

**6 August 2020**

**[131-20]**

**Supporting document 1**

Risk assessment – Application A1198

Food derived from enhanced yield and herbicide-tolerant corn line DP202216

# Executive summary

### Background

A genetically modified (GM) corn line with OECD Unique Identifier DP-202216-6, hereafter referred to as DP202216, has been developed for enhanced yield and herbicide-tolerance. Enhanced yield is achieved by increasing the level and extending the expression time of an endogenous (*Zea mays*) transcription factor, ZMM28. Herbicide-tolerance is conferred through expression of the phosphinothricin-N-acetyltransferase (PAT) protein from *Streptomyces viridochromogenes*, providing tolerance to glufosinate.

In conducting a safety assessment of food derived from DP202216, a number of criteria have been addressed including: characterisation of the transferred genes including their origin, function and stability in the corn genome; the nature of the introduced proteins and their potential to be either allergenic or toxic in humans; compositional analyses and any resultant changes in the whole food. This approach evaluates both the intended and any unintended changes in the plant.

This safety assessment addresses food safety and nutritional issues associated with the GM food. It therefore does not address:

• risks related to the environmental release of GM plants used in food production

• risks to animals that may consume feed derived from GM plants

• the safety of food derived from the non-GM (conventional) plant.

Food derived from the non-GM (conventional) plant (corn) has an accepted history of safe use and is used as the benchmark for the comparative analysis.

### History of use

In terms of food production, corn is the world’s dominant cereal crop. It has a long history of safe use in the food supply, dating back thousands of years. Sweet corn is consumed directly while corn grain-derived products are routinely used in a large number and diverse range of foods (e.g. cornflour, starch products, breakfast cereals and high fructose corn syrup). In Australia and New Zealand, corn is predominantly used as feed.

### Molecular characterisation

The genes encoding ZMM28 (*zmm28*) and PAT (*mo-pat*) were transferred to corn line DP202216 via *Agrobacterium*-mediated transformation. Comprehensive analyses indicate a single copy of the insert containing the *zmm28* and *mo-pat* genes. There were minor truncations of the T-DNA borders, consistent with *Agrobacterium*-mediated transformation. The introduced genetic elements and the expression of new proteins in DP202216 were shown by DNA sequence and phenotypic analyses to be stably inherited from one generation to the next across multiple generations. The pattern of inheritance supports the conclusion that the enhanced yield and herbicide-tolerance traits occur within a single locus in the DP202216 genome and are inherited in accordance with Mendelian principles.

### Characterisation and safety assessment of new substances

Characterisation studies confirmed the identity of the newly expressed ZMM28 and PAT proteins in corn line DP202216. The ZMM28 and PAT proteins *in planta* demonstrated the expected immunoreactivity and molecular weights (28.4 kDa and 20.6 kDa respectively). In the case of PAT, the plants were tolerant to glufosinate treatment. Bioinformatic analyses of ZMM28 and PAT confirmed the sequence shared no similarity to known allergens or toxins. The ZMM28 protein also has a prior history of human consumption as it is a protein that is normally present in sweet corn.

ZMM28 was detected in various tissue analysed from DP202216. Expression of the protein was highest in the root and leaves at specific growth stages. The mean level in the grain used for food was 0.012 µg/g dry weight, which corresponds to 0.000014% of total protein. PAT was also detected in various tissues, with the highest expression in the leaf. The mean level in the grain was 15 µg/g dry weight, which corresponds to 0.01758% of total protein.

### Compositional analyses

Detailed compositional analyses were carried out on grain from DP202216 and the control cultivar grown under normal agricultural conditions over eight field-trial sites in the United States and Canada. The analyses included proximates (protein, fat, ash) amino acids, fatty acids, carbohydrates by calculation, fibre, minerals, vitamins, anti-nutrients and secondary metabolites. The levels of 70 key analytes in DP202216 were compared to those in the control and also to compositional data from a range of commercial non-GM corn varieties available from the published literature and a proprietary database.

Minor differences were found between grain from DP202216 and the control for 5 of the analytes measured and were within the range established for existing commercial corn varieties. Overall, the compositional data support the conclusion that there are no biologically significant differences in the levels of key constituents in grain from DP202216 compared to conventional corn varieties available on the market.

**Conclusion**

No potential public health and safety concerns have been identified in the assessment of the enhanced-yield and herbicide-tolerant corn line DP202216. On the basis of the data provided in the present application, and other available information, food derived from DP202216 is considered to be as safe for human consumption as food derived from conventional corn varieties.

Table of contents

[Executive summary i](#_Toc42761634)

[1 Introduction 1](#_Toc42761635)

[2 History of use 1](#_Toc42761636)

[2.1 Host organism 1](#_Toc42761637)

[2.2 Donor organisms 2](#_Toc42761638)

[3 Molecular characterisation 2](#_Toc42761639)

[3.1 Transformation Method 2](#_Toc42761640)

[3.2 Detailed description of DNA to be introduced 4](#_Toc42761641)

[3.3 Development of the corn line from original transformation 5](#_Toc42761642)

[4 Characterisation and safety assessment of novel substances 8](#_Toc42761643)

[4.1 Description of the ZMM28 protein 8](#_Toc42761644)

[4.2 Description of the PAT protein 11](#_Toc42761645)

[4.4 Novel herbicide metabolites in GM herbicide-tolerant plants 13](#_Toc42761646)

[5 Compositional analysis 14](#_Toc42761647)

[5.1 Key Components 14](#_Toc42761648)

[5.2 Study design 14](#_Toc42761649)

[5.3 Analyses of key components in corn grain 15](#_Toc42761650)

[5.4 Conclusion 21](#_Toc42761651)

[6 Nutritional impact 21](#_Toc42761652)

[7 References 22](#_Toc42761653)

### Index of Figures

|  | Title | Page |
| --- | --- | --- |
| [Figure 1](#Figure1) | Plasmid map of PHP40099 | 3 |
| [Figure 2](#Figure2) | Translated amino acid sequence of the ZMM28 protein | 9 |
| [Figure 3](#Figure3) | Stages of corn growth | 9 |
| [Figure 4](#Figure4) | Translated amino acid sequence of the PAT protein | 12 |

### Index of Tables

|  | Title | Page |
| --- | --- | --- |
| [Table 1](#Table1) | The genetic elements contained in the T-DNA region of PHP40099 used to create DP202216 | 4 |
| [Table 2](#Table2) | Events used in the characterisation studies performed on DP2022161 | 6 |
| [Table 3](#Table3) | Segregation results for DP202216 | 7 |
| [Table 4](#Table4) | Expression of ZMM28 in various tissues | 10 |
| [Table 5](#Table5) | Expression of PAT in various tissues | 13 |
| [Table 6](#Table6) | Summary of consideration of PAT in previous FSANZ safety assessments | 13 |
| [Table 7](#Table7) | Comparison of Proximates and Fibre | 16 |
| [Table 8](#Table8) | Comparison of Amino Acids | 16 |
| [Table 9](#Table9) | Comparison of Vitamins | 17 |
| [Table 10](#Table10) | Comparison of Minerals | 19 |
| [Table 11](#Table11) | Comparison of Fatty Acids | 20 |
| [Table 12](#Table12) | Comparison of Anti-nutrients and Secondary Metabolites | 20 |

### List of Abbreviations

|  |  |
| --- | --- |
| BLOSUM | BLOcks SUbstitution Matrix |
| bp | base pairs |
| COMPARE | COMprehensive Protein Allergen REsource |
| DNA | deoxyribonucleic acid |
| DW | dry weight |
| ELISA | enzyme-linked immunosorbent assay |
| ILSI | International Life Sciences Institute |
| FASTA | Fast alignment search tool – all |
| FAO | Food and Agriculture Organization of the United Nations |
| FSANZ | Food Standards Australia New Zealand |
| GM | genetically modified |
| kDa | kilodalton |
| LLOQ | lower limit of quantification |
| mg | milligram |
| min | minimum |
| MT | million tonnes |
| OECD | Organisation for Economic Co-operation and Development |
| OGTR | Office of the Gene Technology Regulator |
| ORF | open reading frame |
| PCR | polymerase chain reaction |
| SAS | Statistical Analysis Software |
| T-DNA | transfer DNA |
| USDA | United States Department of Agriculture |
| WHO | World Health Organisation |
| zmm / ZMM | Zea mays MADS |

# 1 Introduction

FSANZ has received an application from Dow AgroSciences Australia Pty Ltd to vary Schedule 26 in the *Australia New Zealand Food Standards Code* (the Code). The variation is to add food derived from the genetically modified (GM) enhanced yield and herbicide-tolerant corn line DP202216, with the OECD Unique Identifier DP-202216-6.

Enhanced yield is achieved through increased expression of an endogenous gene *zmm28*, which encodes a transcription factor protein (ZMM28) that regulates the expression of genes associated with floral organ development. Tolerance to the herbicide glufosinate is achieved by the expression of a modified phosphinothricin acetyltransferase (PAT) enzyme, encoded by the maize-optimised *mo-pat* gene, derived from the bacterium *Streptomyces viridochromogenes*. Unlike the PAT protein, the ZMM28 protein has not been assessed previously by FSANZ.

Corn lines containing the DP202216 transformation event will not be cultivated in Australia or New Zealand, therefore food from DP202216 may only be present in the Australian and New Zealand food supply via imported products.

# 2 History of use

## 2.1 Host organism

The information provided here has been summarised from more detailed reports published by the Organisation for Economic Cooperation and Development (OECD 2002), the Grains Research & Development Corporation (GRDC 2017) and the Office of the Gene Technology Regulator (OGTR, 2008). Numerical and statistical data have been sourced from the [FAOSTAT website](http://www.fao.org/faostat/en/#data)[[1]](#footnote-2) of the Food and Agriculture Organization of the United Nations, the United States Department of Agriculture [Foreign Agricultural Service website](https://www.fas.usda.gov/commodities/soybeans)[[2]](#footnote-3) and the International Service for the Acquisition of Agri-Biotech Applications (ISAAA 2017).

