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Request for Extension of Determination of Nonregulated Status to the Additional Regulated Article:

Maize Line HCEM485

The undersigned submits this request under 7 CFR Part 340.6(e) to request an extension of determination of nonregulated status that the article should not be regulated under 7 CFR Part 340.

Submitted by:

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**Revised Document Submitted:
14 February 2011**

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Stine Seed Farm Petition Number 09-063-01p_a3 - Revised

Summary

Glyphosate herbicide-tolerant maize line HCEM485 was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene. DNA introduction was via aerosol beam injector, which is a naked DNA delivery method.

The 6.0 kb fragment contained the endogenous maize *epsps* expression cassette including native promoter, coding sequence, intron, and termination regions. The maize EPSPS coding sequence was specifically modified by site-directed mutagenesis to introduce two single-nucleotide substitutions. These two point mutations resulted in a codon change from threonine→isoleucine at position 102 (relative to the amino acid sequence of the native maize EPSPS enzyme) and a proline→serine change at position 106. These two amino acid substitutions result in a glyphosate-tolerant form of the enzyme and are also present in the modified EPSPS enzyme produced in the antecedent organism, transgenic maize event GA21. Except for the amino acid substitutions at positions 102 and 106, the amino acid sequence of the double-mutated EPSPS (2mEPSPS) enzyme is identical to the native maize EPSPS sequence.

The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-tolerant form of the native EPSPS enzyme. Maize line HCEM485 does not contain any heterologous DNA sequences, either coding or non-coding, from any other species, including those that could be considered a plant pest. In addition, the genetic modification process resulting in maize line HCEM485 did not employ any organism (*e.g.*, *Agrobacterium tumefaciens*) that could be considered a plant pest.

The introduced sequences in maize line HCEM485 are contained within a single genetic locus within the maize genome as demonstrated by Southern blot analysis and Mendelian inheritance studies. The modified maize EPSPS protein expressed in maize line HCEM485 is intact, of the expected molecular weight and there was no evidence of truncated forms of the enzyme. The modified maize EPSPS expressed in HCEM485 maize is also immunochemically cross-reactive with the modified maize EPSPS expressed in the antecedent organism, GA21, and the enzymes from both sources express the same mutations responsible for conferring glyphosate herbicide tolerance.

Agronomic and phenotypic characteristics of an HCEM485 maize hybrid and three control hybrids were evaluated in a series of field trials across 15 United States Corn Belt locations in 2007. The agronomic characteristics chosen for comparison were those typically observed by professional maize breeders and agronomists and represented a broad range of characteristics throughout the development of the maize plant. Results of these trials suggest that there were no biologically significant unintended effects on plant growth habit and general morphology, vegetative vigor, flowering and pollination, grain yield, grain test weight, or disease susceptibility as a result of the genetic modification introduced into maize line HCEM485. These data support the conclusion that HCEM485-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids, and would not display higher rates of outcrossing than unmodified maize.

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Levels of key nutrients, minerals, antinutrients, and secondary metabolites were determined in samples of maize grain and forage derived from HCEM485 and control hybrids collected from up to four field trial locations in 2007. For most analyses, there were no statistically significant differences and in cases where statistically significant differences were observed, the magnitudes of the differences were small and in every case, mean values determined for both HCEM485 and control samples were within the ranges of natural variation as reported in the literature. Overall, no consistent patterns emerged to suggest that biologically significant changes in composition of the grain or forage had occurred as an unintended consequence of the genetic modification resulting in maize line HCEM485. The conclusion based on these data was that grain and forage from HCEM485 maize were substantially equivalent in composition to both the control hybrids included in this study, and to other commercial maize hybrids.

In conclusion, there is no expectation that cultivation of maize line HCEM485 would have any environmental effects different from the cultivation of the antecedent organism, GA21, or other maize lines exhibiting glyphosate tolerance that have also been deregulated by USDA-APHIS (*e.g.*, NK603; MON 88017; and MON 802). Therefore, on the basis of the substantial phenotypic equivalence between maize line HCEM485 and the antecedent organism, GA21, Stine Seed Farm requests that an extension of nonregulated status be granted to maize line HCEM485, any progeny derived from crosses between HCEM485 and conventional maize, and any progeny derived from crosses of HCEM485 with other deregulated maize lines.

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Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner which are unfavorable to the petition.

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Abbreviations Used in This Petition

2mEPSPS	double-mutated EPSP synthase; native maize EPSPS containing Thr-102→Ile and Pro-106→Ser substitutions.
AI	active ingredient
APHIS	Animal and Plant Health Inspection Service
BAC	bacterial artificial chromosome
bp	base pairs
CBI	confidential business information
CTP	chloroplast transit peptide
DW	dry weight
ELISA	enzyme-linked immunosorbent assay
EPSP	5-enolpyruvylshikimate-3-phosphate
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase
FDA	Food and Drug Administration
FW	fresh weight
g	gram
GRAS	generally recognized as safe
HRP	horseradish peroxidase
ILSI	International Life Sciences Institute
kb	kilobases
kg	kilogram
LOQ	limit of quantification
µg	microgram
mg	milligram
µm	micrometer
MW	molecular weight
ND	not determined
NOS	nopaline synthase
OECD	Organization for Economic Cooperation and Development
OTP	optimized transit peptide
PEP	phosphoenolpyruvate
S3P	shikimate-3-phosphate
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
TIPS	threonine to isoleucine mutation at position 102 and proline to serine mutation at position 106, relative to the amino acid sequence of the native maize EPSP synthase enzyme.
USDA	United States Department of Agriculture

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Annexes

Note: The following annexes are included as attachments.

- | | |
|---------|---|
| Annex 1 | Agronomic analysis of maize line HCEM485.
Laboratory Study ID: SSF-07-323. |
| Annex 2 | Morphology and viability of pollen collected from HCEM485 maize.
Laboratory Study ID: SSF-07-288. |
| Annex 3 | Compositional analysis of grain and forage derived from HCEM485
hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098. |

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I. RATIONALE FOR SUBMISSION OF REQUEST FOR EXTENSION OF NONREGULATED STATUS

I.1 BASIS FOR THE REQUEST

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has been given the responsibility, under the Federal Plant Pest Act (7 U.S.C. 150aa–150jj) and the Plant Quarantine Act (7 U.S.C. 151–167), to prevent the introduction and dissemination into the United States or interstate movement of plant pests. Under this authority, APHIS has published regulations found at 7 CFR Part 340 pertaining to the introduction (importation, interstate movement, and release into the environment) of genetically engineered organisms and products derived from known plant pests (regulated articles). An organism is not subject to the regulations when the organism is demonstrated not to present a plant pest risk.

Section 340.6(e) of the regulations provides that APHIS may extend a determination of non-regulated status to additional articles, upon finding that the additional articles do not pose a potential for plant pest risk, and should therefore not be regulated. Such a finding would be made based on an evaluation of the similarity of the additional articles to an antecedent organism, *i.e.*, an organism that has already been the subject of a determination of nonregulated status by APHIS under Section 340.6, and that is used as a reference for comparison to the subject article under consideration.

In its guidance, APHIS has provided the following example of a molecular manipulation that is unlikely to pose new risk issues beyond those that would have been considered in the initial determination of nonregulated status:

- *Modifications in which the amino acid sequence of any encoded proteins is unchanged with respect to the corresponding sequence in the antecedent organism (i.e., synonymous codon changes).*

When applying this guidance it is clear that a request for an extension of determination of nonregulated status for maize line HCEM485 as based upon the previous determination of nonregulated status for Roundup Ready® maize line GA21 (petition 97-099-01p) is appropriate. The glyphosate tolerance of maize line GA21 was imparted by the insertion of a double-mutated form of the maize (*Zea mays* L.) EPSPS encoding gene into the maize genome. In the same manner, the glyphosate tolerance in maize line HCEM485 is also based on expression of the same modified EPSPS enzyme derived from *Z. mays*, with the notable difference that expression of the modified EPSPS enzyme is regulated by endogenous DNA sequences also derived from *Z. mays* rather than regulatory sequences derived from other species.

The specific differences between HCEM485 and its progeny, and the event GA21 in the previous petition are discussed in the appropriate sections and also summarized in Table 1.

As a further basis for this request for a determination of nonregulated status, the petitioner notes that glyphosate tolerant maize line HCEM485 contains only DNA sequences derived from the recipient organism, *Z. mays*, which is not considered a plant pest; it does not contain DNA sequences derived from any organism that could be considered to pose a plant pest risk

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nor was it produced using any organism that could be considered to pose a plant pest risk. On this basis, it can be concluded that maize line HCEM485 is unlikely to pose a plant pest risk and may, therefore, be afforded nonregulated status.

Table 1: Comparison of maize line HCEM485 with event GA21

Characteristic	HCEM485	Event GA21
Crop	maize	maize
Genus and species name	<i>Zea mays</i>	<i>Zea mays</i>
Parent line	Stine 963	Unspecified
Transformation method	Aerosol beam direct DNA transfer	Microparticle acceleration direct DNA transfer
Trait	Tolerance to glyphosate herbicide	Tolerance to glyphosate herbicide
Gene product	double-mutated EPSPS (2mEPSPS)	double-mutated EPSPS
Vector	pHCEM	pDPG434
Transforming DNA	<i>Cla</i> I + <i>Eco</i> RV restriction fragment (<i>ca.</i> 6.0 kb)	<i>Not</i> I restriction fragment (<i>ca.</i> 3.4 kb)
Gene and source	Modified EPSPS-encoding gene from <i>Z. mays</i> including native introns and exons	Modified EPSPS-encoding gene from <i>Z. mays</i>
Targeting sequences	Native chloroplast transit sequences from <i>Z. mays</i> EPSPS-encoding gene	Optimized chloroplast transit sequences derived from <i>Z. mays</i> and <i>Helianthus annuus</i> (sunflower) ribulose-1,5-bisphosphate carboxylase genes
Promoter and source	5' region of the maize (<i>Z. mays</i>) EPSPS-encoding gene containing native promoter sequences	5' region of the rice (<i>Oryza sativa</i>) actin 1 gene containing the promoter and first intron
Terminator and source	3' nontranslated region of the native maize (<i>Z. mays</i>) EPSPS-encoding gene	3' nontranslated region from the nopaline synthase (<i>nos</i>) gene derived from the Ti plasmid of <i>Agrobacterium tumefaciens</i>

I.2 RATIONALE FOR THE DEVELOPMENT OF MAIZE LINE HCEM485

There are no changes in rationale from Section I.A of the previously approved petition number 97-099-01-p, which briefly discusses the benefits of glyphosate tolerant maize.

Prior to commercialization of maize line HCEM485, Stine Seed Farm will seek the following regulatory approvals in the United States:

1. Extension of the existing determination of nonregulated status granted for maize line GA21 (97-099-01p) to maize line HCEM485 and all progenies from crosses between this line and other maize varieties.
2. Maize line HCEM485 is within the scope of the FDA policy statement concerning products derived from new plant varieties, including those genetically engineered, published in the Federal Register on May 29, 1992.

II. THE MAIZE FAMILY

There are no changes from Section II of the previously approved petition number 97-099-01p.

III. DESCRIPTION OF THE TRANSFORMATION SYSTEM

The antecedent organism, maize line GA21, was produced using a particle acceleration method. For the production of maize line HCEM485, DNA introduction was via aerosol beam injector (Held *et al.*, 2004), which is a naked DNA delivery method.

III.1 DONOR GENES AND REGULATORY SEQUENCES

The antecedent organism, maize line GA21, was generated using a particle acceleration transformation system with a gel-isolated *NotI* DNA restriction fragment of plasmid vector pDPG434 containing the modified EPSPS encoding gene. In comparison, glyphosate-tolerant maize line HCEM485 was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene (Held *et al.*, 2006).

The maize BAC library was screened with a DNA probe complementary to a portion of the maize EPSP synthase gene (GenBank Accession No. X63374) and one of the resultant BAC clones containing a 6.0 kb genomic fragment flanked by unique *ClaI* and *EcoRV* restriction endonuclease sites was chosen for further characterization (Figure 1). Nucleotide sequencing of the 6.0 kb fragment revealed that it contained an *epsps* 5' regulatory sequence (before position 1868), an EPSP synthase coding region (positions 1868–5146) comprised of 8 exons (labelled a–h in Figure 1) and 7 introns, and a 3' untranslated region (after position 5146). The EPSP synthase coding region also contained sequences encoding an endogenous N-terminal chloroplast transit peptide (position 1868–2041) as predicted using the PSORT algorithm (Human Genome Center, Institute for Medical Science, University of Tokyo).

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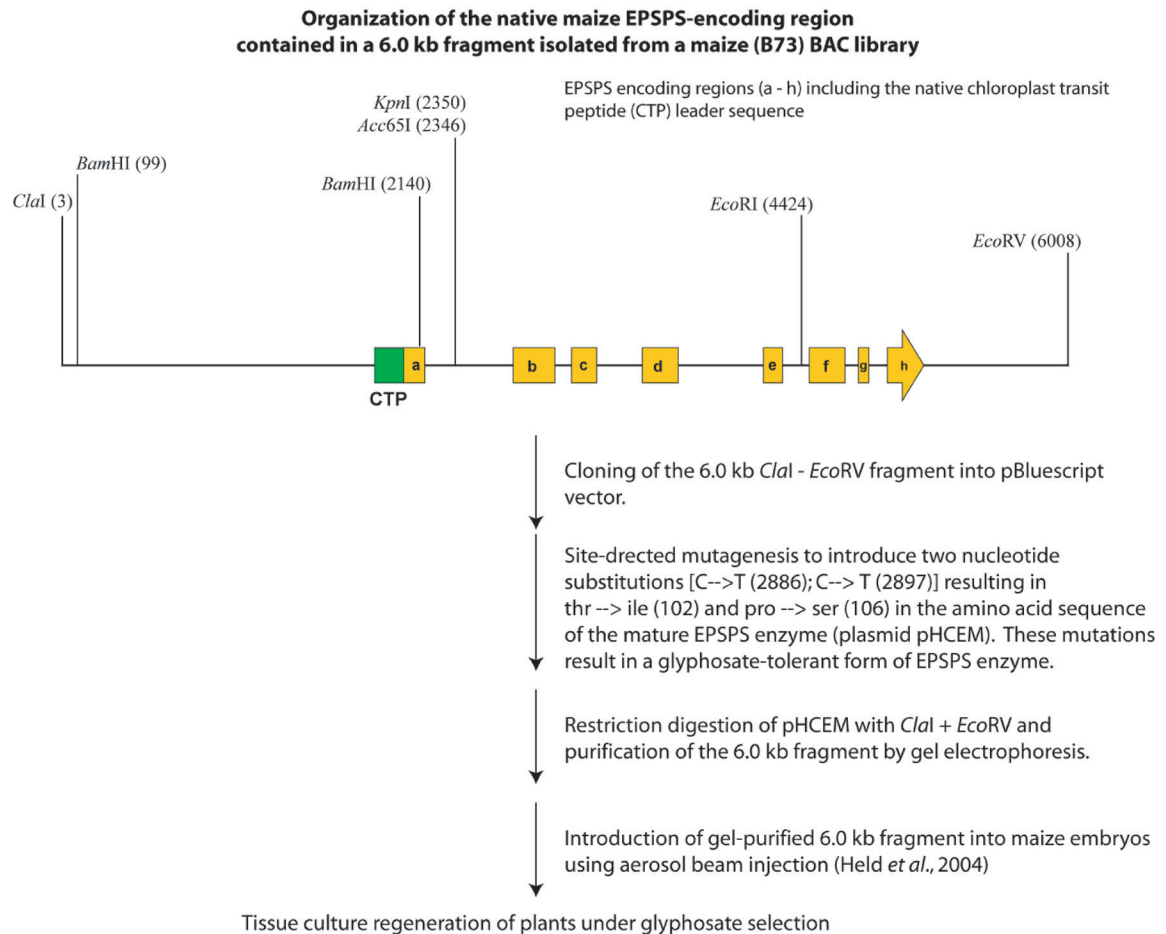


Figure 1: Map of 6.0 kb maize genomic fragment and developmental steps in creating maize line HCEM485.

III.2 THE VECTOR pHCEM

The 6.0 kb maize genomic fragment was cloned into the *Clal* and *EcoRV* sites of pBlueScript vector and subjected to site-directed mutagenesis using the QuikChange Site-Directed Mutagenesis Kit (Stratagene). Two mutations were introduced into the EPSPS coding sequence: a cytosine to thymine substitution at position 2886 and a second cytosine to thymine substitution at position 2897. These two point-mutations resulted in two amino acid changes within the sequence of the mature EPSPS protein, a Thr-102→Ile and Pro-106→Ser substitution. The introduction of the T102I/P106S (TIPS) mutations was based on previous work demonstrating that Class I EPSP synthase variants containing TIPS mutations resulted in functional tolerance to glyphosate-containing herbicides (Spencer *et al.*, 2000; Lebrun *et al.*, 2003). These two mutations are the same mutations as introduced into the modified maize EPSP synthase encoding gene introduced into the antecedent organism, maize line GA21. An amino acid alignment of maize EPSP synthase enzymes illustrating these changes is included in Figure 2.

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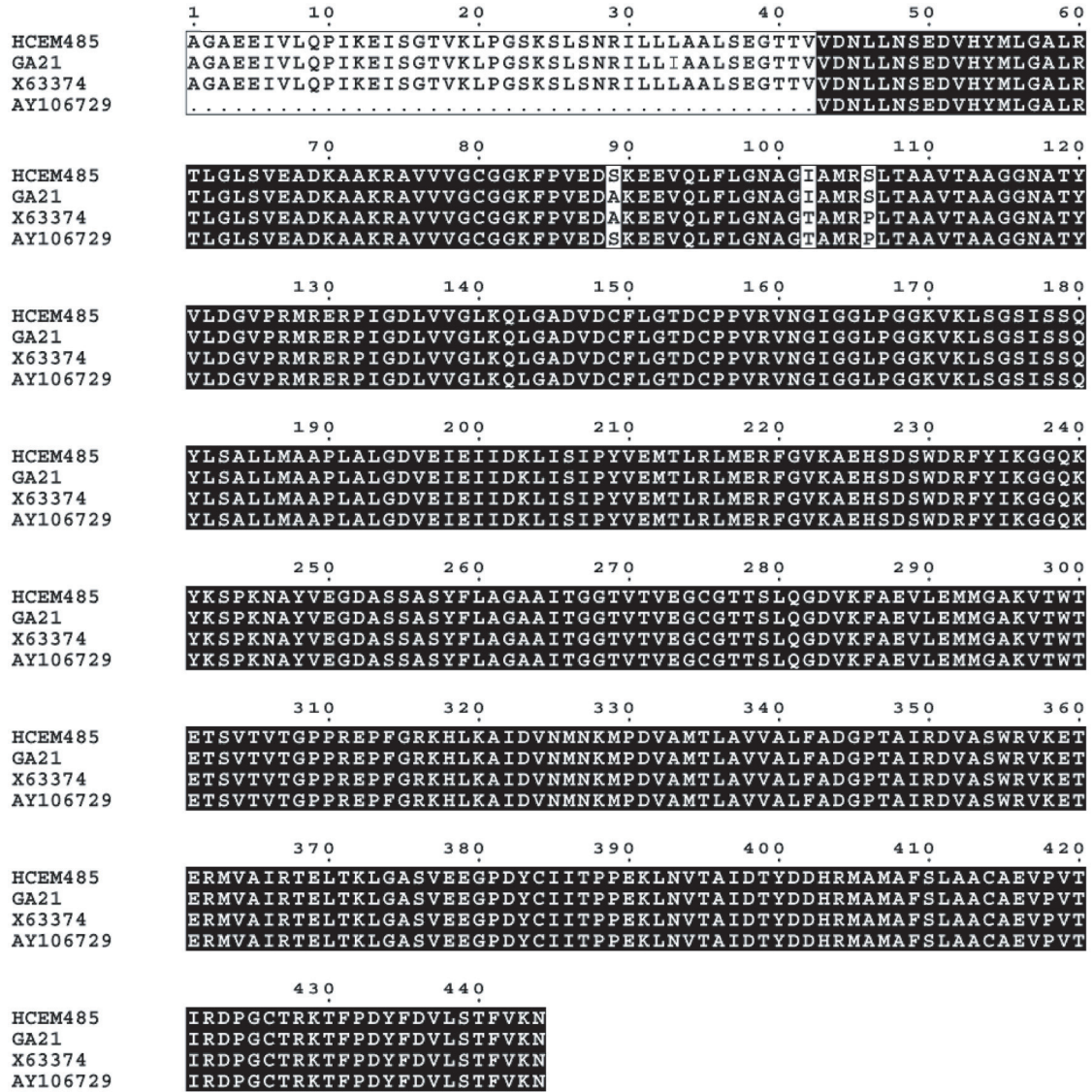


Figure 2: Amino acid sequence alignments of maize EPSP synthase enzymes. HCEM485 is the amino acid sequence of the mutated EPSP synthase expressed in maize line HCEM485 (Held *et al.*, 2006). GA21 is the amino acid sequence of the mutated EPSP synthase expressed in maize event GA21 (Spencer *et al.*, 2000). GenBank Accession No. X63374 corresponds to an EPSP synthase encoding sequence from a maize cell culture (Lebrun *et al.*, 1991). GenBank Accession No. AY106729 was identified from a maize bacterial artificial chromosome (BAC) library as part of a project of expressed sequence tag (EST) assemblies (Gardiner *et al.*, 2004). Positions of the threonine to isoleucine and proline to serine substitutions at positions 102 and 106 (relative to the native enzyme), respectively, are shown. The serine residue at position 89 of the HCEM485 EPSP synthase sequence is identical to the sequence of the native enzyme from GenBank Accession No. AY106729.

The pBlueScript vector containing the 6.0 kb maize genomic fragment with double-mutated EPSPS-encoding gene is designated pHCEM (Figure 3). Nucleotide sequencing of the mutated maize 6.0 kb fragment contained in pHCEM confirmed that no other alterations in sequence had been inadvertently introduced. This sequence is presented in Appendix 1.

