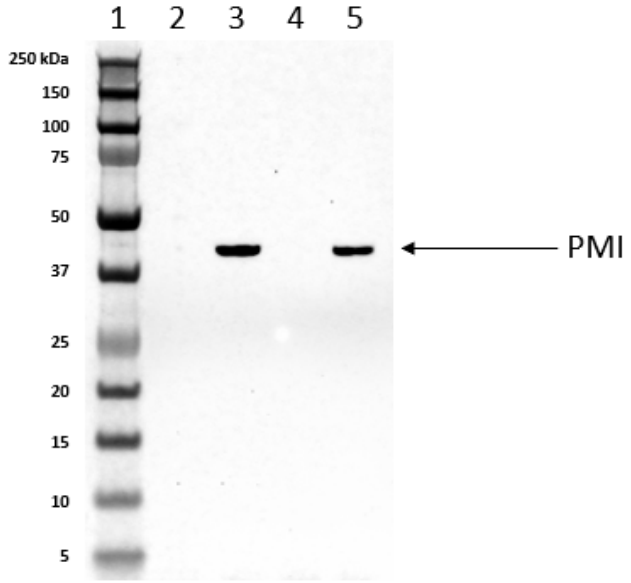
Figure 26. SDS-PAGE Analysis of the PMI Protein

Coomassie blue staining of the SDS-PAGE gel demonstrated the protein migrated as a predominant band consistent with the expected molecular weight for the COR121 maize-derived PMI protein (Lane 3) and the microbially-derived PMI protein (Lane 5).

Western Blot Analysis

Samples of the COR121 maize-derived PMI protein and the microbially derived PMI protein were analyzed by western blot. As expected, the PMI proteins derived from both COR121 maize and the microbial system are immunoreactive and have the expected molecular weight (Figure 27).

Additional details regarding Western blot analytical methods are provided in Appendix E.



Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	COR121 Maize-Derived PMI Protein (diluted 1:175)
4	1X LDS/DTT Sample Buffer Blank
5	PMI Protein Reference Substance (10 ng)

Note: kilodalton (kDa), lithium dodecyl sulfate and dithiothreitol (LDS/DTT), and nanogram (ng).
[a] Molecular weight markers were included to provide a visual estimate of protein migration.

Figure 27. Western Blot Analysis of the PMI Protein

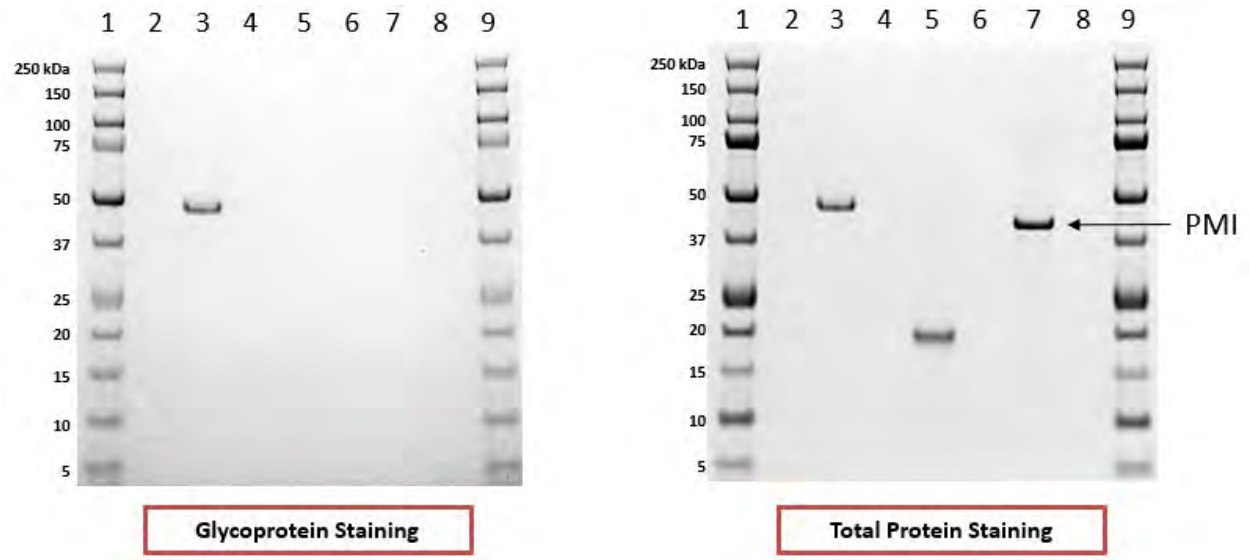
Western blot analysis demonstrated that the PMI protein was immunoreactive to a monoclonal antibody and visible as a predominant band consistent with the expected molecular weight for the COR121 maize-derived PMI protein (Lane 3) and the microbially-derived PMI protein (Lane 5).

Protein Glycosylation Analysis

Samples of the COR121 maize-derived PMI protein were analyzed by SDS-PAGE followed by the glycoprotein staining for glycosylation analysis. Each gel also included a positive control (horseradish peroxidase) and a negative control (soybean trypsin inhibitor). The gel was first stained using a Pierce Glycoprotein Staining Kit to visualize any glycoproteins, imaged, and then stained with the Coomassie blue reagent to visualize all protein bands.

Glycosylation was determined to be negative for the COR121 maize-derived derived PMI proteins (Figure 28). The horseradish peroxidase positive control was clearly visible as a stained band. The soybean trypsin inhibitor negative control was not stained by the glycoprotein stain.

Additional details regarding glycosylation analytical methods are provided in Appendix E.



Lane	Sample Identification
1	Pre-stained Protein Molecular Weight Marker[a]
2	1X LDS/DTT Sample Buffer Blank
3	Horseradish Peroxidase Positive Control (1.0 µg)
4	1X LDS/DTT Sample Buffer Blank
5	Soybean Trypsin Inhibitor Negative Control (1.0 µg)
6	1X LDS/DTT Sample Buffer Blank
7	COR121 Maize-Derived PMI Protein (diluted 1:1.75)
8	1X LDS/DTT Sample Buffer Blank
9	Pre-stained Protein Molecular Weight Marker[a]

Note: kilodalton (kDa), lithium dodecyl sulfate and dithiothreitol (LDS/DTT), and microgram (µg). The gel was stained with glycoprotein staining reagent; following glycoprotein detection, the same gel was then stained with Coomassie for total proteins.

[a] Molecular weight markers were included to provide a visual estimate of protein migration.

Figure 28. Glycosylation Analysis of the COR121 Maize-Derived PMI Protein

A) Glycoprotein staining: Glycosylation was not detected for the COR121 maize-derived PMI protein (Lane 7). The horseradish peroxidase positive control was stained (Lane 3), and the soybean trypsin inhibitor negative control was not stained (Lane 5). **B)** Total protein staining: Subsequent Coomassie blue staining of the same gel for total proteins detected the COR121 maize-derived PMI protein (Lane 7) and both the positive (Lane 3) and negative (Lane 5) control proteins.

Mass Spectrometry Peptide Mapping Analysis

Samples of the COR121 maize-derived PMI protein were analyzed by SDS-PAGE. The gel was stained with Coomassie blue reagent, and the bands containing the PMI protein were excised for each sample. The excised PMI protein bands were digested with trypsin or chymotrypsin. Digested samples were analyzed using liquid chromatography-mass spectrometry (LC-MS). The resulting MS data were used to search and match the peptides from the PMI protein sequence, and the combined sequence coverage was calculated.

The combined sequence coverage of the identified tryptic and chymotryptic peptides for the COR121 maize-derived PMI protein accounts for 96.7% (378/391) of the amino acid sequence (Figure 29).

Additional details regarding peptide mapping analytical methods are provided in Appendix E.

```
  1 MQKLINSVQN YAWGSKTALT ELYGMENPSS QPMAELWMGA HPKSSSRVQN
 51 AAGDIVSLRD VIESDKSTLL GEAVAKRFGE LPFLFKVLCA AQPLSIQVHP
101 NKHNSEIGFA KENAAGIPMD AAERNYKDPN HKPELVFALT PFLAMNAFRE
151 FSEIVSLLQP VAGAHPAIAH FLQQPDAERL SELFASLLNM QGEEKSRALA
201 ILKSALDSQQ GEPWQTIRLI SEFYPEDSGL FSPLLLNVVK LNPGEAMFLF
251 AETPHAYLQG VALEVMANSD NVLRAGLTPK YIDIPELVAN VKFEAKPANQ
301 LLTQPVKQGA ELDFPIPVDD FAFSLHDLSD KETTISQQSA AILFCVEGDA
351 TLWKGSQQLQ LKPGESAFIA ANESPVTVKG HGRLARVYNK L
```

Red type	Red bold type indicates the sequence of the COR121 maize-derived PMI peptides identified using LC-MS analysis against the expected PMI protein sequence.
Amino acid residue abbreviations	Alanine (A), cysteine (C), aspartic acid (D), glutamic acid (E), phenylalanine (F), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), asparagine (N), proline (P), glutamine (Q), arginine (R), serine (S), threonine (T), tryptophan (W), tyrosine (Y), and valine (V).

Note: The N-terminal peptide was acetylated.

Figure 29. Identified Tryptic and Chymotryptic Peptide Amino Acid Sequence of the COR121 Maize-Derived PMI Protein Using LC-MS Analysis

N-Terminal Amino Acid Sequence Analysis

The N-terminal peptide for the COR121 maize-derived PMI protein was identified by LC-MS as MQKLINSVQNY from the chymotryptic digestion, matching the amino acid residues 1-11 of the PMI protein sequence.

Additional details regarding N-terminal amino acid sequencing analytical methods are provided in Appendix E.

Allergenicity and toxicity of the PMI Protein

The PMI protein present in COR121 maize is found in several approved events that are currently in commercial use. In accordance with the FSANZ Application Handbook, only the updated bioinformatics analysis is required for safety assessment of proteins evaluated previously.

Bioinformatic Analysis of PMI Sequence Similarity to Known and Putative Allergens

Assessing newly expressed proteins for potential cross-reactivity with known or putative allergens is an important part of the weight-of-evidence approach used to evaluate the safety of these proteins in genetically modified plant products (Codex Alimentarius Commission, 2009). A bioinformatic assessment of the PMI protein sequence (391 amino acids [aa]) for its sequence similarity with known or putative allergens was conducted according to relevant guidelines (Codex Alimentarius Commission, 2009; FAO/WHO, 2001).

Two separate searches for the PMI protein using the Comprehensive Protein Allergen Resource (COMPARE) 2024 database (February, 2024) (van Ree *et al.*, 2021), compiled by the Health and Environmental Sciences Institute (HESI) and contains 2,748 sequences.

The first allergenicity search was the sliding 80-mer window search, accomplished with an internally developed Perl script running FASTA (v35.04; Pearson and Lipman (1988)) with an *E*-score cutoff set to 100. In a sliding window search, each sequentially overlapping 80-aa subsequence of the overall PMI protein sequence is used as a query against the COMPARE allergen database sequences. The script examined all alignments generated from the query and reported any possessing > 35% identity over an alignment length of ≥ 80 aa. Additionally, the script rescaled the percent identity to an 80-mer window for any alignments possessing an alignment length shorter than 80 aa; the number of identities in these alignments would be divided by 80, then multiplied by 100, and would report any alignment possessing an adjusted percent identity > 35%. The second allergenicity search used EMBOSS Fuzzpro (v6.6.0; Rice *et al.* (2000)) to identify any eight or greater contiguous identical amino acid matches between the PMI protein sequence and the COMPARE allergen sequences.

Results of the search of the PMI protein sequence against the COMPARE database of known and putative allergens found no alignments that were a length of 80 residues or greater with a sequence identity of >35%. One contiguous 8-residue amino acid match (DLSDKETT) was found between the PMI protein sequence and the sequence of an allergen (a putative alpha-parvalbumin from frog, GenBank Accession CAC83047.1; Hilger *et al.* (2002)). Comprehensive analysis of this match strongly indicates that this is a false positive and is unlikely to represent a cross-reactive risk. Collectively, these data indicate that no allergenicity concern arose from the bioinformatics assessment of the PMI protein.

Bioinformatics evaluation of the PMI protein sequence did not generate biologically relevant amino acid sequence similarities to allergens that are harmful to humans.

Bioinformatic Analysis of PMI Sequence Similarity to Known and Putative Protein Toxins

Assessing newly expressed proteins for potential similarity with known or putative protein toxins is an important part of the weight-of-evidence approach used to evaluate the safety of these proteins in genetically modified plant products (Codex Alimentarius Commission, 2009). The potential toxicity of the PMI protein was assessed by comparison of its sequence to the sequences in the National Center for Biotechnology Information (NCBI) non-redundant (nr) protein database (September 2024) with an *E*-value threshold was set to 10^{-4} for searches.

Results of the BLASTP (v2.15.0+) search of the PMI protein against the NCBI nr protein database returned the maximum number of alignments (100), all with *E*-values of zero, to mannose-6-phosphate isomerase proteins, which are predominantly annotated from strains of *E. coli*. None of the alignments returned by the BLASTP search are proteins known to be toxic to humans. Therefore, no toxicity concern arose from the bioinformatics assessment of the PMI protein.

Bioinformatics evaluation of the PMI protein sequence did not generate biologically relevant amino acid sequence similarities to protein toxins that are harmful to humans.

Conclusion on the Safety of the PMI protein in COR121 Maize

Protein characterization results via SDS-PAGE, western blot, peptide mapping, N-terminal amino acid sequence, and glycoprotein analysis have demonstrated that the PMI protein derived from COR121 maize is of the expected molecular weight, immunoreactivity, amino acid sequence and showed a lack of glycosylation.

The amino acid sequence of the PMI protein present in COR121 maize was demonstrated to be identical to the PMI protein found in a number of authorized GM events that are currently commercialized and have a history of safe use (ISAAA, 2025).

The toxicity and allergenicity potential of the PMI protein was evaluated using existing literature, which includes bioinformatic analyses. Updated bioinformatics comparisons of the PMI protein sequence to known or putative allergen and toxin sequences supports that the PMI protein is unlikely to be allergenic or toxic to humans.

Based on this weight of evidence, consumption of the PMI protein is unlikely to cause an adverse effect on humans.

B.3 Other (non-protein substances)

There are no other new substances associated with COR121 maize.

B.4 Novel herbicide metabolites in GM herbicide-tolerant plants

There are no novel herbicide metabolites associated with COR121 maize.

B.5 Compositional analyses of the food produced using gene technology

Trait Expression Assessment

The expression levels of the IPD083Cb and PMI proteins were evaluated in COR121 maize.

COR121 maize plants were grown during the 2024 growing season at six sites in commercial maize growing regions of the United States and Canada. A randomized complete block design with four blocks was utilized at each site.

The following samples were collected: leaf (V6, V9, R1, and R4 growth stages), root (V9, R1, and R4 growth stages), pollen (R1 growth stage), forage (R4 growth stage), and grain (R6 growth stage). Samples were analyzed for the IPD083Cb and PMI protein concentrations using quantitative enzyme linked immunosorbent assay (ELISA) methods.

Concentration results (means, ranges, and standard deviations) are summarized across sites in Tables 11 and 12 for the IPD083Cb and PMI protein, respectively. Individual sample results below the LLOQ were assigned a value equal to the LLOQ for calculation purposes.

Additional details regarding analytical methods and calculations for trait expression analysis are provided in Appendix F.

Table 11. Across-Site Summary of the IPD083Cb Protein Concentrations in COR121 Maize

Tissue (Growth Stage)	ng IPD083Cb/mg Tissue Dry Weight				Number of Samples <LLOQ/ Number of Samples Reported
	Mean	Range	Standard Deviation	Sample LLOQ[a]	
COR121 Maize					
Leaf (V6)	530	290 - 720	140	1.2	0/24
Leaf (V9)	450	260 - 600	89	1.2	0/24
Leaf (R1)	380	250 - 560	77	1.2	0/24
Leaf (R4)	210	100 - 340	72	1.2	0/24
Root (V9)	31	12 - 51	11	0.60	0/24
Root (R1)	24	7.2 - 33	5.8	0.60	0/24
Root (R4)	14	5.1 - 22	5.2	0.60	0/24
Pollen (R1)	0.57[b]	<0.48 - 1.3	0.17[b]	0.48	7/24
Forage (R4)	66	48 - 86	11	0.40	0/24
Grain (R6)	0.99	0.66 - 1.4	0.19	0.60	0/24

Note: Growth stages (Abendroth *et al.*, 2011).
[a] Lower limit of quantification (LLOQ) in ng/mg tissue dry weight.
[b] Some, but not all, sample results were below the LLOQ. A value equal to the LLOQ value was assigned to those samples to calculate the mean and standard deviation.

Table 12. Across-Site Summary of the PMI Protein Concentrations in COR121 Maize

Tissue (Growth Stage)	ng PMI/mg Tissue Dry Weight				Number of Samples <LLOQ/ Number of Samples Reported
	Mean	Range	Standard Deviation	Sample LLOQ[a]	
COR121 Maize					
Leaf (V6)	35	25 - 43	4.7	0.54	0/24
Leaf (V9)	26	17 - 35	4.6	0.54	0/24
Leaf (R1)	24	17 - 31	3.4	0.54	0/24
Leaf (R4)	40	28 - 60	7.0	0.54	0/24
Root (V9)	11	3.6 - 18	3.8	0.27	0/24
Root (R1)	7.6	4.5 - 11	1.7	0.27	0/24
Root (R4)	6.8	4.8 - 12	2.1	0.27	0/24
Pollen (R1)	22	18 - 28	3.1	1.1	0/24
Forage (R4)	17	13 - 22	2.1	1.8	0/24
Grain (R6)	4.3	2.4 - 6.0	0.95	0.27	0/24

Note: Growth stages (Abendroth *et al.*, 2011).
[a] Lower limit of quantification (LLOQ) in ng/mg tissue dry weight.

