FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

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285-24

Supporting document 1

Safety assessment – Application A1287

Food derived from short-stature corn line MON94804

Executive summary

Background

Application A1287 seeks approval for the sale and use of food derived from corn line MON94804 that has been genetically modified (GM) to have reduced overall plant height.

The short stature of MON94804 is due to the presence of one novel substance: the GA20ox_SUP microRNA (miRNA). This miRNA suppresses two genes involved in the synthesis of gibberellin (GA), a plant hormone involved in plant growth and development. The resulting decrease in GA levels in the stalk leads to an overall reduction in plant height. The GA20ox_SUP miRNA has not been previously assessed by FSANZ.

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.

History of use

Corn has a long history of safe use in the food supply. Corn-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.

Molecular characterisation

The DNA sequence encoding the GA20ox_SUP miRNA (*GA20ox_SUP*) was introduced into corn line MON94804 via *Agrobacterium*-mediated transformation. This transformation also introduced a selectable marker cassette that was subsequently removed using the Cre/*lox* recombination system. Molecular analyses indicate that a single copy of the *GA20ox_SUP* cassette is present at a single insertion site in the MON94804 genome. There are no extraneous plasmid sequences or antibiotic resistance marker genes present in this line.

The introduced genetic elements were shown by molecular techniques and phenotypic analyses to be present within a single locus and stably inherited across multiple generations.

Characterisation and safety assessment of new substances

Corn line MON94804 expresses an inverted repeat RNA sequence from the *GA20ox_SUP* cassette which is processed into a mature 21 nucleotide GA20ox_SUP miRNA. The mature miRNA is detectable in a variety of tissues from MON94804, as is the larger unprocessed primary miRNA transcript.

The GA20ox_SUP miRNA exerts its function by suppressing the corn *ZmGA20ox3* and *ZmGA20ox5* genes, which are involved in GA biosynthesis, via RNA interference (RNAi). Statistically significant reductions in mRNA levels from both genes were observed in a number of MON94804 tissues. The suppression of *ZmGA20ox3* and *ZmGA20ox5* by GA20ox_SUP miRNA was also shown to be specific to these target genes.

There are no concerns regarding the safety of the GA20ox_SUP miRNA in MON94804. The data provided do not indicate the miRNA possesses different characteristics or is likely to pose a greater risk than other RNAi mediators naturally present in corn.

Compositional analyses

Detailed compositional analyses were performed on MON94804. Statistically significant differences were found between grain from MON94804 and the non-GM control for 4 of the 68 analytes evaluated, however these differences were small and all within the range established for existing commercial non-GM corn cultivars. Overall, the compositional data support the conclusion that there are no biologically significant differences in the levels of key constituents in grain from MON94804 compared to non-GM corn cultivars available on the market.

Conclusion

No potential public health and safety concerns have been identified in the assessment of short-stature corn line MON94804. On the basis of the data provided in the present application and other available information, food derived from MON94804 is considered to be as safe for human consumption as food derived from conventional non-GM corn cultivars.

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List of Abbreviations

Abbreviation	Description
ADF	acid detergent fibre
AFSI	Agriculture and Food Systems Institute
BLOSUM	BLOcks SUbstitution Matrix
bp	base pair
COMPARE	COMprehensive Protein Allergen Resource
DNA	deoxyribonucleic acid
dw	dry weight
FASTA	fast alignment search tool – all
FSANZ	Food Standards Australia New Zealand
g	Gram
GA	gibberellic acid(s)
GM	genetically modified
HFCS	high fructose corn syrup
HMW	high molecular weight
kDa	Kilodalton
LB	left border
LOQ	limit of quantitation
LMW	low molecular weight
mg	Milligram
miRNA	microRNA
MT	million tons
NCBI	National Centre for Biotechnology Information
NDF	neutral detergent fibre
ng	Nanogram
NGS	next generation sequencing
nt	nucleotide
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
PCR	polymerase chain reaction

Abbreviation	Description
qPCR	quantitative polymerase chain reaction
RB	right border
RF	reading frame
RNA	ribonucleic acid
RNAi	RNA interference
TDF	total dietary fibre
T-DNA	transfer DNA
µg	Microgram
USDA	United States Department of Agriculture
UTR	untranslated region

1 Introduction

FSANZ received an application from Bayer CropScience Proprietary Limited to vary Schedule 26 in the Australia New Zealand Food Standards Code. The variation is to include food from a new genetically modified (GM) corn line MON94804, with the OECD Unique Identifier MON-948Ø4-4. This corn line has reduced overall plant height compared to conventional non-GM corn.

The short stature of MON94804 is due to expression of the GA20ox_SUP microRNA (miRNA). This miRNA suppresses two genes involved in the synthesis of gibberellin (GA), a plant hormone involved in plant growth and development. The resulting decrease in GA levels in the stalk leads to an overall reduction in plant height. The GA20ox_SUP miRNA has not been previously assessed by FSANZ.

If approved, food derived from MON94804 corn line may enter the Australian and New Zealand food supply as imported food products.

2 History of use

2.1 Host organism

The host organism is corn (*Zea mays*) which is also referred to as maize. The inbred corn line HCL301 was used as the parental variety for the genetic modification described in this application.

Corn was one of the first plants to be cultivated by humans (Ranum et al. 2014) and is now the world's dominant cereal crop, with global production of 1,151 MT[1] in 2022/23, ahead of wheat (788 MT) and rice (513 MT) (USDA 2023). Due to its economic importance, corn has been the subject of extensive study[2].

The United States is the world's largest producer of corn, producing 347 MT in 2022/23 (USDA 2023). Of the corn grown in the United States, an estimated 92% is GM[3]. No GM corn is currently grown commercially in Australia or New Zealand.

Relatively small quantities of non-GM corn are grown in Australia and New Zealand. In 2022 these amounted to 0.430 and 0.188 MT respectively (FAOSTAT 2024). To supplement their limited local production of corn, Australia and New Zealand import both corn grain and processed corn products. For example, in 2022 the imported quantities of corn flour into Australia and New Zealand were 14,278 and 1,723 tonnes respectively, while imports of corn oil totalled 688 and 54 tonnes respectively (FAOSTAT 2024).

Corn has a long history of safe human consumption[4]. Food products derived from processing of corn kernels include corn flour, meal, oil, starch and sweeteners such as high fructose corn syrup (HFCS). In Australia and New Zealand, corn starch is used in dessert mixes and canned foods and HFCS is used in breakfast cereals, baking products, corn chips and extruded confectionary.

[1] million tons

[2] Refer to detailed reports published by the OECD (OECD 2002), the Grains Research and Development Corporation (GRDC 2017) and the Office of the Gene Technology Regulator (OGTR 2008).

[3] For more information please see USDA Economic Research Service: http://www.ers.usda.gov/data-products/adoption-of-genetically-engineered-crops-in-the-us.aspx

[4] A large proportion of corn produced is also used as animal feed.

2.2 Donor organisms

2.2.1 Zea mays and Oryza sativa

The *GA20ox_SUP* sequence is derived from coding sequences from the endogenous corn genes *ZmGA20ox3* and *ZmGA20ox5*, and intervening backbone sequences derived from rice (*Oryza sativa*). Both corn and rice have a long history of safe consumption as food.

2.2.4 Other organisms

Genetic elements from several other organisms have been used in the genetic modification of MON94804 (see Table 1 and Appendix 1). These genetic elements are non-coding sequences and are used to regulate the expression of *GA20ox_SUP*.

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

To create corn line MON94804, the conventional corn line HCL301 was transformed using the plasmid PV-ZMAP527892 (Figure 1). During the development of corn line MON94804, a *cp4 epsps* selectable marker was introduced into the genome of an intermediary line and subsequently removed using the Cre/*lox* recombination system. The transformation methodology is outlined in the flowchart in Appendix 2 and summarised below.

Transformation of the HCL301 line was achieved by co-culturing mature embryos excised from a post-pollinated corn ear with *Agrobacterium tumefaciens* containing the PV-ZMAP527892 plasmid (Ye et al. 2022). The embryos were then placed on first bud induction medium for one week, before being transformed to second bud induction medium containing glyphosate to inhibit the growth of untransformed plant cells.

After two weeks on the second bud induction medium, explants were placed on media to encourage shoot and root development. Rooted plants (R0) with normal phenotypes were transferred to soil for further growth, then screened using real-time polymerase chain reaction (PCR) assays to identify R0 plants carrying the transfer DNA (T-DNA) but not the vector backbone (Figure 1). These plants were self-pollinated, producing R1 seed. R1 plants that were homozygous for T-DNA and lacked vector backbone (as judged by real-time PCR) were self-pollinated, giving rise to R2 seed.

Selected R2 plants were crossed with a corn line expressing Cre recombinase protein, which was developed separately with the plasmid PV-ZMOO513642, which contains the *cre* gene (Appendix 3). R2 plants contain the *cp4 epsps* expression cassette, which is part of the T-DNA inserted during transformation and is flanked by *lox*P sites (Figure 1). The Cre/*lox* recombination system allows the site-specific recombination of two *lox*P sites (Gilbertson 2003; Zhang et al. 2003). The introduction of Cre recombinase in the R2 x Cre recombinase

expressing line enabled the removal of the *cp4 epsps* expression cassette in the F1 generation. Subsequently, the *cre* gene and associated PV-ZMOO513642 sequences were segregated away by conventional breeding. Progeny were screened for the absence of the *cp4 epsps* expression cassette, the *cre* gene and other sequences derived from PV-ZMOO513642.

