



**Compositional Analysis of Forage and Grain from Event 5307 Hybrid Maize  
Grown During 2008 in the USA**

**Amended Report No. 2**

Data Requirement                      Not applicable

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Date:                                         Study completed on February 16, 2011

Syngenta Study No.:                      5307-08-101

Report No.:                                 SSB-170-09 A2  
Volume 1 of 2

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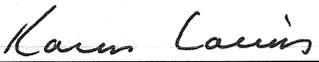
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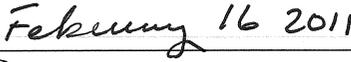
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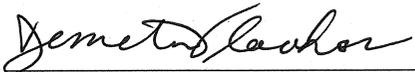
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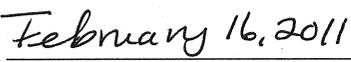
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**Appendix B** Compositional Analysis of 5307-08-101 Maize Forage and Grain. Covance Study No. 8200-888

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## REPORT AMENDMENTS

### **Amendment No 1: May 11, 2010**

This report was amended to present the across-location comparisons using a mixed-model statistical approach, to provide statistical comparison of all components at each location, and to provide statistical comparison of fatty acids reported in the raw data that were not included in the original report.

This amended report replaces in entirety Report No. SSB-170-09 that was issued on December 4, 2009.

### **Amendment No 2: February 16, 2011**

This amended report has the following corrections:

On page 23, a decimal place (0.0) was added to the ILSI mean for potassium to match the value published in the database. This did not change the values.

On page 33, the ILSI mean value for raffinose is corrected to 0.132.

The corrected page in this amended report SSB-170-09 A2 is indicated as “*REVISED*”

This amendment applies only to Volume 1 of this report.

## LIST OF ACRONYMS AND ABBREVIATIONS

ADF	acid detergent fiber
ANOVA	analysis of variance
CFR	Code of Federal Regulations
DW	dry weight
FA	fatty acids
FW	fresh weight
ILSI	International Life Sciences Institute
LOQ	limit of quantitation
NDF	neutral detergent fiber
OECD	Organisation for Economic Co-operation and Development
SEM	standard error of mean
TDF	total dietary fiber
TIU	trypsin inhibitor units

## AMINO ACID ABBREVIATIONS

Ala	alanine
Arg	arginine
Asp	aspartic acid
Cys	cystine
Glu	glutamic acid
Gly	glycine
His	histidine
Ile	isoleucine
Leu	leucine
Lys	lysine
Met	methionine
Phe	phenylalanine
Pro	proline
Ser	serine
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine

## SUMMARY

Key nutritional components in forage and grain from Event 5307 hybrid maize were measured and compared with those from nontransgenic, near-isogenic hybrid maize. Event 5307 hybrid maize produces an eCry3.1Ab protein which is an engineered chimera of mCry3A and Cry1Ab proteins and has activity against certain coleopteran pests, and a phosphomannose isomerase protein that acts as a selectable marker enabling transformed plant cells to utilize mannose as a primary carbon source.

Event 5307 hybrid maize and the corresponding nontransgenic, near-isogenic hybrid maize were grown at six locations in the USA in 2008. At each location, the hybrids were grown in a randomized complete block design, with three replicates per genotype. Forage and grain were harvested and analyzed for key food and feed nutrients, antinutrients, and secondary plant metabolites chosen based on recommendations of the Organisation for Economic Co-operation and Development (OECD 2002) for comparative assessment of the composition of new varieties of maize. Forage was analyzed for proximates, calcium, and phosphorus; and grain was analyzed for proximates, starch, minerals, amino acids, fatty acids, vitamins, secondary metabolites, and antinutrients. Analysis of variance was used to test for genotype effects across locations and at each location. In addition, mean levels of nutritional components were compared with the ranges of variation for conventional hybrid maize published in the International Life Sciences Institute Crop Composition Database (ILSI 2008).

In the forage across-location comparisons, none of the nutritional component levels differed significantly between the two genotypes. In the comparisons at each location, fat, ash, carbohydrates, acid detergent fiber (ADF), and neutral detergent fiber (NDF) were not significantly different at any location. Levels of moisture, protein, calcium, and phosphorus were each significantly different at one location. For all components measured in forage, the mean levels (across locations and at each location) were within the ranges of variation for conventional maize hybrids published in the ILSI Crop Composition Database (2008).

In the grain across-location comparisons, most components were not significantly different. Statistically significant differences between the two genotypes were noted in levels of seven nutritional components: vitamins A, B<sub>6</sub>, and B<sub>9</sub>; and 16:0 palmitic, 18:0 stearic, 18:3 linolenic, and 20:1 eicosenoic fatty acids. Levels below the limit of quantitation precluded across-location statistical comparison of sixteen components (selenium, sodium, furfural, and thirteen fatty acids).

In the comparisons at each location, most components were not significantly different at any location. For the remainder, significant differences were generally intermittent and infrequent. Seventeen components (protein, ash, starch, magnesium, manganese, phosphorus, zinc, vitamin B<sub>6</sub>, eight amino acids, and 20:1 eicosenoic acid) were each significantly different at one location; four components (vitamin A, 16:0 palmitic acid,

18:0 stearic acid, and 18:3 linolenic acid) were each significantly different at two locations. No components were significantly different at more than two locations.

For all quantifiable components measured in grain, the mean levels (across locations and at each location) were within the ranges of variation for conventional maize hybrids published in the ILSI Crop Composition Database (2008) except starch and vitamin B<sub>2</sub>, which were each slightly higher than the ILSI range in the nontransgenic grain from a single location.

The results of this study support the conclusion that no biologically significant changes in composition occurred as an unintended result of the transformation process or expression of the transgenes in Event 5307 hybrid maize. Based on these data, it is concluded that forage and grain from the Event 5307 hybrid maize are not materially different in nutrient composition from forage and grain from both the nontransgenic, near-isogenic hybrid maize and conventional hybrid maize as described in the published literature.

## INTRODUCTION

This study was conducted to measure and compare key food and feed nutrients, antinutrients, and secondary plant metabolites of forage and grain from an Event 5307 hybrid maize and a nontransgenic, near-isogenic hybrid maize, as part of a comparative safety assessment. In addition, mean levels of nutritional components were compared with ranges of levels for conventional hybrid maize published in the International Life Sciences Institute Crop Composition Database (ILSI 2008).

Using the techniques of modern molecular biology, Syngenta has transformed maize (*Zea mays*) to produce Event 5307 maize, a new cultivar that has insecticidal activity against certain corn rootworm (*Diabrotica*) species. Maize plants derived from transformation Event 5307 ("5307 maize") contain the gene *ecry3.1Ab* encoding the eCry3.1Ab protein and the gene *manA* encoding the enzyme phosphomannose isomerase (PMI). The eCry3.1Ab protein is an engineered chimera of modified Cry3A (mCry3A) and Cry1Ab proteins. The gene *manA* (also known as *pmi*) was obtained from *Escherichia coli* strain K-12 and the protein it encodes was utilized as a plant selectable marker during development of 5307 maize.

Conventional agronomic practices were used to plant, maintain, and harvest replicate plots of 5307 maize and the corresponding nontransgenic maize at locations selected to represent agricultural regions where these hybrids typically would be cultivated. Forage and grain were analyzed for various nutritional components, and component levels were statistically compared between 5307 maize and the nontransgenic maize.

## MATERIALS AND METHODS

### Test and Control Substances

The 5307 maize hybrid was the test substance. The control substance was the corresponding nontransgenic hybrid of the same genetic background. Seed lots of the test and control substances were subjected to the appropriate Stewardship Quality Control testing according to current Syngenta standards.

<u>Description</u> Entry	<u>Genotype</u>
Event 5307 hybrid	1 NP2171/NP2460(5307)
Nontransgenic, near-isogenic hybrid	2 NP2171/NP2460

Appendix A shows the pedigree chart for these maize hybrids (Figure A1).

### Plant Material

During 2008, hybrid maize plants were grown according to local agronomic practices at the following six locations in the USA; these locations are representative of the agricultural regions where the hybrid varieties typically would be cultivated (2008 Field Trial 08SUMGSFR1A–5307-08-101):

<u>Location Code</u>	<u>City and State Location</u>	<u>Identifier</u>
L1	Stanton, MN	Stanton 4536
L2 Janesville,	WI	Janesville 5629
L4	New Haven, IN	New Haven 6742
L6 Shirley,	IL	Shirley 7630
L7	Marshall, MO	Marshall 8409
L8	Bloomington, IL	Blm Nursery 761N

At each location, 5307 maize and the corresponding nontransgenic maize were grown in a randomized complete block design, with three replicate plots for each genotype, as shown in the field plot diagrams in Appendix A (Figures A2 through A7).

Both the 5307 maize and the nontransgenic maize were treated with conventional pesticides needed to maintain optimal plant health. Plants were self-pollinated by hand, and the developing ears were bagged to prevent cross-pollination.