Nutrient Composition Assessment

Evaluating crop composition as a component of transgenic crop safety assessments was first proposed in 1993 (OECD, 1993). This proposal was not based on knowledge of the effects of transgenesis on crop composition, but rather on uncertainty about the extent of any compositional changes that could potentially result from transgenesis. In the years following 1993, a substantial volume of knowledge was acquired on the composition of both transgenic and non-transgenic crops, and the numerous factors and interactions that influence crop composition.

The breeding of non-transgenic crops typically involves techniques that result in genetic mutations, deletions, insertions, and rearrangements, and the genotypic variation that is introduced by each of these techniques produces compositional changes in non-transgenic crops (Weber *et al.*, 2012). While compositional changes can also occur in transgenic crops, it is important to understand that gene insertion via transgenesis generally causes substantially fewer and less significant compositional changes compared with traditional breeding (Herman and Price, 2013). It is also worth noting that compositional changes in transgenic crops often result from secondary effects of the traits conferred by the transgenesis (Duke, 2005; Johnson *et al.*, 2012; Mattsson, 2007; Nelson and Renner, 2001). For example, an insect-protected transgenic crop would sustain less injury than the poorly protected non-transgenic crop in the presence of insect pest pressure. The reduced level of biotic stress that would occur in the transgenic crop as a result of the insect protection compared with the non-transgenic crop would then be expected to result in minor differences in composition between the transgenic and non-transgenic crop. Similarly, a reduction in weed interference enabled by herbicide-tolerant crops could potentially increase the health of the transgenic crop, causing minor compositional changes similar to those observed in crops well protected from weed competition by other means.

In addition to these types of compositional changes, it has also been demonstrated that environmental conditions during the production of the crop and storage conditions of the crop after harvest have a far greater influence on the composition of both non-transgenic and transgenic crops than any potential changes that could result from transgenesis (Harrigan *et al.*, 2010; Matthes and Schmitz-Eiberger, 2009; Mpofu *et al.*, 2006; Reynolds *et al.*, 2005; Rotundo and Westgate, 2009; Skogerson *et al.*, 2010; Stevenson *et al.*, 2012).

In the decades that have passed since 1993, hundreds of composition assessments have been conducted on transgenic events and breeding stacks containing a diverse array of traits in numerous crop species (Herman and Price, 2013; USDA-APHIS, 2025). The results from each assessment in this massive body of accumulated scientific evidence supports the conclusion that transgenesis does not have a meaningful impact on crop composition; any minor changes in composition that are observed in the transgenic crop are negligible when compared with the variation in composition that naturally occurs in the comparable commercial non-transgenic crop which has a lengthy history of safe use for food and feed.

Only in the case of transgenic traits where the composition of the crop is intentionally altered would a targeted composition assessment be appropriate. This assessment would likely confirm that the trait confers the expected changes in composition and that no additional hypothesis-driven unintended changes occur.

The composition of COR121 maize was evaluated using tissue samples from COR121 maize, conventional (non-genetically modified [non-GM]) near-isoline control maize, and conventional

reference maize lines generated in a multi-location field study that was conducted in compliance with regulatory requirements. While this assessment was conducted, the need for it was not justified by any hypothesis that the composition of COR121 maize would differ from that of the conventional crop. The traits conferred by the transgenes in COR121 maize do not intentionally alter the composition of the crop and are not expected to do so, which further negates the need for this compositional assessment.

Forage (R4 growth stage) and grain (R6 growth stage) samples were collected during the 2024 growing season at eight sites in commercial maize-growing regions of the United States and Canada. A randomized complete block design with four blocks was utilized at each site. Each block included COR121 maize, control maize, and four reference maize lines.

The samples were assessed for key nutritional components. Proximate, fiber, and mineral analytes were assessed in the forage samples, and grain samples were assessed for proximate, fiber, fatty acid, amino acid, mineral, vitamin, secondary metabolite, and anti-nutrient analytes. The analytes included in the compositional assessment were selected based on the OECD consensus document on compositional considerations for new varieties of maize (OECD, 2002). Procedures and methods for nutrient composition analyses of maize forage and grain were conducted in accordance with the requirements of the U.S. EPA Good Laboratory Practice (GLP) Standards, 40 CFR Part 160. The analytical procedures used were validated methods, with the majority based on methods published by AOAC International, AACC (Cereals & Grains Association), and AOCS (American Oil Chemists' Society).

Statistical analyses were conducted to evaluate and compare the nutrient composition of COR121 maize and the control maize. Across-site analysis was conducted for a total of 71 analytes; 64 analytes were evaluated using mixed model analysis. Two analytes were evaluated using Fisher's exact test as they did not meet the criteria for sufficient quantities of observations above the lower limit of quantification (LLOQ) and were therefore not included in the mixed model analyses. No statistical analysis was conducted for the remaining five analytes, as all data values were below the LLOQ.

For a given analyte in the mixed model analysis, if a statistical difference (P-value < 0.05) was observed between COR121 maize and the control maize, the False Discovery Rate (FDR)-adjusted P-value was examined. In cases where the raw P-value indicated a significant difference, but the FDR-adjusted P-value was non-significant, it was concluded that the difference was likely a false positive. Additionally, three reference ranges representing the conventional maize population with a history of safe use (i.e., tolerance interval, literature range, and in-study reference range) were utilized to evaluate statistical differences in the context of biological variation. If the measured values for COR121 maize for that analyte fell within at least one of the reference ranges, then that analyte would be considered comparable to conventional maize.

The outcome of the nutrient composition assessment is provided in Table 13. Nutrient composition analyses results are provided in Tables 14 to 20.

No statistically significant differences were observed between COR121 maize and the control maize for 59 of the 66 analytes evaluated in the across-site analysis via either mixed model analysis or Fisher's exact test. For the remaining seven analytes (moisture in grain, oleic acid (C18:1), eicosenoic acid (C20:1), calcium, copper, iron, and inositol) evaluated in the across-site analysis, a statistically significant difference (P-value < 0.05), was observed between COR121 maize and the control maize. For each of these analytes, the range of values for COR121 maize were within

one or more of the reference ranges (i.e., tolerance interval, literature range, and in-study reference range) representing the non-GE maize population with a history of safe use, indicating that COR121 maize is within the range of normal variation for these analytes and the statistical differences were not biologically meaningful. Additionally, for all seven analytes, the FDR-adjusted P-value was non-significant, indicating that the identified statistical differences were likely false positives.

The results of the nutrient composition assessment demonstrate that the nutrient composition of forage and grain derived from COR121 maize is comparable to that of conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Additional details regarding the methods and procedures used for the nutrient composition assessment are provided in Appendix G.

Table 13. Outcome of the Nutrient Composition Assessment for COR121 Maize

Subgroup	No Statistical Difference Identified	Statistical Difference Identified					Not Included in Statistical Analysis (All Data Values Below the Lower Limit of Quantification)
		All Data Values Within Tolerance Interval	One or More Data Values Outside Tolerance Interval, or Tolerance Interval Not Available			Adjusted P-Value < 0.05	
			All Data Values Within Literature Range	One or More Data Values Outside Literature Range			
				All Data Values Within Reference Data Range	One or More Data Values Outside Reference Data Range		
Forage (R4 Growth Stage)							
Proximate, Fiber, and Mineral Composition	Moisture (%) Crude Protein Crude Fat ADF NDF Ash Carbohydrates Calcium Phosphorus	--	--	--	--	--	--
Grain (R6 Growth Stage)							
Proximate and Fiber Composition	Crude Protein Crude Fat ADF NDF Total Dietary Fiber Ash Carbohydrates	Moisture (%)	--	--	--	--	--

Table 13. Outcome of the Nutrient Composition Assessment for COR121 Maize (continued)

Subgroup	No Statistical Difference Identified	Statistical Difference Identified					Not Included in Statistical Analysis (All Data Values Below the Lower Limit of Quantification)
		All Data Values Within Tolerance Interval	One or More Data Values Outside Tolerance Interval, or Tolerance Interval Not Available			Adjusted P-Value < 0.05	
			All Data Values Within Literature Range	One or More Data Values Outside Literature Range			
				All Data Values Within Reference Data Range	One or More Data Values Outside Reference Data Range		
Grain (R6 Growth Stage)							
Fatty Acid Composition	Palmitic Acid (C16:0) Palmitoleic Acid (C16:1) Heptadecanoic Acid (C17:0) Heptadecenoic Acid (C17:1) Stearic Acid (C18:0) Linoleic Acid (C18:2) Linolenic Acid (C18:3) Arachidic Acid (C20:0) Behenic Acid (C22:0)	Oleic Acid (C18:1) Eicosenoic Acid (C20:1)	--	--	--	--	Lauric Acid (C12:0) Myristic Acid (C14:0) Eicosadienoic Acid (C20:2)
Amino Acid Composition	Alanine Arginine Aspartic Acid Cystine Glutamic Acid Glycine Histidine Isoleucine Leucine Lysine	--	--	--	--	--	--

Subgroup	No Statistical Difference Identified	Statistical Difference Identified					Not Included in Statistical Analysis (All Data Values Below the Lower Limit of Quantification)
		All Data Values Within Tolerance Interval	One or More Data Values Outside Tolerance Interval, or Tolerance Interval Not Available			Adjusted P-Value < 0.05	
			All Data Values Within Literature Range	One or More Data Values Outside Literature Range			
				All Data Values Within Reference Data Range	One or More Data Values Outside Reference Data Range		
	Methionine Phenylalanine Proline Serine Threonine Tryptophan Tyrosine Valine						

Table 13. Outcome of the Nutrient Composition Assessment for COR121 Maize (continued)

Subgroup	No Statistical Difference Identified	Statistical Difference Identified					Not Included in Statistical Analysis (All Data Values Below the Lower Limit of Quantification)
		All Data Values Within Tolerance Interval	One or More Data Values Outside Tolerance Interval, or Tolerance Interval Not Available			Adjusted P-Value < 0.05	
			All Data Values Within Literature Range	One or More Data Values Outside Literature Range			
				All Data Values Within Reference Data Range	One or More Data Values Outside Reference Data Range		
Grain (R6 Growth Stage)							
Mineral Composition	Magnesium Manganese Phosphorus Potassium Sodium Zinc	Calcium Copper Iron	--	--	--	--	--
Vitamin Composition	β-Carotene Vitamin B1 (Thiamine) Vitamin B3 (Niacin) Vitamin B6 (Pyridoxine) Vitamin B9 (Folic Acid) α-Tocopherol	--	--	--	--	--	Vitamin B2 (Riboflavin)
Secondary Metabolite and Anti-Nutrient Composition	*p*-Coumaric Acid Ferulic Acid Phytic Acid Raffinose	Inositol	--	--	--	--	Furfural

Note: Growth stages (Abendroth et al., 2011).

Proximate, Fiber, and Mineral Assessment of COR121 Maize Forage

Proximates, fiber, and minerals were analyzed in forage derived from COR121 maize and control maize. Results are shown in Table 14. No statistically significant differences were observed between COR121 maize and control maize.

These results demonstrate that the proximate, fiber, and mineral composition of forage derived from COR121 maize is comparable to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 14. Proximate, Fiber, and Mineral Results for COR121 Maize Forage

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Proximate, Fiber, and Mineral Composition (% Dry Weight or as Indicated)						
Moisture (%)	Mean	79.9	79.1	50.1 - 92.5	46.5 - 90.7	65.3 - 86.5
	Range	70.5 - 85.7	68.6 - 84.2			
	Confidence Interval	77.2 - 82.7	76.3 - 81.8			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.201			
Crude Protein	Mean	8.48	8.79	3.86 - 12.2	2.37 - 16.32	4.08 - 11.4
	Range	6.18 - 10.4	6.82 - 11.2			
	Confidence Interval	8.09 - 8.86	8.40 - 9.18			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.153			
Crude Fat	Mean	3.39	3.46	0.957 - 6.62	NQ - 7.500	1.59 - 5.56
	Range	2.14 - 5.96	2.03 - 5.10			
	Confidence Interval	2.85 - 3.92	2.92 - 3.99			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.685			
ADF	Mean	30.9	31.0	16 - 39.7	5.13 - 47.39	21.1 - 40.2
	Range	25.8 - 37.6	23.8 - 37.1			
	Confidence Interval	29.0 - 32.9	29.0 - 32.9			
	Adjusted P-Value	--	0.986			
	P-Value	--	0.955			
NDF	Mean	52.7	51.9	29.5 - 62.9	18.30 - 67.80	37.1 - 62.0
	Range	47.5 - 57.8	41.7 - 60.1			
	Confidence Interval	50.1 - 55.4	49.2 - 54.5			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.354			
Ash	Mean	5.45	5.35	2.44 - 9.36	0.66 - 13.20	3.02 - 8.73
	Range	3.39 - 8.22	3.57 - 8.05			
	Confidence Interval	4.39 - 6.52	4.29 - 6.41			

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
	Adjusted P-Value	--	0.913			
	P-Value	--	0.596			
	Mean	82.6	82.4			
	Range	79.1 - 86.5	78.1 - 85.5			
Carbohydrates	Confidence Interval	81.7 - 83.5	81.5 - 83.3	76.7 - 90.9	73.3 - 92.9	77.6 - 90.0
	Adjusted P-Value	--	0.913			
	P-Value	--	0.603			
	Mean	0.323	0.312			
	Range	0.228 - 0.408	0.200 - 0.475			
Calcium	Confidence Interval	0.296 - 0.350	0.285 - 0.339	0.0801 - 0.536	0.04 - 0.58	0.117 - 0.602
	Adjusted P-Value	--	0.913			
	P-Value	--	0.493			
	Mean	0.246	0.252			
	Range	0.161 - 0.318	0.160 - 0.332			
Phosphorus	Confidence Interval	0.217 - 0.274	0.223 - 0.280	0.0687 - 0.414	0.07 - 0.49	0.129 - 0.515
	Adjusted P-Value	--	0.844			
	P-Value	--	0.382			

Note: Not quantified (NQ): one or more assay values in the published literature references were below the lower limit of quantification (LLOQ) and were not quantified.

Proximate and Fiber Assessment of COR121 Maize Grain

Proximates and fiber were analyzed in grain derived from COR121 maize and control maize. Results are shown in Table 15. No statistically significant differences were observed between COR121 maize and control maize for seven of eight proximate and fiber analytes in forage. For the remaining analyte, moisture, a statistically significant difference (P-value < 0.05) was observed between COR121 maize and control maize. For this analyte the range of values for COR121 maize were within one or more of the reference ranges, indicating COR121 maize is within the range of biological variation for this analyte, and the statistical difference is not biologically meaningful. The non-significant FDR adjusted P-value indicates that the difference was likely a false positive.

These results demonstrate that the proximate and fiber composition of grain derived from COR121 maize is comparable to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 15. Proximate and Fiber Results for COR121 Maize Grain

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Proximate and Fiber Composition (% Dry Weight or as Indicated)						
Moisture (%)	Mean	18.5	18.9	3.71 - 38.3	5.1 - 40.7	12.6 - 30.4
	Range	12.6 - 26.3	13.0 - 27.3			
	Confidence Interval	15.4 - 21.7	15.7 - 22.0			
	Adjusted P-Value	--	0.252			
	P-Value	--	0.0276*			
Crude Protein	Mean	9.39	9.68	6.55 - 13.1	5.72 - 17.26	7.44 - 11.7
	Range	7.59 - 10.7	8.31 - 10.7			
	Confidence Interval	8.89 - 9.90	9.18 - 10.2			
	Adjusted P-Value	--	0.652			
	P-Value	--	0.102			
Crude Fat	Mean	4.31	4.33	2.29 - 5.90	1.363 - 7.830	3.56 - 5.28
	Range	3.15 - 4.87	3.66 - 5.12			
	Confidence Interval	4.13 - 4.48	4.16 - 4.51			
	Adjusted P-Value	--	0.923			
	P-Value	--	0.762			
ADF	Mean	4.05	4.02	2.42 - 5.83	1.41 - 9.56	2.94 - 6.05
	Range	3.04 - 5.53	3.45 - 5.62			
	Confidence Interval	3.83 - 4.27	3.80 - 4.24			
	Adjusted P-Value	--	0.923			
	P-Value	--	0.749			
NDF	Mean	10.2	10.0	7.5 - 16.3	4.28 - 24.30	7.68 - 12.6
	Range	8.77 - 12.4	8.49 - 11.4			
	Confidence Interval	9.73 - 10.6	9.59 - 10.5			
	Adjusted P-Value	--	0.844			

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
	P-Value	--	0.396			
Total Dietary Fiber	Mean	9.85	9.85	2.82 - 19.4	4.44 - 35.31	6.37 - 13.1
	Range	8.51 - 11.5	7.61 - 11.4			
	Confidence Interval	9.47 - 10.2	9.47 - 10.2			
	Adjusted P-Value	--	0.999			
	P-Value	--	0.999			
Ash	Mean	1.41	1.43	0.983 - 1.84	0.616 - 3.220	1.10 - 1.66
	Range	1.20 - 1.58	1.20 - 1.59			
	Confidence Interval	1.34 - 1.48	1.36 - 1.49			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.281			
Carbohydrates	Mean	84.9	84.6	80.7 - 88.7	77.4 - 89.7	82.3 - 87.5
	Range	83.6 - 86.7	82.7 - 86.2			
	Confidence Interval	84.4 - 85.4	84.0 - 85.1			
	Adjusted P-Value	--	0.489			
	P-Value	--	0.0611			

Note: This table provides results from mixed model analysis only.

* A statistically significant difference (P-value < 0.05) was observed.