Subsequent generations were further screened using standard molecular biology techniques, allowing selection of plants with the *GA20ox_SUP* expression cassette, but without unintended DNA insertions. Following the evaluation of insert integrity, trait efficacy, phenotypic characteristics and agronomic performance, corn line MON94804 was selected.

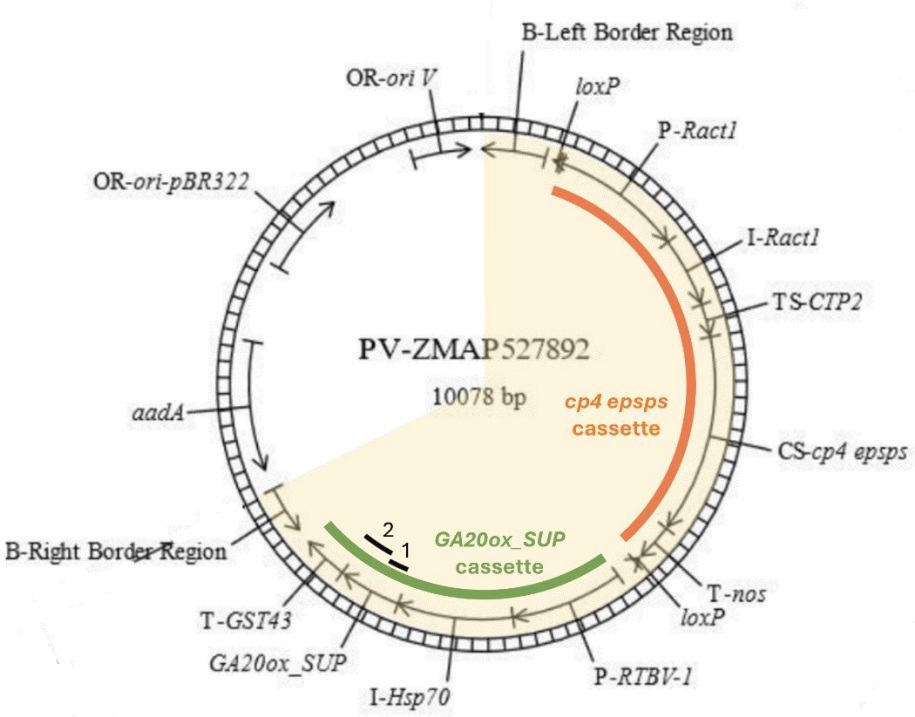

Figure 1. *Map of plasmid PV-ZMAP527892. The T-DNA region between the left and right border regions is shaded yellow and was inserted into the corn genome. This region contains the cp4 epsps and GA20ox_SUP expression cassettes. The vector backbone (unshaded region) contains two origins of replication (ori pBR322, ori V) and the aadA antibiotic resistance gene. The two black lines numbered '1' and '2' indicate the positions of the probes used for the high molecular weight northern blot analysis (see section 3.5).*

3.2 Detailed description of inserted DNA

Corn line MON94804 contains T-DNA from the PV-ZMAP527892 plasmid (Figure 1) and includes the *GA20ox_SUP* expression cassette. The *cp4 epsps* selectable marker cassette, which confers tolerance to the herbicide glyphosate, was also part of the originally inserted T-DNA, but was removed using Cre/*lox* recombination and is not present in MON94804. After removal of the *cp4 epsps* cassette, the final insert was 2,733 bp long and consisted of part of the left border (LB) sequence, one *lox*P site and the *GA20ox_SUP* cassette (Figure 2).

Information on the genetic elements in the T-DNA used for transformation is summarised in

Table 1. Additional detail, including intervening sequences used to assist with cloning, mapping and sequence analysis, can be found in Appendix 1.

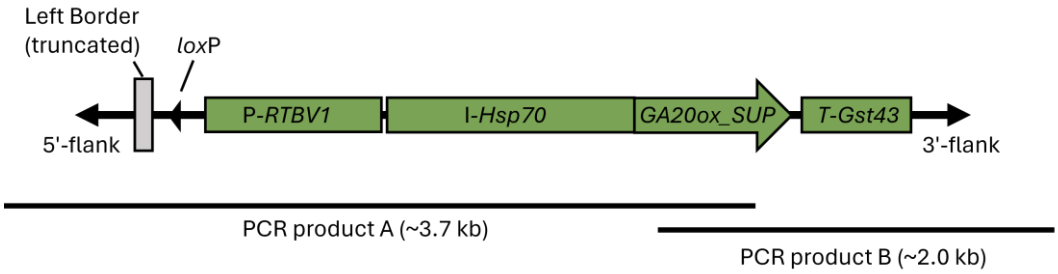

Figure 2: *Schematic of the inserted DNA in MON94804. The components of the GA20ox_SUP cassette are coloured green. PCR products A and B show the positions of the overlapping polymerase chain reaction fragments used for sequencing (see section 3.4.3).*

Table 1: Expression cassettes contained in the T-DNA of PV-ZMAP527892

	Promoter	Enhancer/ Regulatory sequence	Coding sequence	Terminator	Notes
***GA20ox_SUP* cassette**	Promoter and leader from the rice tungro bacilliform virus (RTBV)	Intron and flanking exon sequence of the *hsp70* gene from *Zea mays* (corn)	21nt inverted repeat derived from the *ZmGA20ox3* and *ZmGA20ox5* genes from *Z. mays*, flanked and separated by three *Osa*-miR1425 fragments from *Oryza sativa* (rice).	3' UTR comprised of multiple 3′ UTR sequences from *Z. mays*	When expressed, the inverted repeat sequence is recognised by the endogenous corn RNAi machinery, resulting in suppression of gibberellin activity in the plant.
***cp4 epsps* cassette**	Promoter and leader of the *act1* gene from *O. sativa*	Intron and flanking UTR sequence of the *act1* gene from *O. sativa* Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana*	*aroA* gene from *Agrobacterium tumefaciens* sp. strain CP4	3' UTR of the nopaline synthase (*nos*) gene from the tumour-inducing plasmid from *A. tumefaciens*	Confers glyphosate tolerance. Not present in MON94804 due to removal by Cre/*lox* recombination (Section 3.1)

3.3 Development of the corn line from the original transformation

A breeding programme was undertaken for the purposes of:

- obtaining generations suitable for analysing the characteristics of MON94804
- ensuring that the MON94804 event is incorporated into elite lines for commercialisation.

The breeding history of MON94804, showing the generations used for characterisation studies, is shown in Figure 3. Table 2 indicates the specific generations used in the various analyses of MON94804. As controls, either the non-GM parental line HCL301, or the non-GM cross HCL301 x HCL617 were used (Table 2), based on the most appropriate genetic background for the generation being analysed.

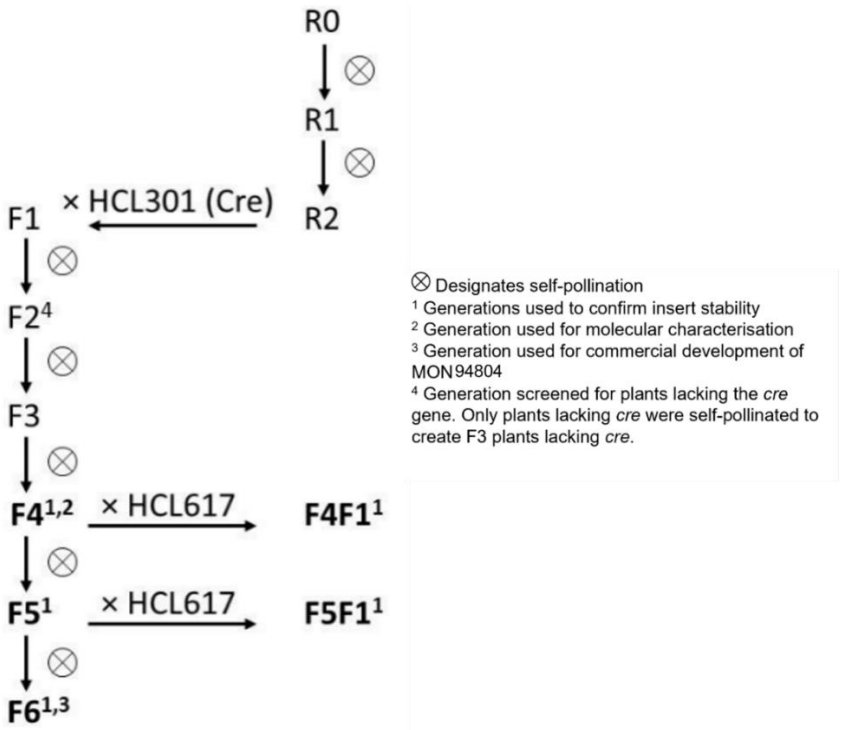

Figure 3. *Breeding path used in the characterisation of MON94804. Bold text indicates generations used to confirm insert stability and for molecular characterisation.*

Table 2: MON94804 generations used for various analyses

Analysis	Section	Generation(s) used	Comparators
Number of integration sites	Section 3.4.1	F4	HCL301
Absence of backbone and other sequences	Section 3.4.2	F4	HCL301
Insert integrity and site of integration	Section 3.4.3	F4	HCL301
Genetic stability	Section 3.4.4.1	F4, F4F1, F5, F5F1, F6	HCL301; HCL301 x HCL617
Mendelian inheritance	Section 3.4.4.2	F4F2, F4F3, F4F4	N/A
Expression of RNA transcripts over several generations	Section 3.5	F4, F4F1, F5, F5F1, and F6	HCL301 (for F4, F5, F6); HCL301 x HCL617 (for F4F1 and F5F1)
Compositional analysis	Section 5	F5F1	HCL301 x HCL617

3.4 Characterisation of the inserted DNA and site(s) of insertion

A range of analyses were undertaken to characterise the genetic modification in MON94804. These analyses focused on the nature and stability of the inserted DNA and whether any unintended re-arrangements or products may have occurred as a consequence of the transformation procedure.