The host organism is conventional corn (*Zea mays*), belonging to the family *Poaceae*. The proprietary corn cultivar PH17AW (Zhao et al. 2010) was used as the parental variety for the genetic modification described in this application.

Corn is grown as a commercial food and feed crop in many countries worldwide. The domestication of corn as a food crop occurred approximately 6,000-10,000 years ago. Archaeological evidence suggests the origin of domestication occurred in the Mexican highlands, indicating this plant has a long history of safe food use (Ranum et al. 2014).

In terms of global production, corn is the dominant cereal crop (2018/19 = 1,125 MT[[3]](#footnote-4)), ahead of wheat (731 MT) and rice (499 MT) (USDA 2019). The United States and China are the largest producers, with production in 2018/19 being 366 and 257 MT, respectively. Corn is not a major crop in Australia or New Zealand, production in 2017 was approximately 0.436 and 0.176 MT, respectively (FAOSTAT 2017). In the U.S. it is estimated that ~92% of all corn planted is GM while in Canada, the estimate of GM corn is ~80% of total corn. No GM corn is currently grown commercially in Australia or New Zealand.

The limited domestic production of corn in Australia and New Zealand is supplemented by importing corn grain and corn-based products that are used widely in processed foods. Imports to Australia and New Zealand included approximately 3,000 and 2,194 tonnes respectively of corn crystalline fructose and high fructose corn syrup and 2,038 and 553 tonnes respectively of corn oil (FAOSTAT 2017; Green Pool 2012). Neither Australia nor New Zealand currently produce fructose (either crystalline or as high fructose corn syrup).

The most likely products to be imported from DP202216 would be wet-milled starch for sweetening products, maize oil and high fructose corn syrup (HFCS). In Australia and New Zealand, maize starch is used in dessert mixes and canned foods and HFCS is used in breakfast cereals, baking products, corn chips and extruded confectionary.

## 2.2 Donor organisms

### 2.2.1 Plant

The source of the *zmm28* gene is the host species *Z. mays.* While corn is known to contain anti-nutrients and allergens (OECD 2002; OGTR 2008), the corn *zmm28* gene is not associated with these known anti-nutrients or allergenic proteins.

### 2.2.1 Bacterial

The source of the *pat* gene is *Streptomyces viridochromogenes*, a gram-positive spore-forming bacterium found in soil and water. This bacterium is not pathogenic to humans and although there is no evidence of use of this bacterium in the food industry, the *pat* gene has been used to confer tolerance to glufosinate ammonium herbicides in food producing crops for over two decades (CERA, 2011).

### 2.2.3 Other organisms

Regulatory and filler sequences used in the genetic modification of DP202216 were sourced from the *Z. mays* host, potato (*Solanum tuberosum*), bacteriophages and *Saccharomyces cerevisiae* (refer to [Table 1](#Table1)). These non-coding sequences are used to drive or enhance expression of the two new genes, mediate cloning and facilitate detection.

# 3 Molecular characterisation

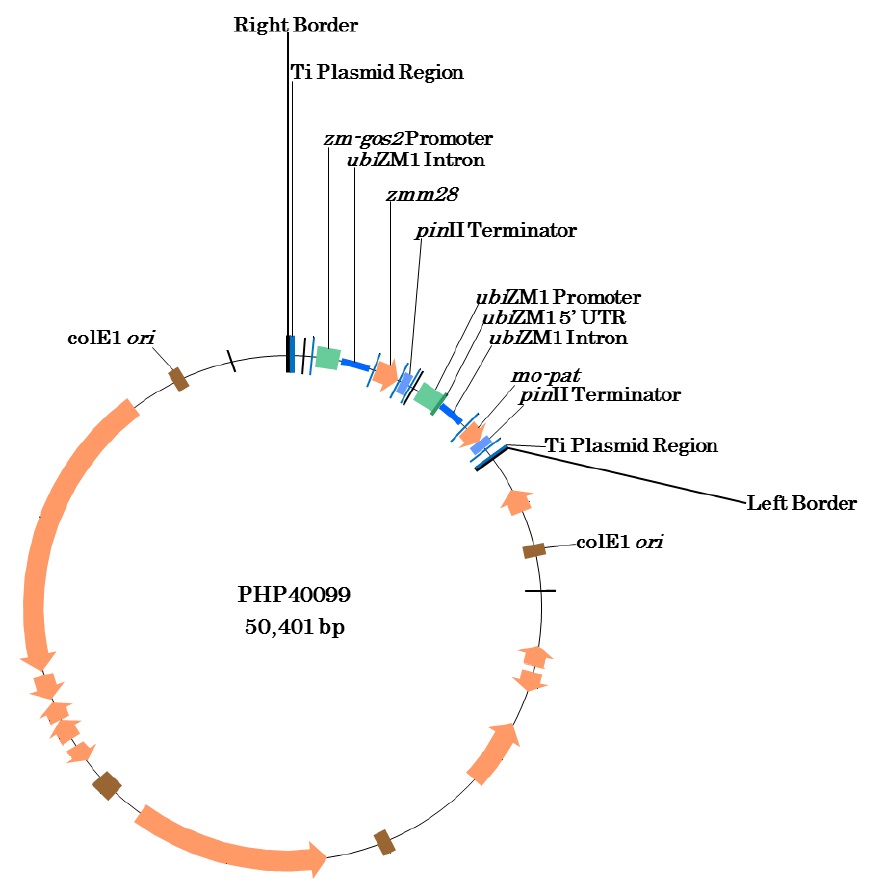
Molecular characterisation is necessary to provide an understanding of the genetic material introduced into the host genome and helps to frame the subsequent parts of the safety assessment. The molecular characterisation addresses three main aspects:

* the transformation method together with a detailed description of the DNA sequences introduced to the host genome
* a characterisation of the inserted DNA, including any rearrangements that may have occurred as a consequence of the transformation
* the genetic stability of the inserted DNA and any accompanying expressed traits.

## 3.1 Transformation Method

In order to create DP202216, plasmid PHP40099 was transformed into the proprietary inbred corn line PH17AW. Plasmid PHP40099 contains two gene cassettes that encode the ZMM28 and PAT proteins ([Figure 1](#Figure1)).

The transformation method involved inoculation of immature corn embryos with a disarmed strain of *Agrobacterium* LBA4404 (Zhao et al. 2001) containing plasmid PHP40099. This plasmid contains the necessary virulence genes required for transformation of strain LBA4404. The virulence genes enhance the transformation process and do not introduce any novel genes into the plant tissue. After infection, the explants were grown on media containing glufosinate to select positive transformants, and carbenicillin, to suppress growth of the agrobacterium. Transformed callus was subsequently cultured in media to encourage shoot and root formation. Rooted plants (T0) were then transferred to soil to grow into mature plants. T0 plants and subsequent generations were evaluated and DP202216 was selected as the commercial candidate.



**Figure 1:** Plasmid map of PHP40099

## 3.2 Detailed description of DNA to be introduced

The plasmid PHP40099 ([Figure 1](#Figure1)), used to generate DP202216, contains two gene cassettes in the T-DNA region. At the right border (RB) is the *zmm28* gene cassette. Expression of the gene is under the control of a constitutive promoter region from the protein translation factor *gos2* gene from *Z. mays* and the proteinase inhibitor II (*pin*II) gene terminator sequence from *S. tuberosum*. An intronic sequence from the *Z. mays* ubiquitin gene 1 (*ubi*ZM1 intron) is also included between the promoter and gene, as this has been shown to enhance gene expression (Assem et al. 2002).

At the left border (LB) is the *mo-pat* gene cassette. Expression of the pat gene with a maize-optimised sequence (*mo-pat*) is under the control of the ubiquitin gene 1 promoter (p*ubi*ZM1) sequence from *Z. mays* and the *pin*II terminator sequence from *S. tuberosum*. The bacterial gene sequence for *pat* has been modified to optimise expression of the gene in corn.

Other non-coding sequences are present in the T-DNA region as outlined in [Table 1](#Table1). This includes intervening and site-specific recombination sequences. These sequences assist with generating the plasmid and the subsequent characterisation and detection of the plasmid and expression cassettes.

Table 1: The genetic elements contained in the T-DNA region of PHP40099 used to create DP202216.