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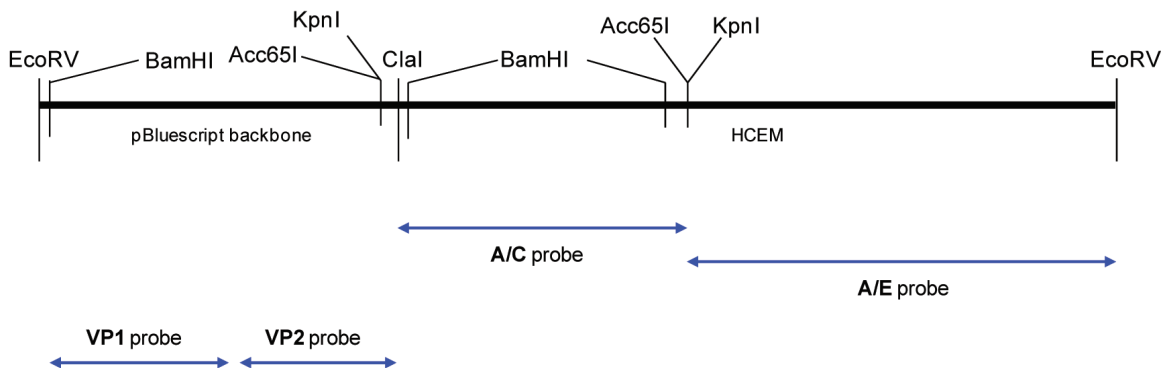


Figure 3: Linear diagram of pHCEM plasmid showing all probes used for Southern hybridization. All restriction enzyme sites corresponding to those enzymes used for Southern hybridizations are indicated.

III.3 PLANT TRANSFORMATION AND REGENERATION

For DNA introduction, pHCEM was digested with *ClaI* and *EcoRV*, subjected to agarose gel electrophoresis (1 percent agarose), and the 6.0 kb band was excised and purified using Qiagen's Qiaquick gel extraction kit. The purified maize DNA fragment was introduced into immature maize embryos derived from the elite inbred line Stine 963 by aerosol beam injection (Held *et al.*, 2004). After 5 days of culture on non-selective medium, embryos were transferred onto medium containing glyphosate (100 mg/l). After two 14-day passages, embryos were transferred onto medium containing successively greater glyphosate concentrations, up to 540 mg/l, and regeneration was carried out as previously described (Held *et al.*, 2004).

The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-resistant form of the native maize EPSP synthase. Except for the introduced TIPS mutations, the amino acid sequence of the double-mutated maize EPSPS (2mEPSPS) enzyme expressed in maize line HCEM485 is identical to the native wild-type maize EPSPS sequence reported by Gardiner *et al.*, 2004 (Figure 2). Maize line HCEM485 does not contain any heterologous DNA sequences, either coding or non-coding, from any other species.

IV. GENETIC ANALYSIS OF MAIZE LINE HCEM485

IV.1 MOLECULAR CHARACTERIZATION

Southern analysis of HCEM485 maize DNA was performed in order to estimate the number of sites of insertion of the introduced DNA. Two probes were used that together spanned the entire 6.0 kb maize DNA fragment introduced into HCEM485. These probes were designated: a) A/C – obtained from a double digest of the pHCEM plasmid with *ClaI* and *Acc65I* (corresponding to positions 1–2346); and b) A/E – obtained from a double digest of the pHCEM plasmid with *Acc65I* and *EcoRV* (corresponding to positions 2347–6010). Probes (*ca.* 50 ng each) were labeled with 50 μ Ci of (α -³²P)-dCTP (3000 Ci/mmol) using a random labeling system (Rediprime™ II, Amersham Piscataway, NJ). Genomic DNA (7 μ g) isolated from

HCEM485 and control Stine 963 maize was digested (37°C, overnight) with different restriction endonuclease enzymes and restriction fragments were separated by agarose gel electrophoresis followed by transfer onto Hybond N+ nylon membrane. A third sample of DNA comprising the control Stine 963 with an amount of the plasmid pHCEM equivalent to a single copy per genome (12.58 pg) was treated in a similar manner to the other samples and included on the gels. Southern hybridizations were performed according to standard procedures using ³²P-labeled probes followed by autoradiography. [

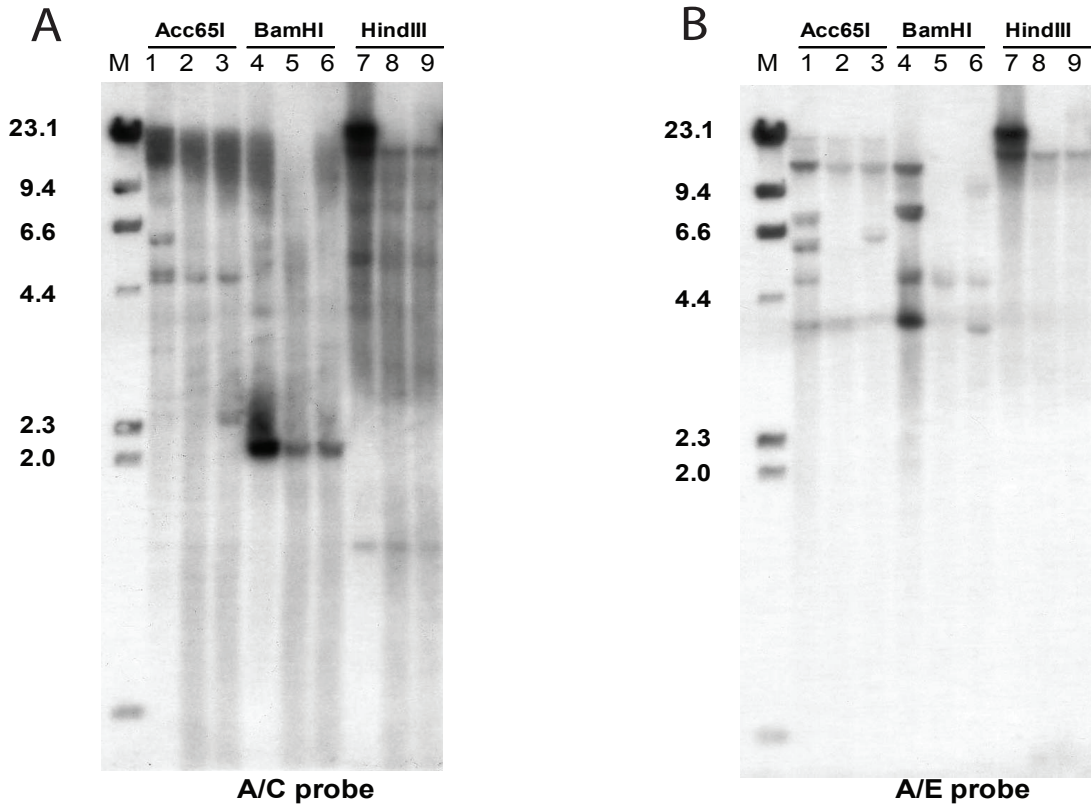


Figure 4: Southern blot hybridisation of HCEM485 with A/E and A/C probes. Samples of genomic DNA (7µg) from HCEM (lanes 1, 4 and 7), negative control Stine 963 maize (lanes 2, 5 and 8) and negative control Stine 963 with 1 copy equivalent of plasmid pHCEM (lanes 3, 6 and 9) were subjected to digestion with the restriction enzymes indicated followed by gel electrophoresis and transfer onto nylon membranes. In panel A, the membrane was hybridized with the A/C probe (Figure 3) and in panel B the membrane was hybridised with the A/E probe. Molecular size markers were included in lanes marked M and the size of these fragments in kb is indicated on the left hand side of each panel.

Southern analysis of HCEM485 genomic DNA using both the A/C and A/E probes following *HindIII* digestion (Figure 4 A and B, lane 7) indicated the presence of a single ≥ 23 kb hybridizing fragment that was unique to HCEM485 (*i.e.*, not present in digests of control Stine 963 maize DNA). This was expected as this enzyme does not cut within the inserted DNA fragment. Digestion with *BamHI* followed by hybridization with the A/C probe (Figure 4A, lane 4) produced a single band of the same size as that obtained in the control DNA. Again, this is as expected given the *BamHI* sites in the inserted DNA (Figure 3), however the greater intensity of this band suggested that multiple copies of the inserted DNA was present. Hybridization with the A/E probe (Figure 4B, lane 4) produced three unique bands and one corresponding to the control DNA. Digestion with *Acc65I* (an isoschizomer of *KpnI* which digests DNA at

the same recognition site) revealed three hybridising bands unique to HCEM485 with both probes (Figure 4 A and B, lane 1). The observed bands are summarised in Table 2 below, together with expected fragment sizes based on the putative organization discussed below. Bands equivalent to those observed in the control DNA lanes are not included in this table. Hybridising fragments from the plasmid DNA includes as a positive control can be seen in the *Acc65I* and *BamHI* digests, however these are not clearly visible in the *HindIII* digested lanes. As *HindIII* does not cut within the plasmid it is likely that there could be multiple bands in this lane corresponding to different forms of the plasmid (supercoiled, relaxed circles and linear) and, together with the low amount (12.58 pg - equivalent to a single copy per haploid genome), can mean that the bands in this lane are not intense enough to be visualised in the background hybridisation seen on these blots.

Table 2: Observed vs. expected hybridising fragments unique to HCEM485 DNA.

	A/C probe		A/E probe	
	Observed	Expected	Observed	Expected
<i>Acc65I</i>	~4.7	4.7	~7.3	7.326
	~6	6.01	~6	6.010
	~8	>2.350	~5	>3.661
<i>BamHI</i>			~7.8	7.738
	~2	2.042	~4	3.969
			~15	>3.871
<i>HindIII</i>	~24	>24	~24	>24

Sizes of fragments observed on the Southern blots shown in Figure 4, given in kb by comparison to the marker lane fragments. Expected sizes are derived from the putative insert organization detailed in Figure 5. Expected fragments listed greater than a certain size are those where only one restriction enzyme site occurs in the inserted DNA, with the other site somewhere in the flanking genomic DNA.

Based on this information, a putative organization of the inserted DNA in HCEM485 has been developed, illustrated in Figure 5. This putative organization suggests 4 complete copies of the fragment are inserted at a single location. The expected fragment sizes included in Table 2 are derived from the putative organization and compared to the observed bands.]

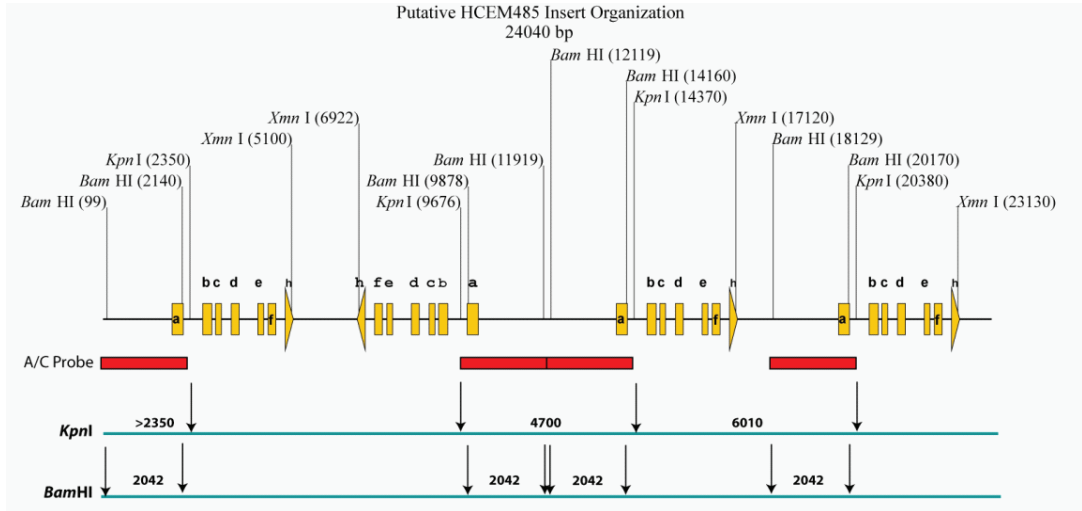
By comparison, the inserted DNA within event GA21 was comprised of two complete copies of the herbicide tolerant gene cassette (Act promoter + intron/OTP/mEPSPS/NOS) and one incomplete fragment within a single DNA *ca.* 18.5 kb segment, which was shown to be stably inherited across multiple generations as a single genetic locus (Monsanto, 1997).

Due to the fact that the inserted DNA in HCEM485 was comprised exclusively of sequences derived from the host organism, maize, and because of the repetitive nature of the DNA insert (*i.e.*, multiple copies of a 6 kb fragment), more elaborate molecular characterization (*e.g.*, nucleotide sequencing) of the insert was not practically feasible. Further evidence supports the single insertion site (segregation analysis) and lack of any truncated coding regions (western blot analysis of expressed proteins).

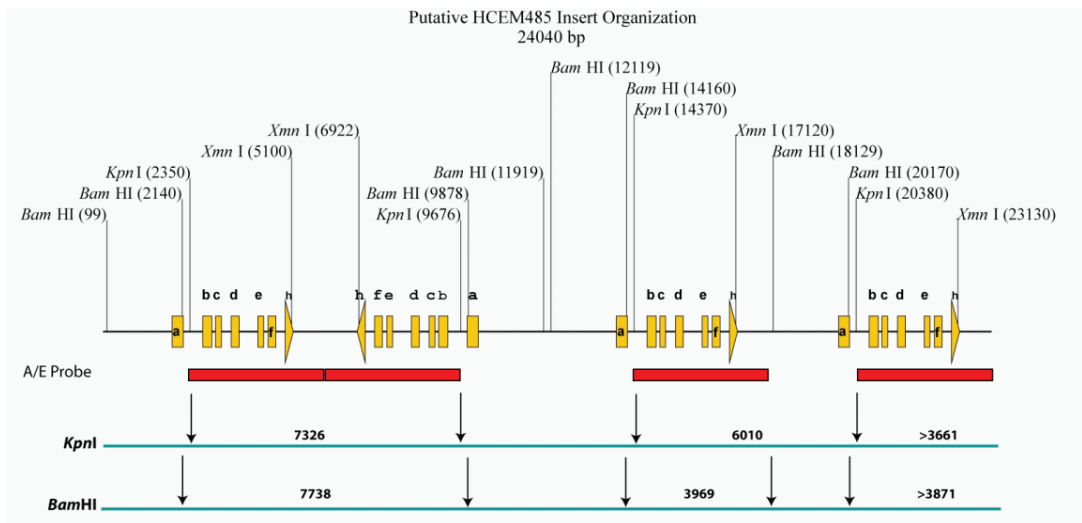
Furthermore, again considering that the introduced DNA was exclusively derived from *Z. mays*, the consequences of any potential genetic rearrangements (*e.g.*, deletions, truncations, rearrangement, or the potential production of chimeric open reading frames) arising from the genetic modification resulting in maize line HCEM485 are not materially different from the

consequences of potential genetic rearrangements arising from natural genetic recombination events or mutations during sexual reproduction in maize.

[



A



B

Figure 5: Putative organization of inserted DNA in HCEM485. Panel A shows the putative organization of the inserted DNA in HCEM485 by comparison with the hybridising bands observed with the enzymes *Kpn*I and *Bam*H and the A/C probe. These fragments can be observed in panel A of Figure 4. Panel B shows a similar comparison based on hybridization with the A/E probe.]

In order to confirm the absence of any plasmid backbone sequences within the HCEM485 genome, samples of genomic DNA (7 µg) isolated from HCEM485, the control Stine 963 maize and control Stine 963 maize DNA with an amount of the plasmid pHCEM equivalent to a single copy per genome (12.58 pg) were digested (37°C, overnight) with different restriction endonuclease enzymes and restriction fragments were separated by agarose gel electrophoresis followed by transfer onto Hybond N+ nylon membrane. Southern hybridization was performed

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using two probes complementary to the plasmid backbone sequences in vector pHCEM (VP1 and VP2 Figure 3). There were no detectable hybridization signals from samples derived from maize line HCEM485 (Figure 6 A and B, lanes 1,4,7 and 10), consistent with the lack of incorporation of any vector backbone derived sequences in the maize genome. Hybridization signals from the plasmid DNA added to the control Stine 963 DNA (Lanes 3, 6, 9 and 12) are clearly seen, demonstrating the sensitivity of the hybridization. As *HindIII* does not cut the plasmid, this band represents a circular form which does not migrate according to its molecular weight.

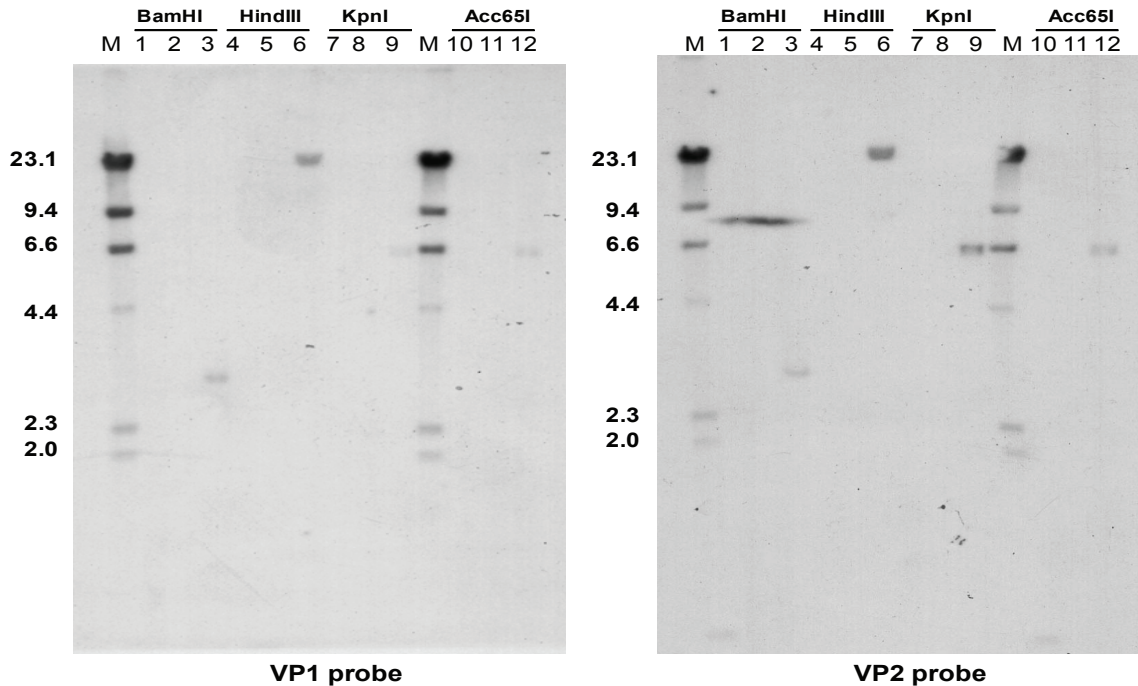


Figure 6: Southern analysis for the presence of vector sequences in HCEM485. Samples of genomic DNA (7µg) from HCEM (lanes 1, 4, 7 and 10), negative control Stine 963 maize (lanes 2, 5, 8 and 11) and negative control Stine 963 with 1 copy equivalent of plasmid pHCEM (lanes 3, 6, 9 and 12) were subjected to digestion with the restriction enzymes indicated followed by gel electrophoresis and transfer onto nylon membranes. In panel A, the membrane was hybridized with the vector probe VP1 (Figure 3) and in panel B the membrane was hybridised with the Vector probe VP2. Molecular size markers were included in lanes marked M and the size of these fragments in kb is indicated on the left hand side of each panel.

IV.2 INHERITANCE AND STABILITY

The inheritance pattern of the glyphosate-tolerance trait has been investigated in F1 hybrid and F2 segregating plant populations derived from maize line HCEM485. The breeding tree for HCEM485 maize is shown below (Figure 7), indicating the derivation of (9289xHCEM485)9032 F1 and (9289xHCEM485)9032 S1F2 plants that were tested for segregation of the herbicide-tolerance trait.

Segregation analysis was conducted on F1 hybrid and F2 segregating plant populations derived from maize line HCEM485 (Figure 7) by screening for glyphosate tolerance. Progeny plants of each generation were grown in the greenhouse and treated with 2.5X the recommended field application rate of glyphosate at approximately the V4 stage of plant development and visually scored for herbicide susceptibility. Numbers of trait positive and trait negative plants from

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each generation are shown in Table 3. The data in Table 3 were used to assess the goodness-of-fit of the observed ratios to the expected ratios using Chi Square analysis with Yates correction factor (Little and Jackson Hills, 1978).

$$\chi^2 = \sum [(\text{Observed} - \text{expected} - 0.5)^2 / \text{expected}]$$

This analysis tested the hypothesis that the introduced trait segregated as a single locus in a Mendelian fashion. The critical value to reject the hypothesis at the 5% level is 3.84. Since the Chi squared value was less than 3.84 (Table 2), the hypothesis that the genetic trait behaved in a Mendelian fashion was accepted.

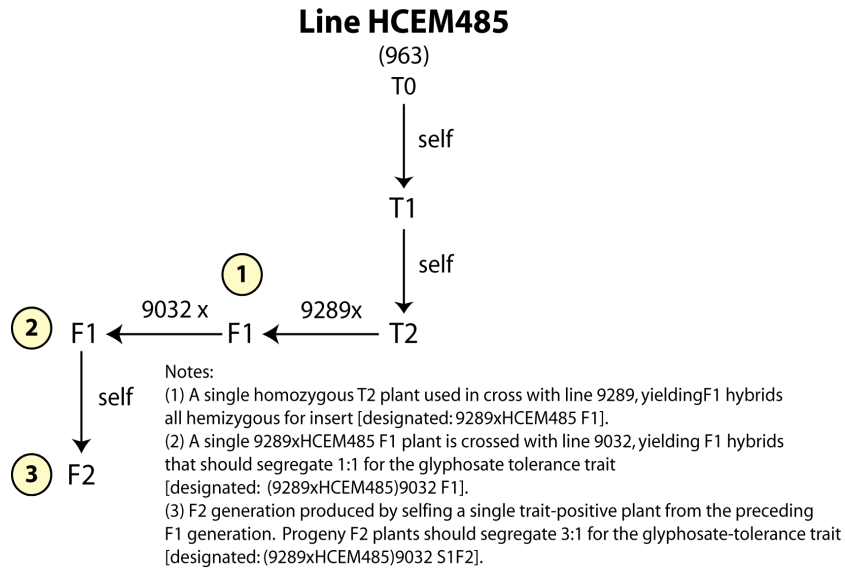


Figure 7: Breeding tree for maize line HCEM485.