Genomic DNA from MON94804 (F4 generation) and from the conventional control (HCL301) was sequenced using next generation sequencing (NGS). This method generates ~150 bp short sequence reads which are randomly distributed throughout the genome and in sufficient number to ensure the genomes are covered comprehensively. In addition, the transformation plasmid PV-ZMAP527892 and the cre-containing plasmid PV-ZMOO513642

were sequenced to serve as references. To assess the sensitivity of the NGS method, plasmid DNA was spiked and sequenced. Sufficient sequence reads were obtained to cover the entire genomes of MON94804 and the HCL301 control, with a depth of coverage ≥75x and an adequate level of sensitivity[5].

3.4.1 Number of integration site(s)

NGS reads from MON94804 (F4 generation) that mapped to the intended T-DNA insert region were analysed and two unique insert-flank junction sites were identified. Each of these sites contained the inserted T-DNA border sequence joined to a flanking sequence in the corn genome. This indicates that a single copy of the intended DNA insert has been integrated into the genome of MON94804 (see Figure 2). No junction sequences were detected in the HCL301 control.

3.4.2 Absence of backbone and other sequences

Absence of selectable marker cassette and transformation plasmid backbone

NGS reads from MON94804 (F4 generation) and the HCL301 control were aligned with the PV-ZMAP527892 transformation plasmid sequence. The HCL301 control contained many reads that mapped to the T-DNA elements P-*Ract1* and I-*Hsp70*. However, these alignments were due to the presence of endogenous sequences in the corn genome that are homologous to these T-DNA encoded elements. As noted in section 3.4.1, no junctions between plasmid and genomic DNA were identified in the HCL301 control, providing further evidence that these reads arose from endogenous sequences.

None of the MON94804 reads mapped to the elements I-*Ract1*, TS-*CTP2*, CS-*cp4 epsps*, and T-*nos* from the *cp4 epsps* selectable marker cassette. There were a small number of reads that mapped to the P-*Ract1* promoter sequence, but these are a result of the similarity of P-*Ract1* to endogenous sequences in the corn genome and do not indicate the presence of the selectable marker cassette. In addition, a single *lox*P site remained in MON94804, as expected. These results confirm the removal of the selectable marker cassette using the Cre/*lox* recombination system.

No MON94804 reads mapped to the vector backbone sequence *aadA* or to either of the two backbone origins of replication, though a small number of reads mapped to the LB region upstream of where the majority of mapped reads were observed. This low number of reads does not indicate the presence of backbone sequence in MON94804 and is likely due to the presence of environmental bacteria in the genomic DNA prepared for NGS, as has been previously described (Yang et al. 2013). The results of the alignment confirmed there was no integration of transformation vector backbone sequences into MON94804, including any antibiotic resistance genes.

Absence of Cre recombinase cassette and *cre* gene-containing plasmid vector

During the breeding process used to develop MON94804, the R2 generation was crossed with a corn line expressing a Cre recombinase protein (Figure 3). This corn line was generated using the PV-ZMOO513642 plasmid (Appendix 3). Subsequent generations were screened for the absence of the *cre* gene and other PV-ZMOO513642 sequences. To confirm the absence of these sequences in MON94804, NGS reads of MON94804 genomic DNA were aligned with the PV-ZMOO513642 plasmid sequence. As a control, reads from

[5] The NGS method was sufficiently sensitive to detect 100% of the spiked plasmid when present at 1/10th of a copy per genome equivalent or greater. Additionally, reads were mapped to a single copy of an endogenous gene and the depth of coverage for MON94804 and the controls was comprehensive.

the HCL301 control were also aligned to the PV-ZMOO513642 sequence.

The results showed that no MON94804 reads mapped to the *cre* gene. A number of reads from MON94804 aligned to the LB region of PV-ZMOO513642. This was expected as PV-ZMOO513642 shares the same LB region as the transformation plasmid PV-ZMAP527892 and is part of the MON94804 insert (see Figure 2).

A small number of reads from MON94804 mapped to the promoter element *Ract1*, which is homologous to a corn sequence in the HCL301 background as noted above, and as such is not indicative of the presence of PV-ZMOO513642 sequences in MON94804. Additionally, a few MON94804 reads aligned with the origin of replication *ori-pBR322* sequence and other sequences in the PV-ZMOO513642 backbone used during cloning. As with the detection of backbone sequences from PV-ZMAP527892, these alignments are most likely due to the presence of DNA from environmental bacteria in the plant tissues used for genomic DNA preparation.

These results indicate that MON94804 (F4 generation) and subsequent generations do not contain the *cre* gene or other sequences from the PV-ZMOO513642 plasmid.

3.4.3 Insert integrity and site of integration

DNA sequence analysis was performed on two overlapping regions covering the full length of the insert and the flanking corn genomic sequences of MON94804 (see positions marked in Figure 2). The sequencing results confirmed that the insertion is 2,733 bp long and that the genetic elements in the inserted *GA20ox_SUP* cassette are intact and organised as expected.

Alignment of the MON94804 insert sequence with the corresponding T-DNA from PV-ZMAP527892 showed that the sequences were identical, with the exception of the border regions: the RB region was absent and the LB region in MON94804 was truncated relative to PV-ZMAP527892, though the remaining portion was identical. Neither of these changes would have a functional impact on the expression of the inserted *GA20ox_SUP* cassette. As expected, the selectable marker cassette (P-*Ract1*, I-*Ract1*, TS-*CTP2*, CS-*cp4 epsps*, and T-*nos*) and one *lox*P site were absent. These results were fully consistent with the NGS dataset.

To examine the T-DNA insertion site, PCR primers flanking the insertion site were used to amplify genomic DNA from MON94804 and from the conventional control HCL301. Comparing the products from MON94804 and HCL301 identified a 41 bp deletion of the corn genomic DNA that occurred during T-DNA integration. All other flanking sequences in MON94804 were identical to those in HCL301. Such changes during T-DNA insertion are common during *Agrobacterium*-mediated plant transformation due to double-stranded break repair mechanisms (Salomon and Puchta 1998; Anderson et al. 2016) and would not affect the expression of the *GA20ox_SUP* cassette.

3.4.4 Stability of the genetic changes in corn line MON94804

The concept of stability encompasses both the genetic and phenotypic stability of the introduced trait over a number of generations. Genetic stability refers to maintenance of the modification (as produced in the initial transformation events) over successive generations. Phenotypic stability refers to the expressed trait remaining unchanged over successive generations.

3.4.4.1 Genetic stability

NGS was used to show the genetic stability of the inserted DNA in MON94804 (F4) by evaluating seed-derived DNA from four additional breeding generations of MON94804 (F4F1, F5, F5F1, F6). Control genomic DNA was isolated from (1) the non-GM parental line HCL301, which shares similar background genetics to the F4, F5 and F6 generations and (2) the non-GM hybrid line HCL301 × HCL617 with similar background genetics to the F4F1 and F5F1 hybrids.

The analysis showed that the two insert-flank junction sequences present in the F4 generation (section 3.4.1) were identical in each of the four additional generations. No other junction sequences were present. These results confirm that a single T-DNA insert is stably integrated in MON94804.

3.4.4.2 Phenotypic stability

Mendelian inheritance

Since the inserted T-DNA resides at a single locus within the MON94804 genome, it would be expected to be inherited according to Mendelian principles. To confirm this, three generations of MON94804 (F4F2, F4F3, and F4F4; see Figure 4) were tested for the presence of T-DNA using a Real Time TaqMan® PCR assay, and a Pearson's chi-square (χ^2) analysis was conducted to compare the observed and expected segregation ratios across these generations.

According to Mendelian inheritance principles, the predicted segregation ratio in all generations was 1:2:1 (homozygous positive: hemizygous positive: homozygous negative). The χ^2 critical value to reject the hypothesis of this ratio at a 5% level of significance was 5.99 (Strickberger 1976). The results of the χ^2 analyses on all three generations tested gave χ^2 values < 5.99 (Table 3), indicating there were no significant differences between the observed and expected segregation ratios in any of the generations. These data support the conclusion that the inserted DNA is present at a single locus in the MON94804 genome and is inherited predictably according to Mendelian inheritance principles.