Fatty Acid Assessment of COR121 Maize Grain

Fatty acids were analyzed in grain derived from COR121 maize and control maize. Results are shown in Table 16. No statistically significant differences were observed between COR121 maize and control maize for 14 of 16 analytes. For the remaining two analytes, oleic acid (C18:1) and eicosenoic acid (C20:1), a statistically significant difference (P-value < 0.05) was observed between COR121 maize and control maize. For each of these analytes the range of values for COR121 maize were within one or more of the reference ranges, indicating COR121 maize is within the range of biological variation for these analytes, and the statistical differences are not biologically meaningful. The non-significant FDR adjusted P-values indicate that the differences were likely false positives.

These results demonstrate that the fatty acid composition of seed derived from COR121 maize is comparable to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 16. Fatty Acid Results for COR121 Maize Grain

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Fatty Acid Composition (% Total Fatty Acids)						
Lauric Acid (C12:0)	Mean	<LLOQ[a]	<LLOQ[a]	0 - 0.423[r]	NQ - 0.698	<LLOQ[a]
	Range	<LLOQ[a]	<LLOQ[a]			
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
Myristic Acid (C14:0)	Mean	<LLOQ[a]	<LLOQ[a]	0 - 0.267[r]	NQ - 0.288	<LLOQ[a]
	Range	<LLOQ[a]	<LLOQ[a]			
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
Palmitic Acid (C16:0)	Mean	13.1	13.2	9.67 - 24.1	6.81 - 26.55	10.5 - 14.5
	Range	12.4 - 13.9	12.2 - 14.0			
	Confidence Interval	12.8 - 13.4	12.9 - 13.5			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.274			
Palmitoleic Acid (C16:1)	Mean	0.111	0.110	0 - 0.397	NQ - 0.453	<LLOQ[a] - 0.167
	Range	0.0876 - 0.138	0.0907 - 0.151			
	Confidence Interval	0.103 - 0.119	0.102 - 0.118			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.394			
Heptadecanoic Acid (C17:0)	Mean	0.0583	0.0479	0 - 0.220	NQ - 0.203	<LLOQ[a] - 0.0919
	Range	<LLOQ[a] - 0.0863	<LLOQ[a] - 0.0795			
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
	Mean	0.0413	<LLOQ[a]	0 - 0.135[r]	NQ - 0.131	

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Heptadecenoic Acid (C17:1)	Range	<LLOQ[a] - 0.0705	<LLOQ[a]			<LLOQ[a] - 0.104
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
Stearic Acid (C18:0)	Mean	1.58	1.54			
	Range	1.35 - 1.77	1.10 - 1.71			
	Confidence Interval	1.51 - 1.65	1.47 - 1.61	1.31 - 3.62	1.02 - 3.83	1.29 - 2.33
	Adjusted P-Value	--	0.844			
	P-Value	--	0.202			
Oleic Acid (C18:1)	Mean	26.2	25.5			
	Range	23.9 - 29.6	22.5 - 28.6			
	Confidence Interval	25.2 - 27.2	24.5 - 26.5	19.7 - 44.5	16.38 - 42.81	23.5 - 33.8
	Adjusted P-Value	--	0.0626			
	P-Value	--	0.000978*			
Linoleic Acid (C18:2)	Mean	56.6	57.0			
	Range	51.5 - 58.6	52.4 - 60.4			
	Confidence Interval	56.3 - 57.0	56.7 - 57.3	34.2 - 65.1	34.27 - 67.68	48.8 - 59.9
	Adjusted P-Value	--	0.652			
	P-Value	--	0.0964			
Linolenic Acid (C18:3)	Mean	1.79	1.79			
	Range	1.62 - 2.16	1.49 - 2.18			
	Confidence Interval	1.67 - 1.91	1.67 - 1.91	0 - 2.11	0.55 - 2.33	1.36 - 2.09
	Adjusted P-Value	--	0.986			
	P-Value	--	0.945			
Arachidic Acid (C20:0)	Mean	0.461	0.458			
	Range	0.412 - 0.532	0.354 - 0.545			
	Confidence Interval	0.435 - 0.486	0.433 - 0.484	0.296 - 0.792	0.267 - 0.993	0.302 - 0.479
	Adjusted P-Value	--	0.913			
	P-Value	--	0.565			
Eicosenoic Acid (C20:1)	Mean	0.325	0.320			
	Range	0.288 - 0.385	0.233 - 0.391			
	Confidence Interval	0.312 - 0.338	0.307 - 0.332	0 - 0.547	NQ - 1.952	0.200 - 0.344
	Adjusted P-Value	--	0.105			
	P-Value	--	0.00500*			

Table 16. Fatty Acid Results for COR121 Maize Grain (continued)

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Fatty Acid Composition (% Total Fatty Acids)						
Arachidic Acid (C20:0)	Mean	0.461	0.458	0.296 - 0.792	0.267 - 0.993	0.302 - 0.479
	Range	0.412 - 0.532	0.354 - 0.545			
	Confidence Interval	0.435 - 0.486	0.433 - 0.484			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.565			
Eicosenoic Acid (C20:1)	Mean	0.325	0.320	0 - 0.547	NQ - 1.952	0.200 - 0.344
	Range	0.288 - 0.385	0.233 - 0.391			
	Confidence Interval	0.312 - 0.338	0.307 - 0.332			
	Adjusted P-Value	--	0.105			
	P-Value	--	0.00500[*]			
Eicosadienoic Acid (C20:2)	Mean	<LLOQ[a]	<LLOQ[a]	0 - 0.825[r]	NQ - 2.551	<LLOQ[a]
	Range	<LLOQ[a]	<LLOQ[a]			
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
Behenic Acid (C22:0)	Mean	0.271	0.271	0 - 0.423	NQ - 0.417	<LLOQ[a] - 0.294
	Range	0.207 - 0.323	0.174 - 0.330			
	Confidence Interval	0.249 - 0.294	0.248 - 0.293			
	Adjusted P-Value	--	0.934			
	P-Value	--	0.812			

Note: This table provides results from mixed model analysis only. Not applicable (NA); mixed model analysis was not performed, or confidence interval was not determined. Not quantified (NQ); one or more assay values in the published literature references were below the lower limit of quantification (LLOQ) and were not quantified.

[a] < LLOQ, one or more fatty acid sample values were below the assay LLOQ.

[*] A statistically significant difference (P-value < 0.05) was observed.

[r] Historical reference data range was provided as tolerance interval was not calculated since the data did not meet the assumptions of any tolerance interval calculation method.

Amino Acid Assessment of COR121 Maize Grain

Amino acids were analyzed in grain derived from COR121 maize and control maize. Results are shown in Table 17. No statistically significant differences were observed between COR121 maize and control maize.

These results demonstrate that the amino acid composition of grain derived from COR121 maize is comparable to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 17. Amino Acid Results for COR121 Maize Grain

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Amino Acid Composition (% Dry Weight)						
Alanine	Mean	0.659	0.699	0.444 - 1.06	0.40 - 1.48	0.494 - 0.979
	Range	0.497 - 0.849	0.532 - 0.977			
	Confidence Interval	0.608 - 0.710	0.648 - 0.749			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.227			
Arginine	Mean	0.439	0.437	0.301 - 0.583	0.12 - 0.71	0.316 - 0.517
	Range	0.355 - 0.516	0.365 - 0.527			
	Confidence Interval	0.422 - 0.457	0.419 - 0.454			
	Adjusted P-Value	--	0.923			
	P-Value	--	0.757			
Aspartic Acid	Mean	0.570	0.602	0.404 - 0.890	0.30 - 1.21	0.359 - 0.801
	Range	0.410 - 0.678	0.466 - 0.831			
	Confidence Interval	0.528 - 0.612	0.561 - 0.644			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.186			
Cystine	Mean	0.195	0.198	0.121 - 0.295	0.09 - 0.51	0.126 - 0.246
	Range	0.153 - 0.244	0.134 - 0.234			
	Confidence Interval	0.184 - 0.207	0.186 - 0.209			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.668			
Glutamic Acid	Mean	1.79	1.90	1.09 - 2.74	0.83 - 3.54	1.24 - 2.40
	Range	1.36 - 2.36	1.29 - 2.71			
	Confidence Interval	1.65 - 1.93	1.76 - 2.05			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.233			
Glycine	Mean	0.378	0.379	0.284 - 0.483	0.184 - 0.685	0.279 - 0.445
	Range	0.310 - 0.425	0.312 - 0.454			
	Confidence Interval	0.363 - 0.392	0.364 - 0.393			
	Adjusted P-Value	--	0.944			
	P-Value	--	0.870			
Histidine	Mean	0.293	0.295	0.192 - 0.384	0.14 - 0.46	0.211 - 0.383
	Range	0.246 - 0.350	0.238 - 0.360			
	Confidence Interval	0.279 - 0.306	0.282 - 0.309			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.678			
Isoleucine	Mean	0.311	0.322	0.209 - 0.493	0.18 - 0.69	0.233 - 0.405
	Range	0.254 - 0.376	0.245 - 0.417			
	Confidence Interval	0.289 - 0.333	0.300 - 0.344			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.401			
Leucine	Mean	1.07	1.12	0.673 - 1.82	0.60 - 2.49	0.778 - 1.48
	Range	0.805 - 1.36	0.813 - 1.52			
	Confidence Interval	0.970 - 1.16	1.02 - 1.22			
	Adjusted P-Value	--	0.844			

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
	P-Value	--	0.312			
	Mean	0.258	0.263			
	Range	0.190 - 0.319	0.211 - 0.357			
Lysine	Confidence Interval	0.242 - 0.274	0.247 - 0.279	0.18 - 0.396	0.127 - 0.668	0.178 - 0.311
	Adjusted P-Value	--	0.913			
	P-Value	--	0.464			
	Mean	0.204	0.208			
	Range	0.144 - 0.242	0.146 - 0.238			
Methionine	Confidence Interval	0.192 - 0.217	0.195 - 0.221	0.103 - 0.313	0.09 - 0.47	0.125 - 0.256
	Adjusted P-Value	--	0.913			
	P-Value	--	0.500			

Table 17. Amino Acid Results for COR121 Maize Grain (continued)

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Amino Acid Composition (% Dry Weight)						
Phenylalanine	Mean	0.452	0.459	0.299 - 0.734	0.24 - 0.93	0.358 - 0.710
	Range	0.348 - 0.541	0.352 - 0.579			
	Confidence Interval	0.424 - 0.480	0.431 - 0.487			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.643			
Proline	Mean	0.785	0.816	0.546 - 1.24	0.46 - 1.75	0.594 - 1.08
	Range	0.650 - 0.990	0.644 - 1.09			
	Confidence Interval	0.734 - 0.837	0.765 - 0.867			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.331			
Serine	Mean	0.462	0.475	0.308 - 0.679	0.15 - 0.77	0.356 - 0.590
	Range	0.375 - 0.566	0.368 - 0.616			
	Confidence Interval	0.432 - 0.492	0.445 - 0.505			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.357			
Threonine	Mean	0.358	0.363	0.25 - 0.484	0.17 - 0.67	0.272 - 0.433
	Range	0.298 - 0.422	0.288 - 0.452			
	Confidence Interval	0.340 - 0.376	0.345 - 0.380			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.577			
Tryptophan	Mean	0.0669	0.0674	0.0375 - 0.0975	0.027 - 0.215	0.0370 - 0.0838
	Range	0.0504 - 0.0802	0.0467 - 0.0841			
	Confidence Interval	0.0626 - 0.0712	0.0631 - 0.0717			
	Adjusted P-Value	--	0.934			
	P-Value	--	0.820			
Tyrosine	Mean	0.282	0.278	0.163 - 0.528	0.10 - 0.73	0.199 - 0.376
	Range	0.203 - 0.333	0.210 - 0.333			
	Confidence Interval	0.263 - 0.301	0.259 - 0.297			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.613			
Valine	Mean	0.436	0.450	0.303 - 0.626	0.27 - 0.86	0.316 - 0.541
	Range	0.362 - 0.530	0.355 - 0.585			
	Confidence Interval	0.410 - 0.461	0.425 - 0.476			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.306			

Note: This table provides results from mixed model analysis only.

Mineral Assessment of COR121 Maize Grain

Minerals were analyzed in grain derived from COR121 maize and control maize. Results are shown in Table 18. No statistically significant differences were observed between COR121 maize and control maize for seven of 10 analytes. For the remaining three analytes, calcium, copper, and iron, a statistically significant difference (P-value < 0.05) was observed between COR121 maize and control maize. For each of these analytes the range of values were within one or more of the reference ranges, indicating COR121 maize is within the range of biological variation for these analytes and the statistical difference is not biologically meaningful. The non-significant FDR adjusted P-values indicate that the differences were likely false positives.

These results demonstrate that the mineral composition of grain derived from COR121 maize is comparable to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 18. Mineral Results for COR121 Maize Grain

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Mineral Composition (% Dry Weight)						
Calcium	Mean	0.00361	0.00340	0.00135 - 0.00748	NQ - 0.101	0.00211 - 0.00678
	Range	0.00266 - 0.00447	0.00267 - 0.00434			
	Confidence Interval	0.00335 - 0.00388	0.00314 - 0.00367			
	Adjusted P-Value	--	0.187			
	P-Value	--	0.0146*			
Copper	Mean	0.000110	0.0000964	<0.0000625[a] - 0.000339	NQ - 0.0021	<0.0000625[a] - 0.000193
	Range	<0.0000625[a] - 0.000219	<0.0000625[a] - 0.000156			
	Confidence Interval	0.0000897 - 0.000131	0.0000759 - 0.000117			
	Adjusted P-Value	--	0.105			
	P-Value	--	0.00410*			
Iron	Mean	0.00182	0.00172	0.00111 - 0.00303	0.0007 - 0.0191	0.00123 - 0.00241
	Range	0.00148 - 0.00226	0.00132 - 0.00215			
	Confidence Interval	0.00171 - 0.00194	0.00161 - 0.00183			
	Adjusted P-Value	--	0.105			
	P-Value	--	0.00655*			
Magnesium	Mean	0.109	0.111	0.0801 - 0.157	0.06 - 0.19	0.0825 - 0.157
	Range	0.0864 - 0.128	0.0842 - 0.131			
	Confidence Interval	0.101 - 0.117	0.103 - 0.119			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.247			
Manganese	Mean	0.000583	0.000604	0.000323 - 0.00118	0.0002 - 0.0014	0.000270 - 0.000876
	Range	0.000444 - 0.000745	0.000422 - 0.000853			
	Confidence Interval	0.000507 - 0.000659	0.000529 - 0.000680			

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
	Adjusted P-Value	--	0.844			
	P-Value	--	0.357			
Phosphorus	Mean	0.338	0.340	0.213 - 0.418	0.13 - 0.55	0.238 - 0.592
	Range	0.269 - 0.502	0.258 - 0.479			
	Confidence Interval	0.312 - 0.364	0.314 - 0.365			
	Adjusted P-Value	--	0.934			
	P-Value	--	0.838			
Potassium	Mean	0.356	0.359	0.235 - 0.504	0.18 - 0.60	0.232 - 0.494
	Range	0.283 - 0.416	0.315 - 0.410			
	Confidence Interval	0.337 - 0.375	0.339 - 0.378			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.679			
Sodium	Mean	0.000100	0.0000914	<LLOQ[a] - 0.0128	NQ - 0.07315	<0.0000625[a] - 0.00135
	Range	<0.0000625[a] - 0.000735	<0.0000625[a] - 0.000473			
	Confidence Interval	0.0000656 - 0.000152	0.0000600 - 0.000139			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.645			
Zinc	Mean	0.00216	0.00214	0.00134 - 0.00344	0.0006 - 0.0043	0.00116 - 0.00271
	Range	0.00156 - 0.00272	0.00130 - 0.00284			
	Confidence Interval	0.00196 - 0.00236	0.00194 - 0.00234			
	Adjusted P-Value	--	0.923			
	P-Value	--	0.717			

Note: This table provides results from mixed model analysis only. Not quantified (NQ); one or more assay values in the published literature references were below the lower limit of quantification (LLOQ) and were not quantified.

[a] < LLOQ, one or more sample values were below the assay LLOQ.

[*] A statistically significant difference (P-value < 0.05) was observed.

Vitamin Assessment of COR121 Maize Grain

Vitamins were analyzed in grain derived from COR121 maize and control maize. Results are shown in Table 19. No statistically significant differences were observed between COR121 maize and control maize.

These results demonstrate that the vitamin composition of grain derived from COR121 maize is comparable to conventional grain represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 19. Vitamin Results for COR121 Maize Grain

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
			Vitamin Composition (mg/kg Dry Weight)			
β-Carotene	Mean	0.453	0.457	0 - 3.39	NQ - 5.81	0.169 - 0.784
	Range	0.222 - 0.722	0.204 - 0.804			
	Confidence Interval	0.332 - 0.617	0.335 - 0.622			
	Adjusted P-Value	--	0.934			
	P-Value	--	0.829			
Vitamin B1 (Thiamine)	Mean	3.12	3.19	0.995 - 4.78	NQ - 40.00	1.78 - 3.87
	Range	2.51 - 4.09	2.59 - 4.34			
	Confidence Interval	2.83 - 3.41	2.90 - 3.49			
	Adjusted P-Value	--	0.844			
	P-Value	--	0.203			
Vitamin B2 (Riboflavin)	Mean	<0.900[a]	<0.900[a]	<0.9[a] - 2.27[r]	NQ - 7.35	<0.900[a]
	Range	<0.900[a]	<0.900[a]			
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
Vitamin B3 (Niacin)	Mean	8.39	8.39	7.63 - 29.7	NQ - 66.00	5.98 - 13.3
	Range	6.94 - 10.5	6.39 - 11.6			
	Confidence Interval	7.62 - 9.24	7.62 - 9.24			
	Adjusted P-Value	--	0.999			
	P-Value	--	0.993			
Vitamin B6 (Pyridoxine)	Mean	4.84	4.85	1.03 - 8.99	NQ - 12.14	3.36 - 6.49
	Range	4.00 - 6.44	3.88 - 6.00			
	Confidence Interval	4.39 - 5.29	4.40 - 5.30			
	Adjusted P-Value	--	0.955			
	P-Value	--	0.896			
Vitamin B9 (Folic Acid)	Mean	0.381	0.378	0.203 - 7.83	NQ - 11.40	0.200 - 0.548
	Range	0.289 - 0.486	0.294 - 0.461			

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
	Confidence Interval	0.347 - 0.414	0.344 - 0.412			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.643			
	Mean	6.65	6.51			
	Range	3.80 - 11.9	3.50 - 11.6			
α-Tocopherol	Confidence Interval	4.78 - 8.52	4.64 - 8.38	0 - 22.6	NQ - 68.67	1.17 - 20.9
	Adjusted P-Value	--	0.913			
	P-Value	--	0.479			

Note: This table provides results from mixed model analysis only. Not applicable (NA); mixed model analysis was not performed, or confidence interval was not determined. Not quantified (NQ); one or more assay values in the published literature references were below the lower limit of quantification (LLOQ) and were not quantified.