### **Forage Sampling and Processing**

The entire above-ground portion of five plants of each genotype was harvested at dough stage (R4), the stage at which silage typically is prepared, from each of the three replicate plots at each location. Plants were pooled to create a composite sample for each plot, then ground with a chipper-shredder. A subsample from each well-mixed composite sample was shipped overnight on ice packs to Syngenta Crop Protection, Inc. (Greensboro, NC, USA). The samples were stored at  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ , then finely ground and shipped on dry ice to Covance Laboratories, Inc., where they were stored at  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$  until they were analyzed.

### **Grain Sampling and Processing**

For each genotype, ears were collected from 15 plants from each replicate plot at each location. Ears were harvested after reaching physiological maturity (R6) and then mechanically dried to approximately 9% to 12% moisture content. (Mechanical drying after harvest is standard agronomic practice for improving storage characteristics of maize grain.) Each sample consisted of grain shelled from ears collected from 15 plants from one plot. A well-mixed subsample of approximately 500 g of grain from each plot was shipped at ambient temperature to Syngenta Crop Protection, Inc., where it was stored at  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ , then finely ground and shipped on dry ice to Covance Laboratories, Inc. The samples were stored at  $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$  until they were analyzed.

### **Compositional Analysis**

The nutritional components measured in maize forage and grain were chosen based on recommendations of the Organisation for Economic Co-Operation and Development (OECD 2002) for comparative assessment of the composition of new varieties of maize. Forage and grain were analyzed for the components shown in Tables 1 and 2.

**Table 1. Components analyzed in maize forage**

Proximates	Minerals
moisture	calcium
protein	phosphorus
fat	
ash	
carbohydrates	
ADF <sup>a</sup>	
NDF <sup>b</sup>	

<sup>a</sup>Acid detergent fiber

<sup>b</sup>Neutral detergent fiber

**Table 2. Components analyzed in maize grain**

Proximates and starch	Minerals	Vitamins	Amino acids	
moisture	calcium	A ( $\beta$ -carotene)	alanine	lysine
protein	copper	B <sub>1</sub> (thiamine)	arginine	methionine
fat	iron	B <sub>2</sub> (riboflavin)	aspartic acid	phenylalanine
ash	magnesium	B <sub>3</sub> (niacin)	cystine	proline
carbohydrates	manganese	B <sub>6</sub> (pyridoxine)	glutamic acid	serine
ADF <sup>a</sup>	phosphorus	B <sub>9</sub> (folic acid)	glycine	threonine
NDF <sup>b</sup>	potassium	E ( $\alpha$ -tocopherol)	histidine	tryptophan
TDF <sup>c</sup>	selenium		isoleucine	tyrosine
starch	sodium		leucine	valine
	zinc			

Fatty acids <sup>d</sup>	Secondary metabolites	Antinutrients
16:0 palmitic	ferulic acid	phytic acid
18:0 stearic	furfural	raffinose
18:1 oleic	inositol	trypsin inhibitor
18:2 linoleic	<i>p</i> -coumaric acid	
18:3 linolenic		
20:0 arachidic		
20:1 eicosenoic		
22:0 behenic		

<sup>a</sup>Acid detergent fiber

<sup>b</sup>Neutral detergent fiber

<sup>c</sup>Total dietary fiber

<sup>d</sup>Maize grain was analyzed for 22 fatty acids; the eight shown were quantifiable and were compared statistically

All compositional analyses were conducted by Covance Laboratories, Inc., according to methods published and approved by AOAC International (AOAC or other industry-standard analytical methods, as described in Volume 2, Appendix B). Component levels

were converted to equivalent units of dry weight (DW) based on the moisture content of each sample.

### **Statistical Analysis**

#### ***Across-location comparisons***

The data for each component were subjected to analysis of variance using the following mixed model:

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

In this model,  $Y_{ijk}$  is the observed response for genotype  $i$  at location  $j$  block  $k$ ,  $U$  is the overall mean,  $T_i$  is the genotype effect,  $L_j$  is the location effect,  $B(L)_{jk}$  is the effect of block within a location,  $LT_{ij}$  is the location-by-genotype interaction effect, and  $e_{ijk}$  is the residual error. Genotype was regarded as a fixed effect, while the effects of location, block within location, and location-by-genotype were regarded as random.

For each quantifiable component, an F test was used to assess the statistical significance of the genotype effect with an alpha level of 0.05 and with the denominator degrees of freedom determined using the Kenward-Roger method (Kenward & Roger 1997). Moisture content of grain was not statistically analyzed because the samples had been mechanically dried.

#### ***Individual-location comparisons***

The data for each component at each location were subjected to an analysis of variance with genotype and block included in the statistical model. Significance was based on an alpha level of 0.05.

Statistical analysis was performed using SAS v. 9.2 (SAS Institute, Inc., Cary NC).

### **Comparison with ILSI Crop Composition Database**

The mean levels of each component for each location and across locations were calculated and compared nonstatistically with means and ranges for forage and grain composition published in the ILSI Crop Composition Database (2008). The ILSI database is the most comprehensive and current source of crop composition data for most nutritional components.

## **RESULTS**

Tables 3 through 10 report the statistical comparisons of nutritional component levels in forage and grain between 5307 maize and nontransgenic maize, the mean levels for each genotype across locations and at each location, and the levels for conventional hybrid maize reported in the ILSI Crop Composition Database (2008).

All data were compared with the ranges reported in the ILSI database to establish whether the results were within the range of natural variation and to provide an indication of whether the results were likely to be of biological significance.

### **Forage Composition**

**Proximates.** As shown in Table 3, in the across-location comparisons, levels of moisture, protein, fat, ash, carbohydrates, ADF, and NDF did not differ significantly between the 5307 maize and the nontransgenic maize. In the comparisons at each location, levels of fat, ash, carbohydrates, ADF, and NDF did not differ significantly between the two genotypes at any of the six locations. Levels of moisture and protein each differed significantly at one location.

The mean levels of all proximates, across locations and for each location were within the ranges reported in the ILSI database.

**Calcium and phosphorus.** As shown in Table 4, in the across-location comparisons, neither calcium nor phosphorus levels differed significantly between the 5307 maize and the nontransgenic maize. In the comparisons at each location, levels of calcium and phosphorus each differed significantly at one location.

The mean levels across locations and for each location were within the ranges reported in the ILSI database.

### **Grain Composition**

**Proximates and starch.** As shown in Table 5, in the across-location comparisons, levels of protein, fat, ash carbohydrates, ADF, NDF, TDF, and starch did not differ significantly between the 5307 maize and the nontransgenic maize. Moisture levels were not compared statistically because grain samples were mechanically dried. In the comparisons at each location, levels of fat, carbohydrates, ADF, NDF, and TDF did not differ significantly between the two genotypes at any of the six locations. Levels of protein, ash, and starch each differed significantly at one location.

The mean levels across locations and for each location were within the ranges reported in the ILSI database, except for starch which was slightly higher than the ILSI range in the nontransgenic hybrid at one location.

**Minerals.** As shown in Table 6, in the across-location comparisons, levels of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, and zinc did not differ significantly between the 5307 maize and the nontransgenic maize. In the comparisons at each location, levels of calcium, copper, iron, and potassium did not differ between the two genotypes at any of the six locations. Levels of magnesium, manganese, phosphorus, and zinc each differed significantly at one location. For selenium and sodium, levels below the limit of quantitation (LOQ) precluded statistical comparison across-locations for both components, at all six locations for sodium, and at four of the six locations for

selenium. At the two locations where statistical analysis was performed for selenium, the levels did not differ significantly.

For all quantifiable components, mean levels across locations and for each location were within the ranges reported in the ILSI database.

**Vitamins.** As shown in Table 7, in the across-location comparisons, levels of vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, and E did not differ significantly between the 5307 maize and the nontransgenic maize. Statistically significant differences were observed in levels of vitamins A, B<sub>6</sub>, and B<sub>9</sub>. In the comparisons at each location, levels of vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, B<sub>9</sub>, and E did not differ between the two genotypes at any of the six locations. Levels of vitamin B<sub>6</sub> differed significantly at one location and levels of vitamin A differed significantly at two locations.

All mean levels across locations and for each location were within the ranges reported in the ILSI database, except for vitamin B<sub>2</sub> which was slightly higher than the ILSI range in the nontransgenic hybrid at one location.

**Amino Acids.** As shown in Table 8, in the across-location comparisons, none of the amino acid levels differed significantly between the 5307 maize and the nontransgenic maize. In the comparisons at each location, levels of eleven amino acids did not differ between the genotypes at any of the six locations. Levels of asparagine, threonine, glutamic acid, glycine, alanine, valine, leucine, and histidine all differed significantly at one single location (L2).

All mean levels across locations and for each location were within the ranges reported in the ILSI database.