Table 3: Observed vs. expected segregants for F1 hybrid and F2 selfed generations derived from HCEM485 maize.

	(9289xHCEM485)9032 F1		(9289xHCEM485)9032 S1F2	
	Observed	Expected	Observed	Expected
Trait Positive ¹	129	124.5	107	108
Trait Negative	120	124.5	37	36
Total	249	249	144	144
Expected Segregation Ratio	1:1		3:1	
Observed Segregation Ratio	1.036:0.964		2.972:1.028	
χ^2	0.930		0.624	

1. Differentiation of trait positive and trait negative plants was based on tolerance to glyphosate. Plants were sprayed at the V4 stage of development with 2.5X the normal rate of glyphosate application (1X = 32 oz/acre).
 2. For significance at the 95% confidence level ($p < 0.05$), the Chi square value should be ≥ 3.841 . Chi square values < 3.841 indicate that the null hypothesis (*i.e.*, observed and expected segregation ratios are not significantly different) should not be rejected at the 95% confidence level.

IV.3 GENE EXPRESSION

IV.3.1 *2mEPSPS Integrity and Equivalence to mEPSPS in Event GA21*

A western blot analysis was conducted with a monoclonal antibody specific to 2mEPSPS to assess the integrity of this protein as expressed in maize line HCEM485 and to assess its equivalence with the modified EPSPS protein expressed in the antecedent organism, GA21, in both leaf and seed tissues.

[The western blot analysis demonstrated that the 2mEPSPS protein expressed in maize leaf tissue from line HCEM485 was intact, with no significant difference in apparent molecular weight between the bacterial and plant-produced forms of the protein (Figure 8, lanes 1 and 3). In addition, the immunoreactive protein detected in samples from HCEM485 corresponded in size to the modified EPSPS protein expressed in event GA21 (Figure 8, lanes 3 and 4). There were no cross-reacting species detected in control samples of parental Stine 963 maize, indicating that the monoclonal antibody used for detection was specific for the modified form of the maize EPSP synthase (Figure 8, lane 2). Similarly, seed tissue of line HCEM485 expressed immunoreactive protein of the same size as that from GA21 (Figure 8, lanes 6 and 8), with no cross-reacting species detected in the control hybrid 9289/5056 (Figure 8, lane 7). The bacterial sample of the protein (Figure 8, lanes 1 and 5) retained the His-tag used to isolate the protein and is thus larger than the plant produced forms. This size difference is less noticeable on the left hand panel (lanes 1 - 4) as the gel used was of lower resolution than the gel used for the right hand panel (4-20% acrylamide compared to 10-20%).

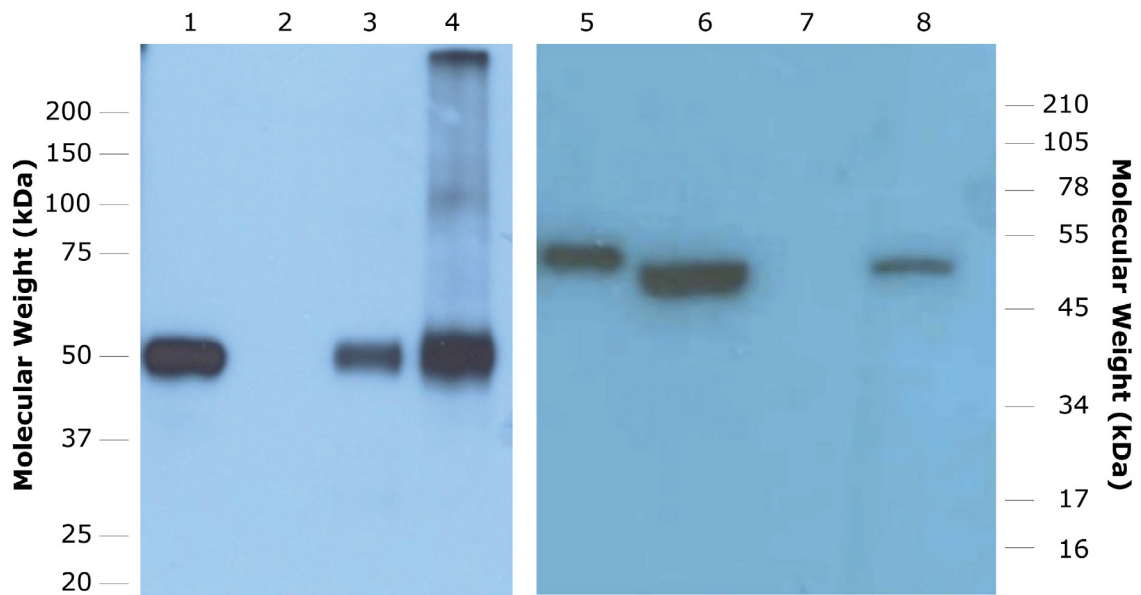


Figure 8: Western immunoblot analysis of 2mEPSPS protein with specific monoclonal antibody. Samples containing purified *E. coli*-expressed 2mEPSPS protein (50 ng; lane 1), leaf tissue extracts prepared from negative control Stine 963 maize (20 µg protein; lane 2), HCEM485 maize (20 µg protein; lane 3), transgenic GA21 maize (20 µg protein; lane 4), *E. coli*-expressed 2mEPSPS protein (25 ng; lane 5), and seed extracts from transgenic GA21 maize (20 µg protein; lane 6), negative control Stine 9289/5056 maize (20 µg protein; lane 7), HCEM485 maize (20 µg protein; lane 8). Samples were subjected to SDS-PAGE followed by electroblotting onto PVDF membrane. The membrane was incubated sequentially with mouse anti-2mEPSPS monoclonal antibody and HRP-conjugated anti-mouse IgG followed by enhanced chemiluminescent detection of bound labeled antibody. The positions of pre-stained MW markers are indicated.]

In summary, the 2mEPSPS protein expressed in maize line HCEM485 is equivalent with respect to molecular weight and immunochemical cross-reactivity to the modified EPSPS protein expressed in maize line GA21.

[A further western blot was performed with a polyclonal antisera to 2mEPSPS raised in rabbits to determine if there were any novel polypeptides produced from the inserted DNA (Figure 9). From this blot it can be seen the only cross-reacting protein in HCEM485 (lane 3) corresponds to the protein present in the control lines 9032 and 963 (lanes 2 and 4, respectively). GA21 (lane 1) produced a large amount of cross-reacting protein, as expected due to the use of a highly active heterologous promoter in this transgenic event. HCEM485 appears to contain a greater amount of the EPSPS protein, as compared to the control samples, due to the presence of an estimated four extra copies of the gene with a native promoter.

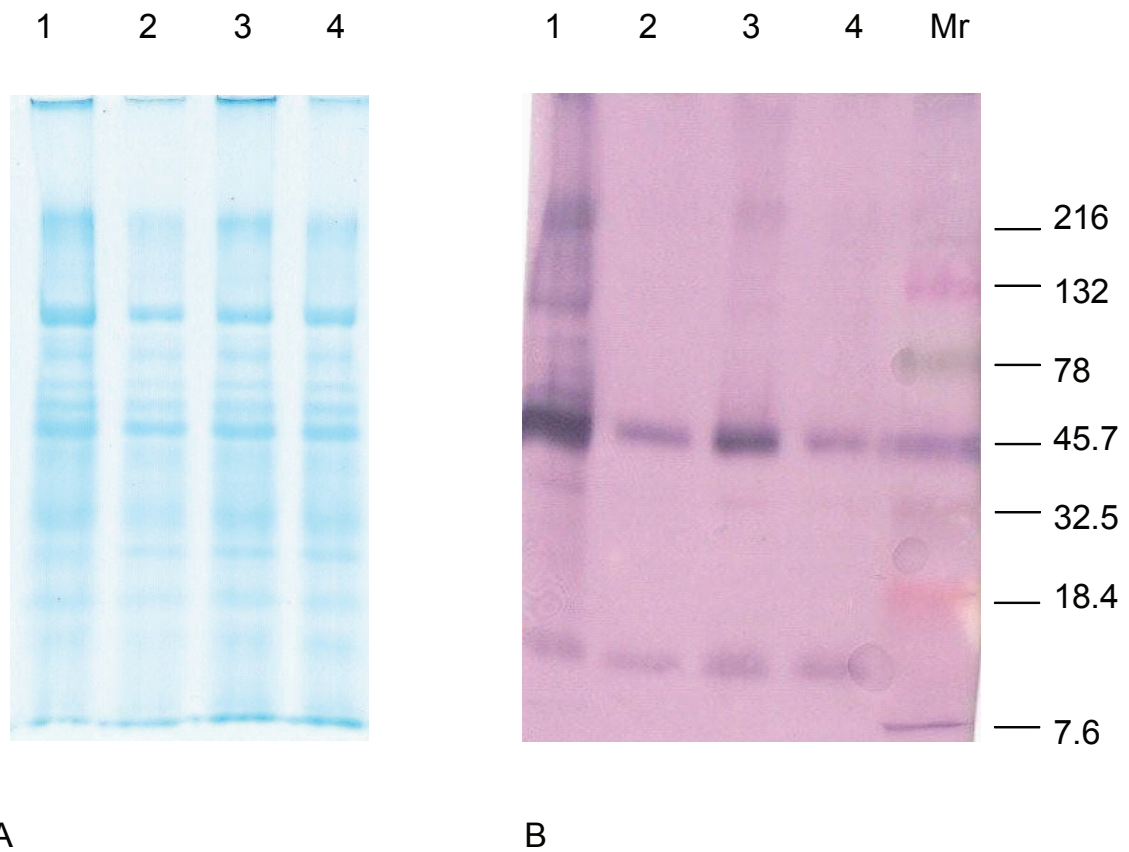


Figure 9: Western immunoblot analysis with polyclonal antisera. Leaf tissue extracts (20 µg protein per lane) were prepared from GA21 (lane 1), control Stine 9032 maize (lane 2), HCEM485 (lane 3), and control Stine 963 maize (lane 4). These samples were subjected to SDS-PAGE and stained with Coomassie Blue stain (panel A). A similar gel was electro-blotted onto PVDF membrane and incubated with rabbit polyclonal antisera to 2mEPSPS (panel B). Kaleidoscope pre-stained molecular weight markers were used (lane Mr) with molecular weights in kDa as shown.]

More detailed analysis of 2mEPSPS protein expression in various plant tissues was judged unnecessary as it would contribute little to the risk assessment. This determination was based on the rationale that: (1) levels of expression in target tissues are likely to be of the same order of magnitude as the endogenous native EPSPS enzyme since expression is driven by the same native regulatory sequences; (2) qualitatively, from western blot analysis, the level of expression

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of 2mEPSPS in HCEM485 is less than that observed in the antecedent organism, GA21 (Figure 9); and (3) the lack of any realistically attainable level of exposure of humans, animals, or non-target organisms to 2mEPSPS protein in plant material or products derived from HCEM485 likely to result in an adverse effect given the demonstrated lack of acute toxicity of modified EPSPS proteins from both plant and bacterial sources (Monsanto, 1997; Monsanto, 2000).

IV.4 2MEPSPS PROTEIN SAFETY

The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; 3-phosphoshikimate 1-carboxyvinyl-transferase; EC2.5.1.19) (Steinrücken and Amrhein, 1980) is the sixth enzyme of the shikimic acid pathway, which is essential for the biosynthesis of aromatic amino acids (L-Phe, L-Tyr and L-Trp) and chorismate-derived secondary metabolites in algae, higher plants, bacteria, and fungi (Kishore and Shah, 1988). EPSPS catalyzes the reaction between phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) to yield EPSP and inorganic phosphate (Pi) (Figure 10, Haslam, 1974; Geiger and Fuchs, 2002). EPSPS enzymes identified from plants and bacteria (Class I EPSPS) have been the most studied with respect to enzyme kinetics and active site analysis.

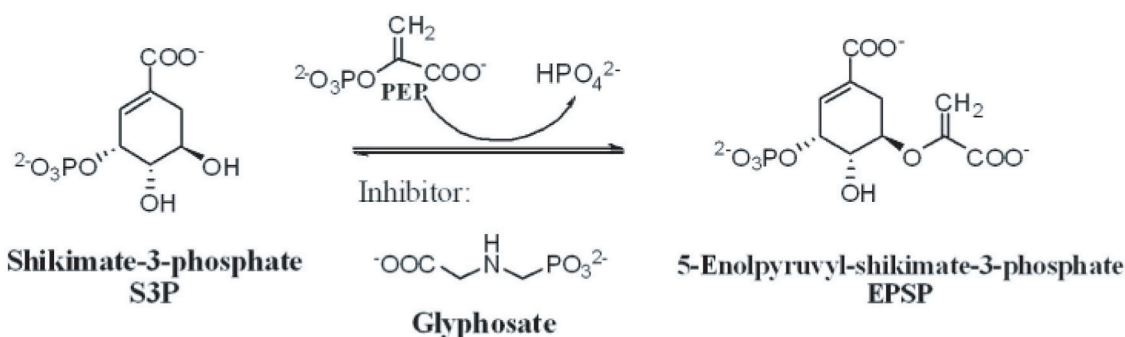


Figure 10: The reaction catalyzed by EPSP synthase.

EPSPS has been identified as the primary target of glyphosate [N-(phosphonomethyl) Gly], which is a nonselective, broad-spectrum, foliar-applied herbicide first commercialized in 1974 and widely used for the management of annual, perennial, and biennial herbaceous species of grasses, sedges, and broadleaf weeds, as well as woody brush and tree species (Bradshaw *et al.*, 1997; Baylis, 2000). In addition to being highly effective on a broad spectrum of annual and perennial weed species common to many cropping systems, glyphosate has very favorable environmental characteristics, such as lack of residual soil activity and very low toxicity to mammals, birds, and fish (Smith and Oehme, 1992; Padgett *et al.*, 1996).

The maize EPSPS enzyme and those from various other plant and microbial food sources have been part of the protein component of human and animal diets over thousands of years, and are not associated with any known health concerns. As the mutations introduced into the maize enzyme involve substitutions with standard amino acids common to all proteins of biological origin, and do not alter the functional properties of the enzyme except for its affinity for glyphosate, the 2mEPSPS protein in HCEM485 maize is not considered to be inherently toxic.

Based on its source (*Zea mays*), deduced amino acid sequence, and equivalence with the modified EPSPS expressed in event GA21, the 2mEPSPS protein expressed in maize line HCEM485

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has the same safety characteristics as the modified EPSPS protein expressed in the antecedent organism, GA21.

IV.5 CONCLUSIONS

Maize line HCEM485 contains additional copies of the maize EPSP synthase encoding gene, modified to express tolerance to glyphosate herbicide, and associated regulatory sequences (e.g., 5' promoter sequences and 3' non-coding termination sequences) also derived from maize, inserted at a single genetic locus within the maize genome as demonstrated by Southern blot analysis and Mendelian inheritance studies. The modified maize EPSP synthase expressed in maize line HCEM485 is intact, of the expected molecular weight and there was no evidence of truncated forms of the enzyme. The modified maize EPSPS expressed in HCEM485 maize is also immunochemically cross-reactive with the modified maize EPSPS expressed in the antecedent organism, GA21, and the enzymes from both sources contain the same mutations responsible for conferring glyphosate herbicide tolerance.

V. PHENOTYPIC AND AGRONOMIC CHARACTERISTICS

It was not possible to evaluate hybrids of HCEM485 in direct comparison to hybrids of the antecedent organism. However as GA21 was determined to be not significantly different to commercially available corn varieties in agronomic and phenotypic characteristics, a comparison of HCEM485 and existing commercial hybrids will be used to establish that HCEM485 is not significantly different to these hybrids and thus demonstrate the similarity to GA21.

Agronomic and phenotypic characteristics of an HCEM485 maize hybrid and three control hybrids were evaluated in a series of field trials across 15 United States Corn Belt locations in 2007. The material used for the field trials was developed from the initial HCEM485 event as detailed in Figure 11. The agronomic characteristics chosen for comparison were those typically observed by professional maize breeders and agronomists and represented a broad range of characteristics throughout the life cycle of the maize plant. Comparisons were made between HCEM485 and control hybrids without glyphosate treatment. Separate plots of HCEM485 were grown with glyphosate treatment at recommended field rates (32 oz/acre), but these were not used for the statistical comparison as it is not valid to compare plants with such different treatments.

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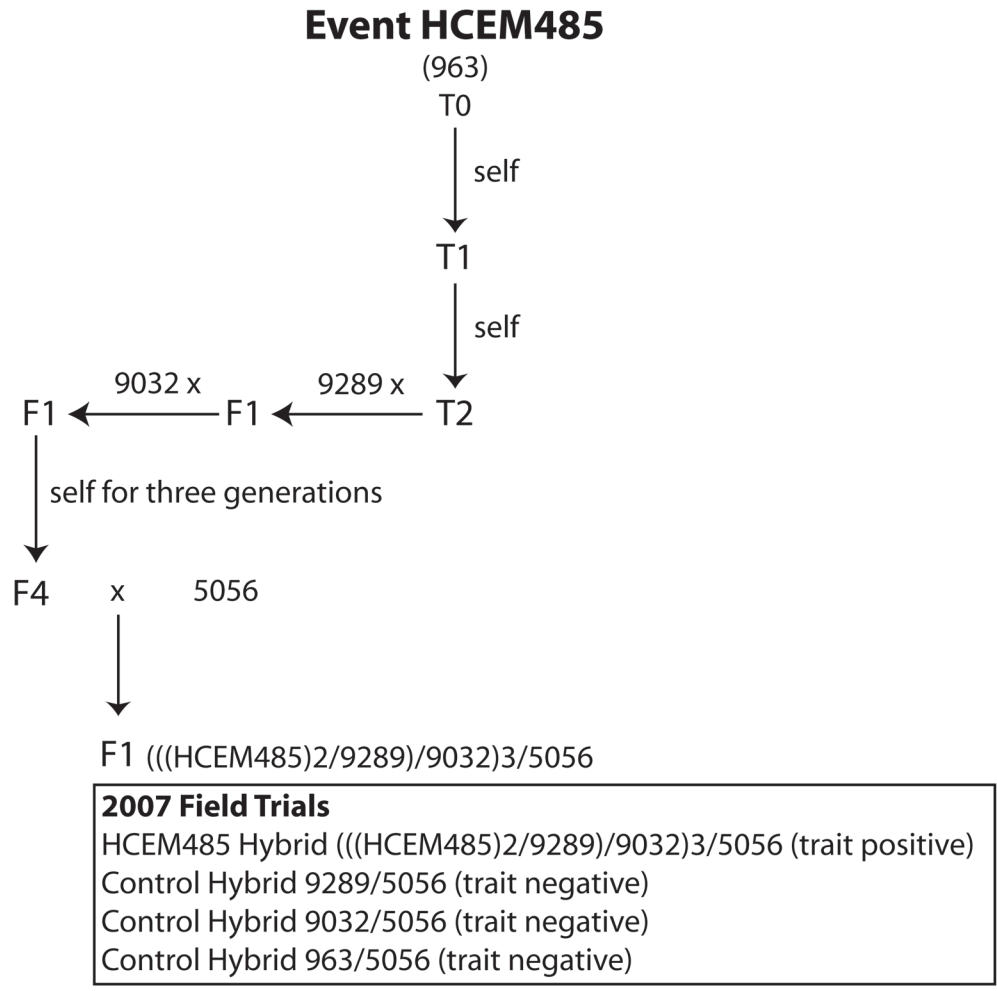


Figure 11: Pedigree chart of HCEM485 seed used in 2007 agronomic trials.

V.1 TRIAL DESIGN AND EVALUATION

V.1.1 Plant Material

Agronomic equivalence trials were conducted using the following hybrid lines:

- HCEM485 hybrid (((HCEM485)2/9289/9032)3/5056) [trait positive]
- Control hybrid 9289x5056 [trait negative]
- Control hybrid 9032x5056 [trait negative]
- Control hybrid 963x5056 [trait negative]

A pedigree map showing the derivation of the HCEM485 hybrid is shown in Figure 11. The control hybrids were produced by crossing the inbred lines Stine 963, 9289 or 9032, each of which were used as parental lines in the breeding of HCEM485, with inbred line 5056, which was also used in creating the HCEM485 hybrid.

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V.1.2 Trial Locations

Field trials were conducted at 15 locations in 2007 under USDA notification # 07-046-110n. These 15 locations covered nine states in the United States Corn Belt (Table 4) and were selected to represent a range of diverse growing environments where HCEM485 maize hybrids are expected to be commercially grown. Field husbandry at all of the trial sites (including irrigation use, fertilization rate, and pest control methods) was consistent with best agronomic practices in the area. Agronomic practices for all genotypes within a trial at a single location were identical.

Table 4: Trial locations and dates.

Location Code	City	State	Planting Date	Harvest Date
ADL1	Adel	IA	5-Jun-2007	NA
ADL2	Adel	IA	5-Jun-2007	17-Oct-2007
ATL	Atlantic	IA	6-Jun-2007	23-Oct-2007
LAU	Laurel	NE	7-Jun-2007	24-Oct-2007
LEN	Lennox	SD	7-Jun-2007	25-Oct-2007
SMI	Smithshire	IL	7-Jun-2007	NA
MAR	Marion	AR	5-Jun-2007	20-Oct-2007
EDM	Edmondson	AR	6-Jun-2007	20-Oct-2007
BLO	Blomkest	MN	5-Jun-2007	26-Oct-2007
BIR	Bird Island	MN	5-Jun-2007	26-Oct-2007
FIT	Fithian	IL	7-Jun-2007	15-Oct-2007
LIN	Lincoln	IL	4-Jun-2007	15-Oct-2007
DUR	Durand	MI	11-Jun-2007	16-Oct-2007
SHE	Sheridan	IN	4-Jun-2007	9-Oct-2007
OH	Spencerville	OH	12-Jun-2007	12-Oct-2007

NA = not applicable. Trial was destroyed prior to harvest.