|  | **Genetic element** | **Relative position** | **Source** | **Description, Function & Reference** |
| --- | --- | --- | --- | --- |
|  | Right border (RB) | 1 - 25 | *Agrobacterium tumefaciens* | Right border repeat from the Ti plasmid (Komari et al. 1996) |
|  | Right border region | 26 - 177 |  |  |
| *zmm28* expression cassette | Intervening sequence | 178 - 435 |  |  |
| *loxP* | 436 - 469 | *Bacteriophage P1* | Cre recombinase recognition site (Dale and Ow, 1990) |
| Intervening sequence | 470 - 698 |  |  |
| *attB4* | 699 - 719 | *Bacteriophage lambda* | Integrase recombination site (Cheo et al. 2004) |
| Intervening sequence | 720 - 753 |  |  |
| *pzm-gos2* | 754 - 1613 | *Zea mays* | promoter sequence from translation initiation factor *gos2* (Taramino et al. 2015) |
| Intervening sequence | 1614 - 1654 |  |  |
| *ubiZM1 intron* | 1655 - 2667 | *Zea mays* | Intronic sequence from ubiquitin gene 1 (Christensen et al. 1992) |
| Intervening sequence | 2668 - 2707 |  |  |
| *attB1* | 2708 - 2731 | *Bacteriophage lambda* | Integrase recombination site from Invitrogen Gateway cloning system (Hartley et al. 2000; Katzen, 2007) |
| Intervening sequence | 2732 - 2748 |  |  |
| *zmm28* | 2749 - 3605 | *Zea mays* | *zmm28* gene, including native 5’UTR (60bp) and 3’UTR (41bp) sequences (Münster et al. 2002; Par̆enicová et al. 2003) |
| Intervening sequence | 3606 – 3621 |  |  |
| *attB2* | 3622 - 3645 | *Bacteriophage lambda* | Integrase recombination site from Invitrogen Gateway cloning system (Hartley et al. 2000; Katzen, 2007) |
| Intervening sequence | 3646 – 3659 |  |  |
| *tpin*II | 3660 - 3967 | *Solanum tuberosum* | Terminator sequence from proteinase inhibitor II gene (An et al. 1989; Keil et al. 1986) |
| Intervening sequence | 3968 - 3997 |  |  |
| *attB3* | 3998 - 4018 | *Bacteriophage lambda* | Integrase recombination site (Cheo et al. 2004) |
| Intervening sequence | 4019 - 4091 |  |  |
| *loxP* | 4092 - 4125 | *Bacteriophage P1* | Cre recombinase recognition site (Dale and Ow, 1990) |
| Intervening sequence | 4126 - 4144 |  |  |
| *mo-PAT* expression cassette | *pubiZM1* | 4145 – 5044 | *Zea mays* | Promoter sequence from ubiquitin gene 1 (Christensen et al. 1992) |
| *ubiZM1 5’UTR* | 5045 - 5127 | *Zea mays* | 5’UTR sequence from ubiquitin gene 1 (Christensen et al. 1992) |
| *ubiZM1 intron* | 5128 - 6140 | *Zea mays* | Intronic sequence from ubiquitin gene 1 (Christensen et al. 1992) |
| Intervening sequence | 6141 - 6168 |  |  |
| *FRT1* | 6169 - 6216 | *Saccharomyces cerevisiae* | Flippase recombination target site (Proteau et al. 1996) |
| Intervening sequence | 6217 - 6242 |  |  |
| *mo-pat* | 6243 - 6794 | *Streptomyces viridochromogenes* | Maize-optimised PAT gene (Wohlleben et al. 1988) |
| Intervening sequence | 6795 - 6801 |  |  |
| *tpin*II | 6802 – 7112 | *Solanum tuberosum* | Terminator sequence from proteinase inhibitor II gene (An et al. 1989; Keil et al. 1986) |
| Intervening sequence | 7113 - 7133 |  |  |
| *FRT87* | 7134 - 7181 | *Saccharomyces cerevisiae* | Modified flippase recombination target site (Tao et al. 2007) |
| Intervening sequence | 7182 – 7388 |  |  |
|  | *Left border region* | 7389 - 7445 | *Agrobacterium tumefaciens* | Left border region from the Ti plasmid (Komari et al. 1996) |
|  | *Left border* | 7446 - 7470 |

## 3.3 Development of the corn line from original transformation

A breeding programme was undertaken for the purposes of:

• obtaining generations suitable for analysing the characteristics of DP202216

• ensuring that the DP202216 event is incorporated into elite lines for commercialisation of enhanced yield and glufosinate-tolerant corn.

The generations analysed for the molecular characterisation are listed in [Table 2](#Table2).

For some of the characterisation studies, the applicant made use of a novel methodology developed in-house, for characterising DP202216. The method combines Southern hybridisation techniques with Next-Generation sequencing and has been termed Southern-by-Sequencing (SbSTM). Details of the methodology and proof of concept work is publically accessible in the following publications: Zastrow-Hayes et al. (2015) and Brink et al. (2019).

Table 2: Generations used in the characterisation studies performed on DP2022161

|  |  |  |
| --- | --- | --- |
| **Analysis** | **Generations analysed** | **Control(s) used** |
| Identifying the number of integration sites (Section 3.4.1) | T1 | PH17AW; PH17AW spiked with Plasmid PHP40099 |
| Detection of backbone sequence  (Section 3.4.2) | T1 | PH17AW; PH17AW spiked with Plasmid PHP40099 |
| Insert integrity and site of integration  (Section 3.4.3) | T1 |  |
| Genetic stability of the inserted DNA  (Section 3.4.4) | T1, T2, BC1F1, BC3F3, BC3F6 |  |
| Mendelian inheritance (Section 3.4.4) | T2, F1 (PH17AW/PHR1J), BC1F1, BC3F3, BC3F6 |  |

### 3.4.1 Identifying the number of insertion sites

SbS was performed on leaf-derived genomic DNA from DP202216-derived plants (n=6) and the parental PH17AW cultivar. A reference sample was generated, where the PH17AW genomic DNA was spiked with an equimolar amount of plasmid PHP40099.

Sequencing libraries were prepared on sheared genomic DNA, with an average fragment size of 400 bp. The DNA was enriched twice by hybridisation, using a series of probes covering the entire T-DNA region of the plasmid. The enriched samples were sequenced using an Illumina platform. Sufficient sequence fragments were obtained to cover the genomes being analysed, with a depth of coverage > 100x. Comparison of the sequence between the PH17AW and DP202216 showed that a single integration event has occurred, with only two junction sites detected.

Background reads were detected in the parental control PH17AW with a coverage of 35x. This was not unexpected because endogenous sequences from *Z. mays* were present in the T-DNA, such as the ubiquitin gene 1 promoter (*pubiZM1*) and *zmm28* gene, that would be present in both PH17AW and DP202216. No junctions between the plasmid DNA and genomic DNA were identified, confirming that the reads were identifying the endogenous sequences and not miscellaneous introduced sequences.

### 3.4.2 Detection of backbone sequence

SbS was performed on DP212216, PH17AW and the plasmid spiked-PH17AW samples using hybridisation probes covering the backbone sequences of the plasmid. Hybridisation against the backbone sequences generated sequence data in the plasmid spiked sample only. This confirms there was no integration of the backbone sequences into DP202216.

### 3.4.3 Insert integrity and site of integration

Sequence data generated from the SbS analyses confirmed that a single insert was present and had not undergone rearrangement. There were minor truncations detected in the left (-22bp) and right (-11bp) border regions of the T-DNA, which occurs due to the presence of nucleases during the *Agrobacterium* transformation process (Kim et al. 2007). The site of integration was also identified.

### 3.4.4 Inheritance and genetic stability of the inserted DNA

Since there was demonstration of the insert being present at a single locus in the DP202216 genome, there is the expectation that the genetic elements within this locus would be inherited according to Mendelian principles.

Chi-square (Χ2) analysis was undertaken over five generations ([Table 2](#Table2)) to confirm the segregation and stability of the T-DNA insert in DP202216. The number for each DP202216-derived plant generation analysed was 100 and for the non-GM near-isoline plants the number was 10. The inheritance pattern was analysed at the genotypic level by quantitative real-time PCR using primers targeting the *zmm28* and *mo-pat* genes and at the phenotypic level by observing plant survival after exposure to glufosinate. Positive plants were those that were glufosinate tolerant and carried both gene inserts.

The expected segregation ratio for T2 is 3:1 and the F1 generations (F1 (PH17AW/PHR1J) and BC1F1) would be 1:1. BC3F3 and BC3F6 are expected to be homozygous for both traits. The critical value to reject the hypothesis of this ratio at the 5% significance level was 3.84 (Strickberger 1976). As the X2 values calculated from these experiments were < 3.84, the results showed there were no significant differences between the observed and expected segregation ratios in any of the generations ([Table 3](#Table3)). These data support the conclusion that the T-DNA is present at a single locus in DP202216 and was inherited predictably according to Mendelian principles in subsequent generations.

Table 3: Segregation results for DP202216

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Generation** | **Expected segregation ratio** | **Observed Segregation ratios** | | | **Statistical Analysis** | | |
| **Positive** | **Negative** | **Total** | | **X2** | ***p*** |
| **T2** | 3:1 | 80 | 20 | 100 | | 1.33 | 0.2482 |
| **F1** | 1:1 | 54 | 46 | 100 | | 0.64 | 0.4237 |
| **BC1F1** | 1:1 | 42 | 58 | 100 | | 2.56 | 0.1096 |
| **BC3F3** | homozygous | 100 | 0 | 100 | | - | - |
| **BC3F6** | homozygous | 100 | 0 | 100 | | - | - |

### 3.4.5 Open reading frame (ORF) analysis

Using an in-house program, all start-to-stop ORFs were identified in the region encompassing the insert and approximately 1000bp flanking genomic DNA. All six reading frames were analysed. ORFs of ≥ 30 amino acids were captured identifying a total of 45 putative proteins. Proteins of ≥ 30 amino acids meet the minimum requirements of a 35% match over an 80 amino acid sequence (Codex 2009). These 45 putative proteins were then used as query sequences in homology searches for known allergens and toxins.

#### 3.4.5.1 Bioinformatic analysis for potential allergenicity

The applicant provided the results of *in silico* analyses comparing the 45 putative proteins to known allergenic proteins listed in the Comprehensive Protein Allergen Resource ([COMPARE](http://comparedatabase.org/database/)[[4]](#footnote-5)) database, from the Health and Environmental Science Institute. At the date of the search, there were 2,081 sequences in the allergen database.

The following analyses were performed for the sequence comparison:

(a) Full length search – a FASTA alignment using a BLOSUM50 scoring matrix and E-value threshold set at 0.0001. Only matches of ≥ 35% similarity over 80 amino acids were considered.

(b) 8-mer exact match search – An in-house program was used to generate all putative 8-amino acid peptides. Only matches of 100% similarity over 8 amino acids were considered.

Of the 45 potential ORFs used to query the COMPARE database, no similarities were found using the full length or 8-mer search to any of the known allergenic proteins.

#### 3.4.5.2 Bioinformatic analysis for potential toxicity

The applicant performed an *in silico* comparative analysis using an in-house database of toxigenic proteins compiled in January 2019. The proteins were identified from the UniProtKB/Swiss-Prot protein databases, using a range of keywords encompassing the function of the protein, such as toxin, vasoactive and hemagglutinin. A FASTA algorithm was used with a BLOSUM62 scoring matrix and the E-value threshold set to 0.0001. No matches were found between the 45 putative proteins and any of the known proteins toxins.

### 3.4.6 Conclusion

The data provided by the applicant shows that a single integration event has occurred at an identified locus. The T-DNA region from PHP40099, containing the *zmm28* and *mo-pat* gene cassettes, has been inserted without rearrangement. Minor truncations were identified at the left and right border regions. No backbone sequences from the transforming plasmid have been incorporated. The introduced DNA was shown to be stably inherited across generations.