**Fatty Acids.** As shown in Table 9, in the across-location comparisons, the proportions of 18:1 oleic acid, 18:2 linoleic acid, 20:0 arachidic acid, and 22:0 behenic acid as fractions of total fatty acids did not differ significantly between the 5307 maize and the nontransgenic maize. Statistically significant differences were observed in proportions of four other fatty acids (16:0 palmitic acid, 18:0 stearic acid, 18:3 linolenic acid, and 20:1 eicosenoic acid). In the comparisons at each location, the proportions of 18:1 oleic acid, 18:2 linoleic acid, 20:0 arachidic acid, and 22:0 behenic acid as fractions of total fatty acids did not differ between the two genotypes at any of the six locations. Statistically significant differences were observed in proportions of 20:1 eicosenoic acid at one location; and 16:0 palmitic acid, 18:0 stearic acid, and 18:3 linolenic acid at two locations. All four fatty acids with statistically significant differences in the individual-location comparisons were different at Location 4 (L4). For 16:1 palmitoleic acid, levels below the LOQ precluded statistical comparison across-locations, and at five of the six locations. At the one location where statistical analysis was performed for 16:1 palmitoleic acid, the proportions did not differ significantly.

For all quantifiable components, mean levels across locations and for each location were within the ranges reported in the ILSI database.

***Secondary Metabolites and Antinutrients.*** As shown in Table 10, in the across-location comparisons, levels of ferulic acid, *p*-coumaric acid, inositol, phytic acid, trypsin inhibitor and raffinose did not differ significantly between the 5307 maize and the nontransgenic maize; nor did these components differ at any of the six locations. For furfural, levels below the LOQ precluded statistical comparison across-locations, and at all six locations.

For all quantifiable components, mean levels across locations and for each location were within the ranges reported in the ILSI database.

## **DISCUSSION AND CONCLUSIONS**

For most components measured in forage and grain, mean levels did not differ significantly between the 5307 maize and nontransgenic maize. Of the seven grain components with statistically significant across-location comparisons, all were significantly different at two or fewer locations. Sixteen components in grain were not compared statistically across locations due to the presence of <LOQ values (selenium, sodium, furfural, and thirteen fatty acids). Moisture in grain was not compared statistically due to mechanical drying of the samples. For all components compared statistically, including those for which statistically significant differences were observed, mean levels were within the ranges for conventional maize hybrids published in the ILSI Crop Composition Database (2008), except starch and vitamin B<sub>2</sub> in grain. Levels of starch and vitamin B<sub>2</sub> were each slightly higher than the ILSI range in the nontransgenic grain, each from a single location. These findings support the conclusion that no biologically significant changes in composition occurred as an unintended result of the transformation process or expression of the transgenes in Event 5307 hybrid maize.

Based on these data, it is concluded that the forage and grain from the Event 5307 maize hybrid are not materially different in composition than forage and grain from both the nontransgenic, near-isogenic maize hybrid and conventional maize hybrids as described in the ILSI Crop Composition Database.

## RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Syngenta Biotechnology, Inc. (3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA).

## CONTRIBUTING SCIENTISTS

Analytical work reported herein was conducted by Covance Laboratories, Inc. Other contributing scientists include those from Syngenta Biotechnology, Inc. (Justin de Fontes and Karen Launis), Syngenta Crop Protection, Inc. (Bill Helke), and Syngenta Ltd. (Keith Ward, Jealott's Hill International Research Centre, Bracknell, U.K.).

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- US EPA. 1989. Good Laboratory Practice Standards. 40 CFR Part 160.

**Table 3. Proximate composition of forage from Event 5307 maize and nontransgenic maize**

 Proximate levels are shown in % DW, except for moisture (% FW). Results significantly different at  $P < 0.05$  are shown in bold italic type.

 For across-location analyses  $N = 18$ .

Location	Data source	Statistic	Moisture	Protein	Fat	Ash	Carbo- hydrates	ADF	NDF
Across all	Event 5307	mean	73.0	7.72	1.90	4.12	86.3	29.1	44.9
		range	66.5–79.5	5.91–10.3	0.893–2.81	2.89–5.35	82.9–88.9	22.3–40.1	35.5–56.1
	Nontransgenic	mean	72.3	7.57	1.89	4.34	86.2	28.6	45.4
		range	66.7–78.0	6.27–10.0	0.843–2.63	3.43–6.18	82.3–89.0	19.0–41.5	32.3–57.4
ANOVA ( <i>F</i> test)									
	Genotype effect	<i>P</i>	0.126	0.525	0.893	0.076	0.895	0.696	0.785
		SEM	1.49	0.449	0.118	0.295	0.64	1.33	1.91
	ILSI (2008)	mean	70.2	7.78	2.039	4.628	85.6	27.00	41.51
		range	49.1–81.3	3.14–11.57	<LOQ–4.570	1.527–9.638	76.4–92.1	16.13–47.39	20.29–63.71
		$N^a$	945	945	921	945	945	945	945

<sup>a</sup> $N$  is the number of ILSI values used to calculate the mean and excludes values <LOQ

**Table 3 (Continued). Proximate composition of forage from Event 5307 maize and nontransgenic maize**

 Proximate levels are shown in % DW, except for moisture (% FW). Results significantly different at  $P < 0.05$  are shown in bold italic type.

 For individual location means  $N = 3$ .

Location	Data source	Statistic	Moisture	Protein	Fat	Ash	Carbo- hydrates	ADF	NDF
L1	Event 5307	mean	70.2	7.98	2.23	3.50	86.3	26.9	36.4
	Nontransgenic	mean	68.8	7.28	1.73	3.67	87.3	26.4	40.1
		<i>P</i>	0.208	0.286	0.415	0.162	0.162	0.887	0.480
		SEM	0.53	0.340	0.346	0.055	0.35	2.48	3.04
L2	Event 5307	mean	72.1	6.96	2.01	4.59	86.4	29.7	44.1
	Nontransgenic	mean	71.4	6.77	2.11	4.82	86.3	33.8	48.9
		<i>P</i>	0.630	0.731	0.772	0.363	0.961	0.433	0.563
		SEM	0.96	0.340	0.207	0.139	0.43	2.98	4.97
L4	Event 5307	mean	72.5	7.46	2.15	3.64	86.8	28.1	46.7
	Nontransgenic	mean	71.3	6.96	2.30	4.01	86.7	26.4	41.1
		<i>P</i>	0.105	<b>0.033</b>	0.795	0.417	0.771	0.738	0.295
		SEM	0.31	0.066	0.350	0.258	0.21	3.13	2.82
L6	Event 5307	mean	76.0	8.40	1.41	4.64	85.5	29.1	47.7
	Nontransgenic	mean	76.7	9.07	1.77	4.41	84.8	27.0	45.5
		<i>P</i>	0.654	0.521	0.192	0.646	0.349	0.092	0.077
		SEM	0.99	0.610	0.131	0.300	0.41	0.49	0.46
L7	Event 5307	mean	78.5	9.35	1.73	5.05	84.0	33.8	48.6
	Nontransgenic	mean	76.7	8.80	1.83	5.47	83.9	32.4	52.9
		<i>P</i>	<b>0.007</b>	0.141	0.695	0.377	0.804	0.672	0.541
		SEM	0.10	0.162	0.162	0.265	0.42	2.06	4.19
L8	Event 5307	mean	68.6	6.19	1.89	3.33	88.6	26.7	45.7
	Nontransgenic	mean	68.7	6.54	1.57	3.65	88.3	25.5	43.7
		<i>P</i>	0.225	0.057	0.323	0.162	0.625	0.688	0.594
		SEM	0.04	0.061	0.174	0.104	0.33	1.88	2.33

**Table 4. Calcium and phosphorus composition of forage from Event 5307 maize and nontransgenic maize**

 Calcium and phosphorus levels shown in mg/kg DW. Results significantly different at  $P < 0.05$  are shown in bold italic type. For across location analyses  $N = 18$ .

Location	Data source	Statistic	Ca	P
Across all	Event 5307	mean	2346	1906
		range	1450–3470	1420–2870
	Nontransgenic	mean	2354	1953
		range	1660–3350	1390–2890
	ANOVA ( <i>F</i> test)			
	Genotype effect	<i>P</i>	0.886	0.491
		SEM	209.3	163.9
	ILSI (2008)	mean	2028.6	2066.1
		range	713.9–5767.9	936.2–3704.1
		<i>N</i>	481	481

**Table 4 (Continued). Calcium and phosphorus composition of forage from Event 5307 maize and nontransgenic maize**

 Calcium and phosphorus levels shown in mg/kg DW. Results significantly different at  $P < 0.05$  are shown in bold italic type. For individual location means  $N = 3$ .

Location	Data source	Statistic	Ca	P
L1	Event 5307	mean	2370	1723
	Nontransgenic	mean	2210	1523
		<i>P</i>	0.208	<b><i>0.003</i></b>
		SEM	61.6	8.2
L2	Event 5307	mean	2130	1500
	Nontransgenic	mean	2223	1457
		<i>P</i>	0.711	0.694
		SEM	154.5	67.4
L4	Event 5307	mean	2217	1783
	Nontransgenic	mean	2303	1813
		<i>P</i>	0.087	0.869
		SEM	19.3	113.1
L6	Event 5307	mean	2497	2493
	Nontransgenic	mean	2407	2677
		<i>P</i>	0.771	0.662
		SEM	191.4	255.6
L7	Event 5307	mean	3287	1947
	Nontransgenic	mean	3190	2043
		<i>P</i>	<b><i>0.032</i></b>	0.585
		SEM	12.5	105.9
L8	Event 5307	mean	1573	1990
	Nontransgenic	mean	1793	2203
		<i>P</i>	0.138	0.076
		SEM	64.8	44.0

**Table 5. Proximate and starch composition of grain from Event 5307 maize and nontransgenic maize**

 Proximate and starch levels shown in % DW, except moisture (% FW). Results significantly different at  $P < 0.05$  are shown in bold italic type.