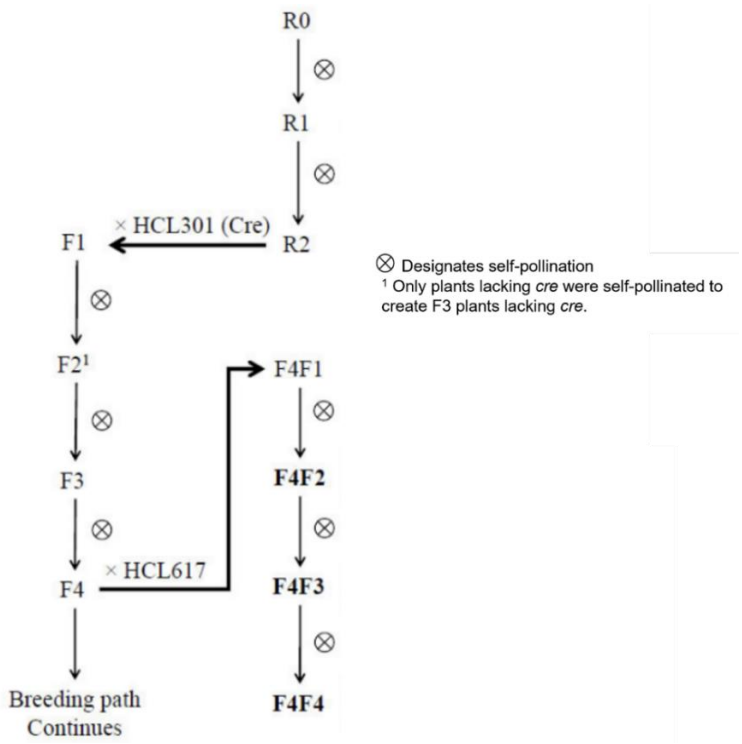

Figure 4. *Breeding path used to assess the inheritance and genetic stability of MON94804. F4 MON94804 was crossed with the proprietary elite inbred line HCL617 to produce a F4F1 hemizygous seed. Self-crossing this line is expected to segregate at a 1:2:1 ratio (homozygous positive: hemizygous positive: homozygous negative). Continual self-crossing of hemizygous progeny is expected to result in the same ratios. Bold text indicates generations whose segregation data was used in the χ^2 analysis.*

Table 3: Segregation results in three generations of MON94804

Generation	Expected segregation ratio	Observed number of plants (expected number)				Statistical analysis	
		Homozygous Positive	Hemizygous positive	Homozygous Negative	Total	χ^2	*P value*
F4F2	1:2:1	65 (84)	180 (168)	91 (84)	336	5.74	0.057
F4F3	1:2:1	73 (61)	110 (122)	61 (61)	244	3.54	0.170
F4F4	1:2:1	98 (91)	174 (182)	92 (91)	364	0.90	0.637

Expression of phenotype over several generations

Refer to section 3.5 – Analysis of expressed RNA transcripts.

3.4.5 Reading frame analysis

A bioinformatic analysis of the MON94804 insert, as well as the flanking DNA regions, was undertaken to identify whether any novel reading frames (RFs) had been created in MON94804 as a result of the DNA insertion, and whether any putative peptides encoded by the identified RFs have the potential for allergenicity or toxicity.

Sequences spanning the 5' and 3' insert-flank junctions of MON94804 were translated *in silico* from stop codon to stop codon (TGA, TAG, TAA) in all six reading frames[6]. A total of 12 putative peptides of eight amino acids or greater in length from the insert-flank junctions were identified. In addition, the entire MON94804 insert DNA was translated in all six reading frames.

The 12 insert-flank junction peptides as well as the six translated insert reading frames were investigated further to determine whether their amino acid sequences showed similarity with known allergen and toxin peptide sequences in established databases. These analyses are theoretical only, as it is highly unlikely that any of the identified putative peptides would be expressed *in planta*.

3.4.5.1 Bioinformatic analysis for potential allergenicity

The putative peptides identified above were compared to known allergenic proteins listed in the COMprehensive Protein Allergen REsource (COMPARE[7]) database, from the Health and Environmental Science Institute (van Ree et al. 2021). At the date of the search, there were 2,463 sequences in the allergen database (AD_2022). Sequences were also compared to the GenBank all protein database (PRT_2022), downloaded from the National Centre for Biotechnology Information (NCBI[8]), which contained 184,933,782 sequences at the date of

[6] Evaluation of sequences stop-to-stop codon is a more conservative approach compared to the evaluation of start-to-stop codon sequences.
[7] http://comparedatabase.org/database/
[8] https://www.ncbi.nlm.nih.gov/protein/

download.

Three types of analyses were performed for this comparison:

(a) Full length sequence search – a FASTA alignment using a BLOSUM50 scoring matrix, which identifies blocks of residues with at least 50% sequence identity. Only matches with E-scores of $\leq 1\times10^{-5}$ were considered.
(b) 80-mer sliding window search – a FASTA alignment was performed comparing all contiguous 80 amino acids to the database entries. Only matches of greater than 35% similarity over ≥ 80 amino acids were considered.
(c) 8-mer exact match search – an in-house algorithm was used to identify whether an 8 amino acid peptide match existed between the query sequences and sequences within the allergen database. Only matches of 100% similarity over 8 amino acids were considered.

The alignment of the 12 putative peptides present in the 5' and 3' insert-flank junctions with database sequences did not identify any matches. A single 8-mer match between a putative peptide encoded by one of the reading frames in the MON94804 insert DNA to a protein in the allergen database was identified. However, the putative peptide containing the 8-mer match does not possess an appropriate upstream methionine for translation initiation, and as such the possibility of this peptide being produced *in planta* is remote.

Given these results, the risk of allergenic proteins with relevance to human safety being produced by novel RFs generated in MON94804 is negligible.

3.4.5.2 Bioinformatic analysis for potential toxicity

The putative peptides encoded by the insert-flank junction and insert sequences were also compared *in silico* to a toxin protein database (TOX_2022). This database is a subset of sequences derived from the UniProtKB/Swiss-Prot protein databases, curated to remove likely non-toxin proteins, and contained 8,131 sequences at the date of analysis. A FASTA algorithm was used with a BLOSUM50 scoring matrix and the E-value threshold conservatively set to 1×10^{-5}. No matches were found between the 12 putative junction peptides and any known protein toxins. Similarly, no matches were identified between the putative peptides encoded by the 6 reading frames in the MON94804 insert and any known protein toxins.

The novel RFs in MON94804 therefore do not present a toxicity concern.

3.5 Analysis of expressed RNA transcripts

The *GA20ox_SUP* cassette in MON94804 expresses an inverted repeat RNA transcript which is recognised by the corn RNA interference (RNAi) machinery and processed into a mature 21 nucleotide (nt) microRNA (miRNA). The unprocessed and partially processed transcripts are known as primary miRNA (pri-miRNA) and precursor miRNA (pre-miRNA) respectively. After processing, the 21 nt GA20ox_SUP miRNA targets the endogenous corn gibberellic acid 20 oxidase (GA20ox) genes *ZmGA20ox3* and *ZmGA20ox5*.

To determine whether the *GA20ox_SUP* cassette is being expressed, northern blot analyses were conducted using total RNA[9] extracted from leaf tissue from five generations of MON94804 (F4, F4F1, F5, F5F1, F6) and comparable conventional controls (see Table 2). Both high molecular weight (HMW) and low molecular weight (LMW) northern blot analyses

[9] Total RNA comprises all RNA extracted from cells, including messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA) and small noncoding RNAs such as miRNA and small interfering RNA (siRNA).

were used in order to detect larger pri- or pre-miRNA transcripts (HMW blots), as well as smaller mature miRNAs (LMW blots).

Northern blot analysis of high molecular weight GA20ox_SUP transcripts in MON94804

For the HMW northern blot analysis, RNA was electrophoresed on an agarose/formaldehyde gel then transferred to a nylon membrane. The blot was hybridised using two oligonucleotide probes labelled with digoxigenin (DIG) that were designed to detect the 5′ and 3′ regions of the *GA20ox_SUP* sequence (Figure 1).The transformation plasmid PV-ZMAP527892 was used as a template for PCR to generate the two probe sequences that were in turn used to prepare the DIG-labelled oligonucleotide probes for hybridisation.

No hybridisation bands were detected for the RNA from the conventional controls. Total RNA from all five MON94804 generations produced a single hybridisation band at ~0.9 kb, which corresponds to the expected size of the pri-miRNA transcript in MON94804. A larger pre-miRNA band was not detected, suggesting that this precursor RNA is rapidly processed.

Northern blot analysis of low molecular weight GA20ox_SUP transcripts in MON94804

For the LMW northern blot analysis, RNA was electrophoresed on a Tris-borate-EDTA (TBE)-urea gel. The RNA was transferred to a nylon membrane then hybridised with a DIG-labelled oligonucleotide probe designed to detect the mature GA20ox_SUP miRNA sequence.

No hybridisation bands were detected for the RNA from the conventional controls. Total RNA from all five MON94804 generations produced a single hybridisation band at ~21 nt, which corresponds to the expected size of the mature GA20ox_SUP miRNA in MON94804.

Refer to Section 4 for information about the mechanism of action of GA20ox_SUP and its expression and activity in multiple MON94804 tissues.