# 4 Characterisation and safety assessment of novel substances

In considering the safety of novel proteins it is important to understand that a large and diverse range of proteins are ingested as part of the normal human diet without any adverse effects. Only a small number of dietary proteins have the potential to impair health, because they have anti-nutrient properties or they can cause allergies in some consumers (Delaney et al. 2008). As proteins perform a wide variety of functions, different possible effects have to be considered during the safety assessment including potential toxic, anti-nutrient or allergenic effects.

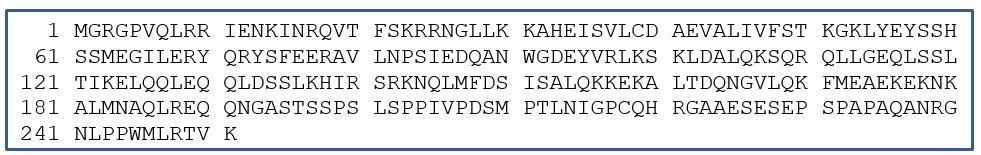
To effectively identify potential hazards, the assessment examined history of human exposure in addition to biochemical and phenotypic analyses. This included an analysis of the concentration levels of the new proteins in the edible components of the plant.

## 4.1 Description of the ZMM28 protein

The *Zea mays* ZMM28 protein is a homeotic MADS[[5]](#footnote-6)-box transcription factor, involved in regulating gene transcription associated with floral organ development. Members of the MADS-box gene family are found in all eukaryotes, including plants and animals used for food (Shore and Sharrocks, 1995; Schilling et al. 2018; Ali et al. 2019). The source of the gene encoding the ZMM28 protein is corn, which has a long history of safe use as food (see [Section 2.1](#_2.1_Host_organism) and [Section 4.1.3](#_4.1.3_Safety_of)).

Examination of corn overexpressing ZMM28 showed increased grain yields (Wu et al. 2019). The increased grain yields were associated with increased plant height, leaf biomass and total leaf area. Biochemical analyses showed increased photosynthesis, over an extended growth period and increased efficiency of nutrient utilisation.

The *zmm28* gene prepared by the applicant encodes a protein of 251 amino acids ([Figure 2](#Figure2)), with an expected mass of 28372.32 Da.

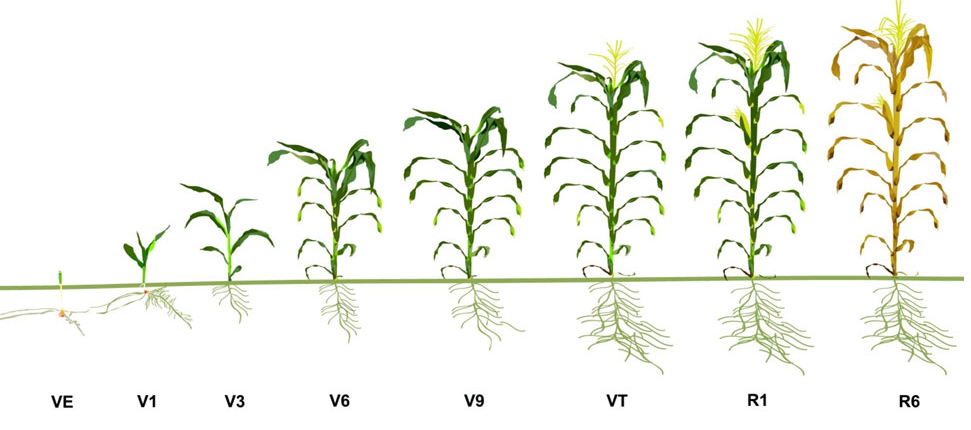
****

**Fig****ure 2:** Deduced amino acid sequence of the ZMM28 protein

### 4.1.1 Characterisation of ZMM28 expressed in DP202216

The amino acid sequence of ZMM28 was determined by an *in silico* translation of the integrated gene sequence in DP202216. The results confirmed the new DNA would encode a protein that matched the expected sequence.

Characterisation of the protein was performed by western blot, using extracts from both DP202216 and the non-GM near-isoline F1(PHR1J/PHW2Z) control. Expression was examined in both leaf at the vegetative growth stage 9 (V9 – ninth leaf is visible) and the grain at the reproductive growth stage 6 (R6 – plant is mature & grain can be harvested) ([Figure 3](#Figure3); Abendroth et al. 2011). Results identified a single protein between 25-37 kDa from leaf and grain of DP202216 that was immunoreactive to a ZMM28 antibody. This protein matched the size of the endogenous protein expressed in leaf tissue from the control. The results also showed the protein expressed in DP202216 was present at higher levels compared to control in the leaf. In the grain, ZMM28 was only detected in DP202216.



**Fi****gure 3:** Stages of corn growth: Image showing the vegetative (VE-VT) and reproductive (R1-R6) growth stages of corn. VE – emergence from soil; VN – N indicates the number of leaf collars present; VT – tasselling; R1 – silking; R6 – full maturity and when grain is harvested. Image from [Staging Corn Growth](https://www.pioneer.com/us/agronomy/staging_corn_growth.html)[[6]](#footnote-7) (Pioneer)

### 4.1.2 Expression of ZMM28 in various tissue samples from DP202216

ZMM28 expression levels were quantified using an ELISA. Various tissue matrices were examined from DP202216 and the non-GM near-isoline F1(PHR1J/PHW2Z) control. Four samplings of each tissue matrix was collected from plants grown across six field-trial sites[[7]](#footnote-8), during the 2017 growing season in the US and Canada.

Results from the ELISA (Table 4) show that ZMM28 was detected in various tissues of the control, with the highest expression in the leaf at the stages of growth when the tassel has formed and silks become visible on the husks (V9-R1; [Figure 3](#Figure3)). This expression pattern was also observed in the whole plant. Examination of expression levels in DP202216 show increased levels at V9 and R1 compared to the control, with an extended period of increased expression in the later reproductive growth stage of R4.

The expression of ZMM28 in corn grain could not be determined using the ELISA due to components in the grain extract interfering with the assay. A semi-quantitative method was therefore developed using a western blotting procedure, using the same reference protein as that for the ELISA. Similar to the data presented in [Section 4.1.1](#_4.1.1_Characterisation_of), ZMM28 was not detected in the grain from the control but was present in DP202216 at a level of 0.012 ng/mg DW ± 0.0070 SD (<LLOQ – 0.029).

Table 4: Expression of ZMM28 (ng/mg DW)1 in various tissues

| **Matrix** | **Growth Stage2** | **DP202216** | | | **Control** | | |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Mean** | **Range** | **SD3** | **Mean** | **Range** | **SD** |
| **Leaf** | V6 | 0.087 | LLOQ4 – 0.33 | 0.098 | 0.062 | LLOQ – 0.28 | 0.081 |
| V9 | 0.28 | 0.066 – 0.72 | 0.18 | 0.21 | 0.060 – 0.56 | 0.13 |
| R1 | 0.32 | 0.084 – 0.66 | 0.15 | 0.22 | LLOQ – 0.44 | 0.11 |
| R4 | 0.12 | LLOQ – 0.22 | 0.049 | 0.079 | LLOQ – 0.14 | 0.037 |
| R6 | <LLOQ | NA5 | NA | <LLOQ | NA | NA |
| **Pollen** | R1 | 0.15 | LLOQ – 0.028 | 0.0029 | <LLOQ | NA | NA |
| **Root** | V9 | 0.031 | LLOQ – 0.078 | 0.018 | 0.019 | LLOQ – 0.051 | 0.011 |
| R1 | 0.015 | LLOQ – 0.029 | 0.0041 | 0.016 | LLOQ – 0.042 | 0.0076 |
| R4 | 0.019 | LLOQ – 0.042 | 0.0091 | <LLOQ | NA | NA |
| R6 | 0.015 | LLOQ – 0.042 | 0.0058 | 0.014 | LLOQ – 0.033 | 0.0040 |
| **Forage** | R4 | 0.049 | LLOQ – 0.12 | 0.02 | 0.029 | LLOQ – 0.058 | 0.013 |
| **Whole plant** | V9 | 0.23 | 0.16 – 0.36 | 0.061 | 0.20 | 0.11 – 0.34 | 0.069 |
| R1 | 0.18 | 0.12 – 0.26 | 0.040 | 0.14 | 0.08 – 0.20 | 0.036 |
| R6 | 0.019 | LLOQ – 0.04 | 0.0045 | 0.019 | LLOQ – 0.044 | 0.0053 |

1. DW - dry weight; 2. Growth Stage abbreviations – see legend for [Figure 3](#Figure3); 3. SD – standard deviation; 4. LLOQ – lower limit of quantification for the assay; 5.. NA – not applicable.

Analysis of the range and standard deviation indicates variable expression levels per tissue. Examination of the data generated from each tissue matrix per plant at the different trial sites indicate the expression of ZMM28 can be influenced by environmental factors, likely soil type and localised agronomical procedures. This is not unexpected considering ZMM28 is a transcription factor that would in turn be responsive to environmental or external input.

### 4.1.3 Safety of ZMM28 in corn grain

### Prior history of human consumption

The donor for the *zmm28* gene is corn. An analysis of 577 in-house commercial lines and six public database sequences for corn grain, showed the sequence for this gene is present in 98% of corn cultivars (Anderson et al. 2019). The remaining 2% expressed a protein with 2 amino acid substitutions. Protein analysis in corn grain at R6 showed that ZMM28 is not detected ([Table 4](#Table4); Anderson et al. 2019). However sweet corn is typically consumed at the R3 stage, where ZMM28 is detected (Anderson et al. 2019), demonstrating the presence of ZMM28 in food.

A BlastP search showed ZMM28 has a high degree of sequence identity (>80%) to MADS-box proteins in other members of the *Poaceae* family traditionally used in food, such as sorghum, rice, barley and wheat. Extending the analysis to fruits and vegetables showed sequence identity >50% (Anderson et al. 2019). While transcripts that code for ZMM28 protein homologues have been identified in edible portions of a range of food crops, no protein analysis has been undertaken to confirm expression or presence of the protein.