[a] < LLOQ, one or more sample values were below the assay LLOQ.

[r] Historical reference data range was provided as tolerance interval was not calculated since the data did not meet the assumptions of any tolerance interval calculation method.

Secondary Metabolite and Anti-Nutrient Assessment of COR121 Maize Grain

Secondary metabolite and anti-nutrients were analyzed in grain derived from COR121 maize and control maize. Results are shown in Table 20. No statistically significant differences were observed between COR121 maize and control maize for five of the six analytes. For the remaining analyte, inositol, a statistically significant difference (P-value < 0.05) was observed between COR121 maize and control maize. The range of values for this analyte were within one or more of the reference ranges, indicating COR121 maize is within the range of biological variation for this analyte, and the statistical differences are not biologically meaningful. The non-significant FDR adjusted P-value for inositol indicates that the difference was likely a false positive.

These results demonstrate that the secondary metabolite and anti-nutrient composition of grain derived from COR121 maize is comparable to conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize.

Table 20. Secondary Metabolite and Anti-Nutrient Results for COR121 Maize Grain

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
Secondary Metabolite and Anti-Nutrient Composition (% Dry Weight or as Indicated)						
p-Coumaric Acid	Mean	0.0255	0.0252	0.00995 - 0.0530	NQ - 0.08	0.0113 - 0.0355
	Range	0.0193 - 0.0340	0.0194 - 0.0337			
	Confidence Interval	0.0232 - 0.0279	0.0229 - 0.0275			
	Adjusted P-Value	--	0.923			
	P-Value	--	0.764			
Ferulic Acid	Mean	0.234	0.232	0.126 - 0.361	0.03 - 0.44	0.169 - 0.294
	Range	0.200 - 0.286	0.196 - 0.298			
	Confidence Interval	0.223 - 0.246	0.221 - 0.244			
	Adjusted P-Value	--	0.913			
	P-Value	--	0.658			
Furfural	Mean	<0.000100[a]	<0.000100[a]	<0.00005[a]	NQ - 0.000634	<0.000100[a]
	Range	<0.000100[a]	<0.000100[a]			
	Confidence Interval	NA	NA			
	Adjusted P-Value	--	NA			
	P-Value	--	NA			
Inositol	Mean	0.0189	0.0169	0.00932 - 0.0589	0.00503 - 0.257	0.0100 - 0.0246
	Range	0.0152 - 0.0242	0.0125 - 0.0206			
	Confidence Interval	0.0177 - 0.0200	0.0158 - 0.0181			
	Adjusted P-Value	--	0.218			
	P-Value	--	0.0204*			
Phytic Acid	Mean	0.995	0.989	0.512 - 1.31	NQ - 1.940	<0.355[b] - 1.40
	Range	0.723 - 1.42	0.733 - 1.46			
	Confidence Interval	0.903 - 1.09	0.897 - 1.08			
	Adjusted P-Value	--	0.934			

Analyte	Reported Statistics	Control Maize	COR121 Maize	Tolerance Interval	Literature Range	Reference Data Range
	P-Value	--	0.847			
	Mean	0.255	0.247			
	Range	0.159 - 0.340	0.108 - 0.364			
Raffinose	Confidence Interval	0.219 - 0.290	0.212 - 0.283	0 - 0.390	NQ - 0.509	0.0983 - 0.390
	Adjusted P-Value	--	0.844			
	P-Value	--	0.409			

Note: This table provides results from mixed model analysis only. Not applicable (NA); mixed model analysis was not performed, or confidence interval was not determined. Not quantified (NQ); one or more assay values in the published literature references were below the lower limit of quantification (LLOQ) and were not quantified.

[a] < LLOQ, one or more sample values were below the assay LLOQ.

[*] A statistically significant difference (P-value < 0.05) was observed.

C. INFORMATION RELATED TO THE NUTRITIONAL IMPACT OF THE FOOD

The composition of COR121 maize was compared with that of a concurrently grown conventional comparator with a history of safe use in food and feed. The results demonstrated that the nutrient composition of forage and grain derived from COR121 maize is comparable to that of conventional maize represented by non-GM near-isoline control maize and non-GM commercial maize. Based on these analyses, the grain and forage of COR121 maize are comparable to conventional maize with respect to nutrient composition.

Therefore, no nutritional impact of COR121 maize is expected.

D. OTHER INFORMATION

Overall Risk Assessment Conclusion for COR121 Maize

This application presents information supporting the safety and nutritional comparability of COR121 maize. The molecular characterization analyses conducted on COR121 maize demonstrated that the introduced genes are integrated at a single locus, stably inherited across multiple generations, and segregate according to Mendel's law of genetics.

The allergenic and toxic potential of the IPD083Cb and PMI proteins were evaluated and found unlikely to be allergenic or toxic to humans. Based on the weight of evidence, consumption of the IPD083Cb and PMI proteins is unlikely to cause an adverse effect on humans. A composition assessment demonstrated that the nutrient composition of COR121 maize forage and grain is comparable to that of conventional maize, represented by non-genetically modified (non-GM) near-isoline maize and non-GM commercial maize.

Overall, data and information contained herein support the conclusion that COR121 maize containing the IPD083Cb and PMI proteins is as safe and nutritious as conventional maize for food and feed uses.

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STUDY INDEX

[redacted] (2025) “Sequence Characterization of Insert and Flanking Genomic Regions of COR-ØØ121-4 Maize” Pioneer Study ID: 2025-1014

Appendix A. Methods for Whole Genome Sequencing Analysis

Test Materials

The test substance is maize event COR-ØØ121-4 contained within five generations ( ) of maize seed. The control substance is non-genetically modified (non-GM) maize (PH184C) seed that has the same genetic background as COR-ØØ121-4 maize.

The laboratory controls are the transformation plasmids .

DNA Extraction and Quantitation

Genomic DNA isolation was performed by Genomics Technologies (Pioneer; Johnston, IA, USA). Leaf tissue of ten COR121 maize plants from each generation ( ) and seven non-GM PH184C control maize plants were pulverized in tubes containing grinding beads using a Geno/Grinder (SPEX SamplePrep). Genomic DNA was isolated using a sbeadex DNA extraction kit (Biosearch Technologies). Following extraction, the DNA was quantified on a Varioskan LUX (Thermo Fisher Scientific).

The WGS sample set included:

- **Five COR121 maize samples** (generations), each prepared by pooling equal amount of DNA from ten individual plants of the corresponding generation.
- **One non-GM PH184C control maize sample** to identify endogenous junctions within the COR121 Intended Insertion, or plasmids .
- **Six positive control samples** (plasmids spiked into non-GM PH184C genomic DNA) to evaluate the WGS detection sensitivity at a single-copy genome equivalent for each plasmid.

Whole Genome Sequencing Analysis of COR121 Maize

1. WGS Library Construction and Sequencing

WGS libraries were prepared by Genomics Technologies (Pioneer Hi-Bred). Genomic DNA from each sample was sheared to an average fragment size of 500 base pairs (bp) using a Covaris E220 focused-ultrasonicator. Sheared DNA was end-repaired, A-tailed, and ligated to NEXTflex-96 DNA Barcodes (PerkinElmer) adapters using the KAPA Hyper Preparation Kit (Roche).

Libraries were PCR-amplified and quality checked using an Agilent TapeStation 4200, pooled, and then size-selected (450-750 bp) with the PippinHT system (SageScience). The final pooled libraries were verified for the size and concentration using the Agilent

TapeStation 4200. Following WGS library construction, the pooled libraries were submitted for NGS (Illumina Novaseq X Plus) as a paired-end sequencing run to achieve an average read depth of 150x of the maize genome.

2. WGS Quality Control and Filtering

Sequencing data were analyzed using WGS Data Analysis Pipeline v3.1.435.0 (Pioneer Hi Bred, International, Inc.), with additional analyses performed by Molecular and Protein Sciences. The sequencing reads were assigned to each corresponding individual sample based on the sample barcode adapter sequence. The sequencing reads for the COR121 [redacted] samples, and non-GM PH184C control maize sample were subsampled to bring them to 150x coverage of the genome. The sequencing data for the positive control samples were subsampled to represent a single copy genome equivalent dataset for each plasmid. After subsampling, if adapter sequence was found in the sequencing read, it was trimmed using Cutadapt, v2.10 (Martin, 2011). All reads less than 50 bp were removed from analysis.

The average sequencing depth of the maize genome was calculated using the Lander–Waterman formula (Lander and Waterman, 1988).

Reads were aligned to the sequences of plasmids and the COR121 Intended Insertion (Figures 1, 2, 3, 4, 6, 8 and 10) using Bowtie2 v2.3.4.2 (Langmead and Salzberg, 2012), allowing both full and partial read alignments. Reads that did not align to the COR121 Intended Insertion or plasmid sequences (genomic reads) were removed, and the duplicate reads were condensed for the remaining reads while preserving abundance data (Zastrow-Hayes *et al.*, 2015).

3. Junction Detection and Characterization

After removal of genomic reads, remaining reads were realigned to the COR121 Intended Insertion and plasmid sequences using Bowtie2 v2.3.4.2 with soft-trimming enabled to identify junctions. A junction is a chimeric sequencing read that partially aligns to the COR121 Intended Insertion or transformation plasmid, containing non-contiguous sequences such as plasmid–plasmid or plasmid–genome junctions. These chimeric reads, referred to as junction reads or junctions, were each assigned a unique identifier. The number of reads supporting each junction and the number of unique junctions for COR121 maize was quantified to identify insertion endpoints and evaluate the insertion copy number and organization.

Sequencing data from the non-GM PH184C control maize was processed through the same WGS pipeline. Unique endogenous junctions identified from non-GM PH184C control maize were used to filter identical junctions from COR121 dataset, ensuring retention of only unique junctions specific to the COR121 Intended Insertion or plasmids remained.

Results for the [redacted] generation of COR121 maize and control maize demonstrating the insertion copy number and organization and absence of unintended plasmid-derived

sequences are presented in the main body (Section A.3(c)) of this document. Results for five generations of transgenic COR121 maize demonstrating stability of the inserted DNA are also presented in Section A.3(c). Results for the positive control plasmids are presented below (Figure A.1).

Figure A.1. WGS Results for Positive Control Samples

The positive control samples consisted of transformation plasmid [redacted] DNA diluted in non-GM PH184C control maize genomic DNA and analyzed at 1 copy per genome. The blue coverage graph shows the number of individual NGS reads aligned at each point on the plasmid using a logarithmic scale at the middle of the graph. A red line in the coverage graph represents the read coverage exceeds the highest threshold. Green bars above the coverage graph indicate endogenous genetic elements are derived from the maize genome (identified by numbers; Table 5), while tan bars indicate genetic elements derived from other sources. Coverage was obtained across the entire length of all plasmids, demonstrating the sensitivity for detecting plasmid DNA in a complex genomic sample.

A) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 1). The two plasmid-plasmid junctions (red arrows) shown at the bottom of the graph are artifacts of aligning sequencing reads from a circular plasmid to a linear map. They show the start and end points (Junctions at bp [redacted] and bp [redacted]) of the plasmid sequence but do not indicate insertions in the genomic DNA of the non-GM PH184C control maize.

A. Alignment of NGS Reads to the Transformation Plasmid [redacted]

B) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 2). The two plasmid-plasmid junctions (red arrows) shown at the bottom of the graph are artifacts of aligning sequencing reads from a circular plasmid to a linear map. They show the start and end points (Junctions at bp [redacted] and bp [redacted]) of the plasmid sequence but do not indicate insertions in the genomic DNA of the non-GM PH184C control maize.

B. Alignment of NGS Reads to the Transformation Plasmid [redacted]

Figure A.1. WGS Results for Positive Control Samples (continued)

C) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 3). The two plasmid-plasmid junctions (red arrows) shown at the bottom of the graph are artifacts of aligning sequencing reads from a circular plasmid to a linear map. They show the start and end points (Junctions at bp [redacted] and bp [redacted]) of the plasmid sequence but do not indicate insertions in the genomic DNA of the non-GM PH184C control maize.

C. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

D) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 4). The two plasmid-plasmid junctions (red arrows) shown at the bottom of the graph are artifacts of aligning sequencing reads from a circular plasmid to a linear map. They show the start and end points (Junctions at bp [redacted] and bp [redacted]) of the plasmid sequence but do not indicate insertions in the genomic DNA of the non-GM PH184C control maize.

D. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Figure A.1. WGS Results for Positive Control Samples (continued)

E) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 6). The two plasmid-plasmid junctions (red arrows) shown at the bottom of the graph are artifacts of aligning sequencing reads from a circular plasmid to a linear map. They show the start and end points (Junctions at bp [redacted] and bp [redacted]) of the plasmid sequence but do not indicate insertions in the genomic DNA of the non-GM PH184C control maize.

E. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

F) Alignment of sequencing reads against plasmid [redacted] ([redacted] bp; Figure 8). The two plasmid-plasmid junctions (red arrows) shown at the bottom of the graph are artifacts of aligning sequencing reads from a circular plasmid to a linear map. They show the start and end points (Junctions at bp [redacted] and bp [redacted]) of the plasmid sequence but do not indicate insertions in the genomic DNA of the non-GM PH184C control maize.

F. Alignment of NGS Reads to the Transformation Plasmid [redacted]

[redacted]

Appendix B. Methods for Multi-Generation Segregation Analysis

Greenhouse Experimental Design

Five generations of COR121 maize ( ) were evaluated using quantitative digital PCR (dPCR) analyses to confirm Mendelian inheritance of genotype. Individual seed chips were collected for each entry from the generations for dPCR analysis with the corresponding remnant seed per entry retained for planting, as needed. All germinated remnant seeds were grown in a controlled environment under suitable conditions for producing maize plants. For generation plants, 96 seeds were analyzed per entry.

Genotypic Analyses

Prior to analysis, seed chip samples were collected from seeds representing all five generations. Using a nail clipper, a small 'chip' of the seed containing seed coat and endosperm was removed from each seed and placed into individual wells of 96-well collection plates. Each seed (now with a chip removed) was placed into a corresponding well within 96-well bubble packs. Each plate and well and corresponding bubble pack were uniquely labeled to allow a given seed chip sample to be tracked back to the originating seed. The seed chip samples were used for genotypic trait confirmation of the target event by quantitative digital PCR or qualitative endpoint PCR. The seed chip samples were used for determination of the copy number of the COR121 maize event and the following genetic elements by dPCR: COR-ØØ121-4, *ipd083Cb.1*, BD-Actin, BSV(AY)TR, and AT-T9 Terminator.

Statistical Analysis

A chi-square test was performed at the 0.05 significance level on the segregation results of and generations of COR121 maize. The observed segregation ratio was determined by genotypic results. Samples were identified as positive if confirmed to contain the COR121 maize event and the associated gene and genetic elements (one or two copy), and samples were identified as negative if not containing the gene and genetic elements (NULL). A chi-square test was performed separately for each generation to compare the observed segregation ratio to the expected segregation ratios (3:1). A chi-square test was not performed for the generations of COR121 maize as all samples were identified as positive as expected for a homozygous generation. Statistical analyses were conducted using SAS software, Version 9.4 (SAS Institute Inc.).

Appendix C. Methods for Event Sequencing Analysis

Test Materials

The test substance in the study was defined COR-ØØ121-4 contained within the maize seed. The control substance was defined as seed from a conventional (non-genetically modified [non-GM]) maize line, PH184C, that has a similar genetic background to the test substance but does not contain the COR121 maize insertion.

DNA Extraction and Quantification

Genomic DNA (gDNA) from COR121 maize and non-GM control maize was extracted from finely ground fresh leaf tissue using the Synergy 2.0 Plant DNA Extraction Kit (OPS Diagnostics). For COR121 maize, approximately 10 mg of leaf tissue from each of five plants was used for extraction. For the non-GM control maize, two replicates of approximately 10 mg of leaf tissue from each of two control plants were used for extraction.

Tissue samples were lysed in OPS Homogenization Buffer with the grinding matrix and heated at 60-65 °C. The resulting supernatants were treated with RNase A, mixed with isopropanol, loaded onto a single silica-based column, washed twice with 70% ethanol, and eluted in EB buffer. The quality of the extracted gDNA was assessed by agarose gel electrophoresis and visualized under ultraviolet light, and the concentration was determined using a Qubit Flex Fluorometer (Thermo Fisher Scientific).

Polymerase Chain Reaction (PCR) Amplification of the Insert and Flanking Genomic Regions in COR121 Maize

All PCR primers were designed based on the sequences of [redacted] recombination fragment region, and the maize genome. PCR primers were designed to amplify three overlapping PCR fragments (A, B, and C) spanning the insert and its 5' and 3' flanking genomic regions. PCR Fragments A and C contained sequence from the insert and either the 5' or 3' flanking genomic region, whereas PCR Fragment B contained sequence from only the COR121 insert. All primers were synthesized by Integrated DNA Technologies.