V.1.3 Agronomic Traits Assessed

Up to 17 separate agronomic characteristics were assessed at each location, but not all traits were assessed at all locations. These agronomic traits covered a broad range of characteristics encompassing the entire life cycle of the maize plant and included data assessing germination and seedling emergence, growth habit, vegetative vigor, days to pollen shed, days to maturity, and yield parameters (Table 5).

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Table 5: List and definitions of agronomic traits assessed in the field trials.

Abbreviation	Trait	Timing	Description
BRRNP	Percent Barren Plants	Harvest	Percent of plants per plot that do not develop an ear.
DROPP	Percent Dropped Ears	Harvest	Percent of plants per plot that have dropped a developed ear prior to harvest.
EAGRR	Early Growth Rating	V6	Early growth rating recorded at V6 on a scale of 1–9, with 9=most vigorous growth.
EMRGP	Early Stand Count	V3	Percent of sowed kernels that resulted in emerged plants within 14 days after planting.
EMRGR	Seedling Vigor	V3	Early emergence vigor rating. Data collected prior to V3 stage of maize development. Rated on a 0-9 scale, where 0=dead and 9=most vigorous growth.
ERHTN	Ear Height	After anthesis	Ear height from base of plant to node where ear connects to plant (cm). Taken at R2-R6 stage of maize development.
ERTLP	Early Root Lodging		A 1-9 rating where a higher score indicates less root lodging potential (1 is very poor, 5 is intermediate, and 9 is very good, respectively, for resistance to root lodging).
GMSTP	Grain Moisture Percent	Harvest	Percent grain moisture measured at harvest.
HAVPN	Final Stand Count	Harvest	Harvest population (plants per acre).
HUP5N	Heat units to 50% pollen shed	Flowering (anthesis)	Heat units to 50% of plants shedding pollen.
HUS5N	Heat units to 50% silking	Flowering (anthesis)	Heat units to 50% of plants extruding silks.
LFCLR	Leaf Color Rating	After anthesis	Leaf color rating taken between R4 and R6 stage of maize development. 5=same as commercial check. 1=darker, 9=severely chlorotic.
PLHTN	Plant Height	After anthesis	Plant height from base of plant to collar of flag leaf (cm). Taken between R2 and R6 stage of maize development.
RTLDR	Root Lodging Rating	Harvest	A 1-9 rating where a higher score indicates less root lodging potential (1 is very poor, 5 is intermediate, and 9 is very good, respectively, for resistance to root lodging).
STKLR	Stalk Lodging Rating	Harvest	This is a 1-9 rating where a higher score indicates less stalk lodging potential (1 is very poor, 5 is intermediate, and 9 is very good, respectively, for resistance to stalk lodging).
TWSMN	Test Weight	Harvest	Grain test weight (pounds/bushel) converted to standard 15% moisture.
YGSMN	Grain Yield	Harvest	Grain yield (bushels/acre) converted to standard 15% grain moisture.

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V.1.4 Experimental Design and Statistical Analysis

Each of the agronomic trials utilized a randomized complete block design with three replications per location. Plot size was ca. 0.002 acres, using 2-row plots, 17.5 feet long with 30 inches between the rows. Each plot was planted to contain approximately 62 plants of the same genotype and there were 12 plots at each location - three replicates of the HCEM485 hybrid and the three control hybrids. Full data for the individual plots is included in Annex 1

Data from each of the three control hybrid lines were treated as a single treatment group, identified as control hybrids, in comparisons with the HCEM485 hybrid. Data for the variates (traits) were subjected to an analysis of variance across locations using the generalized linear model:

$$Y_{ij} = U + T_i + L_j + LT_{ij} + e_{ij}$$

where Y_{ij} is the observed response for genotype i at location j , U is the overall mean, T_i is the treatment (HCEM485 vs. control genotypes) effect, L_j is the location effect, LT_{ij} is the location x treatment (genotype) interaction effect and e_{ij} is the residual error (Annicchiarico, 2002). For each variate, the statistical significance of the genotype effect (*i.e.*, HCEM485 vs. control hybrids) was determined using a standard F-test. An F-test probability of < 0.05 indicates that the difference between the genotypes was statistically significant with 95% confidence. An F-test was also used to assess the significance of the location x genotype interaction – a significant outcome (F-test probability < 0.05) indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations may not be meaningful.

The data for several variates did not lend themselves to formal statistical analysis because they did not conform to the assumptions upon which the validity of the analysis depends. In some cases, the problem was that the data were too discrete, with values taking one of a very limited range of options. In other cases the dataset contained too few non-zero data points on which to base a reasonable estimate of residual error. Consequently, results for such variates are presented as means. Full results and statistical calculations are included in Annex 1 and only summary tables are included with discussion of the results below.

V.2 GROWTH HABIT

In addition to the agronomic characteristics discussed in other sections below, the following parameters are discussed here as indicators of basic morphology and growth habit: ERTLRL (early root lodging rating); STKLR (stalk lodging rating); RTLDR (late season root lodging rating); and LFCLR (leaf color rating). None of these variates were suitable for formal statistical analysis and are presented as a summary of genotype means (Table 6). Overall, there were no remarkable differences indicative of an alteration in plant growth habit.

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[Table 6: Comparison of growth habit characteristics of HCEM485 and control hybrids.

	ERTLR ^a (1-9 rating)	STKLR (1-9 rating)	RTLDR (1-9 rating)	LFCLR (1-9 rating)
HCEM485 hybrid	8.71	8.17	8.55	4.93
Control hybrids	8.69	8.15	8.56	4.91
Mean Difference	-0.02	-0.02	0.01	-0.02
N ^b	15	14	14	15

a. ERTLRL = early root lodging rating; STKLR = stalk lodging rating; RTLDR = late root lodging rating; LFCLR = leaf color rating.

b. N = number of locations with data.]

V.3 VEGETATIVE VIGOR

Comparisons of vegetative vigor between HCEM485-derived and control hybrids were based on assessments of: EMRGR (seedling vigor); EAGRR (early growth rating); ERHTN (ear height); and PLHTN (plant height). The only variates suitable for statistical analysis were ERHTN and PLHTN, both of which showed small but statistically significant increases between HCEM485 and control hybrids (Table 7). The magnitudes of these increases were 2.3% and 2.7%, respectively which is small in relation to the range of values for these traits seen in commercial corn hybrids released by Stine Seed Farms. Ratings of seedling vigor and early growth were similar between HCEM485 and control hybrids.

[Table 7: Comparison of vegetative growth characteristics of HCEM485 and control hybrids.

	EMRGR ^a (0-9 rating)	EAGRR (1-9 rating)	ERHTN (cm)	PLHTN (cm)
HCEM485 hybrid	7.20	7.60	96.4 ± 16.3	238.1 ± 29.7
Control hybrids	7.46	7.54	94.2 ± 15.4	231.7 ± 31.1
Mean Difference	-0.26	0.06	2.2	6.4
F-test genotype			0.043*	<0.001*
F-test genotype x location			0.523	0.178
N ^b	15	15	15	15
Range observed in Stine Seed Farms hybrids			71 - 152	152 - 353

* = indicates that the difference between the genotypes was statistically significant at the 95 percent confidence level (p < 0.05).

a. EMRGR = seedling vigor; EAGRR = early growth rating; ERHTN = ear height; PLHTN = plant height. Mean values are shown. For ERHTN and PLHTN, the mean standard deviation is indicated.

b. N = number of locations with data.]

V.4 REPRODUCTIVE CHARACTERISTICS

The relevant field indicators of potential changes to seed dormancy, pollination or fertility were: EMRGP (percent of emerged plants); HUS5N (heat units to 50 percent silking; HUP5N (heat units to 50 percent pollen shed); and BRRNP (percent barren plants). For those variates

suitable for statistical analysis, there were small but statistically significant decreases in both heat units to 50% silking (-2.5%) and heat units to 50% pollen shed (-2.0%) (Table 8). Once again, these are small differences representing only 1-2 days in the timing of these particular traits and are within the range of commercial hybrids. Furthermore, these are traits which are addressed during breeding process and which will be defined for commercial hybrids produced with HCEM485. Although there was no significant difference in the percent germinated plants (EMRGP) between HCEM485 and control hybrids, the values for both these groups were lower than expected, which was likely due to problems with greenhouse seed production for the trials. Data on percent barren plants (BRRNP) were not suitable to statistical analysis but mean values were not markedly different between HCEM485 and control hybrids.

[Table 8: Comparison of reproductive characteristics of HCEM485 and control hybrids

	EMRGP ^a (%)	HUS5N (heat units)	HUP5N (heat units)	BRRNP (%)
HCEM485 hybrid	49.0 ± 12.1	1358.0 ± 170.3	1467.7 ± 237.3	0.75
Control hybrids	48.4 ± 12.5	1393.5 ± 169.6	1498.4 ± 233.3	0.35
Mean Difference	0.6	-35.5	-30.7	0.4
F-test genotype	0.661	<0.001*	<0.001*	
F-test genotype x location	0.216	0.989	0.918	
N ^b	15	15	10	14
Range observed in Stine Seed Farms hybrids		1012 - 1868	1100 - 1930	

* = indicates that the difference between the genotypes was statistically significant at the 95 percent confidence level (p < 0.05).

a. EMRGP = percent emergence; HUS5N = heat units to 50% silking; HUP5N = heat units to 50% pollen shed; BRRNP = percent barren plants. Mean values are shown. For EMRGP, HUS5N and HUP5N, the mean standard deviation is indicated.

b. N = number of locations with data.]

V.5 YIELD AND GRAIN CHARACTERISTICS

Parameters used to evaluate yield and grain characteristics included: YGSMN (grain yield); HAVPN (plant population at harvest); DROPP (percent dropped ears); TWSMN (grain test weight); and GMSTP (grain moisture percent). Among the variates suitable for statistical analysis, there were no significant differences in average yield, plant population at harvest, grain moisture, or grain test weight between HCEM485 and control hybrids (Table 9). For both yield and plant population at harvest, there were significant genotype x location interactions. Both of these traits are subject to large variance based on initial planting density and germination so a meaningful range of values for commercial hybrids is not available. The mean value seen in HCEM485 is slightly higher in both cases, indicating no deleterious effects from the insertion of the modified EPSPS. Although not subject to statistical analysis, there were no remarkable differences in percent dropped ears between HCEM485 and control genotypes (Table 9).

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[Table 9: Comparison of yield and grain characteristics of HCEM485 and control hybrids

	YGSMN ^a (bu/acre)	HAVPN (plants/acre)	DROPP (%)	GMSTP (%)	TWSMN (lb/bu)
HCEM485 hybrid	115.4 ± 51.2	14976 ± 3410	0.06	18.8 ± 7.7	55.2 ± 1.9
Control hybrids	113.9 ± 50.7	14888 ± 3622	0.04	18.3 ± 7.1	55.2 ± 2.3
Mean Difference	1.5	88	0.02	0.5	0.1
F-test genotype	0.621	0.818		0.051	0.731
F-test genotype x location	0.003**	0.040**		0.463	0.431
N ^b	13	14	14	13	13

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotype averaged across locations is questionable.

a. YGSMN = grain yield; HAVPN = final stand count at harvest; DROPP = percent dropped ears; GMSTP = grain moisture percent; TWSMN = grain test weight. Mean values are shown. For YGSMN, HAVPN and GMSTP, the mean standard deviation is indicated.

b. N = number of locations with data.]

V.6 DISEASE OBSERVATIONS

Natural disease infections were rated in trials where the disease incidence was sufficiently high to warrant assessment. Observations were made at four sites (Adel, Iowa; Laurel, Nebraska; Blomkest, Minnesota; and Lincoln, Illinois) on two separate dates during August and September. These sites were chosen in order to provide a broad range of ecological sites within the maturity range for the hybrids used. Disease incidence was generally low, with no little disease noted during the first inspections. At the time of the second inspection only two sites showed sufficient levels to be scored for Southern rust disease (SRDI) and gray leaf spot (GLSDR), with one site scored for Northern maize leaf blight (NCLBR), common rust (CMRR) and smut (SMTR). Although not suitable for statistical analysis, there were no remarkable differences in any of these ratings between HCEM485 and control hybrids (Table 10).

[Table 10: Comparison of disease ratings for HCEM485 and control hybrids.

	SRDI ^a (0-5 rating)	GLSDR (0-5 rating)	NCLBR (0-5 rating)	CMRR (0-5 rating)	SMTR (0-5 rating)
HCEM485 hybrid	0.93	1.21	0.22	1.00	1.00
Control hybrids	0.90	1.22	0.15	1.08	1.00
Mean Difference	0.03	-0.01	0.07	-0.08	0.0
N ^b	2	2	1	1	1

a. SRDI = Southern rust disease rating; GLSDR = gray leaf spot disease rating; NCLBR = northern maize leaf blight rating; CMRR = common rust rating; and SMTR = smut rating. All ratings were on a 0–5 scale: 0= no incidence; 1= 2-9%; 2= 10-24%; 3= 25-49%; 4= 50-74%; and 5= 75-100%.

b. N = number of locations with data.]

V.7 POLLEN MORPHOLOGY AND VIABILITY

In order to assess whether the presence of the mutated EPSP synthase encoding gene, the gene product, or the genetic modification process altered the pollen characteristics of HCEM485

maize, pollen morphology and viability were investigated by microscopically examining pollen grains that had been fixed and stained according to the method described by Alexander (1969).

Although the viability of HCEM485 pollen (94.6%) was statistically significantly greater than the control (86.0%), both values were within the range that has been reported for other reference samples of maize pollen (Monsanto 2004) and the observed difference was, therefore, considered small and unlikely to be of biological significance (Table 11). There were no readily discernible differences in HCEM485 and control pollen morphology (Figure 11) and no significant difference in average cell diameter was detected between HCEM485 and control pollen samples.

[Table 11: Pollen viability and diameter measurements.

Genotype	Mean Pollen Viability ± SD (%) ^a	Mean Pollen Diameter ± SD (µm)
HCEM485	94.6 ± 2.9	105.5 ± 6.5
Control (9289x5056)	86.0 ± 4.2	104.8 ± 5.2
p-value	0.007*	0.527
N	5	60

a. Mean percent pollen viability and mean diameter measurements are presented with their respective standard deviations (SD). The HCEM485 and control means were compared by a t-test.

* = indicates that the difference between the HCEM485 and control hybrid was statistically significant at $p < 0.05$.

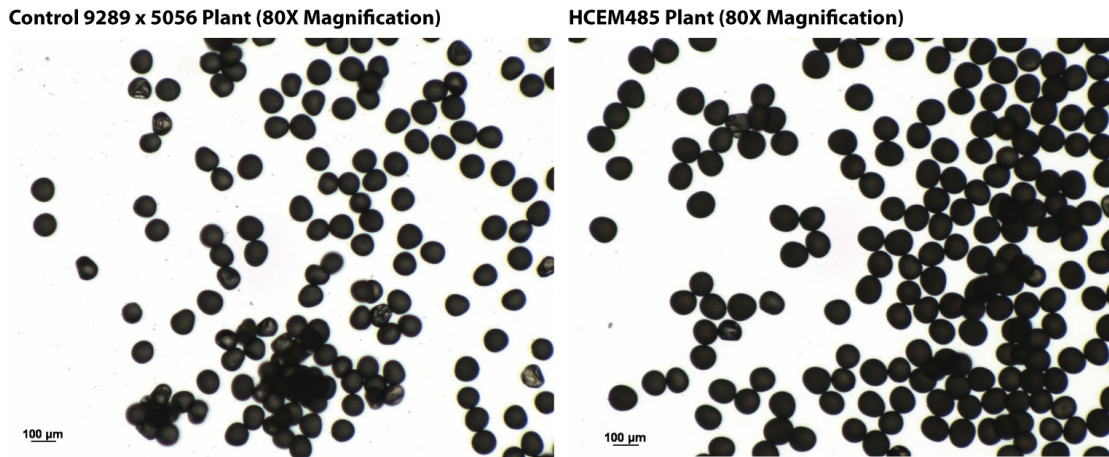


Figure 12: Morphology of pollen from HCEM485 and control hybrids. Representative photomicrographs of control (left) and HCEM485 (right) pollen samples. Pollen samples were stained with Alexander’s stain and examined under light microscopy (80X magnification). The scale bar representing 100 µm is indicated in each photomicrograph.]

V.8 COMPOSITIONAL ANALYSIS

For compositional analysis, grain samples from HCEM485 and control hybrids were obtained from four trial locations (ADL2, FIT, BLO and OH, as identified in (Table 4) and forage samples were obtained from two locations (ADL2 and FIT). HCEM485 samples were from plants treated with glyphosate at the normal commercial application rate (32 oz/acre). Individual

Maize Line HCEM485
USDA Extension Petition

samples of grain and forage from each of the three negative control hybrids were respectively combined into a single composite grain and forage sample from each replicated plot. Triplicate samples obtained from each plot of HCEM485 hybrid and the composited negative controls were analyzed for up to 87 components in grain and 8 components in forage. With the exception of grain total dietary fiber, starch, chromium and selenium, which were analyzed by Eurofins (Des Moines, IA), all compositional analyses were performed by EPL Bio-Analytical Services (Niantic, IL), according to standard methods.

Data for analytes above the limit of quantification (LOQ) were subjected to analysis of variance across all locations with genotype and location as factors. Average values for each analyte were compared to data for forage and grain composition published in both the International Life Sciences Institute (ILSI) crop composition database (ILSI, 2006) and the Organization for Economic Co-operation and Development consensus document on new maize varieties (OECD, 2002) to assess whether any observed variation was within the natural range for cultivated maize forage and grain.

V.8.1 Proximates

Analysis of the major constituents of maize, or proximates, was used to determine the nutritional properties of maize grain and forage from different hybrids. The major constituents of maize grain and forage are carbohydrates, protein, fat and ash. Fiber is the predominant form of carbohydrate present in forage and starch is the major carbohydrate in maize grain. Fiber is measured by the neutral detergent fiber method (NDF), which measures the insoluble fiber: lignin, cellulose and hemicellulose. Total dietary fiber (TDF) consists of the insoluble and soluble fiber (pectin). The soluble fiber fraction in maize is negligible, so the NDF value in maize grain is comparable to that of TDF. The acid detergent fiber (ADF) method solubilizes hemicellulose, measuring only cellulose and lignin (Watson, 1987).

Comparison of the proximate composition of the HCEM485 grain and the negative control grain samples is shown in Table 12. No statistically significant differences were observed for protein, fat, carbohydrates, ADF, NDF, ash, starch or carbohydrate. A statistically significant difference was observed for TDF, however, the magnitude of the difference was small (*ca.* 6.4%). The average values for all proximates measured in grain were within the ranges reported in the literature.

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[Table 12: Proximate composition of grain from HCEM485 and control hybrids.

Samples		Moisture (%FW)	Protein (%DW)	Fat (%DW)	ADF (%DW)	NDF (%DW)
HCEM485	Mean	12.06	10.14	4.36	4.28	13.56
	95%CI	(11.7-12.4)	(9.0-11.2)	(4.2-4.6)	(4.2-4.4)	(13.1-14.1)
Control hybrids	Mean	12.05	10.23	4.52	4.58	13.60
	95%CI	(11.7-12.4)	(9.2-11.3)	(4.2-4.8)	(4.3-4.8)	(13.2-14.0)
Mean Difference (%)		0.08%	-0.91%	-3.42%	-6.57%	-0.27%
F-test probability for genotype			0.453	0.256	0.075	0.891
F-test genotype x location			0.434	0.259	0.391	0.596
Literature Values						
GA21†	Mean	14.60	9.90	3.50	3.90	11.40
ILSI (2006)	Mean	11.30	10.30	3.555	4.05	11.23
	Range	6.1-40.5	6.15-17.26	1.74-5.82	1.82-11.34	5.59-22.64
OECD (2002)	Range	7.0-23.0	6.0-12.7	3.1-5.8	3.0-4.3	8.3-11.9
Samples			TDF (%DW)	Ash (%DW)	Starch (%DW)	CHO (%DW)
HCEM485	Mean		11.29	1.36	60.64	84.14
	95%CI		(10.7-11.9)	(1.3-1.4)	(59.6-61.7)	(83.1-85.2)
Control hybrids	Mean		10.61	1.39	60.12	83.86
	95%CI		(10.3-11.0)	(1.4-1.4)	(59.6-60.6)	(82.7-85.0)
Mean Difference (%)			6.40%	-1.91%	0.87%	0.34%
F-test probability for genotype			0.044*	0.232	0.271	0.225
F-test genotype x location			0.201	0.851	0.008**	0.598
Literature Values						
GA21†	Mean		ND	1.30	ND	85.20
ILSI (2006)	Mean		16.43	1.439	57.7	84.6
	Range		8.85-35.31	0.616-6.282	26.5-73.8	77.4-89.5
OECD (2002)	Range		11.1	1.1-3.9		82.2-82.9

* = indicates that the difference between the genotypes means was statistically significant at (p < 0.05).

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

All values expressed as percent dry weight, except for moisture. Moisture levels in grain not subject to analysis of variance as grain was mechanically dried after harvest.

95%CI = computed 95% confidence interval around the mean value.

CHO = carbohydrate; ADF = acid detergent; NDF = neutral detergent fiber; TDF = total dietary fiber.

ND = Not determined.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.]

Comparison of the proximate composition of the HCEM485 forage and the negative control forage is shown in Table 13. No statistically significant differences were found in five (moisture, ash, carbohydrates, ADF, and NDF) of the seven analytes tested. The only statistically significant differences observed were a higher (*ca.* 6.8%) mean protein content, which was not consistent across locations, and a lower (*ca.* 13.2%) level of total fat in the HCEM485 hybrid

samples than in the control samples. The average values for all proximates in forage, including protein and fat, were within the ranges reported in the literature.

[Table 13: Proximate composition of forage from HCEM485 and control hybrids.