**Post-translational modification**

A comparison of the spectral analyses of microbially expressed ZMM28 to that *in planta* indicates glycosylation has not occurred (Anderson et al. 2019 supplemental data). This is further supported by an analysis of the amino acid sequence for ZMM28, showing absence of consensus motifs for glycosylation (Lowenthal et al. 2016).

### Bioinformatic analyses

The results of *in silico* analyses comparing the ZMM28 amino acid sequence to known allergenic proteins in the COMPARE dataset, as outlined in [Section 3.4.5.1](#_3.4.5.1__Bioinformatic) did not identify any known allergens with similarity to ZMM28.

Results were also provided of *in silico* analyses comparing the amino acid sequence of ZMM28 to proteins identified as toxins as outlined in [Section 3.4.5.2](#_3.4.5.2__Bioinformatic). The search did not identify any known toxins with similarity to ZMM28.

### 4.1.4 Conclusion

Characterisation studies confirm DP202216 overexpresses a protein that is immunoreactive to a ZMM28 antibody and matches the expected size of the endogenous protein expressed by the host. The protein is predominantly found in the leaf tissue, over an extended time period. In particular, expression is maintained into the mid-late reproductive stages, with low levels detected in the grain. Expression was also shown to be influenced by environmental factors, which is expected for a transcription factor.

In the safety assessment, it was recognised that both the gene donor and host are corn, which is a very common food. There has been demonstration that corn-derived ZMM28 is present in the food supply, particularly in sweet corn, indicating a prior history of human consumption. Although ZMM28 is not detected in mature grain in the control line, the expression level in grain from DP202216 averages 0.000014% of total protein, which is very low. The bioinformatic analyses did not identify similarity to any known protein allergens or toxins. Using a weight-of-evidence approach, based on a prior history of human exposure, the low total levels of the protein in grain and the absence of any similarity to known protein allergens or toxins, it can be concluded that the presence of ZMM28 in DP202216 does not raise any safety concerns.

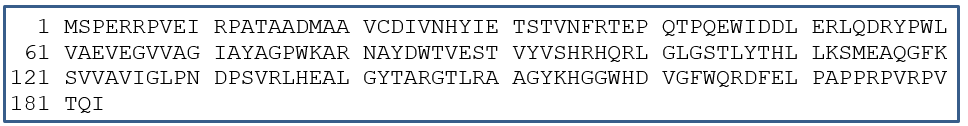
## 4.2 Description of the PAT protein

The *pat* gene from *S. viridochromogenes* encodes the phosphinothricin acetyltransferase (PAT) enzyme (Strauch et al. 1988). This enzyme detoxifies an antibiotic the bacterium co-produces, to reduce competition from neighbouring bacteria (Hara et al. 1991). The antibiotic has been named bialaphos and was first isolated in 1973 (Demain and Sanchez, 2009; CERA 2011).

Bialaphos is a tripeptide, composed of two L-alanine residues and a glutamic acid analogue, L-phosphinothricin (L-PPT). The L-PPT component mediates the antibiotic effect by competitively binding to glutamine synthetase, an essential enzyme required for nitrogen metabolism. By inhibiting enzyme function, there is rapid accumulation of ammonia, leading to cell death. The activity of bialaphos was primarily demonstrated against bacteria but has also been shown to be effective against fungi (fungicide) and plants (herbicide). The common name for bialaphos when used as a herbicide is glufosinate.

The commercialisation of plants engineered for glufosinate-tolerance using the *pat* gene began in the mid-1990s (CERA 2011). The history of use of the enzyme in crops therefore extends about 25 years, with FSANZ having assessed and approved 17 events with *pat* encoded glufosinate-tolerance since 2002 (FSANZ 2002 - A372).

In this application, the gene sequence for *pat* has been codon optimised for expression in corn and has been identified as maize-optimised (*mo-pat*). A translation of the DNA sequence of the *mo-pat* gene in DP202216 gives a protein comprising 183 amino acids ([Figure 4](#Figure4)) with 100% similarity to the enzyme expressed in *S. viridochromogenes*, with a calculated molecular weight of 20618.32 Da.



**Figure 4:** Deduced amino acid sequence of the PAT protein

### 4.2.1 Characterisation of PAT expressed in DP202216

The amino acid sequence of PAT was determined by an *in silico* translation of the integrated gene sequence in DP202216. The results confirmed the new DNA would encode a protein with the expected amino acid sequence.

Characterisation of the expressed protein was performed by western blot, using extracts from both DP202216 and the non-GM near-isoline F1(PHR1J/PHW2Z) control. Two GM corn reference lines expressing PAT were also included: DAS-01507-1 (FSANZ A446 ) and DAS-59122-7 (FSANZ - A543 ). Results identified a single protein in the leaf extract of DP202216 at approximately 20 kDa that was immunoreactive to the PAT antibody. This protein matched the size of the protein expressed in leaf tissue from the two reference lines. The protein was not expressed in the leaf tissue of the control.

The function of the protein was also demonstrated *in planta*. Plantlets were derived from transformed embryonic tissue cultured in glufosinate-containing medium during the selection of transformants ([Section 3.1](#_3.1_Transformation_Method)).

### 4.2.2 Expression of the PAT in DP202216 grain

PAT expression was also examined in the same processed components of corn analysed for ZMM28 ([Section 4.1.2](#_4.1.2_Expression_of)) using an ELISA. Results from the ELISA (Table 5) show that PAT was detected in DP202216, with the highest expression in the leaf in the mid reproductive stage (R4; [Figure 3](#Figure3)) and pollen in the early reproductive stage (R1; [Figure 3](#Figure3)), when pollen is produced. By maturity, PAT is detected in the grain but there is no PAT detected in the leaf tissue in DP202216. There was also no detection of PAT in the non-GM near-isoline F1(PHR1J/PHW2Z) control, as expected because this line does not contain the *pat* gene.

Table 5: Expression of PAT (ng/mg DW)1 in various tissues

| **Matrix** | **Growth Stage2** | **DP202216** | | |
| --- | --- | --- | --- | --- |
| **Mean** | **Range** | **SD3** |
| **Leaf** | V6 | 25 | 14 - 40 | 5.6 |
| V9 | 20 | 9.6 - 46 | 8.2 |
| R1 | 41 | 27 - 56 | 9.3 |
| R4 | 88 | 30- 190 | 36 |
| R6 | < LOQ4 | < LOQ | NA5 |
| **Pollen** | R1 | 76 | 66 - 110 | 10 |
| **Root** | V9 | 17 | 0.072 - 30 | 9.2 |
| R1 | 7.4 | 2.7 - 15 | 3.8 |
| R4 | 11 | 4.5 - 20 | 4.1 |
| R6 | 11 | <LOQ - 23 | 7.1 |
| **Forage** | R4 | 32 | 16 - 48 | 8.1 |
| **Whole plant** | V9 | 26 | 15 - 36 | 5.1 |
| R1 | 32 | 20 - 46 | 6.8 |
| R6 | 21 | 0.52 - 68 | 16 |
| **Grain** | R6 | 15 | 7.5 - 21 | 3.2 |

1. DW - dry weight. 2. Growth Stage abbreviations – see legend for [Figure 3](#Figure3); 3. SD – standard deviation; 4. < LOQ – below the limit of quantification for the assay. 5. NA – not applicable.

### 4.2.3 Safety of PAT in DP202216 grain

FSANZ has previously assessed the safety of PAT in 17 applications of which eight involved corn. A summary of these previous characterisations is provided in Table 6. For information, a reference is provided to the application in which the most recent detailed study or information was considered by FSANZ and is available on the FSANZ website. The overwhelming evidence from these previous studies indicates that PAT is safe for humans at the levels expressed in plants. This is supported by peer-reviewed literature examining the safety of PAT (Herouet et al. 2011).

Table 6: Summary of consideration of PAT in previous FSANZ safety assessments

|  |  |  |
| --- | --- | --- |
| **Consideration** | **Sub-section** | **PAT** |
| Potential toxicity | Amino acid sequence similarity to protein toxins | This application – using an updated search (April 2019) |
| *In vitro* digestibility | A1080 (FSANZ 2013a) |
| Stability to heat | A1080 (FSANZ 2013a) |
| Acute oral toxicity | A1080 (FSANZ 2013a) |
| Potential allergenicity | Source of the protein | A1087 (FSANZ 2013b) |
| Amino acid sequence similarity to allergens | This application – using an updated search (April 2019) |

### Bioinformatic analyses of PAT

The applicant provided updated results from *in silico* analyses comparing the PAT amino acid sequence to known allergenic proteins ([Section 3.4.5.1](#_3.4.5.1__Bioinformatic)) and toxins ([Section 3.4.5.2](#_3.4.5.2__Bioinformatic)). The updated searches did not identify any known allergens or toxins with amino acid sequence similarity to this protein.

### 4.2.4 Conclusion

The data presented by the applicant confirms DP202216 expresses a protein that is immunoreactive to a PAT antibody and matches the expected size of the same recombinant protein expressed in other GM corn lines. The protein is predominantly found in the leaf tissue and pollen, with some expression in grain. Updated *in silico* analyses continue to indicate PAT has no significant similarity with known allergens or toxins.

## 4.4 Novel herbicide metabolites in GM herbicide-tolerant plants

FSANZ has previously assessed the novel herbicide metabolites for glufosinate in corn over eight applications. These previous assessments indicate the spraying of DP202216 with glufosinate ammonium results in the same metabolites that are produced in non-GM corn sprayed with the same herbicide. It is expected that no new glufosinate metabolites would be generated in corn event DP202216.

# 5 Compositional analysis

The main purpose of compositional analyses is to determine if, as a result of the genetic modification, an unexpected change has occurred to the food. These changes could take the form of alterations in the composition of the plant and its tissues and thus its nutritional adequacy. Compositional analyses can also be important for evaluating the intended effect where there has been a deliberate change to the composition of the food.