Two independent PCR reactions were performed for each fragment using gDNA extracted from COR121 maize. All PCR fragments were generated using 100 ng of gDNA as a template with each primer at a final concentration of 0.25 µM in a 50-µl reaction. Phusion Plus Master Mix (Thermo Fisher Scientific) was used to amplify all PCR fragments. PCR conditions optimized to yield targeted PCR products are detailed in Table C.1. The gDNA extracted from non-GM control maize served as a negative control.

Table C.1. PCR Fragment Amplification Conditions for COR121 Maize

Cycles	A	B	C
1×	98 ºC 30"	98 ºC 30"	98 ºC 30"
40×	98 ºC 10" 66 ºC 5" 72 °C 2'30"	98 ºC 10" 68 ºC 5" 72 °C 2'30"	98 ºC 10" 62 ºC 5" 72 °C 2'30"
1×	72 ºC 5' 4 °C ∞	72 ºC 5' 4 °C ∞	72 ºC 5' 4 °C ∞

Note: Time is indicated in minutes (') and seconds (").

Purification and Pooling of PCR Products

PCR products from each fragment were pooled and gel-purified following the QIAquick Gel Extraction Kit Protocol (Qiagen). Gel slices were incubated at 55-65 °C in buffer QG to fully dissolve the agarose, and the resulting solution was applied to a silica column. Following two washes of the column with buffer PE, the DNA was eluted using EB buffer. The concentration of the purified PCR products was then determined using a Qubit Flex Fluorometer.

Amplicon Next-Generation Sequencing and Data Analysis

Purified PCR amplicons were submitted to Genomics Technologies for NGS library preparation and sequencing. Library construction utilized enzymatic fragmentation of PCR products, followed by end-repair and A-tailing using the Watchmaker DNA Library Prep Kit with Fragmentation (Watchmaker Genomics). Dual-indexed adapters (NEXTFLEX Unique Dual Index; Revvity) were ligated to fragments per manufacturer protocols. Post-ligation PCR amplification enabled sample multiplexing via unique adapter sequences.

Library quality was assessed using the Agilent 4200 TapeStation System with D1000 TapeStation Kit (Agilent Technologies) and quantified via Qubit Fluorometer (Thermo Fisher Scientific). The pooled library was denatured and sequenced on the Element Biosciences AVITI system using paired-end 150-bp chemistry. Sequencing read depth targeted a minimum mean of 100x per sample.

High-quality reads were trimmed and aligned to the expected PCR fragment sequence using BWA (v0.7.8; Li *et al.* (2009)). Variations, including SNPs and indels, were analyzed using SAMtools (v1.21; Danecek *et al.* (2021)) and the Genome Analysis Toolkit (GATK) (v4.1.8.0; Poplin *et al.* (2018)). A consensus sequence was generated for each PCR product, with all analyses performed using the Regulatory Amplicon Analysis Pipeline (RAAP v1.1.1).

The consensus sequences of the three overlapping PCR fragments were combined in Geneious Prime (v2025.1.2; GraphPad Software LLC) using default parameters to determine the sequence for COR121 maize, and the determined sequence was compared with the intended landing pad sequences from plasmid [redacted] and the recombination fragment sequence from plasmid [redacted]

Appendix D. Methods for Characterization of the IPD083Cb Protein

Test Materials

COR121 Maize-Derived IPD083Cb Protein

The IPD083Cb protein was isolated from leaf tissue derived from COR121 maize. The leaf tissue was collected at the R1 growth stage (the stage when silks become visible; Abendroth et al., 2011) of development from plants grown at a field location (Johnston, IA, USA). The IPD083Cb protein was extracted from lyophilized maize tissue by homogenization using phosphate-buffered saline containing polysorbate 20 (PBST) with 50 mM L-arginine, 50 mM L-glutamic acid extraction buffer and then clarified by centrifugation and filtration. The filtered extract was purified by immunoaffinity chromatography. Immunoaffinity chromatography (IAC) was used to purify IPD083Cb from the filtered extract, which was eluted using IgG elution buffer (Thermo Scientific). Select elutions were pooled and buffer exchanged in 10 mM CAPS with 50 mM L-arginine, 50 mM L-glutamic acid, pH 10.5, using Econo Pac 10DG desalting columns (BioRad).

Following extraction, purification, and concentration, the final volume in the concentrator was estimated, NuPAGE LDS Sample Buffer (4X) (Life Technologies) was added at 25% along with 10% NuPAGE Reducing Agent (10X) containing DTT (Life Technologies) to the concentrated sample, in the concentrator. The sample in the concentrator was transferred to a microcentrifuge tube, heat-treated, and stored frozen (-20 °C freezer unit).

Tobacco-Expressed IPD083Cb Protein

In order to have sufficient amounts of the purified IPD083Cb protein for the multiple studies required to assess its safety, the IPD083Cb protein was produced by iBio CDMO for Pioneer using a *Nicotiana benthamiana* protein expression system. The protein was first purified by immobilized Metal Affinity Chromatography (IMAC), followed by anion exchange chromatography, then concentrated and buffer exchanged. After lyophilization and mixing, a lot number was assigned.

SDS-PAGE Analysis

The purified COR121 maize-derived IPD083Cb protein sample was thawed, diluted as applicable for the sensitivity of the assay in 1X LDS/DTT, heated, and then loaded into 4-12% Bis-Tris gels along with prestained protein molecular weight markers (Precision Plus Protein Dual Xtra Standards; Bio-Rad). The tobacco-expressed IPD083Cb protein was thawed, diluted in 1X LDS/DTT to approximately the same concentration as the maize-derived IPD083Cb protein sample, and loaded into the same gels to 1 µg for SDS-PAGE and 10 ng for western blot analysis. Electrophoresis was conducted using a pre-cast gel electrophoresis system at a constant 200 volts for 35 minutes.

Upon completion of electrophoresis, the gels were either prepared for protein staining or protein transfer to a membrane for sequencing or western blot analysis.

For Coomassie staining, following electrophoresis, the gel was washed with water and stained with GelCode Blue Stain Reagent (Thermo Scientific). Following staining, the gel was de-stained with water until the gel background was clear. Protein bands were stained on the gel and the gel image was captured electronically using a ChemiDoc MP (Bio-Rad) imaging system.

Western Blot Analysis

Following SDS-PAGE as described previously, the resulting gel was assembled into a nitrocellulose (NC) iBlot Gel Transfer Stack. An iBlot Gel Transfer Device was used to transfer proteins from the gel to the NC membrane.

Following protein transfer, the membrane was blocked in PBST containing 5% weight/volume (w/v) non-fat dry milk. Before and after the blocking step, the membrane was washed with PBST to reduce the background. The blocked membrane was incubated with a rabbit polyclonal antibody R3373 (Pioneer Hi-Bred International, Inc.) diluted 1:5000 in PBST containing 1% w/v non-fat dry milk. Following primary antibody incubation, the membrane was washed in PBST. The membrane was incubated with a secondary antibody (anti-rabbit IgG, horseradish peroxidase conjugate; Promega Corporation) diluted 1:10,000 in PBST containing 1% non-fat dry milk. Following secondary antibody incubation, the membrane was washed in PBST. The membrane remained in PBST prior to incubating with a chemiluminescent substrate (Thermo Scientific). The chemiluminescent signal and the pre-stained markers were detected and captured using a ChemiDoc MP (Bio-Rad) imaging system.

Protein Glycosylation Analysis

COR121 Maize-Derived IPD083Cb Protein

A Pierce Glycoprotein Staining Kit (Thermo Scientific) was used to determine whether the COR121 maize-derived IPD083Cb protein was glycosylated. The purified IPD083Cb protein, a positive control protein (horseradish peroxidase), and a negative control protein (soybean trypsin inhibitor) were run by SDS-PAGE as described previously. For glycosylation staining, the maize derived IPD083Cb protein was loaded on to the gel at approximately the same concentration as the positive and negative control proteins (1 µg).

Following electrophoresis, the gel was washed with water, fixed with 50% methanol, and washed with 3% acetic acid. The gel was then incubated with oxidizing solution and washed with 3% acetic acid. The gel was incubated with glycoprotein staining reagent and then incubated in a reducing reagent. The gel was then washed with 3% acetic acid and then washed in water. Glycoproteins were detected as stained bands on the gel.

Following glycoprotein detection, the image of the gel was captured electronically using a ChemiDoc MP (Bio-Rad) imaging system. The same gel was then stained with GelCode Blue stain reagent (Thermo Scientific) and washed with water to visualize all protein bands. The image of the GelCode stained gel was then captured electronically.

Tobacco-Expressed IPD083Cb Protein

A Pierce Glycoprotein Staining Kit was used to determine whether the tobacco-expressed IPD083Cb protein was glycosylated. For glycosylation staining, 1 µg of IPD083Cb protein was loaded on to the gel. The IPD083Cb protein, a positive control protein (horseradish peroxidase), and a negative control protein (soybean trypsin inhibitor) were run by SDS-PAGE as described above.

Following electrophoresis, the gel was washed with water twice for 5 minutes each wash, fixed with 50% methanol for 30-35 minutes, and washed twice with 3% acetic acid for 10-15 minutes each wash. The gel was then incubated with oxidizing solution for 15-20 minutes and washed three times with 3% acetic acid for 5-7 minutes each wash. The gel was incubated with glycoprotein staining reagent for 15-20 minutes and then incubated in a reducing reagent for 5-7 minutes. The gel was then washed once with 3% acetic acid and once in water for 5 minutes each wash. Glycoproteins were detected as bands stained a magenta color on the gel.

Following glycoprotein detection, the image of the gel was captured electronically using a ChemiDoc MP imaging system. The same gel was then stained with GelCode Blue stain reagent for 60 minutes followed by three washes with water for 5 minutes each wash to visualize all protein bands. The image of the GelCode stained gel was then captured electronically.

Peptide Mapping by Mass Spectrometry

COR121 Maize-Derived IPD083Cb Protein

For mass spectrometry sequencing analyses, the COR121 maize-derived IPD083Cb protein was loaded onto a gel in each of two lanes. Following SDS-PAGE and Coomassie staining using the methods as described previously, protein bands at the expected molecular weight of the COR121 maize-derived IPD083Cb protein were excised from the gel and stored frozen (-20 °C freezer unit). The protein in each gel slice was reduced with DTT, alkylated with iodoacetamide, and then subsequently digested with trypsin or chymotrypsin. The digested samples were separated on a nanoACQUITY UPLC (Waters Corporation) fitted with a Peptide BEH C18, 300 Å, 1.7 µm column (75 µm x 100 mm; Waters Corporation) by gradient elution. Eluent from the column was directed into an electrospray source, operating in positive ion mode, on a TripleTOF 5600+ hybrid quadrupoleTOF-mass spectrometer (AB Sciex; currently Sciex). The resulting mass spectrometry (MS) data were processed using MSConvert to produce a peak list. The peak list was used to perform an MS/MS ion search (Mascot Software Version 2.8.0) and match peptides from the expected IPD083Cb protein sequence (Perkins *et al.*, 1999). The following search parameters were used: peptide and fragment mass tolerance, ± 0.1 daltons (Da); fixed modifications, cysteine carbamidomethyl; variable modifications, methionine oxidation and acetyl protein N-terminal; and maximum missed cleavages, 1 for trypsin and 2 for chymotrypsin. The Mascot-generated peptide ion score threshold was > 13, which indicates identity or extensive homology ($p < 0.05$). The combined sequence coverage was calculated with GPMAW Version 14.02.

Tobacco-Expressed IPD083Cb Protein

For mass spectrometry sequencing analyses, 4 µg of IPD083Cb protein was loaded onto a gel in each of three lanes. Following SDS-PAGE, Coomassie staining, and gel imaging using the methods as described previously, protein bands at the expected molecular weight of IPD083Cb protein were excised from a gel and stored frozen (-20 °C freezer unit). The protein in two of the gel slices was reduced with DTT, alkylated with iodoacetamide, and then subsequently digested with trypsin or chymotrypsin. The digested samples were separated on a nanoACQUITY UPLC (Waters Corporation) fitted with a Peptide BEH C18 300 Å 1.7 µm column (75 µm x 100 mm; Waters Corporation) by gradient elution. Eluent from the column was directed into an electrospray source, operating in positive ion mode, on a TripleTOF 5600+ hybrid quadrupole-TOF mass spectrometer (AB Sciex; currently Sciex). The resulting MS data were processed using MS Data Converter to produce a peak list. The peak list was used to perform an MS/MS ion search (Mascot Software version 2.8.0) and match peptides from the expected IPD083Cb protein sequence (Perkins *et al.*, 1999). The following search parameters were used: peptide and fragment mass tolerance, ± 0.1 Da; fixed modifications, cysteine carbamidomethyl; variable modifications, methionine oxidation, acetyl (protein N-terminal); and maximum missed cleavages, 1 for trypsin and 2 for chymotrypsin. The Mascot-generated peptide ion score threshold was > 13, which indicates identity or extensive homology ($p < 0.05$). The combined sequence coverage was calculated with GPMAW version 12.10.0.

N-Terminal Amino Acid Sequencing Analysis

COR121 Maize-Derived IPD083Cb Protein

The N-terminal amino acid sequence was also determined through peptide mapping as Edman degradation did not obtain any sequence data suggesting the N-terminus is blocked.

Tobacco-Expressed IPD083Cb Protein

The N-terminal amino acid sequence was also determined through peptide mapping as it was determined with previous IPD083Cb protein lots that the N-terminus is blocked, rendering Edman degradation unsuccessful.

Bioactivity Bioassay

The biological activity of IPD083Cb protein was evaluated by conducting a 7-day bioassay using *Chrysodeixis includens* (soybean looper; Lepidoptera: Noctuidae), a species sensitive to the IPD083Cb protein. Eggs were obtained from [redacted] and their identity was recorded by study personnel.

The bioassay was conducted in a 48-well bioassay plate with the plate considered a single block. Treatments were randomized by column with a total of 24 replicates per treatment. *C. includens* larvae were exposed via oral ingestion to one of the following two treatments prepared by surface application of dosing solutions to the targeted protein concentration per cm2 diet:

- Treatment 1: Buffer Control Diet (containing a dosing solution of 25 mM Tris-HC1, pH 8, 100 mM NaCl, 0.01% PS-80; referred to as buffer)

- Treatment 2: Test Diet (targeting 50 µg IPD083Cb protein per cm^2)

The carrier for the *C. includens* bioassay consisted of an agar-based artificial diet prepared by Pioneer by blending dry Southland pre-mix diet and other diet ingredients with molten agar. The blended diet was stirred on a hot plate until ready to dispense. Prior to the beginning of the bioassay, approximately 500 µl of freshly prepared agar diet was dispensed into wells of the bioassay plates and allowed to cool and solidify. Plates were stored refrigerated (~ 4 °C) until use.

An IPD083Cb stock solution was prepared by solubilizing 5.0 mg of test substance in 1.788 ml of chilled buffer to a nominal concentration of 1.6 mg/ml. The stock solution was diluted in buffer to prepare the test dosing solution at a targeted concentration of 1500 ng/µl. The buffer control dosing solution consisted of the buffer used to dilute the test substance. The IPD083Cb stock solution and the test and buffer control dosing solutions were prepared fresh on each day of diet distribution (Day 0 and Day 4). Dosing solutions were maintained chilled (on wet ice) until use.

C. includens eggs were incubated in an environmental chamber until the eggs hatched. Neonates were used in the bioassay within 24 hours of hatching. On Day 0 of the bioassay, dosing solutions were dispensed topically by treatment to assigned wells using a surface application (25 µl per well) and plates were dried under a hood. One *C. includens* neonate was placed in each well containing diet, each bioassay plate was sealed with heat-sealing film, and a small hole was poked over each well to allow for ventilation. The bioassay was conducted in an environmental chamber set at 25 °C, 65% relative humidity, and continuous dark. On Day 4, new bioassay plates were prepared with fresh diet as described for Day 0, organisms were transferred to corresponding wells of the new plates, and the plates were sealed with a hole for ventilation and placed in the environmental chamber. After 7 days, the bioassay was complete, mortality was assessed, and surviving larvae were individually weighed.

The bioassay acceptability criterion indicated the bioassay may be repeated if the combined number of dead and missing organisms exceeds 20% for the buffer control diet (Treatment 1) group. The bioassay met the acceptability criteria (16.7% combined dead and missing).

Thermolability Analysis

The test substance consisted of IPD083Cb protein solubilized in study from a lyophilized powder (lot number PCF-0061A). The carrier consisted of an agar-based artificial diet. The bioassay control dosing solutions used to prepare Treatment 1 consisted of chilled ultrapure (American Society for Testing and Materials [ASTM] Type 1) water. The bulk dosing solutions used to prepare Treatments 2-6 consisted of aliquots of the test substance diluted in chilled ultrapure water to achieve the concentration in each treatment. The dosing solutions used to prepare Treatments 3-6 were incubated for 30-35 minutes at various temperatures.