 For across location analyses  $N = 18$ .

Location	Data source	Statistic	Moisture <sup>a</sup>	Protein	Fat	Ash	Carbo- hydrates	ADF	NDF	TDF	Starch
Across all	Event 5307	mean	10.13	10.86	4.54	1.46	83.1	2.74	8.85	11.8	69.4
		range	9.54–11.4	9.12–12.6	3.85–4.93	1.22–1.60	81.0–85.3	2.23–3.34	7.68–9.52	10.8–13.4	62.0–73.7
	Nontransgenic	mean	10.18	10.92	4.72	1.40	83.0	2.85	8.83	11.7	70.3
		range	9.21–12.2	9.20–13.0	4.43–5.09	1.09–1.67	80.7–84.7	2.47–3.48	7.79–10.2	10.6–13.5	63.1–77.3
ANOVA ( <i>F</i> test)											
	Genotype effect	<i>P</i>	–	0.737	0.053	0.138	0.515	0.281	0.930	0.700	0.589
		SEM	–	0.375	0.067	0.044	0.44	0.069	0.128	0.19	1.21
	ILSI (2008)	mean	11.3	10.30	3.555	1.439	84.6	4.05	11.23	16.43	57.7
		range	6.1–40.5	6.15–17.26	1.742–5.823	0.616–6.282	77.4–89.5	1.82–11.34	5.59–22.64	8.85–35.31	26.5–73.8
		<i>N</i>	1434	1434	1174	1410	1410	1350	1349	397	168

– = not applicable

<sup>a</sup>Grain was mechanically dried after harvest so moisture levels were not subject to ANOVA

**Table 5 (Continued). Proximate and starch composition of grain from Event 5307 maize and nontransgenic maize**

 Proximate and starch levels shown in % DW, except moisture (% FW). Results significantly different at  $P < 0.05$  are shown in bold italic type.

 For individual location means  $N = 3$ .

Location	Data source	Statistic	Moisture <sup>a</sup>	Protein	Fat	Ash	Carbo- hydrates	ADF	NDF	TDF	Starch
L1	Event 5307	mean	10.54	10.93	4.38	1.43	83.2	2.63	8.66	11.2	69.4
		Nontransgenic	mean	11.13	10.60	4.79	1.29	83.3	2.89	9.27	11.9
		<i>P</i>	–	0.405	0.068	<b>0.038</b>	0.578	0.390	0.623	0.456	0.401
		SEM	–	0.225	0.080	0.019	0.14	0.169	0.754	0.54	1.54
L2	Event 5307	mean	10.73	9.38	4.53	1.34	84.8	2.89	9.01	11.7	71.9
		Nontransgenic	mean	11.00	9.97	4.68	1.25	84.1	2.67	8.73	11.2
		<i>P</i>	–	<b>0.011</b>	0.358	0.594	0.070	0.152	0.407	0.431	0.090
		SEM	–	0.044	0.090	0.094	0.13	0.069	0.188	0.41	0.64
L4	Event 5307	mean	9.77	10.97	4.84	1.42	82.8	2.84	9.20	11.6	72.5
		Nontransgenic	mean	9.48	10.50	4.84	1.39	83.3	2.73	8.65	11.9
		<i>P</i>	–	0.630	0.974	0.628	0.621	0.391	0.406	0.710	<b>0.019</b>
		SEM	–	0.586	0.126	0.037	0.65	0.076	0.372	0.50	0.65
L6	Event 5307	mean	9.79	12.37	4.59	1.54	81.5	2.85	9.10	12.5	67.3
		Nontransgenic	mean	9.56	12.50	4.77	1.62	81.1	2.87	8.89	12.4
		<i>P</i>	–	0.801	0.257	0.159	0.593	0.962	0.442	0.978	0.940
		SEM	–	0.327	0.080	0.025	0.41	0.217	0.154	0.75	3.87
L7	Event 5307	mean	10.20	10.60	4.25	1.52	83.6	2.54	8.55	12.0	65.9
		Nontransgenic	mean	10.27	10.67	4.56	1.38	83.4	2.97	8.45	11.4
		<i>P</i>	–	0.868	0.384	0.112	0.805	0.188	0.842	0.135	0.222
		SEM	–	0.249	0.198	0.037	0.42	0.156	0.322	0.19	1.23
L8	Event 5307	mean	9.74	10.90	4.68	1.50	82.9	2.67	8.57	11.7	69.7
		Nontransgenic	mean	9.64	11.27	4.66	1.47	82.6	2.95	8.99	11.4
		<i>P</i>	–	0.053	0.792	0.159	0.057	0.479	0.519	0.189	0.283
		SEM	–	0.062	0.047	0.012	0.05	0.229	0.389	0.11	1.59

 – = not applicable. <sup>a</sup>Grain was mechanically dried after harvest so moisture levels were not subject to ANOVA

**Table 6. Mineral composition of grain from Event 5307 maize and nontransgenic maize**

 Mineral levels shown in mg/kg DW. Results significant at  $P < 0.05$  are shown in bold italic type. For across location analyses  $N = 18$ .

Location	Data source	Statistic	Ca	Cu	Fe	Mg	Mn	P	K	Se <sup>a,c</sup>	Na <sup>b,c</sup>	Zn
Across all	Event 5307	mean	43.9	1.52	23.7	1323	5.65	3228	3758	–	–	23.0
		range	38.6– 49.3	0.89– 4.20	21.2– 28.0	1150– 1430	4.69– 6.61	2620– 3520	3400– 4010	<LOQ– 0.363	< LOQ	19.5– 26.9
	Nontransgenic	mean	44.0	1.89	23.3	1336	5.43	3307	3776	–	–	23.4
		range	40.3– 50.1	1.02– 4.36	20.3– 28.1	1220– 1450	4.43– 6.38	2650– 3600	3240– 4150	<LOQ– 0.400	< LOQ	20.5– 27.9
ANOVA ( <i>F</i> test)												
	Genotype effect	<i>P</i>	0.891	0.058	0.308	0.401	0.131	0.110	0.707	–	–	0.355
		SEM	1.28	0.253	0.85	21.2	0.249	94.7	81.0	–	–	0.78
	ILSI (2008)	mean	46.4	1.75	21.81	1193.8	6.18	3273.5	3842.0	0.20	31.75	21.6
		range	12.7– 208.4	<LOQ– 18.50	10.42– 49.07	594.0– 1940.0	1.69– 14.30	1470.0– 5330.0	1810.0– 6030.0	<LOQ– 0.75	<LOQ– 731.54	6.5– 37.2
		<i>N</i> <sup>d</sup>	1344	1249	1255	1257	1256	1349	1257	89	223	1257

– = not applicable

<sup>a</sup>The LOQ for selenium was 0.055–0.056 mg/kg DW

<sup>b</sup>The LOQ for sodium was 110–114 mg/kg DW

<sup>c</sup>Where some or all values were <LOQ, calculation of the mean and statistical comparison was not possible so only the range is shown

<sup>d</sup>*N* is the number of ILSI values used to calculate the mean and excludes values <LOQ

**Table 6 (Continued). Mineral composition of grain from Event 5307 maize and nontransgenic maize**

 Mineral levels shown in mg/kg DW. Results significant at  $P < 0.05$  are shown in bold italic type. For individual location means  $N = 3$ .