The classic approach to the compositional analyses of GM food is a targeted one. Rather than analysing every possible constituent, which would be impractical, the aim is to analyse only those constituents most relevant to the safety of the food or that may have an impact on the whole diet. Important analytes therefore include the key nutrients, toxicants and anti-nutrients for the food in question. The key nutrients and anti-nutrients are those components in a particular food that may have a substantial impact in the overall diet. They may be major constituents (fats, proteins, carbohydrates or enzyme inhibitors such as anti-nutrients) or minor constituents (minerals, vitamins). Key toxicants are those toxicologically significant compounds known to be inherently present in an organism, such as compounds whose toxic potency and level may be significant to health.

## 5.1 Key Components

The key components to be analysed for the comparison of transgenic and conventional corn are outlined in the OECD Consensus Document on Compositional Considerations for New Varieties of Maize (OECD 2002), and include: proximates and fibre, amino acids, fatty acids and the anti-nutrients phytic acid, raffinose, furfual, and the phenolic acids ferulic acid and *p*-coumaric acid.

## 5.2 Study design

Eight field trials were conducted for DP202216 in the US and Canada in the 2017 growing season. The sites[[8]](#footnote-9) were selected to match the typical geographical and field management styles of the commercial corn growing regions. The agronomic practices and pest control measures used were location-specific and were typical for all aspects of corn cultivation including soil preparation, fertiliser application, irrigation and pesticide-based control methods. The materials tested in the field trials included DP202216, the non-GM near-isoline F1(PHR1J/PHW2Z) control and a total of 16 reference varieties. Four reference varieties were grown at each site and were selected from 34N84, 35F38, 35P12, P0506, P0589, P0760, P0965, P0987, P0993, XL5140, XL5513, XL5828, XL5840, BK5883, XL5939 and BK6076.

The field trials were established in a randomised complete block design, with four replicates of each plot. Plots were separated by a combination of fallow alleyways and planted buffers of conventional non-GM corn.

Corn grains were harvested from all plots at maturity. After harvest, samples were despatched to an analytical laboratory under full identity labelling. The analyses were performed at EPL Bio Analytical Services. The compositional analyses were based on internationally recognised procedures including official methods specified by the Association of Official Analytical Chemists (AOAC), the USDA and published articles or technical notes from industrial-based sources.

A total of 70 different analytes were measured. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC). For each analyte, ‘descriptive statistics’ (mean, range and 95% confidence interval) were generated. A linear mixed model analysis of variance was then applied for combined data, and locations, covering the eight replicated field trial sites. The mixed model analysis was also applied to the data from each site separately. In assessing the significance of any difference between the mean analyte value for DP202216 and the control, a P-value of 0.05 was used. Where statistically significant differences were observed in the combined data from all sites (presented in Tables 7-12), analysis of the data from each site was used to determine if the differences were common to the majority of sites. A further adjusted P-value was determined using the false discovery rate (FDR) method, as a consideration of the chance of false positives being observed with the testing over multiple sites.

In order to complete the statistical analysis for any component in this study, a measured value from an analyte below the limit of quantification (LOQ), was given an arbitrary value of half the LOQ. Any analyte with all observations below the LOQ for that assay, were excluded from the overall summary analysis. Values for all components were expressed on a dry weight basis with the exception of vitamins, expressed as milligrams per kilogram of solid (dry weight), and fatty acids, expressed as percent of total fatty acids.

Compositional data from the non-GM reference varieties grown concurrently in the same trial as DP202216 and the control, were combined across all sites and used to calculate a data range for each component, to define the natural variability in corn varieties grown under the same agronomical conditions. Any statistically significant differences between DP202216 and the control were compared to this data range to assess whether the differences were likely to be biologically meaningful. Further comparisons were performed with an in-house database, containing compositional analyses from 93 non-GM commercial lines cultivated across 88 unique environments in North and South America, from 2003-2015. A final comparison of natural variation was performed from publically available data (Watson 1982; OECD 2002; Codex 2013; Lundry et al, 2013; Cong et al, 2015; ILSI 2016).

## 5.3 Analyses of key components in corn grain

### 5.3.1 Proximates and fibre (7)

There were no statistically significant differences in the proximates and fibre levels between DP202216 and the control ([Table 7](#Table7)).

Table 7: Comparison of Proximates and Fibre (% DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **DP202216 untreated** | **Non-GM reference varieties** | **In-house data** | **Publically available data** |
| **Mean (range)** | **Mean**  **(range)** | **Min-max** | **Min-max** | **Min - max** |
| Ash | 1.27  (0.81-1.43) | 1.30  (0.95-1.54) | 0.83 – 1.63 | 0.98 – 1.80 | 0.62 – 6.28 |
| Carbohydrate | 86.1  (83.6-88.0) | 85.9  (83.9-88.5) | 81.5 – 88.1 | 80.2 – 88.0 | 77.4 – 89.7 |
| Crude protein | 8.36  (7.08-10.5) | 8.58  (7.02-10.6) | 7.12 – 11.7 | 7.18 – 13.2 | 5.72 – 17.26 |
| Crude fat | 4.19  (3.09-5.36) | 4.21  (3.10-5.35) | 2.45 – 5.86 | 2.58 – 6.00 | 1.36 – 7.83 |
| Crude Fibre | 2.36  (1.71-3.14) | 2.39  (1.13-3.06) | 1.18 – 4.04 | 1.44 – 3.48 | 0.49 – 5.50 |
| Acid Detergent Fibre | 4.24  (3.45-5.77) | 4.55  (2.87-6.88) | 2.89 – 7.94 | 2.64 – 6.26 | 1.41 – 11.34 |
| Neutral Detergent Fibre | 9.74  (6.88-11.4) | 9.48  (6.86-11.3) | 5.87 – 12.7 | 7.22 – 20.8 | 4.28 – 22.64 |
| Total Dietary Fibre | 8.88  (6.81-12.7) | 8.94  (6.96-13.2) | 6.53 – 15.2 | 5.91 – 15.2 | 6.68 – 35.31 |

### 5.3.2 Amino acids

Using the raw P-value, a statistically significant increase was observed in DP202216 compared to the control for glycine, methionine and serine ([Table 8](#Table8)). With the FDR adjustment, the P values were no longer significant. The observed values seen in DP202216 fall well within the variance seen in the reference lines grown under the same conditions, the commercial lines and publically available data. Examination of the data from each site identified 1-2 sites where the data for either the control or DP202216 was more variant than the other sites. These data highlight the impact of environmental factors on composition.

Table 8: Comparison of Amino Acids (% DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **DP202216 untreated** | **Non-GM reference varieties** | **In-house data** | **Publically available data** |
| **Mean (range)** | **Mean (range)** | **Min-max** | **Min-max** | **Min - max** |
| Alanine | 0.609  (0.503-0.803) | 0.623  (0.479-0.800) | 0.50 – 0.94 | 0.49 – 1.08 | 0.44 – 1.48 |
| Arginine | 0.380  (0.309-0.429) | 0.390  (0.315-0.450) | 0.31 – 0.50 | 0.32 – 0.57 | 0.12 – 0.71 |
| Aspartic Acid | 0.530  (0.434-0.649) | 0.540  (0.412-0.651) | 0.43 – 0.77 | 0.45 – 0.92 | 0.33 – 1.21 |
| Cysteine | 0.191  (0.124-0.228) | 0.201  (0.126-0.239) | 0.10 – 0.27 | 0.13 – 0.30 | 0.12 – 0.51 |
| Glutamic Acid | 1.53  (1.23-2.03) | 1.57  (1.20-2.03) | 1.24 – 2.38 | 1.04 – 2.70 | 0.97 – 3.54 |
| Glycine | 0.350  (0.304-0.392) | 0.362  (0.303-0.461) | 0.29 – 0.45 | 0.29 – 0.49 | 0.18 – 0.69 |
| Histidine | 0.249  (0.206-0.300) | 0.256  (0.207-0.297) | 0.20 – 0.35 | 0.18 – 0.36 | 0.14 – 0.46 |
| Isoleucine | 0.282  (0.231-0.389) | 0.289  (0.223-0.386) | 0.24 – 0.42 | 0.23 – 0.49 | 0.18 – 0.69 |
| Leucine | 1.01  (0.802-1.46) | 1.03  (0.778-1.45) | 0.84 – 1.62 | 0.76 – 1.85 | 0.64 – 2.49 |
| Lysine | 0.263  (0.198-0.319) | 0.272  (0.220-0.327) | 0.13 – 0.39 | 0.19 – 0.41 | 0.13 – 0.67 |
| Methionine | 0.187  (0.135-0.231) | 0.201  (0.143-0.234) | 0.10 – 0.25 | 0.11 – 0.34 | 0.10 – 0.47 |
| Phenylalanine | 0.418  (0.293-0.570) | 0.430  (0.314-0.567) | 0.32 – 0.63 | 0.34 – 0.74 | 0.24 – 0.93 |
| Proline | 0.780  (0.649-1.01) | 0.798  (0.616-1.01) | 0.63 – 1.11 | 0.60 – 1.25 | 0.46 – 1.75 |
| Serine | 0.430  (0.342-0.526) | 0.446  (0.346-0.609) | 0.36 – 0.60 | 0.30 – 0.68 | 0.18 – 0.91 |
| Threonine | 0.310  (0.265-0.371) | 0.318  (0.260-0.374) | 0.27 – 0.41 | 0.18 – 0.48 | 0.22 – 0.67 |
| Tryptophan | 0.058  (0.036-0.07) | 0.059  (0.037-0.07) | 0.04 – 0.08 | 0.04 – 0.09 | 0.03 – 0.22 |
| Tyrosine | 0.216  (0.162-0.283) | 0.221  (0.157-0.273) | 0.18 – 0.32 | 0.16 – 0.42 | 0.10 – 0.73 |
| Valine | 0.384  (0.329-0.485) | 0.394  (0.316-0.489) | 0.33 - .054 | 0.32 – 0.63 | 0.21 – 0.86 |

1. Cells highlighted in blue show statistically significant data for P < 0.05.

### 5.3.3 Vitamins and minerals

Using the raw P-value, a statistically significant difference was observed in DP202216 compared to the control for vitamin B1 and B3 ([Table 9](#Table9)). With the FDR adjustment, the P values were no longer significant. The observed values seen in DP202216 fall well within the variance seen in the reference lines grown under the same conditions, the commercial lines and publically available data. These changes are considered minor and are not biologically significant. Vitamin B2 and β-tocopherol were excluded from the statistical summary due to levels below the LOQ.