Samples		Moisture (%FW)	Protein (%DW)	Fat (%DW)	ADF (%DW)	NDF (%DW)	Ash (%DW)	CHO (%DW)
HCEM485	Mean	71.41	9.36	3.14	28.29	48.80	4.19	83.31
	95%CI	(70.4-72.4)	(8.5-10.3)	(2.9-3.3)	(22.7-33.9)	(43.0-54.6)	(4.1-4.3)	(82.4-84.2)
Control hybrids	Mean	70.60	8.77	3.61	26.95	46.89	4.00	83.62
	95%CI	(69.7-71.5)	(8.4-9.1)	(3.4-3.9)	(23.7-30.1)	(42.2-51.3)	(3.9-4.2)	(83.0-84.3)
Mean Difference (%)		1.14%	6.81%	-13.17%	4.99%	4.06%	4.73%	-0.37%
F-test probability for genotype		0.271	0.033*	0.024*	0.742	0.604	0.091	0.598
F-test genotype x location		0.925	0.002**	0.908	0.365	0.317	0.665	0.063
Literature Values								
ILSI (2006)	Mean	70.20	7.78	2.04	27.00	41.51	4.63	85.60
	Range	49.1-81.3	3.14-11.57	0.296-4.570	16.13-47.39	20.29-63.71	1.527-9.638	76.4-92.1
OECD (2002)	Range	62.0-78.0	4.7-9.2	1.5-3.2	25.6-34.0	40.0-48.2	2.9-5.7	

* = indicates that the difference between the genotypes means was statistically significant at (p < 0.05).

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

All values expressed as percent dry weight, except for moisture, which is expressed as percent fresh weight.

CHO = carbohydrate; ADF = acid detergent; NDF = neutral detergent fiber.]

V.8.2 Minerals

Several mineral ions are recognized as essential plant nutrients and are required by the plant in significant quantities. These macronutrients include calcium, phosphorous, potassium and sodium. The micronutrient minerals, iron, copper and zinc are incorporated in plant tissues in only trace amounts. Maize is an important source of selenium in animal feed (Watson, 1987), and this analyte was also included in the analyses of grain.

Comparison of the mineral composition of the HCEM485 grain and the negative control grain is shown in Table 14. No statistically significant differences were observed for levels of iron, magnesium, manganese, phosphorus, sodium or zinc. Small but statistically significant differences were noted for calcium, copper, and potassium. For selenium, values that were below the limit of quantification (<LOQ) were distributed equally between the HCEM485 hybrid and control hybrids, where 5 out of 12 total values for each set of samples were <LOQ. Analytes with values <LOQ were not suitable for statistical analysis but quantifiable levels of selenium in the HCEM485 samples (ranging from 0.11–0.21 mg/kg dry weight) were all within ranges reported in the literature. Levels of chromium in HCEM485 and control samples were all <LOQ. For all minerals that were statistically analyzed, including those that showed statistically significant differences, average values were within the ranges reported in the literature.

[Table 14: Mineral composition of grain from HCEM485 and control hybrids.

Samples		Concentration (ppm dry weight)				
		Ca	Cu	Fe	Mg	Mn
HCEM485	Mean	35.40	1.17	25.15	1314.27	6.13
	95%CI	(33.5-37.3)	(1.10-1.24)	(22.9-27.4)	(1255-1373)	(5.62-6.63)
Control hybrids	Mean	37.51	1.34	25.94	1293.83	5.92
	95%CI	(35.9-39.1)	(1.26-1.43)	(23.5-28.4)	(1255-1332)	(5.33-6.51)
Mean Difference (%)		-5.61%	-13.01%	-3.04%	1.58%	3.51%
F-test probability for genotype		0.015*	<0.001*	0.235	0.437	0.166
F-test genotype x location		0.872	0.556	0.555	0.478	0.050
Literature Values						
GA21†	Mean	30.0	ND	ND	ND	ND
ILSI (2006)	Mean	46.4	1.75	21.81	1193.80	6.18
	Range	12.7-208.4	0.73-18.5	10.42-49.07	594-1940	1.69-14.3
OECD (2002)	Range	30-1000	0.9-10	1-100	820-10000	
Samples		P	K	Na	Se	Zn
HCEM485	Mean	3208.79	3739.93	1.44	<LOQ-0.21	19.82
	95%CI	(3088-3330)	(3637-3843)	(1.04-1.83)		(18.4-21.2)
Control hybrids	Mean	3148.27	3600.90	2.25	<LOQ-0.20	20.50
	95%CI	(3047-3249)	(3485-3716)	(1.36-3.15)		(19.2-21.8)
Mean Difference (%)		1.92%	3.86%	-36.15%		-3.34%
F-test probability for genotype		0.336	0.014*	0.199		0.153
F-test genotype x location		0.381	0.138	0.380		0.718
Literature Values						
GA21†	Mean	2900	ND	ND	ND	ND
ILSI (2006)	Mean	3273.5	3842	31.75	0.20	21.6
	Range	1470-5330	1810-6030	0.17-731.54	0.05-0.75	6.5-37.2
OECD (2002)	Range	2340-7500	3200-7200	0-1500	0.01-1.0	12-30

* = indicates that the difference between the genotypes means was statistically significant at $p < 0.05$.

Where some of the sample values were less than the limit of qualification (<LOQ), statistical comparison was not possible, so only the range is shown. Values for chromium in all samples tested were <LOQ.

95%CI = computed 95% confidence interval around the mean value.

Ca=calcium; Cu=copper; Fe=iron; Mg=magnesium; Mn=manganese; P=phosphorous; K=potassium; Na=sodium; Se=selenium; Zn=zinc.

ND = Not determined.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.]

Comparison of the calcium and phosphorus composition of the HCEM485 forage and the control forage samples is shown in Table 15. Only calcium was statistically significantly higher (ca. 13%) in HCEM485 samples than control samples, and mean levels of both calcium and phosphorus were within the ranges reported in the literature.

[Table 15: Mineral composition of forage from HCEM485 and control hybrids.

Samples		Concentration (mg/kg dry weight)	
		Ca	P
HCEM485	Mean	1829.2	2167.7
	95%CI	(1642-2017)	(1935-2401)
Control hybrids	Mean	1617.3	2106.1
	95%CI	(1498-1736)	(1876-2336)
Mean Difference (%)		13.10%	2.93%
F-test probability for genotype		0.006*	0.202
F-test genotype x location		0.065	0.626
Literature Values			
ILSI (2006)	Mean	2028.6	2066.1
	Range	713.9-5767.9	936.2-3704.1
OECD (2002)	Range	1500-3100	2000-2700

* = indicates that the difference between the genotypes means was statistically significant at $p < 0.05$.

Ca=calcium, P=phosphorous.]

V.8.3 Vitamins

Although animal feed formulations are usually supplemented with additional vitamins to achieve nutritional balance, maize contains two fat-soluble vitamins, vitamin-A (β -carotene) and vitamin E, and most of the water-soluble vitamins. Vitamin A occurs in two forms in nature. Its true form, retinol, is present in foods of animal origin such as fish oils and liver. Provitamin A, in the form of the carotenoids β -carotene and cryptoxanthin are found in plants and converted in the body to vitamin A. Vitamin E (tocopherol) occurs in a variety of vegetable, nut, and oilseed crops, and of the various structural isomers (alpha-, beta-, delta- and gamma-tocopherol), α -tocopherol is the most biologically important as a natural antioxidant. Alpha-tocopherol is the only form of vitamin E that is actively maintained in the human body, and has the greatest nutritional significance (Linus Pauling Institute, 2004). The water-soluble vitamins B1 (thiamine) and B6 (pyridoxine) are present in maize grain at quantities sufficient to be important in animal rations (Watson, 1987).

Comparison of the vitamin analysis of grain is shown in Table 16. Statistically significant differences between HCEM485 and control sample means were observed for levels of tocopherols, thiamine (B1), pyridoxine (B6) and folic acid (B9). The magnitudes of these differences were small, ranging from *ca.* 7–18%, and in some cases (*e.g.*, B6, B9, and α -tocopherol), the differences were not consistent across growing locations. Levels of β -cryptoxanthine and riboflavin (B2) were below the limit of quantification in all samples. For all of the quantifiable analytes, the mean values were within the ranges reported in the literature, including those where significant differences were observed between samples from HCEM485 and control hybrids.

[Table 16: Vitamin analysis of grain from HCEM485 and control hybrids.

Samples		Concentration (mg/100g dry weight)					
		A	B1	B3	B5	B6	B9
HCEM485	Mean	<LOQ-1.382	0.264	2.007	0.530	0.795	0.070
	95%CI		(0.25-0.28)	(1.66-2.36)	(0.53-0.54)	(0.73-0.86)	(0.06-0.08)
Control hybrids	Mean	<LOQ-1.377	0.301	1.899	0.537	0.856	0.083
	95%CI		(0.28-0.32)	(1.55-2.24)	(0.53-0.55)	(0.77-0.94)	(0.07-0.09)
Mean Difference (%)			-12.23%	5.67%	-1.29%	-7.14%	-15.25%
F-test genotype			0.002*	0.235	0.106	0.002*	0.018*
F-test genotype x location			0.417	0.022**	0.062	0.004**	0.015**
Literature Values							
ILSI (2006)	Mean	0.684	0.530	2.376		0.644	0.0651
	Range	0.019-4.68	0.126-4.00	1.04-4.69		0.368-1.13	0.015-0.146
OECD (2002)			0.23-0.86	0.93-7.0		0.46-0.96	
Samples		Tocopherols (mg/100g dry weight)					
		alpha	beta	gamma	delta	total	
HCEM485	Mean		1.336	0.112	3.260	0.135	4.843
	95%CI		(1.23-1.44)	(0.11-0.12)	(2.72-3.80)	(0.11-0.16)	(4.37-5.32)
Control hybrids	Mean		1.543	0.119	3.724	0.165	5.551
	95%CI		(1.40-1.68)	(0.12-0.12)	(3.22-4.23)	(0.14-0.19)	(5.14-5.96)
Mean Difference (%)			-13.38%	-5.90%	-12.46%	-18.19%	-12.75%
F-test genotype			<0.001*	0.001*	0.001*	<0.001*	<0.001*
F-test genotype x location			0.028**	0.251	0.789	0.565	0.628
Literature Values							
ILSI (2006)	Mean		1.03	0.701	2.948	0.206	4.040
	Range		0.15-6.87	0.058-2.28	0.646-6.1	0.038-1.61	0.869-13.3

* = indicates that the difference between the genotypes means was statistically significant at the 95% confidence level.

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

Vitamin A is reported as β-carotene. Identity of B vitamins is as follows: B1=thiamine; B2=riboflavin; B3=niacin; B5=pantothenic acid; B6=pyridoxine; B9=folic acid.

95%CI = computed 95% confidence interval around the mean value.

Where some of the sample values were less than the limit of quantification (<LOQ) statistical comparison was not possible, so only the range is shown. Values for riboflavin (B2) and β-cryptoxanthine were <LOQ for all samples and not included in this analysis.]

V.8.4 Amino Acids

The quality of protein produced by different maize hybrids can be determined by measuring the content of different amino acids. Eighteen amino acids commonly found in maize are considered to be important for compositional analysis (EuropaBio, 2003). Levels of methionine and cysteine are important for formulation of animal feed, as are lysine and tryptophan, which cannot be produced by non-ruminant animals such as swine and poultry and are present at low concentrations in maize.

Comparison of the amino acid composition of HCEM485 grain and the control grain is shown in Table 17. The only significant difference was in mean methionine content between HCEM485 and control samples, however, this difference was not consistent across all growing locations.

Average levels of all amino acids, including methionine, were within the ranges reported in the literature.

[Table 17: Amino acid composition of grain from HCEM485 and control hybrids.

Samples		Concentration (mg/g dry weight)								
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
HCEM485	Mean	7.10	3.40	4.63	21.47	9.55	3.70	7.92	2.22	4.97
	95%CI	(6.31-7.90)	(3.10-3.70)	(4.10-5.16)	(18.6-24.3)	(8.36-10.7)	(3.43-3.98)	(6.91-8.92)	(2.00-2.44)	(4.43-5.51)
Control hybrids	Mean	7.13	3.41	4.74	21.87	9.73	3.72	8.11	2.31	5.00
	95%CI	(6.41-7.85)	(3.13-3.69)	(4.21-5.27)	(19.2-24.5)	(8.63-10.8)	(3.45-4.00)	(7.13-9.09)	(2.05-2.56)	(4.50-5.50)
Mean Difference (%)		-0.32%	-0.41%	-2.24%	-1.82%	-1.85%	-0.63%	-2.41%	-3.78%	-0.58%
F-test genotype		0.836	0.742	0.200	0.319	0.267	0.720	0.146	0.477	0.684
F-test genotype x location		0.454	0.711	0.594	0.522	0.676	0.614	0.529	0.384	0.590
Literature Values										
GA21†	Mean	6.60	3.80	5.40	19.40	8.80	3.70	7.70	2.10	4.50
ILSI (2006)	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
OECD (2002)	Range	4.8-8.5	2.7-5.8	3.5-9.1	12.5-25.8	6.3-13.6	2.6-4.9	5.6-10.4	0.8-3.2	2.1-8.5
Samples		Met	He	Leu	Tyr	Phe	Lys	His	Arg	Trp
HCEM485	Mean	2.49	3.38	12.68	1.55	4.62	3.15	2.89	3.79	0.74
	95%CI	(2.26-2.72)	(2.98-3.79)	(10.88-14.47)	(1.41-1.68)	(4.04-5.20)	(2.85-3.46)	(2.62-3.15)	(3.42-4.16)	(0.68-0.79)
Control hybrids	Mean	2.36	3.46	13.07	1.48	4.74	3.14	2.88	3.83	0.73
	95%CI	(2.18-2.54)	3.08-3.84	(11.39-14.75)	(1.37-1.59)	(4.21-5.28)	(2.84-3.44)	(2.66-3.10)	(3.48-4.17)	(0.68-0.78)
Mean Difference (%)		5.30%	-2.08%	-3.01%	4.64%	-2.57%	0.28%	0.20%	-0.92%	1.31%
F-test genotype		0.011*	0.175	0.102	0.140	0.192	0.892	0.890	0.672	0.358
F-test genotype x location		0.013**	0.673	0.710	0.584	0.802	0.387	0.426	0.572	0.597
Literature Values										
GA21†	Mean	2.00	3.50	13.20	4.00	5.10	2.80	7.70	4.00	0.60
ILSI (2006)	Mean	2.09	3.68	13.41	3.36	5.25	3.15	2.96	4.33	0.63
	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
OECD (2002)	Range	1.0-4.6	2.2-7.1	7.9-24.1	1.2-7.9	2.9-6.4	1.5-3.8	0.5-5.5	2.2-6.4	0.4-1.3

* = indicates that the difference between the genotypes means was statistically significant at (p < 0.05).

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

Asp=aspartic acid; Thr=threonine; Ser=serine; Glu=glutamic acid; Pro=proline; Gly=glycine; Ala=alanine; Cys=cysteine; Val=valine;

Met=methionine; Ile=isoleucine; Leu=leucine; Tyr=tyrosine; Phe=phenylalanine; His=histidine; Lys=lysine; Arg=arginine; Trp=tryptophan.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.]

V.8.5 Fatty Acids

Five fatty acids account for nearly 98 percent of the total fatty acids in maize grain (ILSI, 2006), with the most abundant being linoleic (C18:2 Δ9,12; 57.6%) and oleic (C18:1 Δ9; 26.0%) acids. Less abundant, but occurring at measurable levels are palmitic (C16:0; 11.03%), stearic (C18:0; 1.8%) and α-linolenic (C18:3 Δ9,12,15; 1.13%) acids.

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The desaturation of oleic acid to form linoleic acid, and its subsequent desaturation to form α -linolenic acid, occurs only in plants, hence both linoleic and α -linolenic acids are essential fatty acids for mammals. For this reason, it was desirable to measure for any unintended changes in the levels of linoleic and α -linolenic acids, and their key precursors, palmitic, stearic and oleic acids, in grain from HCEM485.

Other polyunsaturated and longer chain polyunsaturated fatty acids, such as γ -linolenic (C18:3 Δ 6,9,12), eicosatrienoic (C20:3 Δ 8,11,14) and arachidonic (C20:4 Δ 5,8,11,14) acids can all be synthesized by mammals from dietary sources of α -linolenic and linoleic acid. Hence, small changes in the levels of these trace fatty acids in HCEM485-derived grain would have little or no biological significance to either humans or animals consuming HCEM485 grain products. The synthesis of palmitoleic (C16:1 Δ 9) and saturated fatty acids with chain lengths greater than 18 (*e.g.*, C20:0, C22:0, C24:0), can be accomplished in mammals through *de novo* fatty acid synthesis without dietary requirements for palmitic and stearic acids, respectively.

The complete fatty acid profile of maize grain from HCEM485 and control hybrids was determined and the results are summarized in Table 18. The concentrations of the following fatty acids were below the limit of quantification in one or more samples and not included in the analysis: caprylic (C8:0); capric (C10:0); lauric (C12:0); myristic (C14:0); myristoleic (C14:1); pentadecanoic (C15:0); pentadecenoic (C15:1); palmitoleic (C16:1); heptadecanoic (C17:0); heptadecenoic (C17:1); gamma-linolenic (C18:3); eicosadienoic (C20:2); arachidonic (C20:4); eicosatrienoic (C20:3); behenic (C22:0); and erucic (C22:1). Statistically significant differences observed for quantifiable fatty acids were for palmitic (C16:0), stearic (C18:0), oleic (C18:1), linolenic (C18:3) and eicosenoic (C20:1), however, the magnitude of these differences was small, ranging between *ca.* 1% and 4.4%. Average levels of all quantifiable fatty acids, including those where significant differences were observed, were within the ranges reported in the literature.

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[Table 18: Fatty acid composition of grain from HCEM485 and control hybrids.

Samples		Amount (% total fatty acids)							
		Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)	Eicosenoic (C20:1)	Lignoceric (C24:0)
HCEM485	Mean	9.79	1.87	24.85	61.35	1.06	0.43	0.25	0.22
	95%CI	(9.7-9.9)	(1.8-1.9)	(24.2-25.5)	(60.5-62.2)	(1.03-1.10)	(0.43-0.44)	(0.25-0.26)	(0.22-0.23)
Control hybrids	Mean	9.69	1.79	25.42	60.87	1.09	0.43	0.26	0.23
	95%CI	(9.5-9.8)	(1.7-1.9)	(25.0-25.8)	(60.3-61.5)	(1.06-1.12)	(0.4-0.4)	(0.26-0.27)	(0.22-0.24)
Mean Difference (%)		1.06%	4.38%	-2.22%	0.80%	-2.40%	0.61%	-3.31%	-2.87%
F-test genotype		0.017*	0.001*	0.01*	0.079	0.008*	0.488	0.01*	0.267
F-test genotype x location		0.292	0.145	0.046**	0.124	0.537	0.685	0.111	0.052
Literature Values									
GA21†	Mean	9.90	1.80	27.1	59.1	1.1	0.40	0.30	ND
ILSI (2006)	Mean	11.5	1.82	25.8	57.6	1.2	0.41	0.3	0.17
	Range	7.94-20.71	1.02-3.40	17.4-40.2	36.2-66.5	0.57-2.25	0.28-0.97	0.17-1.92	0.140-0.230

* = indicates that the difference between the genotypes means was statistically significant at p < 0.05.

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

The concentrations of the following fatty acids were below the limit of quantification (<LOQ) in one or more samples and were not subject to statistical analysis: caprylic (C8:0); capric (C10:0); lauric (C12:0); myristic (C14:0); myristoleic (C14:1); pentadecanoic (C15:0); pentadecenoic (C15:1); palmitoleic (C16:1); heptadecanoic (C17:0); heptadecenoic (C17:1); gamma-linolenic (C18:3); eicosadienoic (C20:2); eicosatrienoic (C20:3); arachidonic (C20:4); behenic (C22:0) and erucic (C22:1).

ND = Not determined.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.]

V.8.6 Secondary Metabolites and Antinutrients

Secondary metabolites are defined as those natural products which do not function directly in the primary biochemical activities that support growth, development and reproduction of the organism in which they occur (EuropaBio, 2003). One class of secondary metabolites, antinutrients, is responsible for deleterious effects related to the absorption of nutrients and micronutrients from foods (Shahidi, 1997). There are generally no recognized antinutrients in maize at levels considered to be harmful, but for the purposes of safety assessment OECD recommends testing for the following secondary metabolites in maize: ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor. These secondary metabolites and antinutrients were analyzed in grain samples from HCEM485 and control hybrids (Table 19).

Phenolic acids — may have beneficial health effects because of their anti-oxidant properties. Ferulic acid and p-coumaric acid are weak anti-oxidants. In vitro tests are equivocal as to whether ferulic acid enhances or inhibits the effects of mutagenic substances (Sasaki *et al.*, 1989; Stich, 1992). Ferulic acid and p-coumaric acid are found in vegetables, fruit and cereals. They are also used as flavoring in foods, as supplements and in traditional Chinese herbal medicine. Daily intake of phenolic acids by humans is estimated to be 0.2–5.2 mg/day (Clifford, 1999; Radtke *et al.*, 1998).

There were no significant differences in mean ferulic acid or p-coumaric acid between grain samples from HCEM485 and control hybrids (Table 19).

[Table 19: Secondary metabolites and antinutrients in grain from HCEM485 and control hybrids.

Samples		Concentration (mg/100g)					Trypsin inhibitor (TIU/mg)
		Ferulic acid	ρ -Coumaric acid	Inositol	Phytic acid	Raffinose	
HCEM485	Mean	222.82	16.52	12.00	800.57	207.73	4.31
	95%CI	(208.7-237.0)	(14.0-19.1)	(10.8-13.2)	(749.9-851.2)	(184.3-231.1)	(4.2-4.4)
Control hybrids	Mean	219.81	16.85	13.85	782.17	205.32	4.17
	95%CI	(212.9-226.7)	(14.9-18.8)	(13.2-14.5)	(747.8-816.5)	(174.0-236.6)	(4.0-4.3)
Mean Difference (%)		1.37%	-1.95%	-13.36%	2.35%	1.17%	3.38%
F-test genotype		0.679	0.598	0.001*	0.533	0.731	0.011*
F-test genotype x location		0.775	0.644	0.139	0.295	0.419	0.012**
Literature Values							
ILSI (2006)	Mean	220.1	21.8	133.2	745	132	2.73
	Range	29.2-388.6	5.34-57.6	8.9-376.5	111-1570	20-320	1.09-7.18
OECD (2002)		20-300	3-30		450-1000	210-310	

* = indicates that the difference between the genotypes means was statistically significant at $p < 0.05$.

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

Levels of furfural in all samples were <LOQ and were not included in this analysis.]