The test system was *Anticarsia gemmatalis* (velvetbean caterpillar; Lepidoptera: Erebidae). The test system was chosen because *A. gemmatalis* is an insect sensitive to IPD083Cb protein. *A. gemmatalis* larvae were exposed via oral ingestion to one of the following six treatments prepared by surface application of dosing solutions to agar-based artificial diet:

- Treatment 1: Bioassay Control Diet with a dosing solution of ultrapure water
- Treatment 2: Control Diet with the unheated IPD083Cb protein dosing solution

- Treatment 3: Test Diet with the IPD083Cb protein dosing solution incubated at 25 °C
- Treatment 4: Test Diet with the IPD083Cb protein dosing solution incubated at 50 °C
- Treatment 5: Test Diet with the IPD083Cb protein dosing solution incubated at 75 °C
- Treatment 6: Test Diet with the IPD083Cb protein dosing solution incubated at 95 °C

Treatments 2-6 were prepared to a targeted protein concentration of 50 µg IPD083Cb protein per cm2 diet. Incubations for the heated test dosing solutions used to prepare Treatments 3-6 were performed for 30-35 minutes. Treatments were arranged in a generalized randomized block design with a total of four blocks. Each block consisted of a 48-well bioassay plate and contained six replicates randomized by column from each treatment. Each treatment was provided to a target of 24 *A. gemmatalis* individuals. The bioassay was conducted in an environmental chamber set at 25 °C, 65% relative humidity, and continuous dark. Larvae were refed on Day 4. After 7 days, the bioassay was complete, mortality was assessed, and surviving organisms were individually weighed.

The bioassay acceptability criteria indicated the bioassay may be terminated and repeated if: the combined number of dead and missing organisms exceeds 20% for the bioassay control (Treatment 1) group and the mortality rate does not exceed 80% in the unheated control (Treatment 2) group. The *A. gemmatalis* bioassay met the acceptability criteria (0% dead and 0% missing in Treatment 1; 100% mortality in Treatment 2). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and densitometry analysis were used to determine the concentration of the IPD083Cb protein test substance and to verify the concentration of IPD083Cb protein in the bulk dosing solutions used to prepare Treatments 2-6. Bias in the *A. gemmatalis* bioassay was controlled through the randomization of treatments within blocks and the use of one or more control diets.

On each day of diet preparation, dosing solutions for Treatments 1-6 were prepared and heat-treated as applicable. Dosing solutions were maintained chilled (in a refrigerator set at 4 °C or on wet ice) until use. *A. gemmatalis* eggs were incubated in an environmental chamber until the eggs hatched. *A. gemmatalis* neonates were used in the bioassay within 24 hours of hatching.

On Day 0 of the bioassay, dosing solutions were dispensed by treatment to assigned wells using a surface application (25 µl per well) and plates were dried under a hood. One *A. gemmatalis* neonate was placed in each well containing a treatment. Each plate was sealed with heat-sealing film and ventilated with a small hole over each well. The plates were placed in an environmental chamber. On Day 4, new bioassay plates were removed from refrigerated storage and prepared with surface applications of the dosing solutions as described for Day 0, living organisms were transferred to the new plates, missing or dead organisms were recorded, and the freshly prepared plates were placed in the environmental chamber. After 7 days, the bioassay was complete, mortality was assessed, and surviving larvae were individually weighed.

Statistical Analysis

Statistical analyses of data were conducted using SAS software, Version 9.4 (SAS Institute Inc.). The response variable of interest was mortality. Statistical comparisons were made between *A. gemmatalis* fed diets containing heated IPD083Cb protein (Treatments 3-6) and those fed a diet

containing unheated IPD083Cb protein (Treatment 2) for mortality. Statistical analysis was conducted using Fisher's exact test to determine if the mortality rate of *A. gemmatalis* fed diets containing the heated IPD083Cb protein (m_T) was less than the mortality rate of those fed the control diet with unheated IPD083Cb protein diet (m_C). The corresponding hypothesis test was:

$$H_0: m_T - m_C = 0 \quad vs. \quad H_a: m_T - m_C < 0.$$

A significant difference was established if the P-value was < 0.05. SAS PROC MULTTEST was utilized to conduct the Fisher's exact test.

Digestibility in Simulated Gastric Fluid (SGF)

Test and control solutions were prepared as follows:

- The gastric control solution was prepared fresh on the day of use and was comprised of 0.2% weight per volume (w/v) NaCl in 0.7% volume per volume (v/v) HCl, with a pH of ~1.2.
- The pepsin digestion solution, referred to as simulated gastric fluid (SGF), was prepared fresh on the day of use by dissolving pepsin (Sigma-Aldrich) into gastric control solution. The SGF was prepared so that the pepsin to protein ratio of the final digestion mixture was approximately 10 units of pepsin per µg of test or control protein.
- The test substance consisted of IPD083Cb protein solubilized from a lyophilized powder (lot number PCF-0061A).
- To prepare the solutions for each of the control proteins (BSA and β-lactoglobulin), a 5.0-mg sub sample of powder was weighed into an individual tube for each control and solubilized by adding 1.0 ml of ultrapure water to a target protein concentration of 5 mg/ml.
- The final concentration of the protein and pepsin in the SGF reaction mixture was 0.25 mg/ml IPD083Cb protein and 2500 units/ml pepsin.

SGF solution (1900 µl) was dispensed into a 7-ml glass vial and placed in a 37 °C water bath for 2-5 minutes prior to the addition of 100 µl of IPD083Cb protein solution at Time 0. The digestion reaction mixture was mixed constantly using a stir bar and a submersible magnetic stirrer.

A 120-µl sub-sample of the IPD083Cb protein digestion reaction mixture was removed from the vial at the following analytical time points (± 10 seconds): 0.25, 1, 2, 5, 10, 30, and 60 minutes. The sub samples were inactivated by neutralization with 139 µl of pre-mixed sample stop solution and heating to 90-100 °C for 5 minutes prior to frozen storage (-20 °C freezer unit).

To prepare control digestion samples at 1 and 60 minutes, a 114-µl sample of the appropriate digestion solution (see table) was pre-warmed in a 37 °C water bath for 2-5 minutes prior to adding 6 µl of the IPD083Cb protein solution, control protein solution, or ultrapure water. The tubes were incubated in the water bath for the allotted time and then inactivated by mixing with 139 µl of the pre-mixed sample solution and heating at 90-100 °C for 5 minutes.

The Time 0 control reaction mixtures were prepared by first neutralizing 114 µl of the appropriate digestion solution (see table) with 139 µl of the pre-mixed sample solution, and then heating at 90 100 °C for 5 minutes. A 6-µl sample of the appropriate IPD083Cb protein solution, control protein solution, or ultrapure water was then added, and the mixture was heated again at 90 100 °C for 5 minutes.

Following digestion and inactivation, all control reaction mixtures were heated at 90-100 °C for 5 minutes prior to frozen storage (-20 °C freezer unit).

Control digestion samples included in the SGF assay are provided in Table D.1.

Table D.1. Control Samples for Simulated Gastric Fluid (SGF) Digestibility Analysis

Protein	Digestion Solution	Digestion Time (min)		
		0	1	60
None (SGF Control – Ultrapure water)	SGF	X	--	X
BSA	SGF	X	X	X
β-lactoglobulin	SGF	X	X	X
IPD083Cb	SGF	X	--	--
IPD083Cb	None (Ultrapure Water)	X	--	X
IPD083Cb	Gastric Control Solution (No Pepsin)	--	--	X

SDS-PAGE Analysis

The IPD083Cb protein digestion time-course samples and control samples were removed from frozen storage, heated at 90-100 °C for 5 minutes, and loaded (10 µl/well) into 4-12% Bis-Tris gels for SDS PAGE analysis. To demonstrate the sensitivity of the SDS-PAGE gel and western blot analyses, an aliquot of the IPD083Cb protein in SGF (Time 0) sample was loaded into the gel at a 1:20 dilution for protein staining, and at a 1:100 dilution for the western blot. Pre-stained protein molecular weight markers (Precision Plus Dual Xtra Standards) were also loaded into each gel. Electrophoresis was conducted using a pre-cast gel electrophoresis system with 1X MES SDS running buffer at a constant 200 volts (V) for 35 minutes.

Upon completion of electrophoresis, the gels were removed from the gel cassettes for use in protein staining or western blot analyses. For protein staining, the gels were washed with water and stained with GelCode Blue Stain Reagent. Following staining, the gels were destained with water until the gel background was clear. Protein bands were stained on the gels. The gel image was captured electronically using a ChemiDoc MP (Bio-Rad) imaging system.

Western Blot Analysis

The IPD083Cb protein digestion time-course samples were also analyzed by western blot. Following SDS PAGE, one of the resulting gels was assembled into a mini nitrocellulose (NC) iBlot Gel Transfer Stack. An iBlot Gel Transfer Device was used to transfer proteins from the gel to the NC membrane.

Following protein transfer, the membrane was blocked in phosphate-buffered saline containing polysorbate 20 (PBST) containing 5% (w/v) non-fat dry milk. Before and after the blocking step, the membrane was washed with PBST to reduce the background. The blocked membrane was incubated with an IPD083Cb polyclonal antibody 3373 (Pioneer Hi Bred International, Inc.) diluted 1:100,000 in PBST containing 1% (w/v) non-fat dry milk. Following primary antibody incubation, the membrane was washed in PBST and incubated with a secondary antibody (anti-rabbit IgG, horseradish peroxidase conjugate; Promega Corporation) diluted 1:100,000 in PBST containing 1% (w/v) non-fat dry milk. The membrane was washed and remained in PBST prior to incubating with a chemiluminescent substrate. The chemiluminescent signal and the pre-stained markers were detected and captured using a ChemiDoc MP imaging system.

Digestibility in Simulated Intestinal Fluid (SIF)

Test and control solutions were prepared as follows:

- The pancreatin digestion solution, referred to as simulated intestinal fluid (SIF), was prepared fresh on the day of use by dissolving 52.6 mg of pancreatin (Sigma-Aldrich) into 5 ml of intestinal control solution (I-Con 1X buffer) to a final concentration of 0.5% weight per volume (w/v) pancreatin and 50 mM KH_2PO4, pH 7.5.
- To prepare the solutions for each of the control proteins (BSA and β-lactoglobulin), a 5.0-mg sub sample of powder was weighed into an individual tube for each control and solubilized by adding 1.0 ml of ultrapure water to a target protein concentration of 5 mg/ml.
- The test substance consisted of IPD083Cb protein solubilized from a lyophilized powder (lot number PCF-0061A).
- The final concentration of the protein and pancreatin in the SIF reaction mixture was 0.25 mg/ml IPD083Cb protein and 1% (w/v) pancreatin.

SIF solution (1900 µl) was dispensed into a 7-ml glass vial and placed in a 37 °C water bath for 2-5 minutes prior to the addition of 100 µl of IPD083Cb protein solution at Time 0. The digestion reaction mixture was mixed constantly using a stir bar and a submersible magnetic stirrer.

A 120-µl sub-sample of the IPD083Cb protein digestion reaction mixture was removed from the vial at the following analytical time points (± 10 seconds): 0.25, 1, 2, 5, 10, 30, and 60 minutes. The sub samples were inactivated by adding them to pre-labeled tubes containing 64 µl of pre-mixed sample solution and heating to 90-100 °C for 5 minutes prior to SDS-PAGE analysis and subsequent frozen storage (-20 °C freezer unit).

To prepare control digestion samples at 1 and 60 minutes, a 114-µl sample of the appropriate digestion solution (see table below) was pre-warmed in a 37 °C water bath for 2-5 minutes prior to adding 6 µl of the IPD083Cb protein solution, control protein solution, or ultrapure water. The tubes were incubated in the water bath for the allotted time and then inactivated by mixing with 64 µl of the pre-mixed sample solution.

The Time 0 control reaction mixtures were prepared by first neutralizing 114 µl of the appropriate digestion solution (see table below) with 64 µl of the pre-mixed sample solution, and then heating at 90-100 °C for 5 minutes. A 6-µl sample of the appropriate IPD083Cb protein solution, control

protein solution, or ultrapure water was then added, and the mixture was heated again at 90-100 °C for 5 minutes.

Following digestion and inactivation, all control digestion samples were analyzed by SDS-PAGE analysis and then stored frozen (-20 °C freezer unit).

Control digestion samples included in the SIF assay are provided in Table D.2.

Table D.2. Control Samples for Simulated Intestinal Fluid (SIF) Digestibility Analysis

Protein	Digestion Solution	Digestion Time (min)		
		0	1	60
None (SIF Control-Ultrapure water)	SIF	X	--	X
BSA	SIF	X	X	X
β-lactoglobulin	SIF	X	X	X
IPD083Cb	SIF	X	--	--
IPD083Cb	None (Ultrapure water)	X	--	X
IPD083Cb	Intestinal Control Solution (No Pancreatin)	--	--	X

SDS-PAGE Analysis

The IPD083Cb protein digestion time-course samples and control samples were heated at 90100 °C for 5 minutes and loaded (10 µl/well) into 4-12% Bis-Tris gels for SDS-PAGE analysis. Prestained protein molecular weight markers (Precision Plus Dual Xtra Standards) were also loaded into each gel. Electrophoresis was conducted using a pre-cast gel electrophoresis system with 1X MES SDS running buffer at a constant 200 volts (V) for 35 minutes.

Upon completion of electrophoresis, the gels were removed from the gel cassettes for use in protein staining or western blot analyses. For protein staining, the gels were washed with water and stained with GelCode Blue Stain Reagent. Following staining, the gels were destained with water until the gel background was clear. Protein bands were stained on the gels. The gel image was captured electronically using a ChemiDoc MP (Bio-Rad) imaging system.

Western Blot Analysis

The IPD083Cb protein digestion time-course samples were also analyzed by western blot. Following SDS PAGE, one of the resulting gels was assembled into a mini nitrocellulose (NC) iBlot Gel Transfer Stack. An iBlot Gel Transfer Device was used to transfer proteins from the gel to the NC membrane.

Following protein transfer, the membrane was blocked in phosphate-buffered saline containing polysorbate 20 (PBST) containing 5% (w/v) non-fat dry milk. Before and after the blocking step, the membrane was washed with PBST to reduce the background. The blocked membrane was incubated with an IPD083Cb polyclonal antibody 3373 (Pioneer Hi Bred International, Inc.) diluted 1:100,000 in PBST containing 1% (w/v) non-fat dry milk. Following primary antibody incubation, the membrane was washed in PBST and incubated with a secondary antibody

(anti-rabbit IgG, horseradish peroxidase conjugate; Promega Corporation) diluted 1:100,000 in PBST containing 1% (w/v) non-fat dry milk. The membrane was washed and remained in PBST prior to incubating with a chemiluminescent substrate. The chemiluminescent signal and the pre-stained markers were detected and captured using a ChemiDoc MP imaging system.

Sequential Digestibility Analysis with Simulated Gastric Fluid (SGF) and Simulated Intestinal Fluid (SIF)

Test solutions were prepared as follows:

- A 2X concentrated pepsin digestion solution, referred to as simulated gastric fluid (SGF), was prepared fresh on the day of use by solubilizing pepsin (Sigma-Aldrich) in a previously prepared 2X gastric control solution. The final concentration of gastric control solution in the final digestion mixture was 0.2% weight per volume (w/v) NaCl and 0.7% volume per volume (v/v) HCl; pH ~1.2. The SGF was prepared so that the pepsin to protein ratio of the final digestion mixture was approximately 8.6 units of pepsin per µg of test substance.
- A 2.5X concentrated pancreatin digestion solution, referred to as simulated intestinal fluid (SIF), was prepared fresh on the day of use by solubilizing pancreatin (Sigma-Aldrich) in 2.5X intestinal control solution. The final concentration of intestinal control solution in SIF was 50 mM KH_2PO_4, pH ~7.5. Pancreatin content in SIF was adjusted so that there was approximately 0.5% (w/v) pancreatin in the final digestion reaction mixture.
- The pre-mixed sample solutions used to inactivate samples were prepared fresh on the day of use. The stop solution for SGF reactions was prepared by mixing 1200 µl of 200 mM Na_2CO_3, 1625 µl of NuPAGE 4X LDS Sample Buffer, and 650 µl NuPAGE 10X Sample Reducing Agent containing DTT. The sample solution for SIF reactions was prepared by mixing 1150 µl NuPAGE 4X LDS Sample Buffer and 450 µl NuPAGE 10X Sample Reducing Agent containing DTT.

In Vitro Pepsin Digestion

IPD083Cb Protein in SGF 10 Minutes Sample for Sequential Digestion: A 1-ml aliquot of the 2X SGF solution was dispensed into a 7-ml glass vial and pre-warmed in the 37 °C water bath for 2-5 minutes prior to addition of 800 µl ultrapure water and 200 µl of the IPD083Cb protein solution. The SGF digestion reaction mixture was incubated and mixed constantly using a stir bar and submersible stir plate for 10 minutes (± 10 seconds) after adding the IPD083Cb protein. At the end of the time period, a 1.5-ml sample of the IPD083Cb SGF digestion reaction mixture was transferred to a separate vial and inactivated by neutralization with 0.3 ml of 0.5 N NaOH. This sample was used for the sequential SIF digestion.

IPD083Cb Protein in SGF 10 Minutes: A sample (120 µl) was collected from the IPD083Cb SGF digestion reaction mixture at the end of 10 minutes (± 10 seconds) and inactivated by neutralization with 139 µl of pre-mixed SGF sample stop solution. The neutralized sample was heated for 5 minutes at 90-100 °C prior to frozen storage (-20 °C freezer unit).

IPD083Cb Protein in SGF Time 0: An SGF "Time 0" control sample (IPD083Cb protein in SGF) was prepared by first neutralizing a 60 µl sample of 2X SGF and 49 µl ultrapure water with 139 µl of SGF sample stop solution and then adding 12 µl of IPD083Cb protein. The neutralized sample was then heated at 90-100 °C for 5 minutes.

SGF-Only 10 Minutes Incubation: An SGF only control sample (without IPD083Cb protein) was prepared by mixing 60 µl of 2X SGF with 49 µl ultrapure water and then pre-warming in a 37 °C water bath for 2-5 minutes. After pre-warming, 12 µl of ultrapure water was added and the SGF solution was incubated in the water bath for 10 minutes. At the end of the time period, 139 µl of

SGF sample stop solution was added and the neutralized sample was then heated at 90-100 °C for 5 minutes.