Table 9: Comparison of Vitamins (mg/kg DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **DP202216 untreated** | **Non-GM reference varieties** | **In-house data** | **Publically available data** |
| **Mean (range)** | **Mean (range)** | **Min-max** | **Min-max** | **Min - max** |
| β-carotene | 0.983  (0.429–2.08) | 0.962  (0.413-2.30) | 0.249 – 3.51 | <LOQ – 2.06 | 0.3 – 5.4 |
| Vitamin B1 | 2.38  (2.08-3.08) | 2.54  (1.99-3.23) | 1.97 – 3.11 | 1.71 – 5.38 | ND2 – 40.0 |
| Vitamin B3 | 14.7  (10.9-22.7) | 13.5  (9.33-16.2) | 9.49 – 66.0 | 7.86 – 25.2 | ND - 70 |
| Vitamin B5 | 5.11  (3.62-7.10) | 4.71  (3.16-6.22) | 3.08 – 6.51 | 3.05 – 7.66 | 3.0 – 1.4 |
| Vitamin B6 | 4.54  (2.81-9.48) | 4.44  (2.23-8.15) | 2.51 – 10.7 | 1.37 – 8.67 | ND – 12.14 |
| Vitamin B9 | 0.923  (0.565-2.50) | 0.854  (0.235-1.72) | 0.461 – 2.70 | 0.319 – 2.41 | ND – 3.50 |
| α-tocopherol | 4.28  (0.969-7.63) | 4.44  (1.07-8.92) | <LOQ – 21.3 | 0 – 25.1 | ND – 68.67 |
| β-tocopherol | <LOQ | <LOQ | <LOQ | <LOQ – 1.10 | ND – 19.80 |
| γ-tocopherol | 25.9  (10.8-35.6) | 26.9  (11.4-36.3) | 3.06 – 42.7 | 0 – 46.5 | ND – 58.61 |
| δ-tocopherol | 0.519  (<LOQ-1.16) | 0.533  (<LOQ-1.13) | <LOQ – 1.14 | <LOQ – 2.61 | ND – 14.61 |
| total-tocopherol | 31.0  (12.3-42.2) | 32.1  (13.6-42.8) | 5.33 – 52.1 | 0 – 61.0 | ND – 89.91 |

1. Cells highlighted in blue show statistically significant data. 2. ND – not detected; 3. LOQ – limit of quantification.

There were no statistically significant differences in the mineral levels between DP202216 and the control ([Table 10](#Table10)).

Table 10: Comparison of Minerals (mg/kg DW)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **DP202216 untreated** | **Non-GM reference varieties** | **In-house data** | **Publically available data** |
|  | **Mean (range)** | **Mean (range)** | **Min-max** | **Min-max** | **Min - max** |
| Calcium | 0.00342  (0.00285-0.00435) | 0.00340  (0.00271-0.00408) | 0.00212 – 0.00595 | 0.00131 – 0.00784 | ND1 – 0.101 |
| Copper | 0.000128  (<LOQ-0.00024) | 0.000125  (<LOQ-0.00021) | <LOQ2 – 0.000169 | <LOQ – 0.000617 | ND – 0.0021 |
| Iron | 0.00168  (0.00151-0.00195) | 0.00173  (0.00146-0.00220) | 0.00120 – 0.00218 | 0.00118 – 0.00261 | 0.0000712 – 0.0191 |
| Magnesium | 0.108  (0.0876-0.137) | 0.110  (0.0904-0.136) | 0.082 – 0.147 | 0.079 – 0.163 | 0.004 – 1.000 |
| Manganese | 0.000556  (0.00035-0.00080) | 0.000571  (0.00027-0.00085) | 0.0003 – 0.0010 | 0.0003 – 0.00131 | 0.00003 - 0.0054 |
| Phosphorus | 0.296  (0.209-0.367) | 0.298  (0.205-0.351) | 0.189 – 0.410 | 0.204 – 0.429 | 0.010 – 0.750 |
| Potassium | 0.399  (0.306-0.459) | 0.395  (0.316-0.451) | 0.276 – 0.511 | 0.222 – 0.541 | 0.180 – 0.720 |
| Sodium | 0.000158  (<LOQ-0.000961) | 0.000101  (<LOQ-0.000726) | <LOQ – 0.00207 | 0.000003 – 0.00366 | ND – 0.150 |
| Zinc | 0.00226  (0.00183-0.00277) | 0.00226  (0.00166-0.00282) | 0.00150 – 0.00295 | 0.0014 – 0.0037 | 0.00003 – 0.0043 |

1. ND – not detected; 2. LOQ – limit of quantification.

### 

### 5.3.4 Fatty Acids

There were no statistically significant differences in the fatty acid levels between DP202216 and the control ([Table 11](#Table11)). The following fatty acids were excluded from the statistical summary due to levels below the LOQ: 12:0 lauric acid, 14:0 myristic acid, C17:0 heptadecanoic acid, C17:1 heptadecenoic acid and 20:2 eicosadienoic acid.

Table 11: Comparison of Fatty Acids (% Total Fatty Acids)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **DP202216 untreated** | **Non-GM reference varieties** | **In-house data** | **Publically available data** |
| **Mean (range)** | **Mean (range)** | **Min-max** | **Min-max** | **Min - max** |
| C16:0 Palmitic acid | 10.6  (1.03-11.7) | 1.06  (10.3-11.3) | 10.0 – 14.2 | 9.23 – 26.0 | 6.81 – 39.0 |
| C16:1 Palmitoleic acid | 0.0775  (0.037-0.105) | 0.0787  (0.039-0.107) | 0.035 – 0.136 | 0 – 0.463 | ND1 – 0.67 |
| C18:0 Stearic acid | 2.06  (1.77-2.40) | 2.09  (1.66-2.42) | 1.39 – 2.54 | 1.31 – 3.94 | ND – 4.9 |
| C18:1 Oleic acid | 29.9  (28.3-32.3) | 29.9  (27.5-32.5) | 22.4 – 34.3 | 18.9 – 39.4 | 16.4 – 42.8 |
| C18:2 Linoleic acid | 55.0  (51.3-56.7) | 54.9  (51.2-57.3) | 45.5 – 60.6 | 28.9 – 61.4 | 13.1 – 67.7 |
| C18:3 Linolenic acid | 1.33  (1.20-1.53) | 1.33  (1.16-1.56) | 0.92 – 2.21 | 0.04 – 2.15 | ND – 2.33 |
| C20:0 Arachidic acid | 0.388  (0.337-0.498) | 0.390  (0.344-0.526) | 0.296 – 0.558 | 0.296 – 0.916 | 0.267 – 1.2 |
| C20:1 Eicosenoic acid | 0.256  (0.234-0.290) | 0.258  (0.236-0.304) | 0.224 – 0.521 | 0.038 – 0.693 | ND – 1.952 |
| C22:0 Behenic acid | 0.0873  (0.070-0.182) | 0.0871  (0.071-0.204) | 0.069 – 0.314 | 0 – 0.453 | ND – 0.5 |
| C24:0 Lignoceric acid | 0.165  (0.071-0.258) | 0.167  (0.071-0.283) | 0.080-0.391 | 0 – 0.639 | ND – 0.91 |

1. ND – not detected.

### 5.3.5 Anti-nutrients and Secondary Metabolites

There were no statistically significant differences in the anti-nutrient and secondary metabolite levels between DP202216 and the control ([Table 12](#Table12)).

Table 12: Comparison of Anti-nutrients and Secondary Metabolites

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Non-GM counterpart** | **DP202216 untreated** | **Non-GM reference varieties** | **In-house data** | **Publically available data** |
| **Mean (range)** | **Mean (range)** | **Min-max** | **Min-max** | **Min - max** |
| Phytic acid | 0.895  (0.50-1.27) | 0.878  (0.46-1.24) | <LOQ1 – 1.34 | 0.516 – 1.37 | ND2 – 1.94 |
| Inositol | 0.0248  (0.0175-0.0351) | 0.0236  (0.0160-0.0362) | 0.013 – 0.034 | 0.007 – 0.051 | 0.006 – 0.480 |
| Raffinose | 0.0995  (<LOQ-0.183) | 0.104  (<LOQ-0.246) | <LOQ – 0.30 | 0 – 0.44 | ND – 0.47 |
| Trypsin inhibitors | 1.69  (1.22-3.25) | 1.66  (1.05-2.83) | 1.03 – 3.01 | 1.02 – 5.68 | ND – 3.01 |
| Furfural | <LOQ | <LOQ | <LOQ | <LOQ | ND |
| Ferulic acid | 0.207  (0.170-0.249) | 0.213  (0.190-0.254) | 0.135 – 0.324 | 0.109 – 0.359 | 0.02 – 0.44 |
| *p*-coumaric acid | 0.0233  (0.0182-0.0296) | 0.0242  (0.0200-0.0297) | 0.0150 – 0.0505 | 0.0072 – 0.0521 | ND – 0.08 |

1. LOQ – limit of quantification; 2. ND – not detected.

## 5.4 Conclusion

Of the 70 analytes measured in corn grain, mean values were provided for 62 analytes. Minor differences were reported with 5 analytes, where p < 0.05 but the FDR adjusted P was not significant. These differences fall well within the reference ranges of the commercial non-GM lines. Like any food crop, nutrient and anti-nutrient composition of corn grain can be impacted by cultivation site and agricultural practices. The differences reported here are consistent with the normal biological variability that exists in corn.

Overall, the compositional data are consistent with the conclusion that there are no biologically significant differences in the levels of key constituents in DP202216 when compared with conventional corn cultivars already available in agricultural markets.