Location	Data source	Statistic	Ca	Cu	Fe	Mg	Mn	P	K	Se <sup>a</sup>	Na <sup>a</sup>	Zn
L1	Event 5307	mean	47.6	2.66	23.6	1347	5.66	3163	3803	<LOQ–0.086	<LOQ	22.3
	Nontransgenic	mean	45.4	2.89	22.0	1330	5.03	3103	3680	0.079	<LOQ	22.1
		<i>P</i>	0.198	0.556	0.187	0.755	0.096	0.712	0.381	–	–	0.915
		SEM	0.81	0.232	0.55	33.0	0.149	99.7	78.1	–	–	0.78
L2	Event 5307	mean	41.6	1.35	22.4	1307	5.16	2857	3473	0.113	<LOQ	20.4
	Nontransgenic	mean	41.0	1.88	23.1	1337	5.31	2903	3403	0.125	<LOQ	22.1
		<i>P</i>	0.657	0.200	0.482	0.644	0.669	0.866	0.724	0.211	–	0.138
		SEM	0.87	0.198	0.63	39.4	0.219	172.4	121.7	0.0046	–	0.49
L4	Event 5307	mean	39.7	1.45	27.2	1297	5.09	3293	3657	0.348	<LOQ	25.7
	Nontransgenic	mean	41.2	1.28	27.5	1283	5.11	3413	3760	0.364	<LOQ	26.8
		<i>P</i>	0.200	0.453	0.701	0.732	0.910	0.230	0.446	0.569	–	<b>0.045</b>
		SEM	0.59	0.133	0.42	23.9	0.128	49.7	77.7	0.0160	–	0.16
L6	Event 5307	mean	46.7	0.95	22.5	1347	6.44	3383	3920	<LOQ–0.058	<LOQ	22.1
	Nontransgenic	mean	47.7	1.09	22.5	1353	6.25	3487	3930	<LOQ–0.063	<LOQ	21.4
		<i>P</i>	0.118	0.271	1.000	0.900	0.369	0.335	0.946	–	–	0.559
		SEM	0.27	0.066	0.27	33.2	0.117	58.1	92.7	–	–	0.78
L7	Event 5307	mean	46.8	1.36	24.3	1243	6.32	3227	3750	<LOQ–0.063	<LOQ	24.1
	Nontransgenic	mean	45.5	2.09	23.4	1297	6.14	3417	3883	<LOQ–0.107	<LOQ	23.9
		<i>P</i>	0.156	0.302	0.459	<b>0.047</b>	0.366	<b>0.033</b>	0.231	–	–	0.841
		SEM	0.40	0.372	0.73	8.5	0.108	24.8	55.4	–	–	0.83
L8	Event 5307	mean	40.8	1.34	22.1	1400	5.25	3443	3947	<LOQ–0.061	<LOQ	23.5
	Nontransgenic	mean	42.9	2.14	21.0	1417	4.73	3520	3997	<LOQ–0.062	<LOQ	24.2
		<i>P</i>	0.152	0.156	0.200	0.588	<b>0.018</b>	0.323	0.286	–	–	0.417
		SEM	0.66	0.252	0.39	18.4	0.049	41.7	24.5	–	–	0.51

– = not applicable

<sup>a</sup>Where some or all values were <LOQ, calculation of the mean and statistical comparison was not possible so only the range is shown

**Table 7. Vitamin composition of grain from Event 5307 maize and nontransgenic maize**

 Vitamin levels shown in mg/100 g DW except as indicated for vitamin E (mg/g). Results significant at  $P < 0.05$  are shown in bold italic type.

 For across location analyses  $N = 18$ .

Location	Data source	Statistic	Vitamin A $\beta$ -carotene	Vitamin B <sub>1</sub> Thiamine	Vitamin B <sub>2</sub> Riboflavin	Vitamin B <sub>3</sub> Niacin	Vitamin B <sub>6</sub> Pyridoxine	Vitamin B <sub>9</sub> Folic Acid	Vitamin E <sup>a</sup> $\alpha$ -tocopherol
Across all	Event 5307	mean	0.155	0.449	0.198	3.13	0.692	0.0397	0.0093
		range	0.133–0.185	0.399–0.511	0.156–0.264	2.53–4.11	0.587–0.769	0.0305–0.0460	0.00719–0.0111
	Nontransgenic	mean	0.176	0.458	0.198	3.18	0.737	0.0382	0.0090
		range	0.155–0.216	0.408–0.518	0.152–0.318	2.51–3.70	0.621–0.815	0.0289–0.0463	0.00607–0.0110
ANOVA ( <i>F</i> test)									
	Genotype effect	<i>P</i>	<b><i>&lt;0.001</i></b>	0.146	0.941	0.674	<b><i>0.005</i></b>	<b><i>0.031</i></b>	0.074
		SEM	0.0049	0.0126	0.0096	0.104	0.0167	0.00199	0.00055
	ILSI (2008)	mean	0.684	0.530	0.125	2.376	0.644	0.0651	0.0103
		range	0.019–4.681	0.126–4.000	0.050–0.236	1.037–4.694	0.368–1.132	0.0147–0.1464	0.0015–0.0687
		<i>N</i>	276	894	704	415	415	895	863

<sup>a</sup>Original units of mg/100 g reported by Covance Laboratories were converted to mg/g

**Table 7 (Continued). Vitamin composition of grain from Event 5307 maize and nontransgenic maize**

 Vitamin levels shown in mg/100 g DW except as indicated for vitamin E (mg/g). Results significant at  $P < 0.05$  are shown in bold italic type.

 For individual location means  $N = 3$ .

Location	Data source	Statistic	Vitamin A $\beta$ -carotene	Vitamin B <sub>1</sub> Thiamine	Vitamin B <sub>2</sub> Riboflavin	Vitamin B <sub>3</sub> Niacin	Vitamin B <sub>6</sub> Pyridoxine	Vitamin B <sub>9</sub> Folic Acid	Vitamin E <sup>a</sup> $\alpha$ -tocopherol
L1	Event 5307	mean	0.152	0.473	0.219	3.27	0.611	0.0448	0.0075
	Nontransgenic	mean	0.171	0.476	0.205	3.29	0.716	0.0434	0.0069
		<i>P</i>	0.297	0.939	0.612	0.963	0.105	0.399	0.176
		SEM	0.0096	0.0220	0.0162	0.225	0.0261	0.00091	0.00022
L2	Event 5307	mean	0.151	0.456	0.175	3.53	0.660	0.0422	0.0082
	Nontransgenic	mean	0.167	0.476	0.202	3.33	0.684	0.0424	0.0078
		<i>P</i>	0.082	0.232	0.587	0.657	0.645	0.807	0.313
		SEM	0.0035	0.0083	0.0294	0.279	0.0321	0.00059	0.00018
L4	Event 5307	mean	0.141	0.488	0.197	3.16	0.739	0.0393	0.0093
	Nontransgenic	mean	0.159	0.504	0.169	3.20	0.748	0.0360	0.0090
		<i>P</i>	<b>0.016</b>	0.548	0.222	0.903	0.609	0.093	0.289
		SEM	0.0017	0.0164	0.0112	0.239	0.0098	0.00078	0.00011
L6	Event 5307	mean	0.172	0.432	0.213	2.89	0.699	0.0427	0.0095
	Nontransgenic	mean	0.188	0.424	0.246	2.85	0.749	0.0407	0.0094
		<i>P</i>	0.061	0.218	0.403	0.826	0.484	0.506	0.743
		SEM	0.0029	0.0033	0.0222	0.104	0.0421	0.00176	0.00028
L7	Event 5307	mean	0.157	0.434	0.183	3.16	0.721	0.0317	0.0107
	Nontransgenic	mean	0.176	0.435	0.172	3.25	0.759	0.0299	0.0109
		<i>P</i>	0.218	0.808	0.519	0.709	0.076	0.346	0.733
		SEM	0.0076	0.0009	0.0100	0.153	0.0079	0.00104	0.00030
L8	Event 5307	mean	0.160	0.410	0.203	2.77	0.723	0.0377	0.0105
	Nontransgenic	mean	0.195	0.432	0.191	3.17	0.767	0.0368	0.0098
		<i>P</i>	<b>0.025</b>	0.077	0.560	0.498	<b>0.003</b>	0.744	0.454
		SEM	0.0040	0.0045	0.0123	0.345	0.0018	0.00176	0.00056

<sup>a</sup>Original units of mg/100 g reported by Covance Laboratories were converted to mg/g

**Table 8. Amino acid composition of grain from Event 5307 maize and nontransgenic maize**

 Amino acid levels shown in mg/g DW. Results significant at  $P < 0.05$  are shown in bold italic type. For across location analyses  $N = 18$ .

Location	Data source	Statistic	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
Across all	Event 5307	mean	6.93	3.80	5.14	20.4	9.23	3.95	8.21	2.33	5.12
		range	5.82–8.15	3.19–4.39	4.10–6.10	16.4–24.9	7.55–11.0	3.52–4.39	6.64–9.97	2.07–2.50	4.33–6.08
	Nontransgenic	mean	6.88	3.79	5.17	20.6	9.24	3.97	8.24	2.36	5.13
		range	6.00–8.20	3.36–4.47	4.44–6.28	17.4–25.3	7.84–10.9	3.61–4.35	7.06–10.0	2.14–2.59	4.28–6.01
ANOVA ( <i>F</i> test)											
	Genotype effect	<i>P</i>	0.625	0.908	0.736	0.715	0.973	0.761	0.846	0.284	0.877
		SEM	0.236	0.123	0.203	0.90	0.375	0.087	0.345	0.043	0.179
	ILSI (2008)	mean	6.88	3.75	5.12	20.09	9.51	3.85	7.90	2.21	4.90
		range	3.35–12.08	2.24–6.66	2.35–7.69	9.65–35.36	4.62–16.32	1.84–5.39	4.39–13.93	1.25–5.14	2.66–8.55
		<i>N</i>	1350	1350	1350	1350	1350	1350	1350	1350	1350

**Table 8 (Continued). Amino acid composition of grain from Event 5307 maize and nontransgenic maize**

 Amino acid levels shown in mg/g DW. Results significant at  $P < 0.05$  are shown in bold italic type. For individual location means  $N = 3$ .