Sequential Pancreatin Digestion

For the sequential SIF digestion time-course, a 1.2-ml sample of the NaOH-neutralized IPD083Cb SGF digestion reaction mixture was dispensed into a 7-ml glass vial and placed in a 37 °C water bath for 2-5 minutes prior to addition of 800 µl of 2.5X SIF solution. The SIF digestion reaction mixture was mixed constantly using a stir bar and a submersible stir plate. A 120-µl sub sample of the SIF digestion reaction mixture was removed from the vial at each of the following analytical time points (± 10 seconds): 0.25, 1, 2, 5, 10, 20, and 30 minutes. Each sub-sample was inactivated by adding it to a pre-labeled tube containing 64 µl of pre-mixed SIF sample solution and heating at 90-100 °C for 5 minutes. After heating, all SIF reaction samples were stored frozen (-20 °C freezer unit).

SDS-PAGE Analysis

The IPD083Cb protein digestion time-course samples and control samples were removed from frozen storage, heated at 90-100 °C for 5 minutes, and loaded (10 µl/well) into a 4-12% Bis-Tris gel for SDS PAGE analysis. Pre-stained protein molecular weight markers (Precision Plus Dual Xtra Standards) were also loaded into the gel. Electrophoresis was conducted using a pre-cast gel electrophoresis system with 1X MES SDS running buffer at a constant 200 volts (V) for 35 minutes.

Upon completion of electrophoresis, the gel was removed from the gel cassette for use in protein staining. For protein staining, the gel was washed with water and stained with GelCode Blue Stain Reagent. Following staining, the gel was destained with water until the gel background was clear. Protein bands were stained on the gel. The gel image was captured electronically using a ChemiDoc MP (Bio-Rad) imaging system.

Appendix E. Methods for Characterization of the PMI Protein

Test Materials

The PMI protein was isolated from maize stalk tissue derived from COR121 maize. The stalk tissue was collected at the R1 growth stage (the stage when silks become visible; Abendroth et al., 2011) of development from plants grown at a field location (Johnston, IA, USA). The PMI protein was extracted from lyophilized maize tissue by homogenization in phosphate buffered saline containing polysorbate 20 (PBST) and EDTA-free protease inhibitor. The extract was then clarified by centrifugation and filtration. The filtered extract was purified by ammonium sulfate (AS) precipitation and the 45–60% saturation fraction was collected based on calculations from an online tool (EnCor Biotechnology, 2025). The resulting pellets were stored frozen (-80 °C freezer unit). Pellets were then solubilized and buffer-exchanged into phosphate-buffered saline using Econo-Pac 10DG desalting columns (Bio-Rad). Following desalting, the sample underwent further purification by immunoaffinity chromatography. The PMI protein was eluted from the column with IgG elution buffer (Thermo Scientific). Selected elution fractions were pooled, concentrated, and buffer-exchanged into 50 mM Tris buffer, pH 8.

Following extraction, purification, and concentration, an equal volume of 2X LDS/DTT sample buffer (50% NuPAGE LDS Sample Buffer [4X], 20% NuPAGE Sample Reducing Agent containing DTT [10X], and 30% ultrapure [American Society for Testing and Materials (ASTM) Type 1] water [referred to as water]) was added to the concentrated sample. The sample was heat-treated and stored frozen (-20 °C freezer unit).

SDS-PAGE Analysis

The purified COR121 maize-derived PMI protein sample was thawed, diluted as applicable for the sensitivity of the assay in 1X LDS/DTT, heated, and then loaded into 4-12% Bis Tris gels along with pre stained protein molecular weight markers (Precision Plus Protein Dual Xtra Standards; Bio-Rad). The reference substance was thawed, heated, and loaded into the same gels to 1 µg for SDS-PAGE and 10 ng for western blot analysis. Electrophoresis was conducted using a pre-cast gel electrophoresis system at a constant 200 volts for 35 minutes.

Upon completion of electrophoresis, the gels were either prepared for protein staining or protein transfer to a membrane for western blot analysis.

For Coomassie staining, following electrophoresis, the gel was washed with water and stained with GelCode Blue Stain Reagent (Thermo Scientific). Following staining, the gel was de-stained with water until the gel background was clear. Protein bands were stained on the gel and the gel image was captured electronically using a ChemiDoc MP (Bio-Rad) imaging system.

Western Blot Analysis

Following SDS-PAGE as described above, the resulting gel was assembled into a nitrocellulose (NC) iBlot Gel Transfer Stack. An iBlot Gel Transfer Device was used to transfer proteins from the gel to the NC membrane.

Following protein transfer, the membrane was blocked in PBST containing 5% weight/volume (w/v) non-fat dry milk. Before and after the blocking step, the membrane was washed with PBST to reduce the background. The blocked membrane was incubated with a PMI mouse monoclonal antibody 13D11.F11.C12 (Pioneer Hi-Bred International, Inc.) conjugated to horseradish peroxidase diluted 1:10,000 in PBST containing 1% w/v non-fat dry milk. Following antibody incubation, the membrane was washed with PBST. The membrane remained in PBST prior to incubating with a chemiluminescent substrate (Thermo Scientific). The chemiluminescent signal and the pre-stained markers were detected and captured using a ChemiDoc MP (Bio Rad) imaging system.

Protein Glycosylation Analysis

A Pierce Glycoprotein Staining Kit (Thermo Scientific) was used to determine whether the COR121 maize-derived PMI protein was glycosylated. The purified PMI protein, a positive control protein (horseradish peroxidase), and a negative control protein (soybean trypsin inhibitor) were run by SDS-PAGE as described above. For glycosylation staining, the maize-derived PMI protein was loaded on to the gel at approximately the same concentration as the positive and negative control proteins (1 µg).

Following electrophoresis, the gel was washed with water, fixed with 50% methanol, and washed with 3% acetic acid. The gel was then incubated with oxidizing solution and washed with 3% acetic acid. The gel was incubated with glycoprotein staining reagent and then incubated in a reducing reagent. The gel was then washed with 3% acetic acid and then washed in water. Glycoproteins were detected as stained bands on the gel.

Following glycoprotein detection, the image of the gel was captured electronically using a ChemiDoc MP (BioRad) imaging system. The same gel was then stained with GelCode Blue stain reagent (Thermo Scientific) and washed with water to visualize all protein bands. The image of the GelCode stained- gel was then captured electronically.

Peptide Mapping and N-Terminal Peptide Identification by Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

For mass spectrometry sequencing analyses, the COR121 maize-derived PMI protein was loaded onto gels. Following SDS-PAGE and Coomassie staining using the methods as described previously, protein bands at the expected molecular weight of the COR121 maize-derived PMI protein were excised from the gels and stored frozen (-20 °C freezer unit). The protein in two of the gel slices was reduced with DTT, alkylated with iodoacetamide, and then subsequently digested with trypsin or chymotrypsin. The digested samples were separated on a nanoACQUITY UPLC (Waters Corporation) fitted with a Peptide BEH C18, 300 Å, 1.7 µm column (75 µm x 100 mm; Waters Corporation) by gradient elution. Eluent from the column was directed into an electrospray source, operating in positive ion mode, on a TripleTOF 5600+ hybrid

quadrupole-TOF mass spectrometer (AB Sciex; currently Sciex). The resulting mass spectrometry (MS) data were processed using MSConvert to produce a peak list. The peak list was used to perform an MS/MS ion search (Mascot Software Version 2.8.0) and match peptides from the expected PMI protein sequence (Perkins *et al.*, 1999). The following search parameters were used: peptide and fragment mass tolerance, ± 0.1 daltons (Da); fixed modifications, cysteine carbamidomethyl; variable modifications, methionine oxidation and acetyl protein N-terminal; and maximum missed cleavages, 1 for trypsin and 2 for chymotrypsin. The Mascot-generated peptide ion score threshold was > 13, which indicates identity or extensive homology ($p < 0.05$). The combined sequence coverage was calculated with GPMAW Version 14.02. The N-terminal amino acid sequence was also determined through peptide mapping.

Appendix F. Methods for Trait Expression Analyses

Field Trial Experimental Design

A multi-site field trial was conducted during the 2024 growing season at six sites in commercial maize-growing regions of the United States (one site in Iowa, Minnesota, Nebraska, Pennsylvania, and Wisconsin) and Canada (one site in Ontario). A randomized complete block design with four blocks was utilized at each site.

Sample Collection

The following samples were collected: leaf (V6, V9, R1, and R4 growth stages), root (V9, R1, and R4 growth stage), pollen (R1 growth stage), forage (R4 growth stage), and grain (R6 growth stage). Growth stage descriptions are provided in Table F.1. One sample per plot was collected for each tissue at the applicable growth stages. All samples were collected from impartially selected, healthy, representative plants to minimize potential bias.

Table F.1. Maize Growth Stage Descriptions

Growth Stage	Description
VE	The stage when the plant first emerges from the soil.
V1	The stage when the collar of the first leaf becomes visible.
V2	The stage when the collar of the second leaf becomes visible.
V3	The stage when the collar of the third leaf becomes visible.
V4	The stage when the collar of the fourth leaf becomes visible.
V5	The stage when the collar of the fifth leaf becomes visible.
V6	The stage when the collar of the sixth leaf becomes visible.
V7	The stage when the collar of the seventh leaf becomes visible.
V8	The stage when the collar of the eighth leaf becomes visible.
V9	The stage when the collar of the ninth leaf becomes visible.
V10	The stage when the collar of the tenth leaf becomes visible.
VT	The stage when the last branch of tassel is completely visible.
R1	The stage when silks become visible.
R2	The stage when kernels are white on the outside and resemble a blister in shape.
R3	The stage when kernels are yellow on the outside and the inner fluid is milky white.
R4	The stage when the material within the kernel produces a doughy consistency.
R5	The stage when all or nearly all the kernels are dented or denting.
R6	Typical grain harvest would occur. This stage is regarded as physiological maturity.

Note: Growth stages (Abendroth *et al.*, 2011).

Samples were collected as follows:

- Each V6 growth stage leaf sample was obtained by pruning the youngest two leaves that had emerged at least 8 in. (20 cm) in length from the plant. Each V9, R1 and R4 growth stage sample was obtained by pruning the youngest healthy leaf that was at least 8 in. (20 cm) in length from the plant. The tissue was cut into sections of 1 in. (2.5 cm) or less in length and collected into a pre labeled vial.

- Each V9, R1, and R4 growth stage root sample was obtained by cutting a circle 10-15 in. (25-38 cm) in diameter around the base of the plant to a depth of 7-9 in. (18-23 cm). The roots were thoroughly cleaned with water and removed from the plant. No above ground brace roots were included in the sample. The root tissue was cut into sections of 1 in. (2.5 cm) or less in length and collected to fill a pre labeled vial, unpacked, no more than ½ - ¾ full.
- Each R1 growth stage pollen sample was obtained by bagging and shaking a selected tassel to dislodge the pollen. The tassel selected for sampling had the tassel's main spike shedding pollen. For some plots, pollen may have been pooled from multiple plants within the same plot in order to collect the appropriate amount. The pollen was screened for anthers and foreign material and then collected to fill approximately 50-100% of the conical area of a pre-labeled vial.
- Each R4 growth stage forage sample consisted of stalks, leaves, cobs, husks, and grain, and was obtained by cutting a plant approximately 4-6 in. (10-15 cm) above the soil surface line. The aerial portion of the plant was chopped into sections of 3 in. (7.6 cm) or less in length and collected into a pre labeled, plastic-lined, cloth bag. The plants selected for forage sampling contained self pollinated ears.
- Each R6 growth stage grain sample was obtained by husking and shelling the grain from one primary ear that had previously been self-pollinated. For each sample, a representative sub sample of 10 kernels was collected into an individual pre-labeled vial.

Sample Processing, Shipping, and Storage

Each sample was placed on dry ice within 10 minutes of collection in the field and transferred to frozen storage (≤ 0 °C) until shipment. Expressed trait protein samples were then shipped frozen in a freezer truck to Pioneer Hi-Bred International, Inc. for processing and analysis. Samples were received frozen, intact, and in good condition. Upon arrival, samples were stored frozen. Forage samples were coarsely homogenized prior to lyophilization. All samples were lyophilized under vacuum until dry. Following lyophilization, pollen samples were stored frozen until analysis and leaf, root, forage, and grain samples were finely homogenized and stored frozen until analysis. Lyophilized samples were moved from freezer storage and placed on wet ice directly before protein extraction.

Protein Concentration Determination

The concentrations of IPD083Cb and PMI proteins were determined using quantitative ELISA methods that have been internally validated to demonstrate method suitability.

Processed tissue sub-samples were weighed at the following target weights: 5 mg for pollen, 10 mg for leaf, and 20 mg for root and grain; and 30 mg for forage. Samples were extracted with 0.60 ml of chilled phosphate-buffered saline containing 0.05% polysorbate 20 (PBST). Extracted samples were centrifuged, and then supernatants were removed and prepared for analysis.

ELISA Methods

ELISA methods were performed as follows:

IPD083Cb Protein ELISA Method: Prior to analysis, samples were diluted as applicable in PBST. Standards (typically analyzed in triplicate wells) and diluted samples (typically analyzed in duplicate wells) were incubated in a plate pre-coated with an IPD083Cb-specific antibody. Following incubation, unbound substances were washed from the plate. A different IPD083Cb-specific antibody conjugated to the enzyme horseradish peroxidase (HRP) was added to the plate and incubated. Unbound substances were washed from the plate. Detection of the bound IPD083Cb-antibody complex was accomplished by the addition of substrate, which generated a colored product in the presence of HRP. The reaction was stopped with an acid solution and the optical density (OD) of each well was determined using a plate reader.

PMI Protein ELISA Method: Prior to analysis, samples were diluted as applicable in PBST. Standards (typically analyzed in triplicate wells) and diluted samples (typically analyzed in duplicate wells) were incubated in a plate pre-coated with a PMI-specific antibody. Following incubation, unbound substances were washed from the plate and the bound PMI protein was incubated with a different PMI-specific antibody conjugated to the enzyme HRP. Unbound substances were washed from the plate. Detection of the bound PMI-antibody complex was accomplished by the addition of substrate, which generated a colored product in the presence of HRP. The reaction was stopped with an acid solution and the OD of each well was determined using a plate reader.

Calculations for Determining IPD083Cb, and PMI Protein Concentrations

SoftMax Pro GxP Version 7.2 (Molecular Devices) microplate data software was used to perform the calculations required to convert the OD values obtained for each set of sample wells to a protein concentration value.

A standard curve was included on each ELISA plate. The equation for the standard curve was derived by the software, which used a quadratic fit to relate the OD values obtained for each set of standard wells to the respective standard concentration (ng/ml).

The sample concentration values were adjusted for a dilution factor expressed as 1:N by multiplying the interpolated concentration by N.

Adjusted Concentration = Interpolated Sample Concentration x Dilution Factor

Adjusted sample concentration values obtained from SoftMax Pro GxP software were converted from ng/ml to ng/mg sample weight as follows:

$$\text{Sample Concentration (ng protein/mg sample weight)} = \text{Sample Concentration (ng/ml)} \times \frac{\text{Extraction Buffer Volume (ml)}}{\text{Sample Target Weight (mg)}}$$

The reportable assay lower limit of quantification (LLOQ) in ng/ml was calculated as follows for the IPD083Cb protein:

Reportable Assay LLOQ (ng/ml) = (lowest standard concentration) x minimum dilution

The reportable assay lower limit of quantification (LLOQ) in ng/ml was calculated as follows for the PMI protein:

Reportable Assay LLOQ (ng/ml) = (lowest standard concentration - 10%) x minimum dilution

The LLOQ, in ng/mg sample weight, was calculated as follows:

$$\text{LLOQ} = \text{Reportable Assay LLOQ (ng/ml)} \times \frac{\text{Extraction Buffer Volume (ml)}}{\text{Sample Target Weight (mg)}}$$

Statistical Analysis

Statistical analysis of the protein concentration results consisted of the calculations of means, ranges, and standard deviations. Individual sample results below the LLOQ were assigned a value equal to the LLOQ for calculation purposes.

Appendix G. Methods for Nutrient Composition Analysis

Field Trial Experimental Design

A multi-site field trial was conducted during the 2024 growing season at eight sites in commercial maize-growing regions of the United States (one site in Illinois, Minnesota, Nebraska, Pennsylvania, and Wisconsin, and two sites in Iowa) and Canada (one site in Ontario). A randomized complete block design with four blocks was utilized at each site. Each block included COR121 maize, control maize, and four reference maize lines.

Sample Collection

Forage (R4 growth stage) and grain (R6 growth stage) samples were collected (one sample per plot) from self-pollinated plants at the applicable growth stage. All samples were collected from impartially selected, healthy, representative plants. Reference maize and control maize samples were collected prior to the collection of COR121 maize samples to minimize the potential for contamination. Each sample was uniquely labeled with a sample identification number and barcode for sample tracking and is traceable by site, entry, block, tissue, and growth stage.

Each forage sample (combination of three plants) was obtained by cutting the aerial portion of the plants from the root system approximately 4-6 in. (10-15 cm) above the soil surface. The plants were chopped into sections of 3 in. (7.6 cm) or less in length, pooled, and approximately one-third of the chopped material was collected in a pre-labeled, plastic-lined, cloth bag. Each grain sample was obtained from five ears at typical harvest maturity. The ears were husked and shelled, and the pooled grain was collected into a pre-labeled, plastic, resealable bag and then placed into a pre-labeled, plastic-lined, cloth bag. Samples were placed in chilled storage (e.g., coolers with wet ice, artificial ice, or dry ice) within 30 minutes of collection and were transferred to a freezer (≤ -10 °C) or placed on dry ice within three hours of collection.

Samples from each site were shipped frozen to [redacted] for analyses.