# 6 Nutritional impact

In assessing the safety of a GM food, a key factor is the need to establish that the food is nutritionally adequate and will support typical growth and wellbeing. In most cases, this can be achieved through a detailed understanding of the genetic modification and its consequences, together with an extensive compositional analysis of the food, such as that presented in [Section 5](#_5_Compositional_analysis) of this report.

Where a GM food has been shown to be compositionally equivalent to conventional varieties, the evidence to date indicates that feeding studies using target livestock or other animal species will add little to the safety assessment (Bartholomaeus et al. 2013; OECD 2003). If the compositional analysis indicates biologically significant changes, either intended or unintended, to the levels of certain nutrients in the GM food, additional nutritional studies should be undertaken to assess the potential impact of the changes on the whole diet.

DP202216 is the result of a simple genetic modification to confer enhanced yield and glufosinate-tolerance, with no intention to significantly alter nutritional parameters in the food. The compositional analyses have demonstrated that the genetic modification has not altered the nutritional adequacy of DP202216 as a source of food when compared with that of conventional corn varieties. The introduction of foods derived from DP202216 into the food supply is therefore expected to have negligible nutritional impact.

# 7 References

Anderson J, Brustkern S, Cong B, Deege L, Delaney B, Hong B, Lawit S, Mathesius C, Schmidt J, Wu J, Zhang J, Zimmermann C (2019) Evaluation of the History of Safe Use of the Maize ZMM28 Protein. Journal of Agricultural and Food Chemistry 67: 7466-7474

Assem SK, El-Itriby HA, Hussein EHA, Saad ME, Madkour MA (2002) Comparison of the efficiency of some novel maize promoters in monocot and dicot plants. Arab J Biotech 5(1):57-66

Bartholomaeus A, Parrott W, Bondy G, Walker K (2013) The use of whole food animal studies in the safety assessment of genetically modified crops: Limitations and recommendations. Crit Rev Toxicol 43(S2):1-24

Brink K, Anitha SC, Beatty MK, Anderson J, Lyon M, Weaver J, Dietrich N (2019) Comparison of Southern-by-Sequencing (SbSTM) technology and Southern blot analysis for molecular characterization of genetically modified crops. J Regul Sci 7:1-14

CERA (2011) A review of the environmental safety of the PAT protein. Center for Environmental Risk Assessment, ILSI Research Foundation. Environ Biosafety Res 10:73–101

Codex (2009) Foods derived from modern biotechnology, 2nd edition. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome.

Cong B, Maxwell C, Luck S, Vespestad D, Richard K, Mickelson J, Zhong C (2015) Genotypic and Environmental Impact on Natural Variation of Nutrient Composition in 50 Non Genetically Modified Commercial Maize Hybrids in North America. Journal of Agricultural Food Chemistry 63:5321-5334

Delaney B, Astwood JD, Cunny H, Eichen Conn R, Herouet-Guicheney C, MacIntosh S, Meyer LS, Privalle LS, Gao Y, Mattsson J, Levine M, ILSI (2008) Evaluation of protein safety in the context of agricultural biotechnology. Food Chem Toxicol 46:S71-S97

Demain A, Sanchez S (2009) Microbial drug discovery: 80 years of progress. J Antibiot 62:5-16

FSANZ (2002) Application A372 - Glufosinate ammonium tolerant canola Topas 19/2. Report prepared by Food Standards Australia New Zealand. <https://www.foodstandards.gov.au/code/applications/Pages/applicationa372glufo948.aspx>

FSANZ (2013a) Application A1080 – Food derived from Herbicide-tolerant Cotton Line MON88701. Report prepared by Food Standards Australia New Zealand.

<https://www.foodstandards.gov.au/code/applications/Pages/A1080-Food-derived-from-Herbicide-tolerant-Cotton-Line-MON88701.aspx>

FSANZ (2013b) A1087 – Food derived from Insect-protected Soybean Line DAS-81419-2. Report prepared by Food Standards Australia New Zealand. <https://www.foodstandards.gov.au/code/applications/Pages/A1087-Food-derived-from-Insect-protected-Soybean-Line-DAS-81419-2.aspx>

GRDC (2017) Maize: Northern region. GrowNotes. Grains Research and Development Corporation. [https://grdc.com.au/GN-Maize-North](https://grdc.com.au/GN-Maize-North?utm_source=website&utm_medium=short_url&utm_term=North&utm_content=Maize%20Northern%20Region%20-%20GrowNotes™) (accessed April 2020)

Hara O, Murakami T, Imai S, Anzai H, Itoh R, Kumada Y, Takano E, Satoh E, Satoh A, Nagaoka K (1991) The bialaphos biosynthetic genes of *Streptomyces viridochromogenes*: cloning, heterospecific expression, and comparison with the genes of *Streptomyces hygroscopicus*. J Gen Microbiol 137(2):351-359

Hoekema A, Hirsch P, Hooykaas P, Schilperoot RA (1983) A binary plant vector strategy based on separation of vir- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303:179–180

ILSI (2016) ILSI Crop Composition Database, Version 6.0. International Life Sciences Institute, <http://www.cropcomposition.org/> (accessed April 2020)

ISAAA (2017) Global Status of Commercialized Biotech/GM Crops in 2017: Biotech Crop Adoption Surges as Economic Benefits Accumulate in 22 Years. The International Service for the Acquisition of Agri-biotech Applications Brief No. 53. ISAAA: Ithaca, NY.

Kim SI, Gelvin SB (2007) Genome‐wide analysis of *Agrobacterium* T‐DNA integration sites in the *Arabidopsis* genome generated under non‐selective conditions. Plant J 51:779-791

Lundry DR, Burns JA, Nemeth MA, Riordan SG (2013) Composition of Grain and Forage from Insect-Protected and Herbicide-Tolerant Corn, MON 89034 x TC1507 x MON 88017 x DAS-59122-7 (SmartStax), Is Equivalent to That of Conventional Corn (Zea mays L.). Journal of Agricultural and Food Chemistry 61: 1991-1998

OECD (2001) Guideline for testing of chemicals. Acute oral toxicity – fixed dose procedure. Test Guideline 420

OECD (2002) Consensus document on compositional considerations for new varieties of maize (*Zea mays*): Key food and feed nutrients, anti-nutrients, toxicants and secondary plant metabolites. ENV/JM/MONO(2002)25. Organisation for Economic Cooperation and Development, Paris.

OECD (2003) Considerations for the Safety Assessment of Animal Feedstuffs Derived from Genetically Modified Plants. Series on the Safety of Novel Foods and Feeds, No. 9. Organisation for Economic Cooperation and Development, Paris.

OGTR (2008) The biology of *Zea mays* L. ssp *mays* (maize or corn). Office of the Gene Technology Regulator. Canberra, ACT

Ranum P, Peña-Rosas JP, Garcia-Casal MN (2014) Global maize production, utilization, and consumption. Ann N Y Acad Sci 1312:105–112

Shaner DL (2004) Herbicide safety relative to common targets in plants and mammals. Pest Manag Sci 60(1):17-24

Strickberger MW (1976) Probability and statistical testing in Genetics, 2nd ed. New York: Macmillan Publishing Company. pp. 140-163

Taramino G, Sakai H, Tingey SV, Luck A, Tomes D, Niu X (2015) Plants with altered root architecture, related constructs and methods involving genes encoding exostosin family polypeptides and homologs thereof. Patent US9115203B2. <http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=1&f=G&l=50&co1=AND&d=PTXT&s1=9115203&OS=9115203&RS=9115203> (accessed April 2020)

Thomas K, Aalbers M, Bannon GA, Bartels M, Dearman RJ, Esdaile DJ, Fu T-J, Glatt CM, Hadfield N, Hatzos C, Hefle SL, Heylings JR, Goodman RE, Henry B, Herouet C, Holsapple M, Ladics GS, Landry TD, MacIntosh SC, Rice EA, Privalle LS, Steiner HY, Teshima R, Van Ree R, Woolhiser M, Zawodny J (2004) A multi-laboratory evaluation of a common in vitro pepsin digestion assay protocol used in assessing the safety of novel proteins. Regulatory Toxicology and Pharmacology 39:87–98

Watson SA (1982) Corn: Amazing Maize. General Properties. In IA Wolff, ed, CRC Handbook of Processing and Utilization in Agriculture, Vol 2. CRC Press, Boca Raton, pp 3-29

Zastrow-Hayes GM, Lin H, Sigmund AL, Hoffman JL, Alarcon CM, Hayes KR, Richmond TA, Jeddeloh JA, May GD, Beatty MK (2015) Southern-by-Sequencing: a robust screening approach for molecular characterization of genetically modified crops. Plant Genome 8(1): e2014.08.0037

Zhao ZY, Wang N, Nubel DS, Zhen S (2010) Highly transformable elite doubled haploid line PH17AW. Patent US20100017908A1 <http://appft.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/srchnum.html&r=1&f=G&l=50&d=PG01&s1=20100017908.PGNR> (accessed March 2020).

1. <http://www.fao.org/faostat/en/#data> [↑](#footnote-ref-2)
2. <https://www.fas.usda.gov/commodities/corn> [↑](#footnote-ref-3)
3. MT - million tons [↑](#footnote-ref-4)
4. <http://comparedatabase.org/database/> [↑](#footnote-ref-5)
5. MADS is an abbreviation of the first four factors identified:- Minichromosome maintenance 1 (MCM1), AGAMOUS (AG), DEFICIENS (DEF A) and serum-response factor (SRF). [↑](#footnote-ref-6)
6. [www.pioneer.com/us/agronomy/staging\_corn\_growth.html](http://www.pioneer.com/us/agronomy/staging_corn_growth.html) [↑](#footnote-ref-7)
7. Field trial sites for testing protein expression levels: Johnston, IA; Sheridan, IN; Fisk, MO; York, NE; Germansville, PA; in the USA and Guelph, ON in Canada. [↑](#footnote-ref-8)
8. The location of the eight field trial sites: Johnston, IA; York, NE; Sheridan, IN; Stewardson, IL; Germansville, PA; Fisk, MO; and Groom, TX in the USA and Guelph, ON in Canada. [↑](#footnote-ref-9)