Location	Data source	Statistic	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
L1	Event 5307	mean	7.07	3.82	5.09	21.1	9.25	3.86	8.42	2.33	5.25
	Nontransgenic	mean	6.74	3.71	4.98	19.9	8.51	3.80	7.97	2.34	5.00
		<i>P</i>	0.133	0.197	0.431	0.239	0.408	0.059	0.212	0.707	0.179
		SEM	0.096	0.043	0.082	0.51	0.499	0.011	0.176	0.016	0.088
L2	Event 5307	mean	5.94	3.26	4.28	16.8	7.78	3.56	6.78	2.12	4.40
	Nontransgenic	mean	6.27	3.48	4.63	18.3	8.18	3.75	7.30	2.26	4.66
		<i>P</i>	<b>0.030</b>	<b>0.018</b>	0.118	<b>0.010</b>	0.380	<b>0.028</b>	<b>0.010</b>	0.148	<b>0.012</b>
		SEM	0.041	0.021	0.092	0.10	0.255	0.024	0.037	0.045	0.020
L4	Event 5307	mean	6.96	3.81	5.25	20.5	9.08	4.04	8.23	2.36	5.17
	Nontransgenic	mean	6.97	3.84	5.32	20.9	9.78	4.13	8.40	2.36	5.30
		<i>P</i>	0.961	0.833	0.761	0.591	0.247	0.485	0.591	0.940	0.367
		SEM	0.128	0.088	0.149	0.48	0.307	0.075	0.186	0.028	0.079
L6	Event 5307	mean	7.89	4.31	5.94	24.0	10.90	4.28	9.60	2.47	5.82
	Nontransgenic	mean	7.73	4.22	5.95	23.9	10.14	4.18	9.48	2.54	5.72
		<i>P</i>	0.747	0.668	0.976	0.967	0.301	0.606	0.849	0.277	0.820
		SEM	0.306	0.123	0.206	1.01	0.390	0.124	0.393	0.033	0.272
L7	Event 5307	mean	6.69	3.68	4.98	19.3	8.82	3.95	7.85	2.32	4.91
	Nontransgenic	mean	6.48	3.61	4.93	19.0	8.92	3.86	7.68	2.32	4.74
		<i>P</i>	0.514	0.680	0.885	0.772	0.763	0.554	0.725	0.840	0.533
		SEM	0.192	0.109	0.186	0.78	0.219	0.084	0.292	0.021	0.167
L8	Event 5307	mean	7.03	3.89	5.30	20.9	9.55	4.02	8.36	2.39	5.16
	Nontransgenic	mean	7.09	3.88	5.19	21.5	9.89	4.08	8.59	2.36	5.38
		<i>P</i>	0.835	0.933	0.519	0.661	0.584	0.594	0.648	0.311	0.508
		SEM	0.189	0.075	0.097	0.83	0.368	0.064	0.306	0.019	0.198

**Table 8 (Continued). Amino acid composition of grain from Event 5307 maize and nontransgenic maize**

 Amino acid levels shown in mg/g DW. Results significant at  $P < 0.05$  are shown in bold italic type. For across location analyses  $N = 18$ .

Location	Data source	Statistic	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
Across all	Event 5307	mean	2.29	3.92	13.8	3.18	5.50	3.10	2.99	4.81	0.570
		range	1.97–2.51	3.19–4.77	10.8–17.1	1.57–4.18	4.34–6.68	2.76–3.36	2.57–3.44	3.72–5.56	0.381–0.704
	Nontransgenic	mean	2.36	3.91	13.8	3.26	5.52	3.09	3.01	4.82	0.557
		range	2.08–2.56	3.23–4.71	11.5–17.3	1.67–3.98	4.73–6.70	2.74–3.38	2.57–3.43	4.20–5.32	0.380–0.700
ANOVA ( <i>F</i> test)											
	Genotype effect	<i>P</i>	0.102	0.947	0.789	0.711	0.883	0.902	0.684	0.892	0.722
		SEM	0.049	0.163	0.66	0.153	0.239	0.059	0.088	0.144	0.0298
	ILSI (2008)	mean	2.09	3.68	13.41	3.36	5.25	3.15	2.96	4.33	0.627
		range	1.24–4.68	1.79–6.92	6.42–24.92	1.03–6.42	2.44–9.30	1.72–6.68	1.37–4.34	1.19–6.39	0.271–2.150
		<i>N</i>	1350	1350	1350	1350	1350	1350	1350	1350	1350

**Table 8 (Continued). Amino acid composition of grain from Event 5307 maize and nontransgenic maize**

 Amino acid levels shown in mg/g DW. Results significant at  $P < 0.05$  are shown in bold italic type. For individual location means  $N = 3$ .

Location	Data source	Statistic	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
L1	Event 5307	mean	2.32	4.03	14.3	2.88	5.63	3.08	3.01	4.43	0.497
	Nontransgenic	mean	2.32	3.74	13.3	3.47	5.26	3.06	2.92	4.61	0.601
		<i>P</i>	0.915	0.150	0.231	0.334	0.283	0.319	0.175	0.429	0.552
		SEM	0.039	0.092	0.39	0.330	0.177	0.014	0.031	0.132	0.1039
L2	Event 5307	mean	2.02	3.26	11.1	2.57	4.49	2.81	2.62	4.19	0.494
	Nontransgenic	mean	2.23	3.49	12.2	3.02	4.86	2.95	2.80	4.44	0.539
		<i>P</i>	0.144	0.066	<b>0.007</b>	0.228	0.079	0.072	<b>0.009</b>	0.161	0.656
		SEM	0.061	0.043	0.06	0.187	0.077	0.029	0.012	0.083	0.0605
L4	Event 5307	mean	2.28	3.95	13.8	3.16	5.50	3.19	3.03	5.02	0.680
	Nontransgenic	mean	2.36	4.01	14.1	3.52	5.63	3.22	3.11	5.29	0.588
		<i>P</i>	0.279	0.535	0.589	0.622	0.515	0.762	0.402	0.343	0.169
		SEM	0.037	0.057	0.30	0.441	0.114	0.068	0.058	0.157	0.0307
L6	Event 5307	mean	2.42	4.56	16.4	3.54	6.46	3.31	3.34	5.23	0.624
	Nontransgenic	mean	2.52	4.46	16.3	3.06	6.31	3.21	3.28	4.92	0.658
		<i>P</i>	0.115	0.793	0.931	0.524	0.724	0.578	0.721	0.523	0.427
		SEM	0.027	0.229	0.72	0.446	0.255	0.100	0.109	0.279	0.0238
L7	Event 5307	mean	2.37	3.74	13.0	3.43	5.27	3.08	2.91	5.02	0.554
	Nontransgenic	mean	2.39	3.58	12.7	3.39	5.18	2.97	2.82	4.84	0.502
		<i>P</i>	0.823	0.538	0.761	0.895	0.781	0.333	0.589	0.555	0.403
		SEM	0.037	0.154	0.61	0.189	0.193	0.063	0.100	0.178	0.0350
L8	Event 5307	mean	2.32	3.98	14.0	3.48	5.68	3.12	3.04	4.95	0.573
	Nontransgenic	mean	2.32	4.21	14.5	3.08	5.88	3.15	3.13	4.80	0.454
		<i>P</i>	0.919	0.477	0.644	0.665	0.592	0.607	0.509	0.703	0.134
		SEM	0.021	0.187	0.61	0.562	0.231	0.031	0.086	0.236	0.0342

**Table 9. Fatty acid composition<sup>a</sup> of grain from Event 5307 maize and nontransgenic maize**

 Fatty acids shown as % of total fatty acids. Results significant at  $P < 0.05$  are shown in bold italic type. For across location analyses  $N = 18$ .

Location	Data source	Statistic	16:0 Palmitic	16:1 Palmitoleic <sup>b</sup>	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenoic	22:0 Behenic
Across all	Event 5307	mean	15.7	–	1.74	24.5	55.6	1.60	0.392	0.250	0.220
		range	15.1–16.1	<LOQ–0.137	1.50–2.04	22.0–27.0	53.2–58.1	1.48–1.71	0.353–0.453	0.238–0.265	0.186–0.252
	Nontransgenic	mean	15.2	–	1.81	24.9	55.7	1.50	0.387	0.242	0.213
		range	14.6–15.9	<LOQ–0.450	1.54–2.17	22.6–26.4	53.8–58.4	1.40–1.57	0.361–0.437	0.232–0.261	0.194–0.247
ANOVA ( <i>F</i> test)											
	Genotype effect	<i>P</i>	<b>&lt;0.001</b>	–	<b>0.038</b>	0.108	0.599	<b>&lt;0.001</b>	0.186	<b>&lt;0.001</b>	0.243
		SEM	0.07	–	0.059	0.54	0.60	0.017	0.0098	0.0029	0.0056
<hr/>											
	ILSI (2008)	mean	11.50	0.154	1.82	25.8	57.6	1.20	0.412	0.297	0.176
		range	7.94– 20.71	<LOQ– 0.447	1.02– 3.40	17.4– 40.2	36.2– 66.5	0.57– 2.25	0.279– 0.965	0.170– 1.917	<LOQ– 0.349
		<i>N</i>	1344	596	1344	1344	1344	1344	988	987	924

– = not applicable

<sup>a</sup> Where some or all values were <LOQ, % of total fatty acids could not be calculated and statistical analysis could not be performed. Levels <LOQ were observed for all replicates at all locations for 8:0 caprylic, 10:0 capric, 12:0 lauric, 14:0 myristic, 14:1 myristoleic, 15:0 pentadecanoic, 15:1 pentadecenoic, 17:0 heptadecanoic, 17:1 heptadecenoic, 20:2 eicosadienoic, 20:3 eicosatrienoic, and 20:4 arachidonic fatty acids

<sup>b</sup> Some values were <LOQ so only the range is shown

**Table 9 (Continued). Fatty acid composition of grain from Event 5307 maize and nontransgenic maize**

 Fatty acids shown as % of total fatty acids. Results significant at  $P < 0.05$  are shown in bold italic type. For individual location means  $N = 3$ .