3.6 Conclusion

Corn line MON94804 contains a single copy of the intended DNA insertion, integrated at a specific locus in the corn genome. DNA sequencing confirmed that the *GAox20_SUP* cassette has been inserted with the expected organisation. The *cp4 epsps* selectable marker cassette, which was inserted as part of the T-DNA transformation, is absent in MON94804 as a result of Cre/*lox* recombination. No backbone sequences from the plasmids used in the transformation are present, including any antibiotic resistance genes.

The inserted DNA is stably inherited across several breeding generations of MON94804. Northern blot analysis of MON94804 RNA shows that two populations of GA20ox_SUP RNA, corresponding to the 0.9 kb pri-miRNA and the 21 nt mature miRNA are produced upon expression of the *GA20ox_SUP* cassette and maintained across five breeding generations. Bioinformatics analyses of the new ORFs created by the modification did not raise any allergenicity or toxicity concerns.

4 Characterisation and safety assessment of novel substances

One novel substance is expressed in MON94804: the GA20ox_SUP miRNA which is designed to downregulate the expression of the endogenous corn *ZmGA20ox3* and *ZmGA20ox5* genes. This is the first time FSANZ has assessed the safety of the GA20ox_SUP miRNA.

In considering the safety of newly expressed substances it is important to note that a large and diverse range of RNAs are ingested as part of the normal human diet without any adverse effects.

4.1 GA20ox_SUP miRNA

4.1.1 Mechanism of action

Gibberellins or gibberellic acids (GAs) are plant hormones which regulate an array of growth and development processes in plants, including stem elongation, seed germination and flowering (Richards et al. 2001; Hedden and Thomas 2016). The GA biosynthetic pathway consists of multiple steps (Hedden 2020; Figure 5). In the later stages of GA biosynthesis, the *gibberellin 20-oxidase* (*GA20ox*) genes regulate the production of bioactive GAs, including the major GA which controls stem elongation in corn: GA_1 (Phinney 1985; Figure 5). A reduction in GA_1 in corn has been shown to lead to a reduction in overall plant height (Chen et al. 2020).

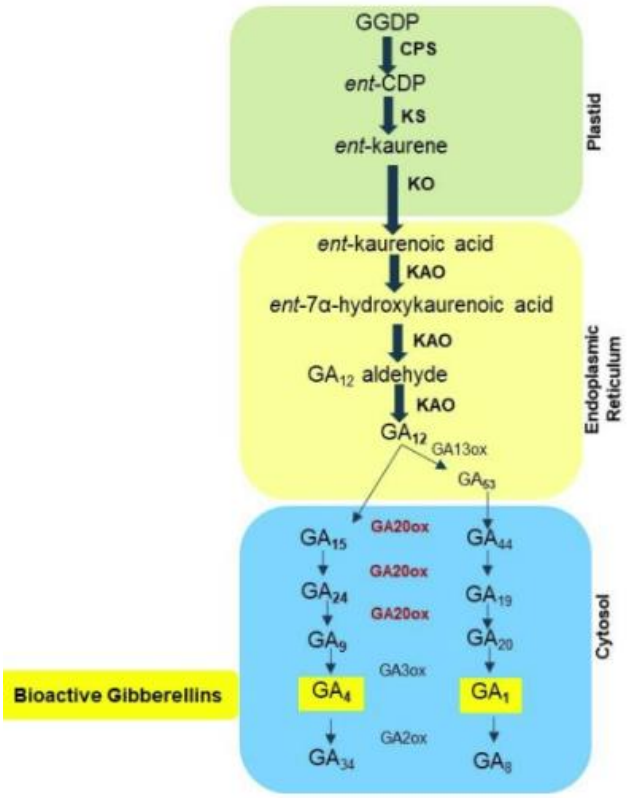

Figure 5. *Gibberellin biosynthesis pathway in plants. GGDP: geranylgeranyl diphosphate; ent-CDP: ent- copalyl diphosphate; CPS: ent-copalyl diphosphate synthase; KS: ent-kaurene synthase ; KO: ent-kaurene oxidase; KAO: ent-kaurenoic acid oxidase; GA20ox (in red): gibberellin 20-oxidase enzyme encoded by the ZmGA20ox gene family. Figure adapted from Binenbaum et al. (2018).*

In MON94804, the *GA20ox_SUP* cassette expresses an inverted repeat RNA sequence which folds into a stem-loop structure known as pri-miRNA, before being processed into a duplex pre-miRNA by DICER LIKE 1 (DCL1). Finally, a mature 21 nt GA20ox_SUP miRNA is incorporated into the ARGONAUTE (AGO) complex, where it carries out sequence-specific downregulation of the target *ZmGA20ox3* and *ZmGA20ox5* genes via miRNA-directed cleavage of the messenger RNA (mRNA) transcripts. This miRNA-mediated mechanism of action is similar to the RNAi mechanism triggered by other small non-coding RNAs such as small interfering RNAs (siRNAs) (Bartel 2004; Carthew and Sontheimer 2009).

Suppression of *ZmGA20ox3* and *ZmGA20ox5* by GA20oxSUP miRNA leads to reduced levels of bioactive GAs within the stalk internodes, resulting in reduced stalk internode length and reduced overall plant height (Paciorek et al. 2022). Both the *ZmGA20ox3* and *ZmGA20ox5* genes share significant homology with the rice *SD1* gene (*OsGA20ox2*), the key semi-dwarfing gene used in rice breeding (Monna et al. 2002). The short-stature of MON94804 offers agronomic benefits, including reduced crop lodging[10] compared to conventional corn, which in turn leads to higher yields, improved crop quality and ease of harvesting (Paciorek et al. 2022).

4.1.2 Expression levels of GA20ox_SUP in multiple MON94804 tissues

Northern blots were performed to analyse the RNA produced from the *GA20ox_SUP* cassette in MON94804 in multiple tissue types. As with the analyses described in section 3.5, both HMW and LMW northern blotting were performed in order to detect the presence or absence of pri-miRNA, pre-miRNA, and fully processed GA20ox_SUP miRNA in over season leaf (OSL1), over season root (OSR1), stalk, forage, and grain tissues collected from MON94804. The same assay conditions and probes as those described in section 3.5 were used.

The results of the northern blot analyses showed that in MON94804 OSL1, OSR1, stalk, and forage tissues, two populations of RNA were present, consistent with the expected 0.9 kb pri-miRNA and 21 nt mature miRNA. In MON94804 grain tissue, only the smaller RNA, corresponding to the mature miRNA, was detected.

4.1.3 Specificity and efficacy of RNA interference

Plant miRNA machinery is intrinsically highly specific, requiring a very high level of complementarity between an miRNA and its target mRNA (Schwab et al. 2006). The GA20ox_SUP miRNA is perfectly complementary to *ZmGA20ox3*, and has only a single bp mismatch with *ZmGA20ox5*, which is outside the critical miRNA recognition site required for successful miRNA/target mRNA pairing (Paciorek et al. 2022; Figure 6). In addition, alignment of the GA20ox_SUP miRNA sequence with the other seven *ZmGA20ox* genes identified in corn shows that they all contain multiple mismatches in this critical region (Figure 6), suggesting that these genes are unlikely to be suppressed by GA20ox_SUP miRNA (Paciorek et al. 2022).

[10] Lodging is the displacement of stems from their vertical position.

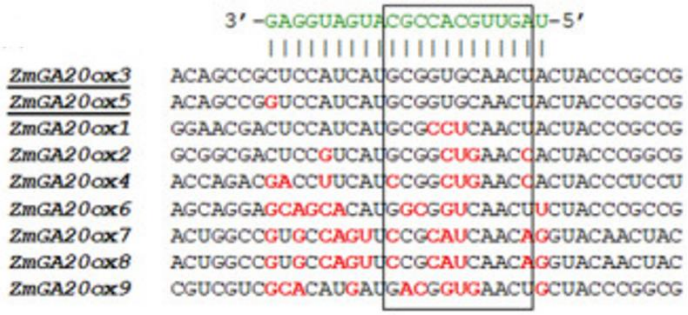

Figure 6. *Nucleotide alignment of the miRNA recognition sites from nine corn ZmGA20ox genes with the GA20ox_SUP miRNA (green). The black outlined box indicates the critical 'seed-region' required for miRNA/target mRNA pairing. Mismatched nucleotides are highlighted in red; the two target genes, ZmGA20ox3 and ZmGA20ox5, are underlined. Figure from Paciorek et al. (2022).*

ZmGA20ox3 and *ZmGA20ox5* are both preferentially expressed in vegetative tissues (i.e. stalk and leaf) compared to reproductive tissues such as grain (Paciorek et al. 2022). In MON94804, GA20ox_SUP miRNA is expressed under the control of the rice tungro bacilliform virus (RTBV) promoter (see Figure 3), which is preferentially expressed in vascular bundles (Paciorek et al. 2022). The rationale for the use of the RTBV promoter is to further localise the suppression of target genes to vegetative tissues.

To assess the expression of *ZmGA20ox3* and *ZmGA20ox5* in various MON94804 tissues compared to those from the conventional control, total RNA was extracted from OSL1, OSR1, stalk, forage, and grain. A real-time TaqMan quantitative PCR (qPCR) assay was used to determine the relative mRNA levels of *ZmGA20ox3* and *ZmGA20ox5* in each tissue type.