Furfural — is a heterocyclic aldehyde which occurs in several vegetables, fruits and cereals. It is used as a pesticide, but also in foodstuff as flavoring. Furfural is generally recognized as safe (GRAS) under conditions of intended use as a flavor ingredient. Field maize generally contains < 0.01 ppm (< 0.001 mg/100g) furfural (Adams *et al.*, 1997). Furfural was below the lower limit of quantification in all grain samples (Table 19).

Phytic acid — (myo-inositol 1,2,3,4,5,6-hexakis[dihydrogenphosphate]) is considered to be an antinutrient due to its ability to bind minerals, proteins and starch at physiological pH (Rickard and Thompson, 1997). Phytic acid is present in maize germ and binds 60–75 percent of phosphorous in the form of phytate (NRC, 1998), decreasing the bioavailability of phosphorous in maize for non-ruminant animals. Phytic acid levels in maize grain vary from 0.45–1.0 percent of dry matter (Watson, 1982).

There was no significant difference in mean phytic acid level between grain samples from HCEM485 or control hybrids, although average inositol levels were significantly lower (*ca.* -13%) in HCEM485 grain samples (Table 19). In both cases, the average values were well within the ranges reported in the literature for these two analytes.

Alpha-galactosides — of sucrose, including raffinose, are widely distributed in higher plants (Naczki *et al.*, 1997). Due to the absence of alpha-galactosidase activity in human and animal mucosa, raffinose cannot be broken down by enzymes in the gastrointestinal tract and is considered an antinutrient, although it is not toxic. No statistically significant differences were detected in raffinose levels between the HCEM485 and control grain samples and all values were within ranges reported in the literature (Table 19).

Protease inhibitors — are found in abundance in raw cereals and legumes, especially soybeans. Trypsin inhibitors in soybean give rise to inactivation and loss of trypsin in the small

intestine, triggering the induction of excess trypsin in the pancreas at the expense of sulfur-containing amino acids (Shahidi, 1997). Maize contains low levels of trypsin and chymotrypsin inhibitors, neither of which is considered nutritionally significant (White and Pollak, 1995). A small, but statistically significant increase (*ca.* 3.4%) in mean trypsin inhibitor activity was observed from HCEM485 grain samples compared with control samples (Table 19), but this difference was not consistent across all growing locations and levels of trypsin inhibitor for all samples were within the range reported in the literature.

V.8.7 *Phytosterols*

Phytosterols are cholesterol-like molecules found in all plant foods, with the highest concentrations occurring in vegetable oils. They are absorbed only in trace amounts but have the beneficial effect of inhibiting the absorption of dietary cholesterol (Ostlund, 2002). Phytosterols are not endogenously synthesized in the body but are derived solely from the diet (Rao and Koratkar, 1997).

There were no significant differences in mean levels of cholesterol, campesterol, stigmasterol, β -sitosterol, stigmastanol, or total phytosterols between grain samples from HCEM485 or control hybrids (Table 20). Since phytosterols are not commonly included in compositional analyses of *Z. mays*, there no ranges for these given in the OECD consensus document for maize (OECD, 2002). One reference (Ryan *et al*, 2007) gives a single value (mean +/- standard error of three independent extractions on the same sample) and so does not provide a range to determine biological significance of the different values seen in the control maize hybrids.

[Table 20: Phytosterol composition of grain from HCEM485 and control hybrids.

Samples		Concentration (mg/100g)					Total
		Cholesterol	Campesterol	Stigmasterol	β -sitosterol	Stigmastanol	
HCEM485	Mean	0.232	9.376	2.961	54.412	10.879	77.860
	95%CI	(0.21-0.26)	(8.5-10.2)	(2.7-3.2)	(53.1-55.7)	(10.3-11.4)	(76.1-79.6)
Control hybrids	Mean	0.234	9.508	3.099	55.692	10.739	79.273
	95%CI	(0.23-0.24)	(8.7-10.3)	(2.9-3.3)	(54.0-57.4)	(10.11-11.4)	(77.2-81.3)
Mean Difference (%)		-1.11%	-1.39%	-4.44%	-2.30%	1.30%	-1.78%
F-test genotype		0.870	0.591	0.134	0.170	0.513	0.307
F-test genotype x location		0.598	0.470	0.424	0.544	0.761	0.575
Literature Values							
Ryan <i>et al.</i> (2007)			9.1+/-0.5	0.4+/-0.0	34.1+/-1.1		

95%CI = computed 95% confidence interval around the mean value.]

V.8.8 *Nutritional Impact*

Compositional analysis is the cornerstone of the nutritional assessment of a food derived from a new plant variety. When compositional equivalence between the new food and its conventional counterpart has been established, the results of numerous published livestock feeding trials with genetically modified varieties of maize, soybean, canola, cotton, or sugar beet, have confirmed no significant differences in digestibility of nutrients, animal health or animal performance (Flachowsky *et al.*, 2005). Therefore, once compositional and phenotypic equivalence

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has been established, nutritional equivalence may be assumed, and livestock feeding trials add little to the safety assessment (OECD, 2003; EFSA, 2006). As the HCEM485-derived hybrid was determined to be compositionally equivalent to control parental lines, additional livestock feeding studies are not necessary.

V.9 CONCLUSIONS

The agronomic performance and phenotypic data generated for the HCEM485-derived hybrid and control hybrids suggest that the genetic modification resulting in maize line HCEM485 did not have any biologically significant unintended effect on plant growth habit and general morphology, vegetative vigor, flowering and pollination, grain yield, grain test weight, disease susceptibility, or pollen morphology. These data support the conclusion that HCEM485-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids, and would not display higher rates of out crossing than unmodified maize. With this conclusion, similarity to GA21, the antecedent organism, is demonstrated.

Levels of key nutrients, minerals, antinutrients, and secondary metabolites were determined in samples of maize grain and forage derived from HCEM485 and control hybrids collected from up to four field trial locations in 2007. For most analyses, there were no statistically significant differences and in cases where statistically significant differences were observed, the magnitudes of the differences were small and in every case, mean values determined for both HCEM485 and control samples were within the ranges of natural variation as reported in the literature. Overall, no consistent patterns emerged to suggest that biologically significant changes in composition of the grain or forage had occurred as an unintended consequence of the genetic modification resulting in maize line HCEM485. The conclusion based on these data was that grain and forage from HCEM485 maize were substantially equivalent in composition to both the control hybrids included in this study, to other commercial maize hybrids and - by extension - to the antecedent organism.

**VI. ENVIRONMENTAL IMPACT OF INTRODUCTION OF MAIZE
LINE HCEM485**

There are no changes from Section VI of the previously approved petition 97-099-01p in terms of the description of glyphosate herbicide, current uses of maize herbicides, weediness potential of glyphosate tolerant maize, cross pollination to wild and cultivated related species and transfer of genetic material to species to which maize cannot interbreed (*e.g.*, horizontal gene transfer). There is no expectation that cultivation of maize line HCEM485 would have any environmental effects different from the cultivation of the antecedent organism, GA21, or other maize lines exhibiting glyphosate tolerance that have also been deregulated by USDA-APHIS (*e.g.*, NK603; MON 88017; and MON 802).

VII. ADVERSE CONSEQUENCES OF INTRODUCTION

Stine Seed Farm knows of no study results or observations associated with maize line HCEM485 that would be anticipated to result in adverse environmental consequences from its introduction. Therefore, on the basis of the substantial phenotypic equivalence between maize line HCEM485 and the antecedent organism, GA21, Stine Seed Farm requests that an extension of nonregulated status be granted to maize line HCEM485.

VIII. REFERENCES

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IX. APPENDIX 1: HCEM NUCLEOTIDE SEQUENCE

[

LOCUS HCEM 6010 bp DNA linear
 DEFINITION *Zea mays* fragment containing EPSPS encoding sequences, following site-directed mutagenesis
 ACCESSION Not-Specified.
 VERSION Not-Specified.
 KEYWORDS
 SOURCE *Zea mays*
 ORGANISM

FEATURES
 source Location/Qualifiers
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 5' UTR 1..1867
 /note="epsps regulatory sequences"
 transit_peptide 1868..2041
 /gene="CTP"
 CDS 1868..2167
 /gene="epsps exon 1"
 CDS 2696..2944
 /gene="epsps exon 2"
 CDS 3044..3193
 /gene="epsps exon 3"
 CDS 3467..3679
 /gene="epsps exon 4"
 CDS 4189..4302
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 CDS 4463..4675
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 3' UTR 5147..6010

BASE COUNT 1581 a 1375 c 1288 g 1766 t
 ORIGIN

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121 ctctcccctc ggttcgtttg acatttggtg tggagtgact aacctgctaa caccctgcaa
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241 cacaggacac gcacaggaca cgcaaacagt ttcagactca tgcacacgca catcagtttc
301 agactcaggc acacgcacat caaatcacct tcgcttgctg atgagtcgca gccgcacgt
361 acaatggcga ttttaccgac gataaggcat gggagcacga gccgtcgccg tcgcttgctg
421 agacgacggg agcgatctct cccttcattt aatctcttcc acgtcagggt attttgctga
481 gatggcagta tacagacggc aaagttaatg ccggtgtaca tgccttaga ctctccgctc
541 accaactcac ttagattttt acaacggaac ataaggttcg cttgcagact tacatataag
601 gtatagttgc ataataatcg ccttatgctg tacattgcca caccgtaaa tattcgatga
661 aatattagta cacaatatta aataagaacg aacaatacat atattatcat tgatcttagt
721 atctcctttt gctcctcgta gaacaattct gtgtaaatga tgcgtaaaa tcgaggacca
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841 ttttcttgct tataaaagtt tccaaaagta ccattttgga tgaaaaaacg gaaaacaacg
901 ctggtctact tgtaaaattg gtatgtgacat ttgggaccgt ctagacacga cctaaaaata
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6001 cgttgatatc  
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**X. APPENDIX 2: USDA APHIS RELEASE NOTIFICATIONS
RELEVANT TO THE FIELD TESTING OF MAIZE LINE HCEM485**

USDA #	Internal ID	County and State of Release
05-060-09n	SSF2005-006	Dallas, IA; Marshall, IA; Madison, IA; Lincoln, IL; Warren, IL; Vermillion, IL; Tipton, IN; Hamilton, IN; Boone, IN; Blue Earth, MN; Clinton, MO; Saline, MO; Valley, NE; Dodge, NE; Paulding, OH.
06-047-09n	SSF2006-001	Dallas, IA; Cass, IA; Logan, IL; Vermillion, IL; Tipton, IN; Hamilton, IN; Boone, IN; Lincoln, SD; Renville, MN; Valley, NE; Arkansas, AR; Paulding, OH.
07-046-110n	SSF2007-003	Dallas, IA; Tipton, IN; Hamilton, IN; Vermillion, IL; Logan, IL; Defiance, OH; Shiawassee, MI; Renville, MN; Kandiyohi, MN; Crittenden, AR; Lincoln, SD; Cedar, NE; Cass, IA; Warren, IL.
08-046-109n	SSF2008-003	Dallas, IA.

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Annex 1

Agronomic analysis of maize line HCEM485

Laboratory Study ID: SSF-07-323

STUDY TITLE

Agronomic Analysis of Maize Line HCEM485

LABORATORY STUDY ID

SSF-07-323

STUDY COMPLETED ON

30 November 2007

PERFORMING FACILITY

**Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003
USA**

SUBMITTED BY

**Stine Seed Farm Inc.
22555 Laredo Trail
Adel, Iowa 50003
USA**

Summary

Agronomic and phenotypic characteristics of a HCEM485 maize hybrid and three control hybrids were evaluated in a series of field trials across 15 United States Corn Belt locations in 2007. HCEM485 maize produces a form of the maize 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme that was specifically modified through site-directed mutagenesis to confer tolerance to glyphosate-containing herbicides.

Up to 17 separate agronomic characteristics were assessed at each location, but not all traits were assessed at all locations. These agronomic traits covered a broad range of characteristics encompassing the entire life cycle of the maize plant and included data assessing germination and seedling emergence, growth habit, vegetative vigor, days to pollen shed, days to maturity, and yield parameters

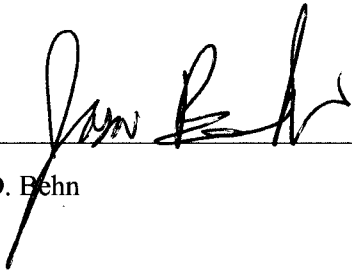
Results of these trials suggest that there were no biologically significant unintended effects on plant growth habit and general morphology, vegetative vigor, flowering and pollination, grain yield, grain test weight, or disease susceptibility as a result of the genetic modification introduced into maize line HCEM485. These data support the conclusion that HCEM485-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids, and would not display higher rates of outcrossing than unmodified maize.

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Statement of Good Laboratory Practices

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

PRINCIPAL INVESTIGATORS:



J.D. Behn

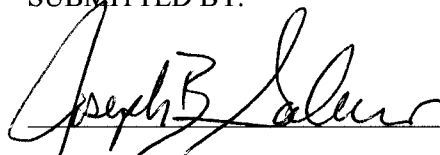
2-24-09
Date



J.T. Mason

2/24/09
Date

SUBMITTED BY:



Joseph B. Safuri
Vice President

Feb. 24, 2009
Date

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11. INTRODUCTION

Maize line HCEM485 was developed by Stine Seed Farm to incorporate the trait of tolerance to glyphosate-containing herbicides. The line was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene (Held *et al.*, 2006). The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-resistant form of the native maize EPSP synthase. Except for the introduced mutations, the amino acid sequence of the double-mutated maize EPSPS (2mEPSPS) enzyme expressed in maize line HCEM485 is identical to the native wild-type maize EPSPS sequence reported by Gardiner *et al.* 2004. Maize line HCEM485 does not contain any heterologous DNA sequences, either coding or non-coding, from any other species.

Small field trials were conducted in the United States during the 2007 growing season to compare an HCEM485-derived hybrid with three conventional hybrids derived from parental inbred lines used in the development of the HCEM485 hybrid. Grain yield and other agronomic and phenotypic measurements were compared in the HCEM485-derived hybrid and concurrently grown control hybrids. The results of these trials are summarized in this report.

12. MATERIALS AND METHODS

12.1 PLANT MATERIAL

Agronomic equivalence trials were conducted using the following hybrid lines:

HCEM485 hybrid	(((HCEM485)2/9289/9032)3/5056) [trait positive]
Control hybrid	9289x5056 [trait negative]
Control hybrid	9032x5056 [trait negative]
Control hybrid	963x5056 [trait negative]

A pedigree map showing the derivation of the HCEM485 hybrid is shown in Figure 1. The control hybrids were produced by crossing the inbred lines Stine 963, 9289 or 9032, each of which were used as parental lines to produce HCEM485, with inbred line 5056, which was also used in creating the HCEM485 hybrid.

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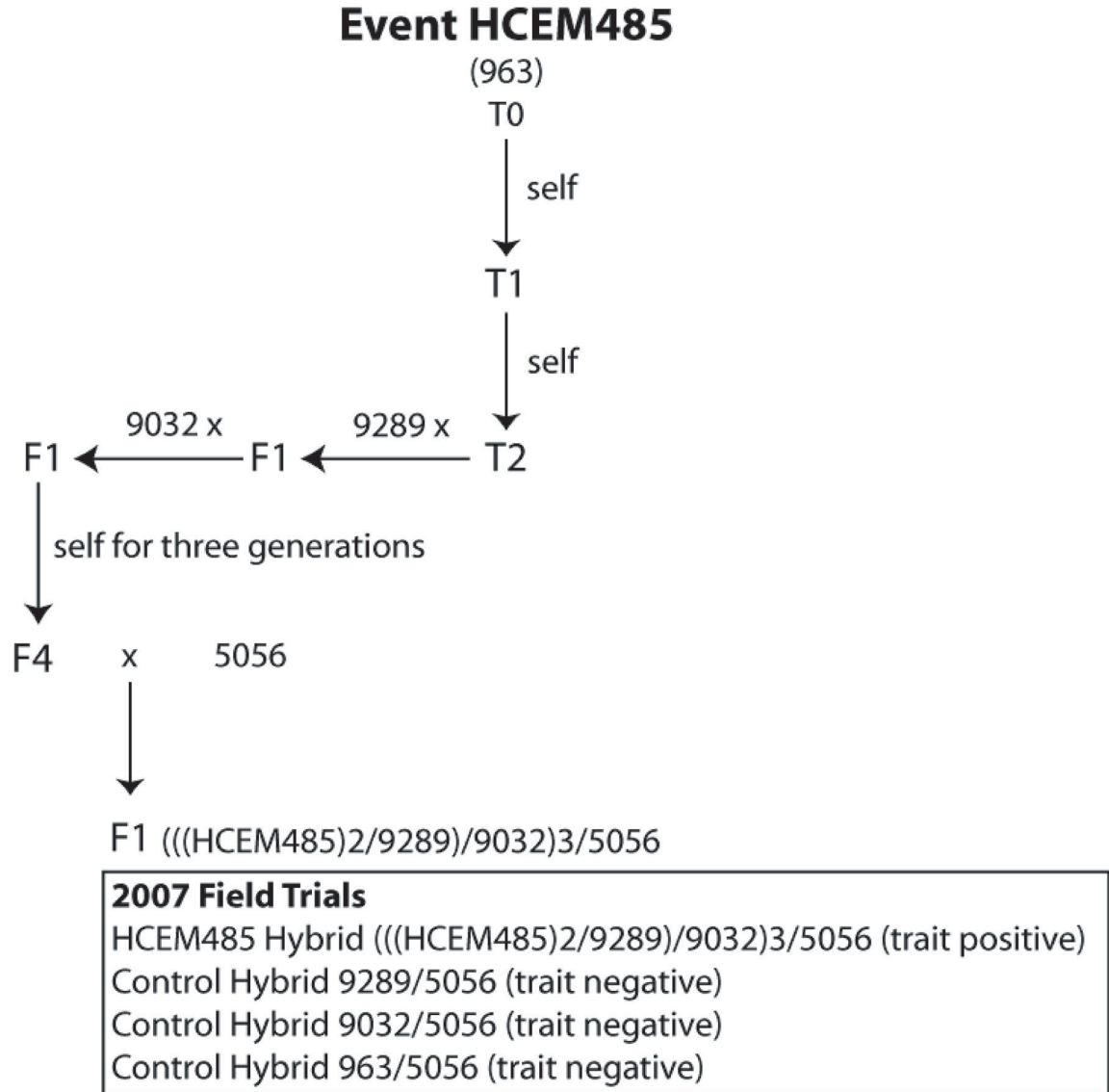


Figure 1: Pedigree chart of HCEM485 seed used in 2007 agronomic trials.

12.2 TRIAL LOCATIONS

Field trials were conducted at 15 locations in 2007 under USDA notification # 07-046-110n. These 15 locations covered nine states in the United States Corn Belt (Table 1) and were selected to represent a range of diverse growing environments where HCEM485 maize hybrids are expected to be commercially grown. Field husbandry at all of the trial sites (including irrigation use, fertilization rate, and pest control methods) was consistent with best agronomic practices in the area. Agronomic practices for all genotypes within a trial at a single location were identical.

All seed and grain material for these trials was packaged and shipped in accordance with Stine Seed Farm guidelines described in Standard Operating Procedure (SOP) No. 07-116-01. This includes appropriate sanitized primary, secondary and tertiary containers and inclusion of all

necessary and appropriate documentation with each shipment. All seed and grain material for these trials was stored according to Stine Seed Farm SOP No. 07-116-02. After each trial location was harvested, residual plant material was destroyed according to Stine Seed Farm guidelines described in SOP No. 07-116-04.

Table 1: Trial locations and dates.

Location Code	City	State	Planting Date	Harvest Date
ADL1	Adel	IA	5-Jun-2007	NA
ADL2	Adel	IA	5-Jun-2007	17-Oct-2007
ATL	Atlantic	IA	6-Jun-2007	23-Oct-2007
LAU	Laurel	NE	7-Jun-2007	24-Oct-2007
LEN	Lennox	SD	7-Jun-2007	25-Oct-2007
SMI	Smithshire	IL	7-Jun-2007	NA
MAR	Marion	AR	5-Jun-2007	20-Oct-2007
EDM	Edmondson	AR	6-Jun-2007	20-Oct-2007
BLO	Blomkest	MN	5-Jun-2007	26-Oct-2007
BIR	Bird Island	MN	5-Jun-2007	26-Oct-2007
FIT	Fithian	IL	7-Jun-2007	15-Oct-2007
LIN	Lincoln	IL	4-Jun-2007	15-Oct-2007
DUR	Durand	MI	11-Jun-2007	16-Oct-2007
SHE	Sheridan	IN	4-Jun-2007	9-Oct-2007
OH	Spencerville	OH	12-Jun-2007	12-Oct-2007

NA = not applicable. Trial was destroyed prior to harvest.

12.3 AGRONOMIC TRAITS ASSESSED

Up to 17 separate agronomic characteristics were assessed at each location, but not all traits were assessed at all locations. These agronomic traits covered a broad range of characteristics encompassing the entire life cycle of the maize plant and included data assessing germination and seedling emergence, growth habit, vegetative vigor, days to pollen shed, days to maturity, and yield parameters (Table 2).

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Table 2: List and definitions of agronomic traits assessed in the field trials.