Location	Data source	Statistic	16:0 Palmitic	16:1 Palmitoleic <sup>a</sup>	18:0 Stearic	18:1 Oleic	18:2 Linoleic	18:3 Linolenic	20:0 Arachidic	20:1 Eicosenoic	22:0 Behenic
L1	Event 5307	mean	15.5	<LOQ	1.53	22.4	58.1	1.63	0.374	0.247	0.210
	Nontransgenic	mean	15.1	<LOQ	1.58	22.7	58.2	1.51	0.371	0.237	0.204
		<i>P</i>	0.225	–	<b>0.013</b>	0.286	0.199	0.122	0.641	0.061	0.730
		SEM	0.16	–	0.004	0.15	0.06	0.033	0.0043	0.0019	0.0107
L2	Event 5307	mean	15.8	<LOQ	1.77	23.5	56.4	1.67	0.397	0.242	0.216
	Nontransgenic	mean	15.5	<LOQ–0.450	1.93	24.3	55.8	1.54	0.388	0.238	0.202
		<i>P</i>	0.149	–	0.202	0.120	0.406	<b>0.017</b>	0.467	0.476	0.100
		SEM	0.10	–	0.060	0.22	0.43	0.012	0.0077	0.0038	0.0035
L4	Event 5307	mean	15.7	0.134	1.76	25.3	54.8	1.57	0.391	0.245	0.200
	Nontransgenic	mean	15.2	0.132	1.86	25.5	55.0	1.47	0.391	0.240	0.212
		<i>P</i>	<b>0.013</b>	0.372	<b>0.023</b>	0.319	0.478	<b>0.019</b>	0.960	<b>0.047</b>	0.373
		SEM	0.04	0.0017	0.011	0.14	0.16	0.010	0.0042	0.0009	0.0075
L6	Event 5307	mean	15.8	<LOQ–0.132	1.68	24.5	55.5	1.60	0.368	0.246	0.228
	Nontransgenic	mean	15.1	<LOQ–0.134	1.72	24.7	56.1	1.54	0.370	0.239	0.206
		<i>P</i>	0.069	–	0.270	0.560	0.210	0.203	0.606	0.242	0.072
		SEM	0.14	–	0.019	0.17	0.25	0.023	0.0027	0.0029	0.0045
L7	Event 5307	mean	15.5	<LOQ	1.98	26.4	53.6	1.55	0.441	0.263	0.242
	Nontransgenic	mean	15.4	<LOQ–0.143	1.96	26.1	54.1	1.47	0.427	0.253	0.235
		<i>P</i>	<b>0.038</b>	–	0.701	0.493	0.291	0.086	0.336	0.132	0.678
		SEM	0.02	–	0.032	0.26	0.23	0.017	0.0077	0.0030	0.0098
L8	Event 5307	mean	15.7	<LOQ–0.137	1.71	24.9	55.1	1.59	0.380	0.254	0.221
	Nontransgenic	mean	15.0	<LOQ–0.124	1.80	25.9	54.9	1.45	0.378	0.247	0.220
		<i>P</i>	0.053	–	0.082	0.211	0.678	0.093	0.878	0.294	0.946
		SEM	0.13	–	0.020	0.39	0.29	0.032	0.0054	0.0037	0.0061

– = not applicable

<sup>a</sup>Where some or all values were <LOQ, % of total fatty acids could not be calculated, statistical analysis could not be performed, and only the range is shown

**Table 10. Secondary metabolite and antinutrient composition of grain from Event 5307 maize and nontransgenic maize**

 Analyte units as in column headings. Results significant at  $P < 0.05$  are shown in bold italic type. For across location analyses  $N = 18$ .

Location	Data source	Statistic	Ferulic acid (mg/kg DW)	<i>p</i> -Coumaric acid (mg/kg DW)	Inositol (ppm DW)	Phytic acid (% DW)	Trypsin inhibitor (TIU/mg DW)	Furfural <sup>a,b</sup> (mg/kg DW)	Raffinose (% DW)
Across all	Event 5307	mean	1906	186	2510	0.910	3.34	–	0.156
		range	1670–2190	153–229	2120–3160	0.671–1.03	2.39–4.42	< LOQ	0.115–0.199
	Nontransgenic	mean	1889	186	2504	0.942	3.46	–	0.163
		range	1620–2090	148–226	1980–3060	0.729–1.06	2.22–3.94	< LOQ	0.119–0.188
ANOVA ( <i>F</i> test)		<i>P</i>	0.691	0.926	0.951	0.216	0.393	–	0.066
Genotype effect									
		SEM	52.4	9.1	86.1	0.0261	0.118	–	0.0087
ILSI (2008)		mean	2201.1	218.4	1331.5	0.745	2.73	3.697	0.132
		range	291.9–3885.8	53.4–576.2	89.0–3765.4	0.111–1.570	<LOQ–7.18	<LOQ–6.340	<LOQ–0.320
		<i>N</i> <sup>c</sup>	817	817	504	1196	696	14	701

– = not applicable

<sup>a</sup>The LOQ for furfural was 0.55–0.57 mg/kg DW

<sup>b</sup>All values were <LOQ and therefore statistical comparison was not possible

<sup>c</sup>*N* is the number of ILSI values used to calculate the mean and excludes values <LOQ

**Table 10 (Continued). Secondary metabolite and antinutrient composition of grain from Event 5307 maize and nontransgenic maize**

 Analyte units as in column headings. Results significant at  $P < 0.05$  are shown in bold italic type. For individual location means  $N = 3$ .

Location	Data source	Statistic	Ferulic acid (mg/kg DW)	p-Coumaric acid (mg/kg DW)	Inositol (ppm DW)	Phytic acid (% DW)	Trypsin inhibitor (TIU/mg DW)	Furfural <sup>a</sup> (mg/kg DW)	Raffinose (% DW)
L1	Event 5307	mean	1770	169	2323	0.903	3.31	<LOQ	0.179
	Nontransgenic	mean	1883	187	2160	0.878	3.58	<LOQ	0.174
		<i>P</i>	0.579	0.494	0.062	0.577	0.124	–	0.707
		SEM	122.2	15.9	30.1	0.0268	0.073	–	0.0082
L2	Event 5307	mean	1747	197	2517	0.840	2.90	<LOQ	0.166
	Nontransgenic	mean	1827	207	2230	0.848	3.48	<LOQ	0.178
		<i>P</i>	0.463	0.375	0.323	0.938	0.111	–	0.607
		SEM	62.8	6.5	155.8	0.0592	0.149	–	0.0133
L4	Event 5307	mean	2137	226	2790	1.000	3.58	<LOQ	0.164
	Nontransgenic	mean	1977	209	2760	1.006	3.73	<LOQ	0.175
		<i>P</i>	0.186	0.193	0.896	0.818	0.702	–	0.093
		SEM	57.2	6.3	143.5	0.0171	0.241	–	0.0026
L6	Event 5307	mean	2017	177	2527	0.842	3.44	<LOQ	0.167
	Nontransgenic	mean	1990	185	2523	0.965	3.64	<LOQ	0.173
		<i>P</i>	0.829	0.702	0.959	0.156	0.412	–	0.234
		SEM	76.6	12.8	40.9	0.0390	0.140	–	0.0027
L7	Event 5307	mean	1800	159	2470	0.912	3.40	<LOQ	0.139
	Nontransgenic	mean	1753	154	2627	0.984	3.28	<LOQ	0.156
		<i>P</i>	0.340	0.225	0.691	0.053	0.861	–	0.091
		SEM	26.6	2.0	241.1	0.0123	0.403	–	0.0039
L8	Event 5307	mean	1967	186	2433	0.964	3.41	<LOQ	0.122
	Nontransgenic	mean	1903	175	2727	0.971	3.02	<LOQ	0.123
		<i>P</i>	0.604	0.601	0.081	0.864	0.311	–	0.893
		SEM	73.5	13.0	62.8	0.0230	0.205	–	0.0031