Of the five tissues analysed, statistically significant ($p < 0.05$) reductions in the mRNA levels for both *ZmGA20ox3* and *ZmGA20ox5* in MON94804 compared to the conventional control were observed for OSL1, stalk, and grain (Figure 7). No statistically significant change in *ZmGA20ox3* or *ZmGA20ox5* mRNA levels was observed for OSR1 or forage tissues of MON94804 compared to the conventional control (Figure 7).

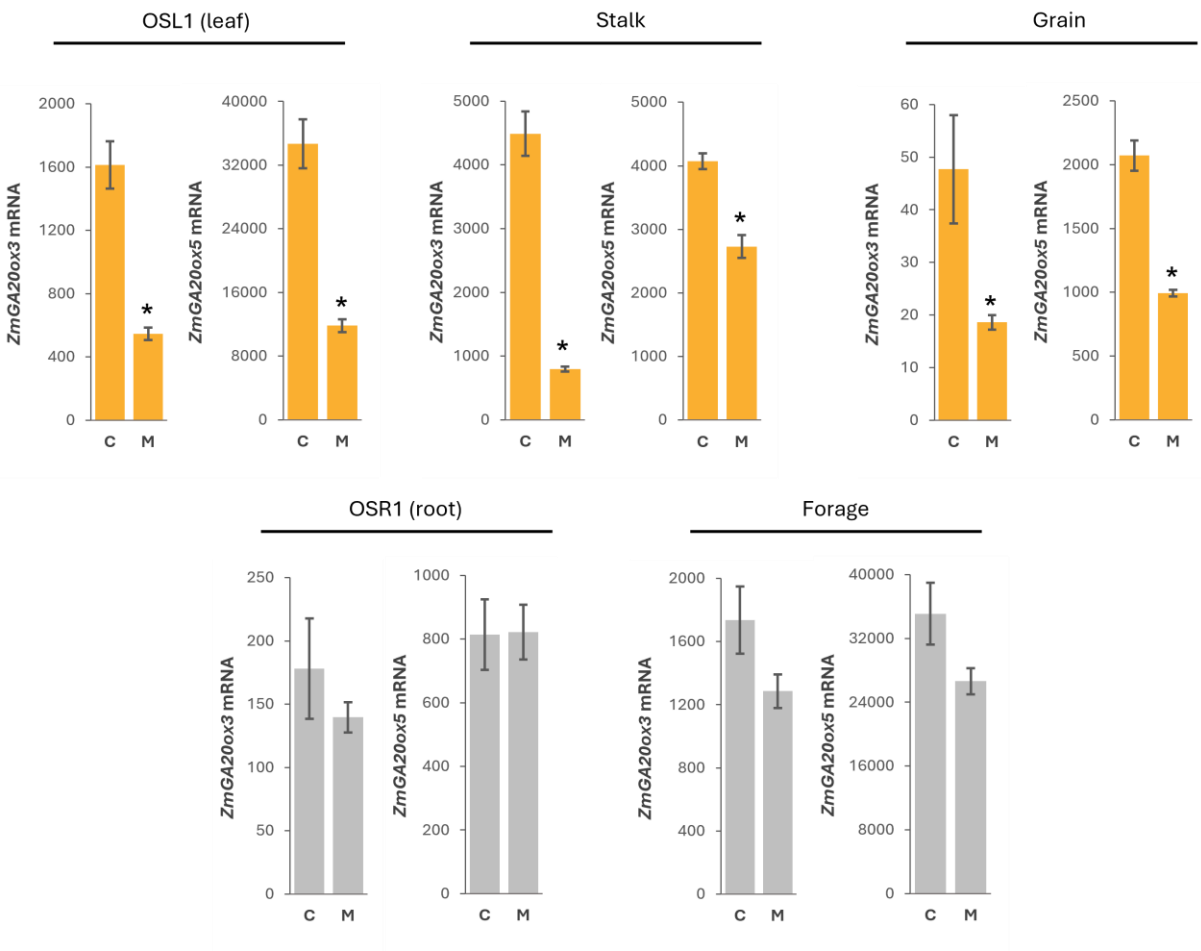

Figure 7. *Relative mean (n = 8) mRNA levels for ZmGA20ox3 and ZmGA20ox5 in OSL1, stalk, grain, OSR1, and forage tissues from a conventional control corn (C) and MON94804 (M). Asterisks indicate a significant difference ($p < 0.05$) between the control and MON94804. Error bars represent standard error.*

In addition to these results, published data demonstrates the predicted specificity of the GA20ox_SUP miRNA. Paciorek et al. (2022) quantifies the level of *ZmGA20ox1* mRNA, the closest homolog to the two target genes in corn. No significant impact on the expression of *ZmGA20ox1* was detected in the tissues tested, which included stalk, leaf, and grain.

In the same study, the major bioactive GAs, GA_1 and GA_4, were quantified in a range of MON94804 tissues. The levels of GA_1 and GA_4 were significantly reduced in stalk and leaf tissues, but no significant decrease was detected in reproductive tissues (Paciorek et al. 2022).

Together, these results show that expression of the GA20ox_SUP miRNA from the GA20ox_SUP cassette results in the suppression of its target genes in a number of MON94804 tissues. This suppression is specific to the two target genes, as published data demonstrates that the expression of the closely related gene *ZmGA20ox1* is not impacted. Further, the suppression of *ZmGA20ox3* and *ZmGA20ox5* results in reduced levels of bioactive GAs in the stalk and leaf of MON94804, which leads to a reduction in stalk length and plant height.

4.1.4 Safety of the GA20ox_SUP miRNA

There are no concerns regarding the safety of the GA20ox_SUP miRNA in MON94804.

Humans and other animals do not possess a GA biosynthetic pathway, and the target genes are found only in plants (Salazar-Cerezo et al. 2018; Keswani et al. 2022). In addition, the silencing of these target genes is highly specific (section 4.1.3). The data provided do not indicate this RNA possesses different characteristics or is likely to pose a greater risk than other RNAi mediators naturally present in corn.

A history of safe human consumption of RNAi mediators exists, including those with homology to human genes. The evidence published to date also does not indicate that dietary uptake of these RNAs from plant food is a widespread phenomenon in vertebrates (including humans), or if it does occur, that sufficient quantities are taken up to exert a biologically relevant effect[11].

4.1.5 Conclusion

Corn line MON94804 expresses an inverted repeat RNA sequence from the *GA20ox_SUP* cassette which is processed into a mature 21 nt GA20ox_SUP miRNA. The mature miRNA is detectable in a variety of tissues from MON94804, as is the larger unprocessed pri-miRNA.

The GA20ox_SUP miRNA exerts its function by targeting the endogenous corn *ZmGA20ox3* and *ZmGA20ox5* genes, which are involved in GA biosynthesis. Statistically significant reductions in the mRNA levels from both genes are observed in the OSL1, stalk, and grain tissues of MON94804 compared to the conventional control. Suppression of the mRNA transcripts from these genes leads to reduced levels of bioactive GAs in vegetative tissues and a reduction in plant height.

Due to the high level of complementarity required for plant miRNA silencing, the suppression of *ZmGA20ox3* and *ZmGA20ox5* by GA20ox_SUP miRNA is specific to these target genes. The closely related homolog of these genes, *ZmGA20ox1*, is not impacted by the expression of GA20ox_SUP.

There are no concerns regarding the safety of the GA20ox_SUP miRNA in MON94804.

[11] For a detailed response see FSANZs Response to Heinemann et al on the regulation of GM crops and foods developed using gene silencing: https://www.foodstandards.gov.au/sites/default/files/consumer/gmfood/Documents/Heinemann%20Response%20210513.pdf

5 Compositional analysis

The main purpose of compositional analyses is to determine if, as a result of the genetic modification, any 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 GM and conventional corn are outlined in the OECD Consensus Document on Compositional Considerations for New Varieties of Maize (OECD 2002), and include: proximates (protein, fat, fibre, ash, carbohydrates), amino acids, fatty acids, minerals, vitamins and the anti-nutrients phytic acid, and raffinose, and the phenolic acids ferulic acid, furfural and *p*-coumaric acid.

5.2 Study design

MON94804 (F5F1 generation) and a non-GM control of similar genetic background (HCL301 x HCL617) were grown and harvested from five field trial sites in the United States during the 2020 growing season[12]. The sites were representative of corn growing regions suitable for commercial production. The field sites were established in a randomised complete block design with four replicates per site. Plants were grown under agronomic field conditions typical for each growing region.

At maturity, grain was harvested from all plots and shipped to an analytical laboratory at ambient temperature, before being frozen at -20°C until analysis. Compositional analyses were based on internationally recognised procedures including official methods specified by the Association of Official Analytical Chemists (AOAC), the Analytical Oil Chemists' Society (AOCS), methods specified by the manufacturer of the equipment used for analysis, or other published scientific methods.

68 different analytes were measured in grain (see Figure 8 for a complete list). Moisture was also measured and used to convert the analyte values from fresh to dry weight. Analytes were expressed as either percent dry weight (% dw), µg/g dw or as a percentage of total fatty acids (% total FA), as shown in Figure 9. Of the 68 components measured, 15 had more than 50% of observations below the limit of quantification (LOQ) (listed in grey in Figure 8) and were excluded from the statistical analyses, leaving a total of 53 components that were fully analysed in grain.