Abbreviation	Trait	Timing	Description
BRRNP	Percent Barren Plants	Harvest	Percent of plants per plot that do not develop an ear.
DROPP	Percent Dropped Ears	Harvest	Percent of plants per plot that have dropped a developed ear prior to harvest.
EAGRR	Early Growth Rating	V6	Early growth rating recorded at V6 on a scale of 1–9, with 9=most vigorous growth.
EMRGP	Early Stand Count	V3	Percent of sowed kernels that resulted in emerged plants within 14 days after planting.
EMRGR	Seedling Vigor	V3	Early emergence vigor rating. Data collected prior to V3 stage of corn development. Rated on a 0-9 scale, where 0=dead and 9=most vigorous growth.
ERHTN	Ear Height	After anthesis	Ear height from base of plant to node where ear connects to plant (cm). Taken at R2-R6 stage of corn development.
ERTLP	Early Root Lodging		A 1-9 rating where a higher score indicates less root lodging potential (1 is very poor, 5 is intermediate, and 9 is very good, respectively, for resistance to root lodging).
GMSTP	Grain Moisture Percent	Harvest	Percent grain moisture measured at harvest.
HAVPN	Final Stand Count	Harvest	Harvest population (plants per acre).
HUP5N	Heat units to 50% pollen shed	Flowering (anthesis)	Heat units to 50% of plants shedding pollen.
HUS5N	Heat units to 50% silking	Flowering (anthesis)	Heat units to 50% of plants extruding silks.
LFCLR	Leaf Color Rating	After anthesis	Leaf color rating taken between R4 and R6 stage of corn development. 5=same as commercial check. 1=darker, 9=severely chlorotic.
PLHTN	Plant Height	After anthesis	Plant height from base of plant to collar of flag leaf (cm). Taken between R2 and R6 stage of corn development.
RTLDR	Root Lodging Rating	Harvest	A 1-9 rating where a higher score indicates less root lodging potential (1 is very poor, 5 is intermediate, and 9 is very good, respectively, for resistance to root lodging).
STKLR	Stalk Lodging Rating	Harvest	This is a 1-9 rating where a higher score indicates less stalk lodging potential (1 is very poor, 5 is intermediate, and 9 is very good, respectively, for resistance to stalk lodging).
TWSMN	Test Weight	Harvest	Grain test weight (pounds/bushel) converted to standard 15% moisture.
YGSMN	Grain Yield	Harvest	Grain yield (bushels/acre) converted to standard 15% grain moisture.

12.4 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Each of the agronomic trials utilized a randomized complete block design with three replications per location. Plot size was *ca.* 0.002 acres, using 2-row plots, 17.5 feet long with 30 inches between the rows. Each plot was planted to contain approximately 62 plants.

Data from each of the three control hybrid lines were treated as a single treatment group, identified as control hybrids, in comparisons with the HCEM485 hybrid. Data for the variates (traits) were subjected to an analysis of variance across locations using the generalized linear model:

$$Y_{ij} = U + T_i + L_j + LT_{ij} + e_{ij}$$

where Y_{ij} is the observed response for genotype i at location j , U is the overall mean, T_i is the treatment (HCEM485 vs. control genotypes) effect, L_j is the location effect, LT_{ij} is the location x treatment (genotype) interaction effect and e_{ij} is the residual error. For each variate, the statistical significance of the genotype effect (*i.e.*, HCEM485 vs. control hybrids) was determined using a standard F-test. An F-test probability of < 0.05 indicates that the difference between the genotypes was statistically significant with 95% confidence. An F-test was also used to assess the significance of the location x genotype interaction – a significant outcome (F-test probability < 0.05) indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations may not be meaningful.

The data for several variates did not lend themselves to formal statistical analysis because they did not conform to the assumptions upon which the validity of the analysis depends. In some cases, the problem was that the data were too discrete, with values taking one of a very limited range of options. In other cases the dataset contained too few non-zero data points on which to base a reasonable estimate of residual error. Consequently, results for such variates are presented as means. Hybrid-by-location means are included in Appendix B and the individual plot data are included in Appendix C.

13. RESULTS

13.1 GROWTH HABIT

In addition to the agronomic characteristics discussed in other sections below, the following parameters are discussed here as indicators of basic morphology and growth habit: ERTLRL (early root lodging rating); STKLR (stalk lodging rating); RTLDR (late season root lodging rating); and LFCLR (leaf color rating). None of these variates were suitable for formal statistical analysis and are presented as a summary of genotype means (Table 3). Overall, there were no remarkable differences indicative of an alteration in plant growth habit.

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Table 3: Comparison of growth habit characteristics of HCEM485 and control hybrids.

	ERTLR^a (1–9 rating)	STKLR (1–9 rating)	RTLDR (1–9 rating)	LFCLR (1–9 rating)
HCEM485 hybrid	8.71	8.17	8.55	4.93
Control hybrids	8.69	8.15	8.56	4.91
Mean Difference	-0.02	-0.02	0.01	-0.02
N ^b	15	14	14	15

a. ERTLRL = early root lodging rating; STKLR = stalk lodging rating; RTLDR = late root lodging rating; LFCLR = leaf color rating.

b. N = number of locations with data.

13.2 VEGETATIVE VIGOR

Comparisons of vegetative vigor between HCEM485-derived and control hybrids were based on assessments of: EMRGR (seedling vigor); EAGRR (early growth rating); ERHTN (ear height); and PLHTN (plant height). The only variates suitable for statistical analysis were ERHTN and PLHTN, both of which showed small but statistically significant increases between HCEM485 and control hybrids (Table 4). The magnitudes of these increases were 2.3% and 2.7%, respectively. Ratings of seedling vigor and early growth were similar between HCEM485 and control hybrids.

Table 4: Comparison of vegetative growth characteristics of HCEM485 and control hybrids.

	EMRGR^a (0–9 rating)	EAGRR (1–9 rating)	ERHTN (cm)	PLHTN (cm)
HCEM485 hybrid	7.20	7.60	96.4 ± 16.3	238.1 ± 29.7
Control hybrids	7.46	7.54	94.2 ± 15.4	231.7 ± 31.1
Mean Difference	-0.26	0.06	2.2	6.4
F-test genotype			0.043*	<0.001*
F-test genotype x location			0.523	0.178
N ^b	15	15	15	15

* = indicates that the difference between the genotypes was statistically significant at the 95 percent confidence level (p < 0.05).

a. EMRGR = seedling vigor; EAGRR = early growth rating; ERHTN = ear height; PLHTN = plant height. Mean values are shown. For ERHTN and PLHTN, the mean standard deviation is indicated.

b. N = number of locations with data.

13.3 REPRODUCTIVE CHARACTERISTICS

The relevant field indicators of potential changes to seed dormancy, pollination or fertility were: EMRGP (percent of emerged plants); HUS5N (heat units to 50 percent silking; HUP5N (heat units to 50 percent pollen shed); and BRRNP (percent barren plants). For those variates suitable for statistical analysis, there were small but statistically significant decreases in both heat units to 50% silking (-2.5%) and heat units to 50% pollen shed (-2.0%) (Table 5). Although there was no significant difference in the percent germinated plants (EMRGP) between HCEM485 and control hybrids, the values for both these groups were lower than expected,

which was likely due to problems with greenhouse seed production for the trials. Data on percent barren plants (BRRNP) were not suitable to statistical analysis but mean values were not markedly different between HCEM485 and control hybrids.

Table 5: Comparison of reproductive characteristics of HCEM485 and control hybrids.

	EMRGP ^a (%)	HUS5N (heat units)	HUP5N (heat units)	BRRNP (%)
HCEM485 hybrid	49.0 ± 12.1	1358.0 ± 170.3	1467.7 ± 237.3	0.75
Control hybrids	48.4 ± 12.5	1393.5 ± 169.6	1498.4 ± 233.3	0.35
Mean Difference	0.6	-35.5	-30.7	0.4
F-test genotype	0.661	<0.001*	<0.001*	
F-test genotype x location	0.216	0.989	0.918	
N ^b	15	15	10	14

* = indicates that the difference between the genotypes was statistically significant at the 95 percent confidence level ($p < 0.05$).

a. EMRGP = percent emergence; HUS5N = heat units to 50% silking; HUP5N = heat units to 50% pollen shed; BRRNP = percent barren plants. Mean values are shown. For EMRGP, HUS5N and HUP5N, the mean standard deviation is indicated.

b. N = number of locations with data.

13.4 YIELD AND GRAIN CHARACTERISTICS

Parameters used to evaluate yield and grain characteristics included: YGSMN (grain yield); HAVPN (plant population at harvest); DROPP (percent dropped ears); TWSMN (grain test weight); and GMSTP (grain moisture percent). Among the variates suitable for statistical analysis, there were no significant differences in average yield, plant population at harvest, grain moisture, or grain test weight between HCEM485 and control hybrids (Table 6). For both yield and plant population at harvest, there were significant genotype x location interactions. Although not subject to statistical analysis, there were no remarkable differences in percent dropped ears between HCEM485 and control genotypes (Table 6).

Table 6: Comparison of yield and grain characteristics of HCEM485 and control hybrids.

	YGSMN ^a (bu/acre)	HAVPN (plants/acre)	DROPP (%)	GMSTP (%)	TWSMN (lb/bu)
HCEM485 hybrid	115.4 ± 51.2	14976 ± 3410	0.06	18.8 ± 7.7	55.2 ± 1.9
Control hybrids	113.9 ± 50.7	14888 ± 3622	0.04	18.3 ± 7.1	55.2 ± 2.3
Mean Difference	1.5	88	0.02	0.5	0.1
F-test genotype	0.621	0.818		0.051	0.731
F-test genotype x location	0.003**	0.040**		0.463	0.431
N ^b	13	14	14	13	13

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

a. YGSMN = grain yield; HAVPN = final stand count at harvest; DROPP = percent dropped ears; GMSTP = grain moisture percent; TWSMN = grain test weight. Mean values are shown. For YGSMN, HAVPN and GMSTP, the mean standard deviation is indicated.

b. N = number of locations with data.

13.5 DISEASE OBSERVATIONS

Natural disease infections were rated in trials where the disease incidence was sufficiently high to warrant assessment. Ratings were included for Southern rust disease (SRDI), gray leaf spot (GLSDR), Northern corn leaf blight (NCLBR), common rust (CMRR) and smut (SMTR). Although not suitable for statistical analysis, there were no remarkable differences in any of these ratings between HCEM485 and control hybrids (Table 7).

Table 7: Comparison of disease ratings for HCEM485 and control hybrids.

	SRDI^a (0-5 rating)	GLSDR (0-5 rating)	NCLBR (0-5 rating)	CMRR (0-5 rating)	SMTR (0-5 rating)
HCEM485 hybrid	0.93	1.21	0.22	1.00	1.00
Control hybrids	0.90	1.22	0.15	1.08	1.00
Mean Difference	0.03	-0.01	0.07	-0.08	0.0
N ^b	2	2	1	1	1

a. SRDI = Southern rust disease rating; GLSDR = gray leaf spot disease rating; NCLBR = northern corn leaf blight rating; CMRR = common rust rating; and SMTR = smut rating. All ratings were on a 0–5 scale: 0= no incidence; 1= 2–9%; 2= 10–24%; 3= 25–49%; 4= 50–74%; and 5= 75–100%.
b. N = number of locations with data.

14. CONCLUSIONS

The agronomic performance and phenotypic data generated for the HCEM485-derived hybrid and control hybrids suggest that the genetic modification resulting in maize line HCEM485 did not have any biologically significant unintended effect on plant growth habit and general morphology, vegetative vigor, flowering and pollination, grain yield, grain test weight or disease susceptibility. These data support the conclusion that HCEM485-derived hybrids are unlikely to form feral persistent populations, or to be more invasive or weedy than conventional maize hybrids, and would not display higher rates of outcrossing than unmodified maize.

15. RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Stine Seed Farm, Inc., 22555 Laredo Trail, Adel, Iowa 50003.

16. REFERENCES

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17. APPENDIX A: PLOTS OF HYBRID-BY-LOCATION MEANS

The following illustrations present mean values by location for HCEM485 and control hybrids for each of the parameters suitable for statistical analysis. In each of the illustrations, mean values of the respective parameter for HCEM485 are represented by open triangles while the corresponding mean values for the group of control hybrids are represented by open circles. The error bars represent the standard deviation around each mean value.

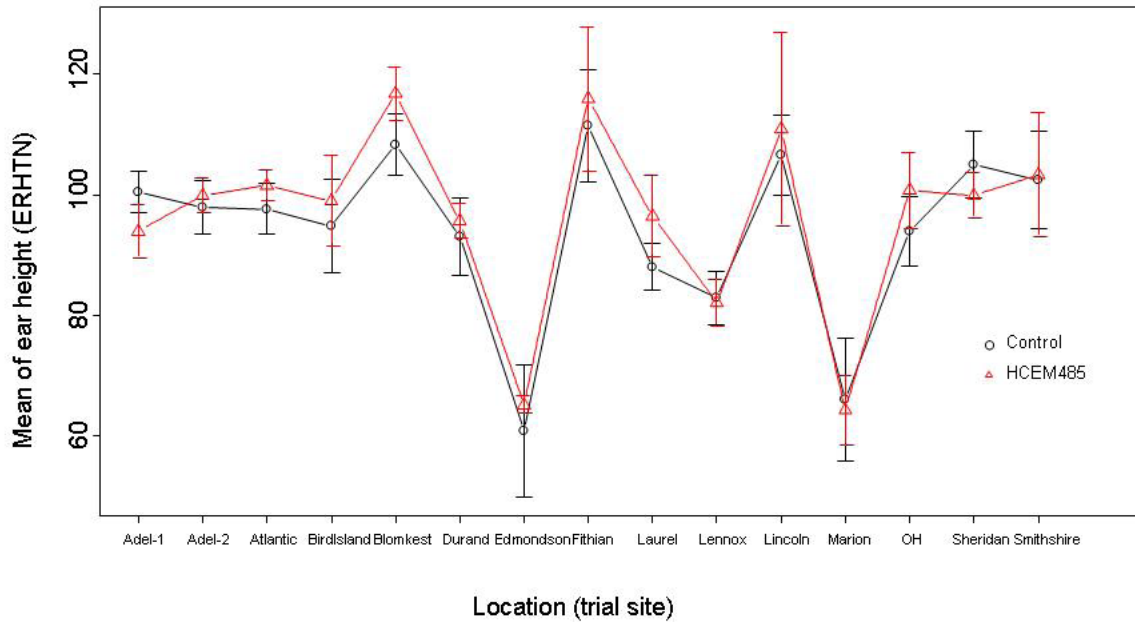


Illustration 1: Plot of means by location for plant ear height (ERHTN).

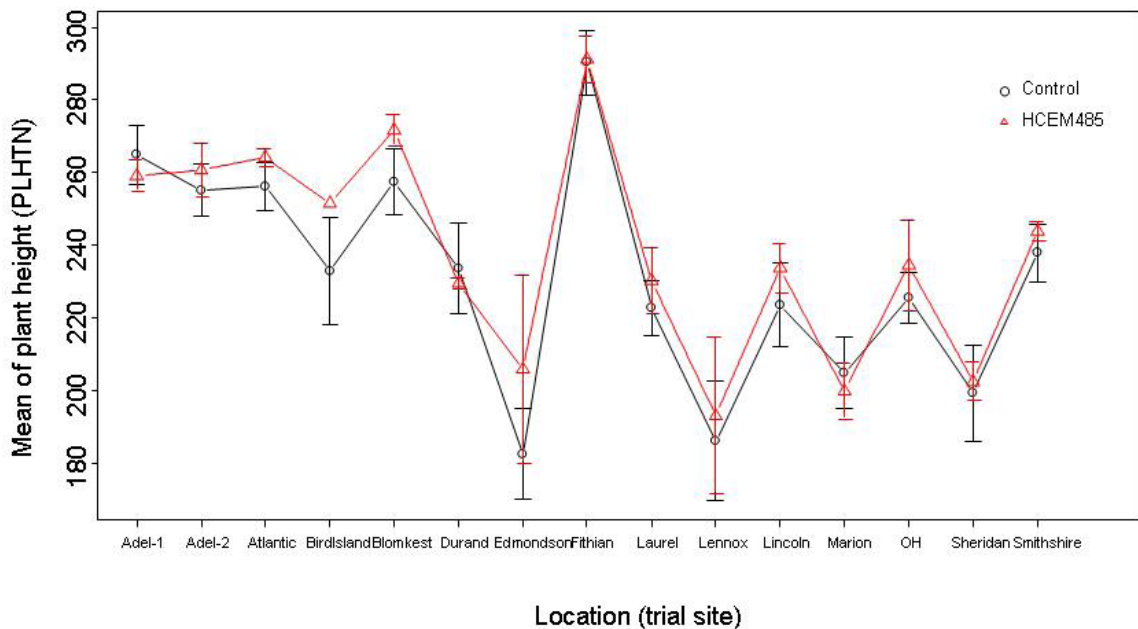


Illustration 2: Plot of means by location for plant height (PLHTN).

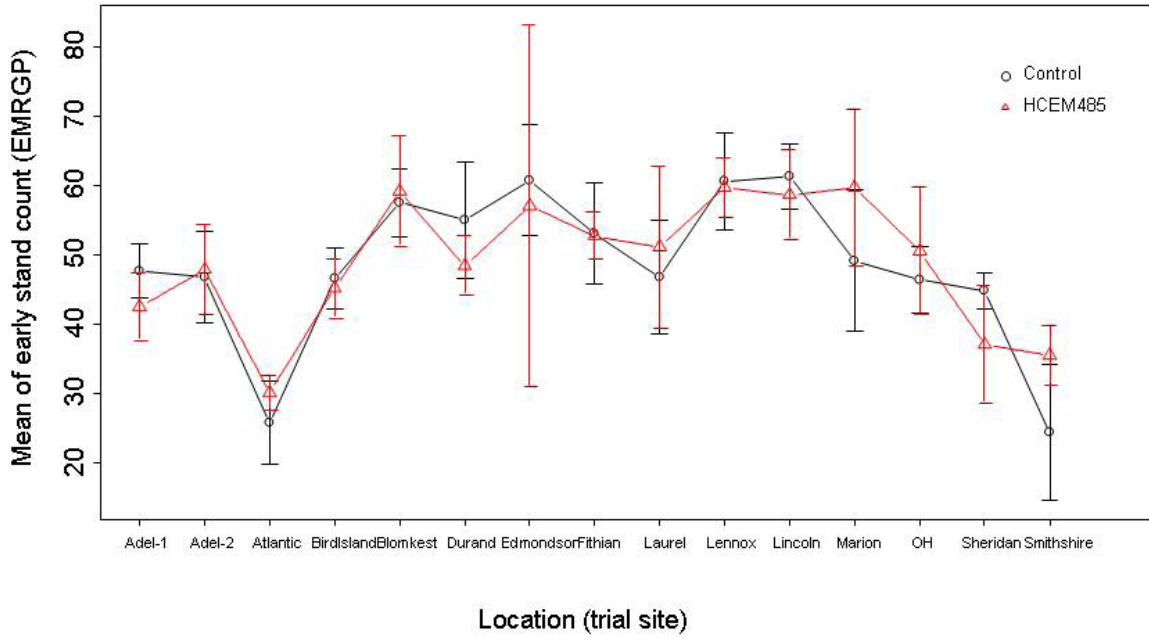


Illustration 3: Plot of means by location for early stand count (EMRGP).

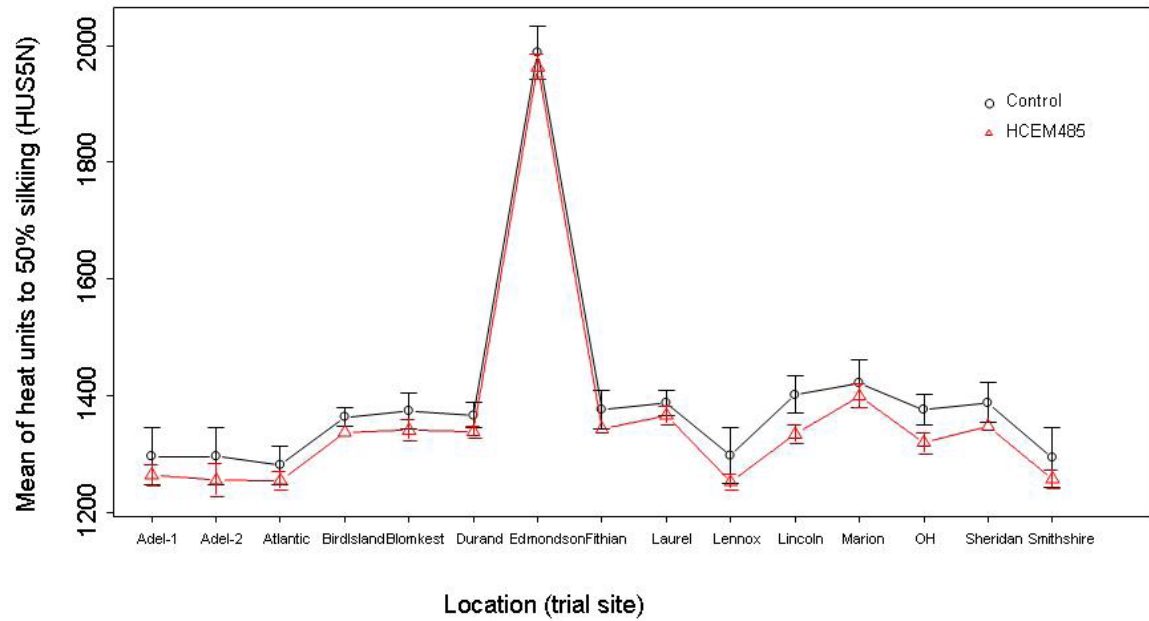


Illustration 4: Plot of means by location for heat units to 50% silking (HUS5N).

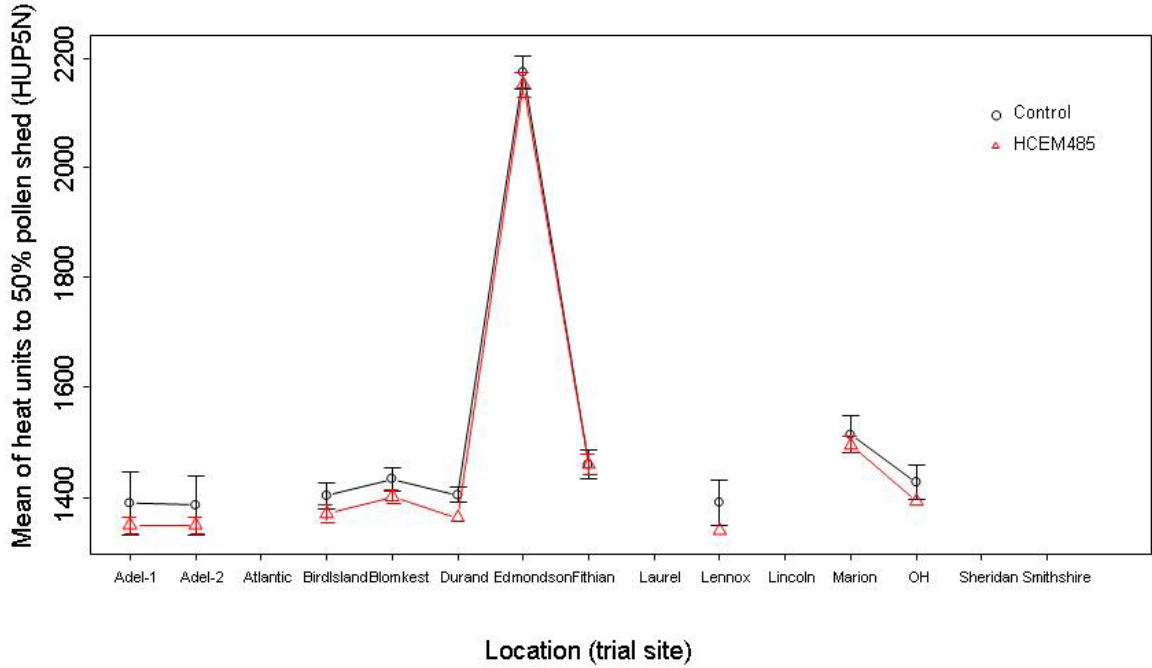


Illustration 5: Plot of means by location for heat units to 50% pollen shed (HUP5N).