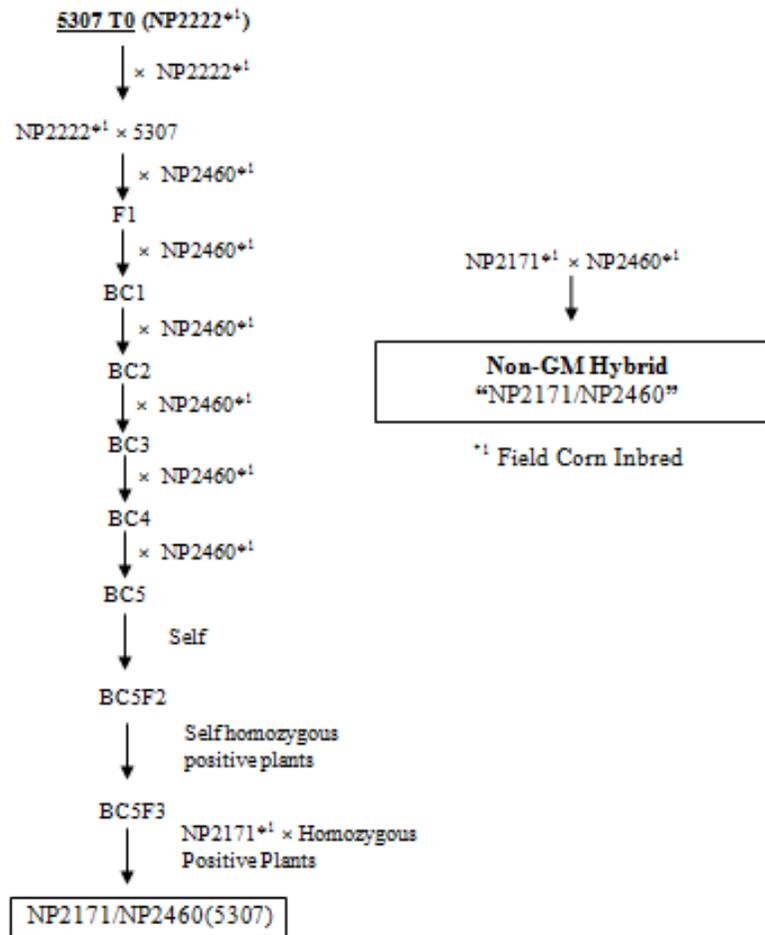
– = not applicable

<sup>a</sup> All values were <LOQ and therefore statistical comparison was not possible

## **APPENDIX A**

### **Pedigree Chart of Event 5307 Maize and Field Plot Diagrams Used in 5307-08-101 Compositional Study**

Figure A1. Pedigree chart of Event 5307 maize used in 5307-08-101 composition study



**Figure A2. Event 5307-08-101 Compositional analysis field plot–location 01 Stanton, MN**

Trial 08SUMGSFR1A4356 – 5307-08-101

		Row Number											
		31	32	33	34	35	36	37	38	39	40	41	42
Range Number	22	1001 Entry 2 Replicate 1	1001 Entry 2 Replicate 1	1001 Entry 2 Replicate 1	1001 Entry 2 Replicate 1					1003 Entry 1 Replicate 1	1003 Entry 1 Replicate 1	1003 Entry 1 Replicate 1	1003 Entry 1 Replicate 1
	23	2003 Entry 2 Replicate 2	2003 Entry 2 Replicate 2	2003 Entry 2 Replicate 2	2003 Entry 2 Replicate 2					2001 Entry 1 Replicate 2	2001 Entry 1 Replicate 2	2001 Entry 1 Replicate 2	2001 Entry 1 Replicate 2
	24	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3					3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3

**Figure A3. Event 5307 Compositional analysis field plot–location 2 Janesville, WI**

Trial 08SUMGSFR1A5629 – 5307-08-101

		Row Number											
		69	70	71	72	73	74	75	76	77	78	79	80
2		1001	1001	1001	1001	1002	1002	1002	1002				
		Entry 2	Entry 2	Entry 2	Entry 2	Entry 1	Entry 1	Entry 1	Entry 1				
		Replicate 1											
		Row Number											
		81	82	83	84	85	86	87	88	89	90	91	92
2						2002	2002	2002	2002	2003	2003	2003	2003
						Entry 1	Entry 1	Entry 1	Entry 1	Entry 2	Entry 2	Entry 2	Entry 2
						Replicate 2							
		Row Number											
		81	82	83	84	85	86	87	88	89	90	91	92
3						3002	3002	3002	3002	3001	3001	3001	3001
						Entry 2	Entry 2	Entry 2	Entry 2	Entry 1	Entry 1	Entry 1	Entry 1
						Replicate 3							

**Figure A4. Event 5307 Compositional analysis field plot–location 4 New Haven, IN**

Trial 08SUMGSFR1A6742– 5307-08-101

		Row Number											
		5	6	7	8	9	10	11	12	13	14	15	16
Range Number	41					1002 Entry 1 Replicate 1	1002 Entry 1 Replicate 1	1002 Entry 1 Replicate 1	1002 Entry 1 Replicate 1	1003 Entry 2 Replicate 1	1003 Entry 2 Replicate 1	1003 Entry 2 Replicate 1	1003 Entry 2 Replicate 1
	42					2002 Entry 1 Replicate 2	2002 Entry 1 Replicate 2	2002 Entry 1 Replicate 2	2002 Entry 1 Replicate 2	2001 Entry 2 Replicate 2	2001 Entry 2 Replicate 2	2001 Entry 2 Replicate 2	2001 Entry 2 Replicate 2
	43	3001 Entry 1 Replicate 3	3001 Entry 1 Replicate 3	3001 Entry 1 Replicate 3	3001 Entry 1 Replicate 3					3003 Entry 2 Replicate 3	3003 Entry 2 Replicate 3	3003 Entry 2 Replicate 3	3003 Entry 2 Replicate 3

**Figure A5. Event 5307 Compositional analysis field plot–location 6 Shirley, IL**

Trial 08SUMGSFR1A7630 – 5307-08-101

		Row Number											
		1	2	3	4	5	6	7	8	9	10	11	12
Range Number	27	1001 Entry 1 Replicate 1	1001 Entry 1 Replicate 1	1001 Entry 1 Replicate 1	1001 Entry 1 Replicate 1					1003 Entry 2 Replicate 1	1003 Entry 2 Replicate 1	1003 Entry 2 Replicate 1	1003 Entry 2 Replicate 1
	28	2003 Entry 1 Replicate 2	2003 Entry 1 Replicate 2	2003 Entry 1 Replicate 2	2003 Entry 1 Replicate 2	2002 Entry 2 Replicate 2	2002 Entry 2 Replicate 2	2002 Entry 2 Replicate 2	2002 Entry 2 Replicate 2				
	29	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3					3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3

**Figure A6. Event 5307 Compositional analysis field plot–location 7 Marshall, MO**

Trial 08SUMGSFR1A8409 – 5307-08-101

		Row Number							
		79	80	81	82	83	84	85	86
5		2002	2002	2002	2002	2001	2001	2001	2001
		Entry 2	Entry 2	Entry 2	Entry 2	Entry 1	Entry 1	Entry 1	Entry 1
		Replicate 2							
		Row Number							
		91	92	93	94	95	96	97	98
5		1002	1002	1002	1002	1001	1001	1001	1001
		Entry 1	Entry 1	Entry 1	Entry 1	Entry 2	Entry 2	Entry 2	Entry 2
		Replicate 1							
		Row Number							
		79	80	81	82	83	84	85	86
6						3001	3001	3001	3001
						Entry 1	Entry 1	Entry 1	Entry 1
						Replicate 3	Replicate 3	Replicate 3	Replicate 3
		Row Number							
		91	92	93	94	95	96	97	98
6		3003	3003	3003	3003				
		Entry 2	Entry 2	Entry 2	Entry 2				
		Replicate 3	Replicate 3	Replicate 3	Replicate 3				

**Figure A7. Event 5307 Compositional analysis field plot–location 8 Bloomington, IL**

Trial 08SUMGSFR1A761N – 5307-08-101

		Row Number							
		4	5	6	7	8	9	10	11
Range Number	10	1001 Entry 2 Replicate 1	1001 Entry 2 Replicate 1	1001 Entry 2 Replicate 1	1001 Entry 2 Replicate 1	1002 Entry 1 Replicate 1	1002 Entry 1 Replicate 1	1002 Entry 1 Replicate 1	1002 Entry 1 Replicate 1
	11								
	12	2002 Entry 1 Replicate 2	2002 Entry 1 Replicate 2	2002 Entry 1 Replicate 2	2002 Entry 1 Replicate 2	2003 Entry 2 Replicate 2	2003 Entry 2 Replicate 2	2003 Entry 2 Replicate 2	2003 Entry 2 Replicate 2
	13					3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3	3001 Entry 2 Replicate 3
14	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3	3003 Entry 1 Replicate 3					