[12] The states in which the five field trial sites were located: Iowa, Illinois, Indiana, Nebraska and Ohio

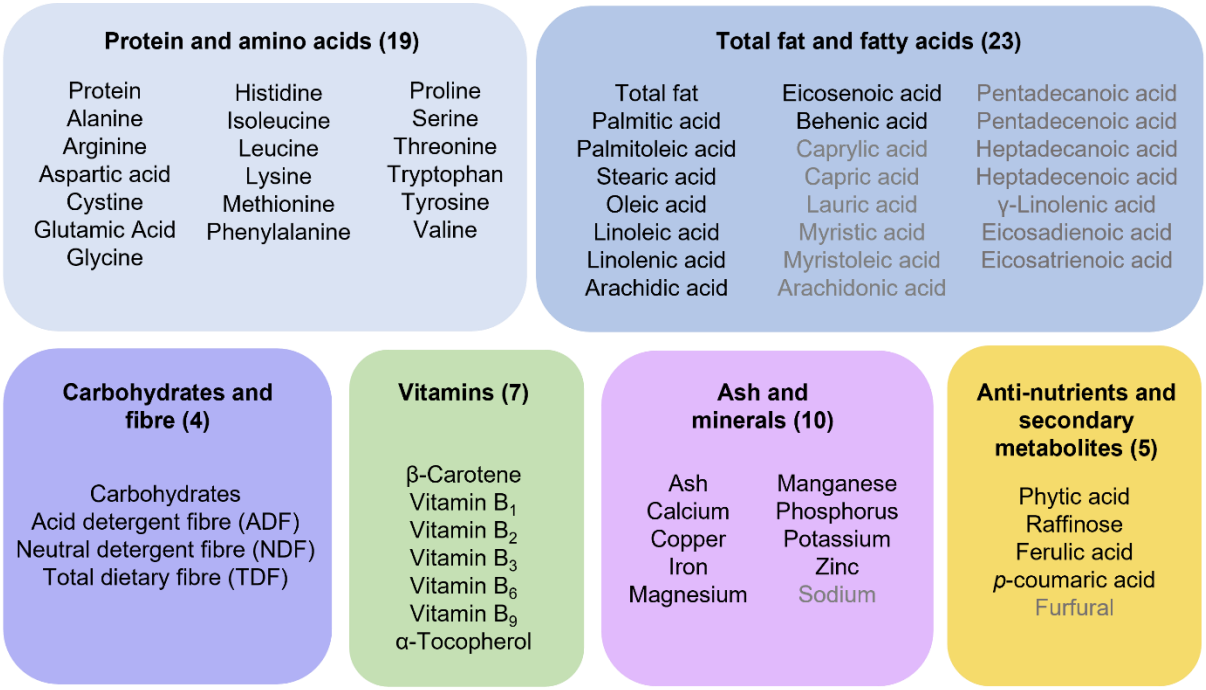

Figure 8. *Analytes measured in MON94804 grain samples. Analytes listed in grey text had >50% of samples below the LOQ and were excluded from statistical analysis. The 53 analytes listed in black text were analysed fully.*

A linear mixed model analysis of variance was applied on data combined across the five replicated field trial sites. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina 2012). For each analyte, 'descriptive statistics' (mean, standard error (SE), and range) were generated.

In assessing the statistical significance of any difference between MON94804 and the conventional control, a *p*-value of 0.05 was used. Any statistically significant differences were evaluated further to assess whether they were likely to be biologically meaningful. The magnitude of differences in mean values between MON94804 and the control were determined, and this difference was compared to the variation observed within the control grown at multiple sites.

The natural variation of analytes from the literature and from the AFSI Crop Composition Database (AFSI CCDB) was also considered (Egesel et al. 2003, Harrigan et al. 2009; Ridley et al. 2011, AFSI 2023). The ranges derived from these values account for variability present in non-GM corn varieties due to a wider range of agronomic and environment conditions, as well as different genetic backgrounds.

Key analyte levels were also analysed in forage but the results are not included in this report.

5.3 Analyses of key components in grain

Of the 53 analytes for which mean values were provided (Figure 8), there were 4 for which there was a statistically significant difference ($p < 0.05$) between corn line MON94804 and the non-GM control: stearic acid, total dietary fibre (TDF), calcium, and ferulic acid. A summary of these 4 analytes is provided in Figure 9. For the complete data set, including values for the analytes for which no statistically significant differences were found, refer to the Application

dossier[13] (pages 92-104).

For all 4 analytes where a statistically significant difference was found, the deviation of the MON94804 mean from the control mean was less than 15% (Figure 9a). However, as can be observed in Figure 9 (panels b-e), the MON94804 mean for each of these components was within the control range value, indicating that MON94804 has a smaller impact on the levels of these analytes than does natural variation within the conventional control. In addition, the observed MON94804 means fall well within the natural variability seen in the publicly-available data ranges (grey bars, Figure 9, b-e). The differences reported here are therefore 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 MON94804 when compared with conventional non-GM corn varieties already available in agricultural markets. Grain from MON94804 can therefore be regarded as equivalent in composition to grain from conventional non-GM corn.

[13] https://www.foodstandards.gov.au/food-standards-code/applications/a1287-food-derived-short-stature-corn-line-mon94804

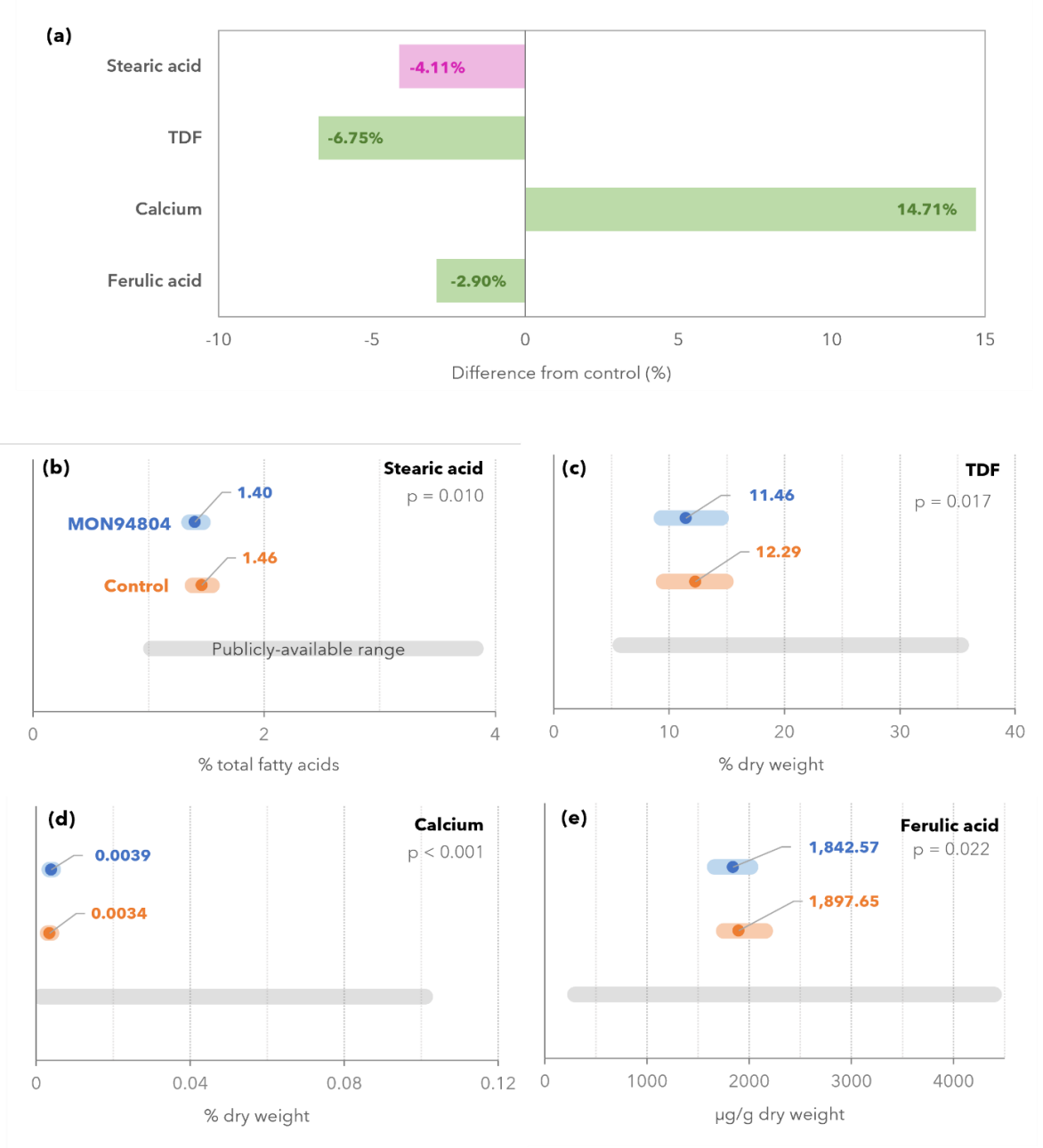

Figure 9. *Visual summary of statistically significant compositional differences between MON94804 and the conventional control corn.* **(a)** *Percentage deviation of the mean MON94804 value from the mean control value for each of the 4 analytes for which a statistically significant difference was found.* **(b) – (e)** *Measured means (dots) and ranges (coloured bars) for MON94804 (blue) and the conventional control (orange) for the 4 analytes as labelled. The grey bars represent the publicly-available range of values for each analyte. Note that the x-axes vary in scale and unit for each component. TDF: total dietary fibre.*

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.