Nutrient Composition Analyses

Nutrient composition analyses of forage and grain were conducted by [redacted] and included the determination of the following analytes:

- *Forage proximate and fiber composition:* moisture, crude protein, crude fat, acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, carbohydrates, calcium and phosphorus.
- *Grain proximate and fiber composition*: moisture, crude protein, crude fat, acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), ash, and carbohydrates.
- *Grain fatty acid composition*: lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:0), heptadecenoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid

(C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), eicosadienoic acid (C20:2), behenic acid (C22:0)

- *Grain amino acid composition*: alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine
- *Grain mineral composition*: calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc
- *Grain vitamin composition*: β-carotene, vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B6 (pyridoxine), vitamin B9 (folic acid), α-tocopherol
- *Grain secondary metabolite and anti-nutrient composition: p*-coumaric acid, ferulic acid, furfural, inositol, phytic acid, raffinose

Nutrient composition analytical methods and procedures are summarized in Table G.1.

Table G.1. Methods for Compositional Analysis

Nutritional Analyte	Method
Moisture	The analytical procedure for moisture determination was based on a method published by Association of Official Analytical Chemists (AOAC) International. Samples were assayed to determine the percentage of moisture by gravimetric measurement of weight loss after drying in a forced air oven (forage) and a vacuum oven (grain).
Ash	The analytical procedure for ash determination was based on a method published by AOAC International. Samples were analyzed to determine the percentage of ash by gravimetric measurement of the weight loss after ignition in a muffle furnace.
Crude Protein	The analytical procedure for crude protein determination utilized an automated Kjeldahl technique based on a method provided by the manufacturer of the titrator unit (Foss-Tecator) and AOAC International. Ground samples were digested in the presence of a catalyst. The digestate was then distilled and titrated with a Foss-Tecator Kjeltec Analyzer unit.
Crude Fat	The analytical procedure for crude fat determination was based on methods provided by the American Oil Chemists' Society (AOCS) and the manufacturer of the hydrolysis and extraction apparatus (Ankom Technology). Samples were hydrolyzed with 3N hydrochloric acid at 90 °C for 80 minutes for forage and 60 minutes for grain. The hydrolysates were extracted with a petroleum ether/ethyl ether/ethyl alcohol solution at 90 °C for 60 minutes. The ether extracts were evaporated and the fat residue remaining determined gravimetrically.
Carbohydrates	The carbohydrate content in maize forage and grain on a dry weight basis was calculated using a formula obtained from the United States Department of Agriculture "*Energy Value of Foods*," in which the percent dry weight of crude protein, crude fat, and ash was subtracted from 100%.
Neutral Detergent Fiber	The analytical procedure for neutral detergent fiber (NDF) determination was based on a method provided by the manufacturer of the extraction apparatus (Ankom Technology), AOAC International, and the *Journal of AOAC International*. Samples were analyzed to determine the percentage of NDF by digesting with a neutral detergent solution, sodium sulfite, and alpha amylase. The remaining residue was dried and weighed to determine the NDF content.
Acid Detergent Fiber	The analytical procedure for acid detergent fiber (ADF) determination was based on a method provided by the manufacturer of the extraction apparatus (Ankom Technology) and AOAC International. Samples were analyzed to determine the percentage of ADF by digesting with an acid detergent solution and washing with reverse osmosis water. The remaining residue was dried and weighed to determine the ADF content.

Table G.1. Methods for Compositional Analysis (continued)

Nutritional Analyte	Method
Total Dietary Fiber	The analytical procedure for the determination of total dietary fiber in grain was based on methods provided by the manufacturer of the extraction apparatus (Ankom Technology), AOAC International, and the manufacturer of the protein titrator unit (Foss-Tecator). Duplicate samples were gelatinized with heat stable α-amylase, enzymatically digested with protease and amyloglucosidase to remove protein and starch, respectively, and then soluble dietary fiber precipitated with ethanol. The precipitate (residue) was quantified gravimetrically. Protein analysis was performed on one of the duplicate samples while the other duplicate sample was analyzed for ash. The weight of the protein and ash was subtracted from the weight of the residue divided by sample dry weight.
Minerals	The analytical procedure for the determination of minerals is based on methods published by AOAC International and CEM Corporation. The maize forage minerals determined were calcium and phosphorus. Additional grain minerals determined were calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc. The samples were digested in a microwave-based digestion system and the digestate was diluted using DI water. Both the diluted and undiluted portions for maize grain and diluted portions for maize forage were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES).
Tryptophan	The analytical procedure for tryptophan determination was based on an established lithium hydroxide hydrolysis procedure with reverse phase ultra performance liquid chromatography (UPLC) with ultraviolet (UV) detection published by the *Journal of Micronutrient Analysis*.
Cystine and Methionine	The analytical procedure for cystine and methionine determination was based on methods obtained from Waters Corporation, AOAC International, and *Journal of Chromatography*. The procedure converts cystine to cysteic acid and methionine to methionine sulfone, after acid oxidation and hydrolysis, to the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatives which are then analyzed by reverse phase UPLC with UV detection.
Additional Amino Acids	Along with tryptophan, cystine, and methionine, 15 additional amino acids were determined. The analytical procedure for analysis of these amino acids was based on methods obtained from Waters Corporation and the *Journal of Chromatography*. The procedure converts the free acids, after acid hydrolysis, to the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivatives, which are analyzed by reverse phase UPLC with UV detection.
Fatty Acids	The analytical procedure for determination of fatty acids was based on methods published by AOAC International and AOCS. The procedure converts the free acids, after ether extraction and base hydrolysis, to the fatty acid methyl ester (FAME) derivatives, which are analyzed by gas chromatography with flame ionization detection (GC/FID). Results are reported as percent total fatty acids but presented in the raw data as percent fresh weight.

Table G.1. Methods for Compositional Analysis (continued)

Nutritional Analyte	Method
Thiamine (Vitamin B_1) and Riboflavin (Vitamin B_2)	The analytical procedure for the determination of thiamine (vitamin B_1) and riboflavin (vitamin B_2) was based on a method published by the American Association of Cereal Chemists (AACC). The samples were extracted with 10% acetic acid/4.3% trichloroacetic acid solution. A 50-fold dilution was performed and then the samples were analyzed by reverse phase high pressure liquid chromatography (UPLC) tandem mass spectrometry (MS/MS).
Niacin (Vitamin B_3)	The analytical procedure for the determination of niacin (vitamin B_3) was based on a method published by r-Biopharm. Niacin (vitamin B_3) was extracted from the sample using a citric acid buffer and enzyme treatment. After heating, filtering, and diluting the extract, niacin was assayed microbiologically using microtiter plates coated with standardized, lyophilized *Lactobacillus plantarum* provided in the VitaFast® Niacin assay kit from r-Biopharm. The growth of *Lactobacillus plantarum* was proportional to the amount of niacin in the extract. The turbidity produced by the organism was measured using a spectrophotometer set to measure absorbance at 630 nm.
Pyridoxine (Vitamin B_6)	The analytical procedure for the determination of pyridoxine (vitamin B_6) was based on a method from r-Biopharm. Pyridoxine (vitamin B_6) was extracted from the matrix using a citric acid buffer and enzyme treatment. After heating, filtering, and diluting the extract, vitamin B_6 was assayed microbiologically using the lyophilized organism *Saccharomyces cerevisiae* provided in the VitaFast® Vitamin B_6 assay kit from r-Biopharm. The growth of *Saccharomyces cerevisiae* was proportional to the amount of vitamin B_6 in the extract. The turbidity (optical density) produced by the organism was measured using a spectrophotometer set to measure absorbance at 630 nm.
Folic Acid (Vitamin B_9)	The analytical procedure for determination of folic acid was based on a microbiological assay from r-Biopharm. Samples were hydrolyzed and digested by chicken pancreatin. After heating, filtering, and diluting the extract, folic acid was assayed microbiologically using the lyophilized organism *Lactobacillus rhamnosus* provided in the VitaFast® Folic Acid assay kit from r-Biopharm. The turbidity produced by the organism was measured using a spectrophotometer set to measure absorbance at 630 nm.
α-Tocopherols	The analytical procedure for the determination of alpha-tocopherol in maize grain was based on methods from the *Journal of the American Oil Chemists' Society* and *Analytical Sciences*. Alpha-tocopherol was extracted with BHT in hexane and the extracts were analyzed by normal phase UPLC with fluorescence detection.

Table G.1. Methods for Compositional Analysis (continued)

Nutritional Analyte	Method
β-Carotene	The analytical procedure for determination of beta-carotene was based on a method published by AOAC International. Samples were extracted using a 40:60 acetone:hexane with tert-butylhydroquinone (TBHQ) solution then analyzed by UPLC-UV.
Inositol and Raffinose	The analytical procedure for the determination of inositol and raffinose was based on a gas chromatography (GC) method published in the *Handbook of Analytical Derivatization Reactions*, an AACC method, and a method from the *Journal of Agricultural and Food Chemistry*. Extracted inositol was derivatized with butylboronic acid and analyzed by GC/FID. Extracted raffinose was analyzed by reverse phase UPLC with refractive index detection.
Furfural	The analytical procedure for the determination of furfural was based on methods published in the *Journal of Agricultural and Food Chemistry*. Ground maize grain were extracted in methanol and analyzed for furfural content by reverse phase UPLC with UV detection.
p-Coumaric and Ferulic Acid	The analytical procedure for the determination of *p*-coumaric and ferulic acids was developed based on methods published in *Journal of Agricultural and Food Chemistry* and *The Journal of Chemical Ecology*. Ground maize grain was analyzed to determine the amounts of *p*-coumaric acid and ferulic acid by separating the total content of phenolic acids using reverse phase UPLC and UV detection.
Phytic Acid	The analytical procedure for the determination of phytic acid was based on a method published by AOAC International. The samples were analyzed to determine the amount of phytic acid by extracting the phytic acid with dilute hydrochloric acid (HCl) and isolating it using an aminopropyl silica solid phase extraction column. Once isolated and eluted, the phytic acid was analyzed for elemental phosphorus by ICP-OES.

Statistical Analysis of Nutrient Composition Data

Prior to statistical analysis, the data were processed as follows:

Values Below Lower Limit of Quantification

For statistical analysis, nutrient composition values reported as below the assay lower limit of quantification (LLOQ) were each assigned a value equal to half the LLOQ.

Conversion of Fatty Acid Assay Values

The raw data for all fatty acid analytes were provided by [redacted] in units of percent fresh weight (%FW). Any fatty acid values below the %FW LLOQ were set to half the LLOQ

value, and then all assay values were converted to units of % total fatty acids for statistical analyses.

For a given sample, the conversion to units of % total fatty acids was performed by dividing each fatty acid analyte value (%FW) by the total fresh weight of all fatty acids for that sample; for analyte values below the LLOQ, the half LLOQ value was used as the analyte value. Half LLOQ values were also included in the total fresh weight summations. After the conversion, a fixed LLOQ value was not available for a given individual fatty acid analyte on the % total fatty acids basis.

Statistical analyses were conducted using SAS software, Version 9.4 (SAS Institute, Inc.). The following rules were implemented for each analyte:

- If both COR121 maize and the control maize had < 50% of samples below the LLOQ, then an across-site mixed model analysis was conducted.
- If either COR121 maize or the control maize had ≥ 50% samples below the LLOQ, but not both entries had 100% of samples below the LLOQ across sites, then Fisher's exact test was conducted. The Fisher's exact test assessed whether there was a significant difference (P-value < 0.05) in the proportion of samples below the LLOQ between these two maize lines across sites.
- If both COR121 maize and the control maize had 100% of samples below the LLOQ, then statistical analyses were not performed.

Statistical Model for Across-Site Analysis

For a given analyte, data were analyzed using the following linear mixed model:

$$y_{ijk} = \mu_i + \ell_j + r_{k(j)} + (\mu\ell)_{ij} + \varepsilon_{ijk}$$

$$\ell_j \sim iid\ N(0, \sigma^2_{Site}),\ r_{k(j)} \sim iid\ N(0, \sigma^2_{Rep}),\ (\mu\ell)_{ij} \sim iid\ N(0, \sigma^2_{Ent\times Site}),\ \text{and}\ \varepsilon_{ijk} \sim iid\ N(0, \sigma^2_{Error})$$

where μ_i denotes the mean of the i^{th} entry (fixed effect), ℓ_j denotes the effect of the j^{th} site (random effect), $r_{k(j)}$ denotes the effect of the k^{th} block within the j^{th} site (random effect), $(\mu\ell)_{ij}$ denotes the interaction between the entries and sites (random effect), and ε_{ijk} denotes the effect of the plot assigned the i^{th} entry in the k^{th} block of the j^{th} site (random effect or residual). Notation $\sim iid\ N(0, \sigma^2_a)$ indicates random variables that are identically independently distributed (*iid*) as normal with zero mean and variance σ^2_a. Subscript *a* represents the corresponding source of variation.

The residual maximum likelihood estimation procedure was utilized to generate estimates of variance components and entry means across sites. The estimated means are known as empirical best linear unbiased estimators (hereafter referred to as LS-Means). The statistical comparison was conducted by testing for a difference in LS-Means between COR121 maize and the control maize. The Kenward-Roger method (Kenward and Roger, 2009) was used to estimate the variance

of LS-Means and approximate degrees of freedom for statistical tests. A significant difference was identified if a P-value was < 0.05.

For each analyte, goodness-of-fit of the model was assessed in terms of meeting distributional assumptions of normally, independently distributed errors with homogeneous variance. Deviations from assumptions were addressed using an appropriate transformation or allowing for heterogeneous error variance among sites ($\varepsilon_{ijk} \sim iid\ N(0, \sigma^2_{Error,j})$).

False Discovery Rate Adjustment

The false discovery rate (FDR) method (Benjamini and Hochberg, 1995; Westfall *et al.*, 1999) was used to control for false positive outcomes across all analytes analyzed using linear mixed models. A false positive outcome occurs if the difference in means between two entries is declared significant, when in fact the two means are not different. Since its introduction in the mid-1990s, the FDR approach has been widely employed across a number of scientific disciplines, including genomics, ecology, medicine, plant breeding, epidemiology, dairy science, and signal/image processing (e.g., Pawitan *et al.*, 2005; Spelman and Bovenhuis, 1998). In the FDR method, the false discovery rate is held at 5% across comparisons of multiple analytes via an adjustment to the P-value and is not inflated by the number of analytes in the comparison.

Interpretation of Statistical Results

For a given analyte, when a statistically significant difference (P-value from mixed model analysis < 0.05, or Fisher's exact test P-value < 0.05) was identified in the across-site analysis, the respective range of individual values from COR121 maize was compared to a tolerance interval. Tolerance intervals are expected to contain at least 99% of the values for corresponding analytes of the conventional maize population with a 95% confidence level (Hong *et al.*, 2014). The tolerance intervals were derived from Pioneer proprietary accumulated data from conventional maize lines, which were grown in commercial maize-growing regions between 2003 and 2021 in the United States, Canada, Chile, Brazil, and Argentina. The combined data represent 206 commercial maize lines and 214 unique environments. The selected non-GM commercial maize lines represent the conventional maize population with a history of safe use, and the selected environments (site and year combinations) represent maize growth under a wide range of environmental conditions (i.e., soil texture, temperature, precipitation, and irrigation) and maize maturity group zones.

If the range of COR121 maize contained individual values outside the tolerance interval, it was then compared to the respective literature range obtained from published literature (AFSI, 2022). Literature ranges complement tolerance intervals in that they are composed of non-proprietary data from additional conventional maize lines and growing environments, which are not included in Pioneer's proprietary database.

If the range of COR121 maize contained individual values outside the literature range, it was then compared to the respective in-study reference range comprised of all individual values across-sites from all reference maize lines included in this portion of the study. In-study reference data ranges complement tolerance intervals and literature ranges in that they provide additional context of biological variation specific to the current study.

In cases when a raw P-value indicated a significant difference, but the FDR-adjusted P-value was > 0.05, it was concluded that the difference was likely a false positive.

Reported Statistics

The statistical results for transformed data were back-transformed to the original data scale for reporting purposes. For analytes examined using mixed model analysis, the following statistical results were reported: LS-Means, ranges, 95% confidence intervals, FDR-adjusted P-values, and non-adjusted P-values. For the remaining analytes that were not examined using mixed model analysis, the following statistical results were reported: arithmetic means, ranges, and P-value (if Fisher's exact test was conducted). Additionally, three reference ranges (tolerance interval, literature range, and in-study reference range) were provided as available. For fatty acid analytes, LLOQ values were not fcortavailable on a % total fatty acids basis; therefore, when all sample values were below the LLOQ for a given analyte, mean and range were reported as "<LLOQ". Table G.2. shows the number of samples below the LLOQ.

Table G.2. Number of Sample Values Below the Lower Limit of Quantification

Analyte	Number of Samples Below the LLOQ		Statistical Analysis	Fisher's Exact Test P-Value
	Control Maize (n=32)	COR121 Maize (n=32)		
Fatty Acid Composition				
Lauric Acid (C12:0)	32	32	Not Analyzed	--
Myristic Acid (C14:0)	32	32	Not Analyzed	--
Heptadecanoic Acid (C17:0)	19	26	Fisher's Exact Test	0.0994
Heptadecenoic Acid (C17:1)	31	32	Fisher's Exact Test	1.00
Eicosadienoic Acid (C20:2)	32	32	Not Analyzed	--
Mineral Composition				
Copper[a]	2	4	Mixed Model	--
Sodium[a]	6	10	Mixed Model	--
Vitamin Composition				
Vitamin B2 (Riboflavin)	32	32	Not Analyzed	--
Secondary Metabolite and Anti-Nutrient Composition				
Furfural	32	32	Not Analyzed	--

[a] This analyte had < 50% of sample values below the lower limit of quantification (LLOQ) in each maize line and was subjected to the mixed model analysis.