Where a GM food has been shown to be compositionally equivalent to conventional cultivars, the evidence to date indicates that feeding studies using target livestock or other animal species will add little to the safety assessment (OECD 2003; Bartholomaeus et al. 2013). 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.

MON94804 is the result of a genetic modification to reduce overall plant height, with no intention to significantly alter nutritional parameters in the food. The compositional analyses have demonstrated that the genetic modification has not altered the nutrient composition MON94804 compared with conventional non-GM corn cultivars. The introduction of food derived from MON94804 into the food supply is therefore expected to have negligible nutritional impact.

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Appendix 1

PV-ZMAP527892-derived genetic elements in T-DNA region

Genetic Element	Location in Plasmid Vector	Description, source and reference	Present in MON94804?
Left Border Region	1-442	DNA regions from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al. 1983)	Yes[tr]
Intervening sequence	443-509	Sequence used in DNA cloning	Yes
loxP	510-543	Sequence from Bacteriophage P1 for the *loxP* recombination site recognized by the Cre recombinase (Russell et al. 1992)	No*
Intervening sequence	544-557	Sequence used in DNA cloning	No
***cp4 epsps* cassette**			
***Ract1* promoter**	558-1478	Promoter and leader of the *act1* gene from *Oryza sativa* (rice) encoding the rice Actin 1 protein (McElroy et al. 1990) that directs transcription in plant cells	No
***Ract1* intron**	1479-1956	Intron and flanking UTR sequence of the *act1* gene from *O. sativa* encoding rice Actin 1 protein (McElroy et al. 1990) that is involved in regulating gene expression	No
Intervening sequence	1957-1965	Sequence used in DNA cloning	No
***CTP2* targeting sequence**	1966-2193	Targeting sequence of the *ShkG* gene from *Arabidopsis thaliana* encoding the EPSPS transit peptide region that directs transport of the protein to the chloroplast (Herrmann, 1995; Klee et al. 1987)	No
***cp4 epsps* coding sequence**	2194-3561	Coding sequence of the *aroA* gene from *Agrobacterium tumefaciens* sp. strain CP4 encoding the CP4 EPSPS protein that provides herbicide tolerance (Barry et al. 2001; Padgette et al. 1996)	No
Intervening sequence	3562-3576	Sequence used in DNA cloning	No
***nos* terminator**	3577-3829	3' UTR sequence of the *nopaline synthase (nos)* gene from *A. tumefaciens* pTi encoding NOS that directs polyadenylation (Bevan et al. 1983; Fraley et al. 1983)	No
Intervening sequence	3830-3850	Sequence used in DNA cloning	Partially*
loxP	3851-3884	Sequence from Bacteriophage P1 for the *loxP* recombination site recognized by the Cre recombinase (Russell et al. 1992)	Yes
Intervening sequence	3885-4039	Sequence used in DNA cloning	Yes

***GA20ox_SUP* cassette**			
***RTBV1* promoter**	4040-4805	Promoter and leader from the rice tungro bacilliform virus (RTBV) (Yin and Beachy 1995) that directs transcription in plant cells	Yes
Intervening sequence	4806-4825	Sequence used in DNA cloning	Yes
***Hsp70* intron**	4826-5629	Intron and flanking exon sequence of the *hsp70* gene from *Zea mays* (corn) encoding the heat shock protein 70 (HSP70) (Rochester et al. 1986) that is involved in regulating gene expression (Brown and Santino, 1997)	Yes
GA20ox_SUP	5630-6037	Sequence composed of an inverted repeat (Plasmid location: 57425762 and 57975817) derived from coding sequence of *ZmGA20ox3* and *ZmGA20ox5* genes from *Z. mays* that encodes the gibberellic acid 20 oxidase 3 and 5 proteins (Song et al. 2011), flanked and separated by three OsamiR1425 fragments (Plasmid location: 56305741, 57635796, 58186037) from *O. sativa* (Lacombe et al. 2008)	Yes
Intervening sequence	6038-6069	Sequence used in DNA cloning	Yes
***GST43* terminator**	6070-6369	A 3' UTR that has been developed from multiple 3' UTR sequences from *Z. mays* (To et al. 2021) that directs polyadenylation of the mRNA	Yes
Intervening sequence	6370-6486	Sequence used in DNA cloning	Yes
Right Border Region	6487-6817	DNA region from *A. tumefaciens* containing the right border sequence used for transfer of the T–DNA (Depicker et al. 1982)	No[ab]

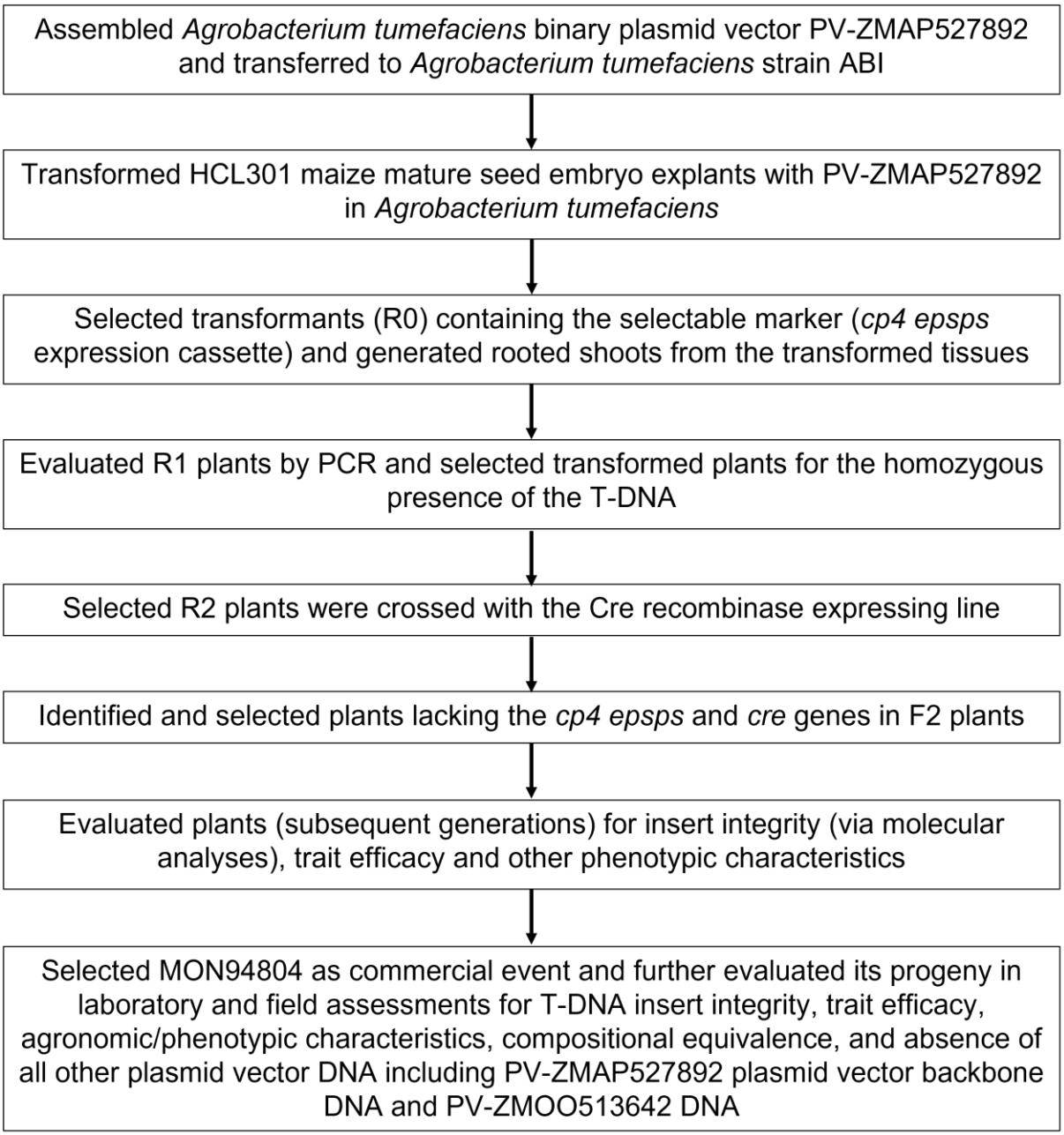
***** Bases 544 through 3,844 of the PV-ZMAP527892 T-DNA were excised from an intermediary line in the development of MON94804. This removed the selectable marker cassette (*Ract1* promoter, *Ract1* intron, *cp4 epsps* coding sequence, and *nos* terminator) and one *lox*P site. One 34 bp *lox*P site remained in MON94804 (bases 3851-3884).

[tr] The sequence of the left border region in MON94804 is truncated relative to its sequence in PV-ZMAP527892

[ab] The right border region from PV-ZMAP527892 is absent in MON94804

Appendix 2

Development of MON94804



Appendix 3

Map of Cre recombinase-expressing plasmid PV-ZMOO513642