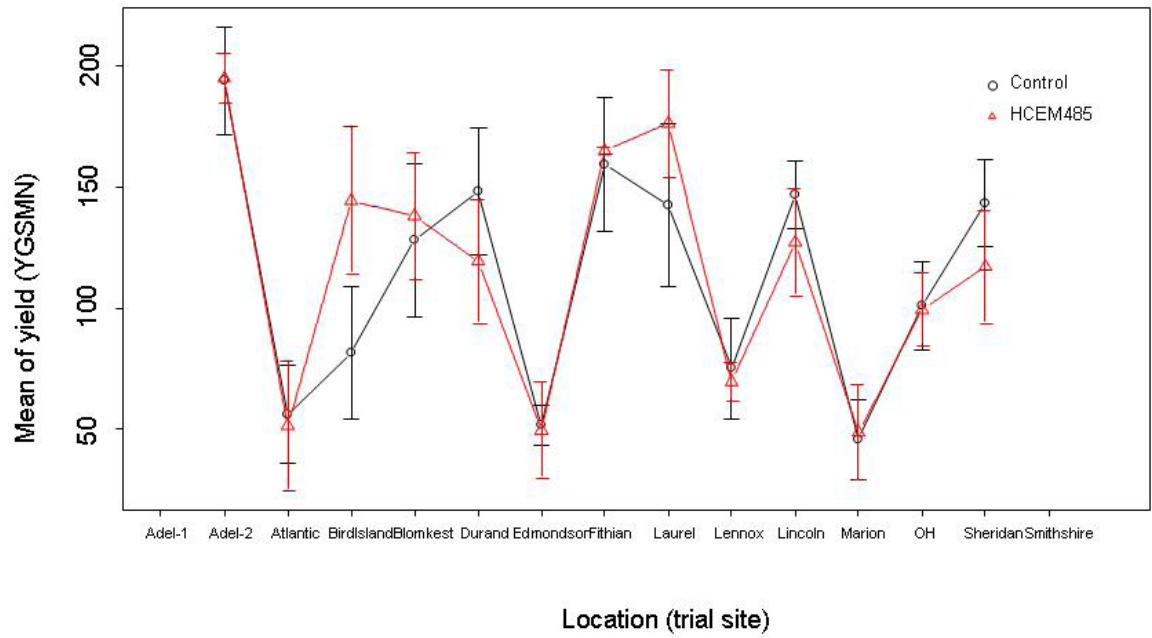


Illustration 6: Plot of means by location for yield (YGSMN).

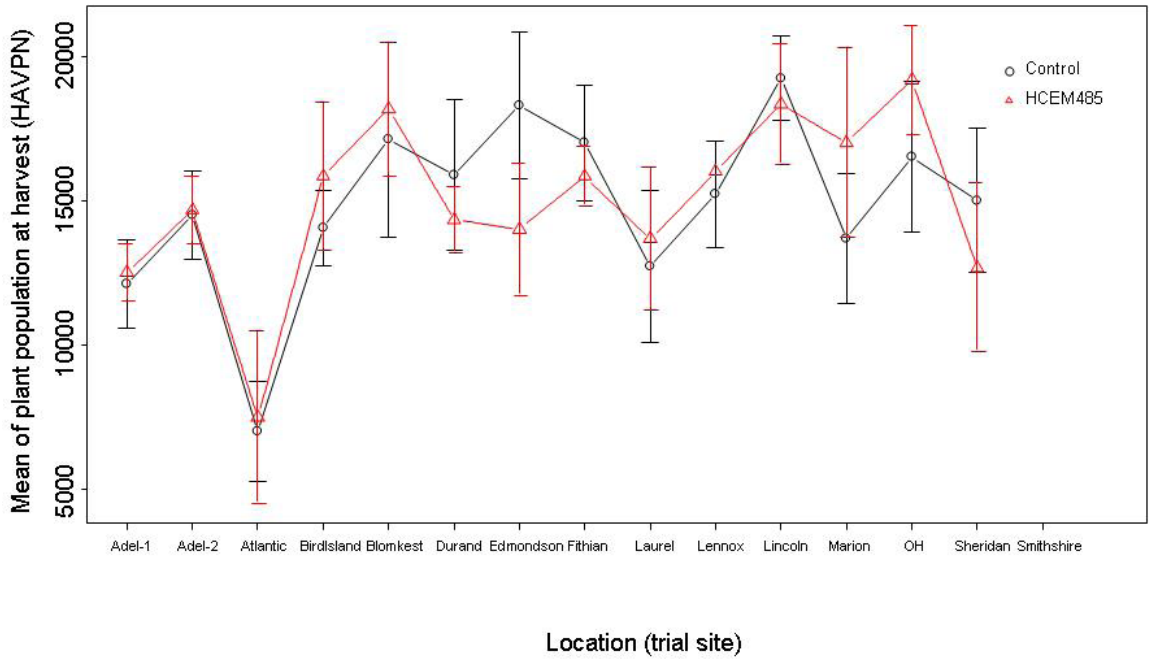


Illustration 7: Plot of means by location for plant population at harvest (HAVPN).

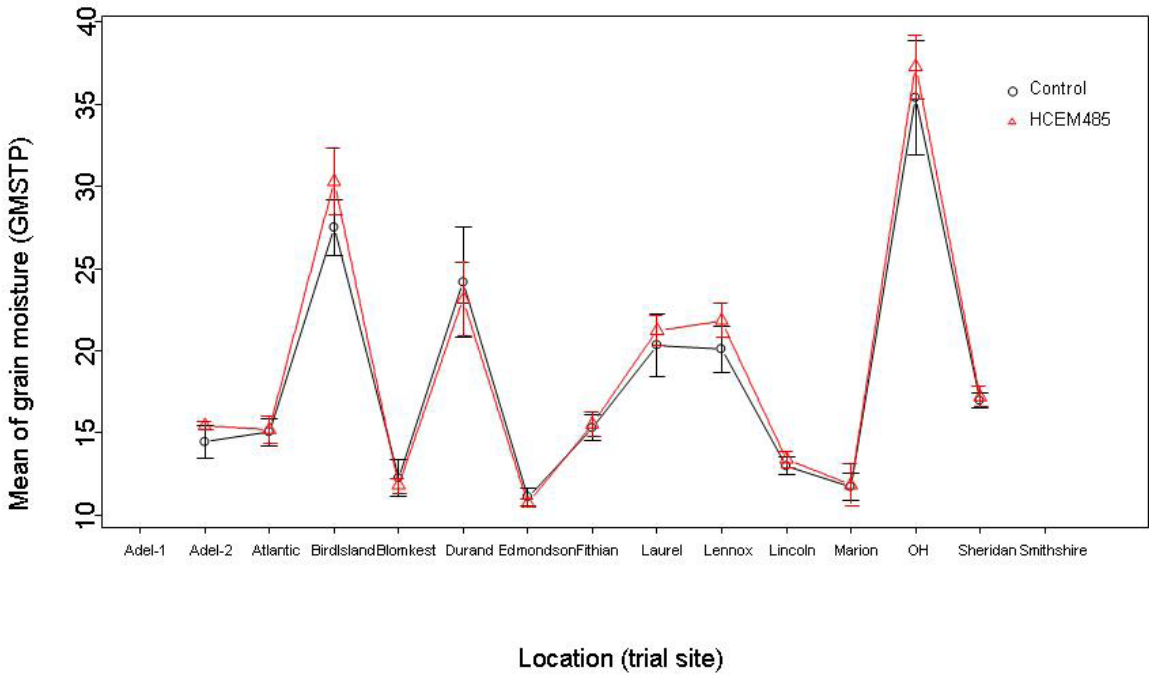


Illustration 8: Plot of means by location for grain moisture (GMSTP).

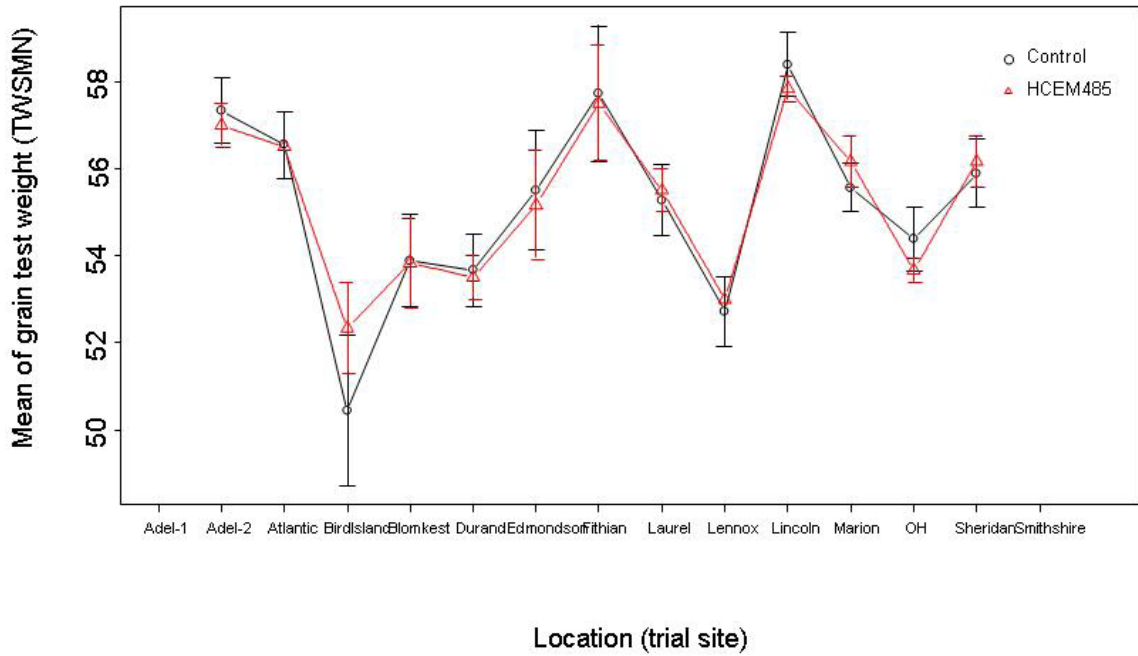


Illustration 9: Plot of means by location for grain test weight (TWSMN).

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18. APPENDIX B: HYBRID-BY-LOCATION MEANS

Location Code	Treatment	ERTLR (1-9 rating)	STKLR (1-9 rating)	RTLDR (1-9 rating)	LF CLR (1-9 rating)	EMRGR (1-9 rating)
ADL1	HCEM485 hybrid	9.0	6.3	9.0	4.7	7.0
	Control hybrids	9.0	6.7	9.0	5.0	7.7
	Mean Difference	0.0	-0.3	0.0	-0.3	-0.7
ADL2	HCEM485 hybrid	9.0	8.3	9.0	5.0	7.0
	Control hybrids	9.0	8.6	9.0	5.0	7.6
	Mean Difference	0.0	-0.2	0.0	0.0	-0.6
ATL	HCEM485 hybrid	9.0	9.0	9.0	5.0	7.3
	Control hybrids	9.0	9.0	9.0	5.0	7.1
	Mean Difference	0.0	0.0	0.0	0.0	0.2
BIR	HCEM485 hybrid	9.0	8.7	9.0	5.0	8.0
	Control hybrids	9.0	7.9	9.0	5.0	7.1
	Mean Difference	0.0	0.8	0.0	0.0	0.9
BLO	HCEM485 hybrid	9.0	9.0	9.0	5.0	8.0
	Control hybrids	9.0	9.0	9.0	5.0	7.8
	Mean Difference	0.0	0.0	0.0	0.0	0.2
DUR	HCEM485 hybrid	9.0	6.3	9.0	5.0	7.3
	Control hybrids	9.0	6.7	9.0	4.9	7.4
	Mean Difference	0.0	-0.3	0.0	0.1	-0.1
EDM	HCEM485 hybrid	9.0	8.0	9.0	5.0	6.7
	Control hybrids	9.0	8.2	9.0	5.3	7.6
	Mean Difference	0.0	-0.2	0.0	-0.3	-0.9
FIT	HCEM485 hybrid	9.0	6.7	9.0	5.3	7.0
	Control hybrids	9.0	6.9	9.0	5.2	7.9
	Mean Difference	0.0	-0.2	0.0	0.1	-0.9
LAU	HCEM485 hybrid	8.0	9.0	9.0	5.0	7.7
	Control hybrids	8.0	9.0	9.0	5.0	7.2
	Mean Difference	0.0	0.0	0.0	0.0	0.4
LEN	HCEM485 hybrid	5.7	9.0	2.7	5.0	7.0
	Control hybrids	5.3	9.0	2.9	4.9	7.7
	Mean Difference	0.3	0.0	-0.2	0.1	-0.7
LIN	HCEM485 hybrid	9.0	8.3	9.0	5.0	7.0
	Control hybrids	9.0	7.8	9.0	4.9	7.9
	Mean Difference	0.0	0.6	0.0	0.1	-0.9
MAR	HCEM485 hybrid	9.0	8.0	9.0	5.3	7.3
	Control hybrids	9.0	8.3	9.0	4.5	7.7
	Mean Difference	0.0	-0.3	0.0	0.8	-0.3
OH	HCEM485 hybrid	9.0	8.7	9.0	3.7	6.3
	Control hybrids	9.0	8.7	9.0	3.7	6.4
	Mean Difference	0.0	0.0	0.0	0.0	-0.1
SHE	HCEM485 hybrid	9.0	9.0	9.0	5.0	7.7
	Control hybrids	9.0	8.6	9.0	5.4	8.0
	Mean Difference	0.0	0.4	0.0	-0.4	-0.3
SMI	HCEM485 hybrid	9.0	NA	NA	5.0	6.7
	Control hybrids	9.0	NA	NA	4.8	6.9
	Mean Difference	0.0	NA	NA	0.2	-0.2

Location Code	Treatment	EAGRR (1-9 rating)	ERHTN (cm)	PLHTN (cm)	EMRGP (%)	HUSSN (heat units)
ADL1	HCEM485 hybrid	8.67	93.58	259.08	42.47	1264.00
	Control hybrids	8.11	100.47	265.01	47.67	1296.33
	Mean Difference	0.56	-6.49	-5.93	-5.20	-32.33
ADL2	HCEM485 hybrid	8.00	99.51	260.77	47.85	1255.00
	Control hybrids	8.33	97.53	255.13	46.77	1296.33
	Mean Difference	-0.33	1.98	5.54	1.08	-41.33
ATL	HCEM485 hybrid	7.33	101.60	264.16	37.11	1254.50
	Control hybrids	7.33	97.65	256.26	25.81	1281.56
	Mean Difference	0.00	3.95	7.90	4.30	-27.06
PIR	HCEM485 hybrid	6.33	99.06	251.46	45.16	1337.00
	Control hybrids	5.00	94.83	232.83	46.59	1363.11
	Mean Difference	1.33	4.23	18.63	-1.43	-26.11
PLO	HCEM485 hybrid	6.67	116.84	271.78	59.14	1340.83
	Control hybrids	6.11	108.37	257.39	57.53	1373.39
	Mean Difference	0.56	8.47	14.39	1.61	-32.56
DUR	HCEM485 hybrid	8.33	95.67	229.45	48.39	1337.83
	Control hybrids	8.67	93.13	233.68	55.02	1365.94
	Mean Difference	-0.33	2.54	-4.23	-6.63	-28.11
EDM	HCEM485 hybrid	6.67	65.19	205.74	56.99	1963.00
	Control hybrids	7.44	60.56	182.32	67.75	1988.00
	Mean Difference	-0.78	4.23	23.42	-3.76	-25.00
FIT	HCEM485 hybrid	7.67	115.99	291.25	52.69	1343.00
	Control hybrids	7.78	111.48	290.41	53.05	1375.89
	Mean Difference	-0.11	4.51	0.84	-0.36	-32.89
LAU	HCEM485 hybrid	8.00	96.52	230.29	51.08	1366.17
	Control hybrids	7.44	88.05	222.67	46.77	1387.06
	Mean Difference	0.56	8.47	7.62	4.30	-20.89
LEN	HCEM485 hybrid	7.67	82.13	193.04	59.68	1252.17
	Control hybrids	8.11	82.57	185.98	67.57	1297.83
	Mean Difference	-0.44	-0.85	7.06	-0.90	-45.67
LIN	HCEM485 hybrid	7.67	110.91	233.68	58.60	1334.00
	Control hybrids	8.00	106.68	223.52	61.29	1401.33
	Mean Difference	-0.33	4.23	10.16	-2.69	-67.33
MAR	HCEM485 hybrid	7.67	64.25	199.81	59.68	1399.17
	Control hybrids	7.75	66.04	204.79	49.10	1421.69
	Mean Difference	-0.08	-1.69	-4.97	13.57	-22.52
OH	HCEM485 hybrid	7.67	100.75	234.53	57.54	1319.33
	Control hybrids	7.44	93.58	225.50	46.42	1375.72
	Mean Difference	0.22	6.77	9.03	4.12	-56.39
SHE	HCEM485 hybrid	7.67	99.51	202.35	37.10	1347.50
	Control hybrids	8.11	104.99	199.25	44.80	1387.06
	Mean Difference	-0.44	-5.08	3.10	-7.71	-39.56
SMI	HCEM485 hybrid	8.00	103.29	243.84	35.48	1257.00
	Control hybrids	7.56	102.45	237.91	24.37	1294.11
	Mean Difference	0.44	0.85	5.93	11.11	-37.11

Location Code	Treatment	HUP5N (heat units)	BRRNP (%)	YGSMN (bu/acre)	HAVPN (plants/acre)	DROPP (%)
ADL1	HCEM485 hybrid	1350.00	0.00	NA	12500.0	0.00
	Control hybrids	1389.56	0.00	NA	12111.1	0.00
	Mean Difference	-39.56	0.00	NA	388.9	0.00
ADL2	HCEM485 hybrid	1350.00	0.00	194.98	14666.7	0.00
	Control hybrids	1386.33	0.00	193.97	14500.0	0.00
	Mean Difference	-36.33	0.00	1.01	166.7	0.00
ATL	HCEM485 hybrid	NA	0.00	51.20	7500.0	0.00
	Control hybrids	NA	0.00	55.82	7000.0	0.00
	Mean Difference	NA	0.00	-4.62	500.0	0.00
BIR	HCEM485 hybrid	1370.67	0.00	144.30	15833.3	0.00
	Control hybrids	1403.72	0.00	81.44	14055.6	0.00
	Mean Difference	-33.06	0.00	62.87	1777.8	0.00
BLO	HCEM485 hybrid	1401.67	0.00	137.99	18166.7	0.00
	Control hybrids	1433.67	0.00	128.18	17111.1	0.00
	Mean Difference	-32.00	0.00	9.81	1055.6	0.00
DUR	HCEM485 hybrid	1363.00	0.00	119.39	14333.3	0.00
	Control hybrids	1404.78	0.00	148.13	15888.9	0.00
	Mean Difference	-41.78	0.00	-28.74	-1555.6	0.00
EDM	HCEM485 hybrid	2151.83	0.00	49.26	14000.0	0.00
	Control hybrids	2173.89	0.00	51.47	18277.8	0.00
	Mean Difference	-22.06	0.00	-2.21	-4277.8	0.00
FIT	HCEM485 hybrid	1460.33	1.11	164.99	15833.3	0.00
	Control hybrids	1460.11	0.63	159.28	17000.0	0.00
	Mean Difference	0.22	0.49	5.71	-1166.7	0.00
LAU	HCEM485 hybrid	NA	0.00	176.32	13666.7	0.00
	Control hybrids	NA	0.00	142.52	12722.2	0.00
	Mean Difference	NA	0.00	33.80	944.4	0.00
LEN	HCEM485 hybrid	1340.50	0.00	69.46	16000.0	0.00
	Control hybrids	1390.89	0.00	75.07	15222.2	0.00
	Mean Difference	-50.39	0.00	-5.60	777.8	0.00
LIN	HCEM485 hybrid	NA	0.00	127.07	18333.3	0.88
	Control hybrids	NA	0.00	146.95	19222.2	0.60
	Mean Difference	NA	0.00	-19.88	-888.9	0.28
MAR	HCEM485 hybrid	1495.67	0.00	48.42	17000.0	0.00
	Control hybrids	1514.81	0.00	45.56	13687.5	0.00
	Mean Difference	-19.15	0.00	2.86	3312.5	0.00
OH	HCEM485 hybrid	1393.00	9.32	99.33	19166.7	0.00
	Control hybrids	1427.67	4.25	100.88	16500.0	0.00
	Mean Difference	-34.67	5.07	-1.55	2666.7	0.00
SHE	HCEM485 hybrid	NA	0.00	117.01	12666.7	0.00
	Control hybrids	NA	0.00	143.43	15000.0	0.00
	Mean Difference	NA	0.00	-26.43	-2333.3	0.00
SMI	HCEM485 hybrid	NA	NA	NA	NA	NA
	Control hybrids	NA	NA	NA	NA	NA
	Mean Difference	NA	NA	NA	NA	NA

Annex 1 - Agronomic analysis of maize.
 Laboratory Study ID: SSF-323
 Maize Line HCEM485
 USDA Extension Petition

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Location Code	Treatment	GMSTP (%)	TWSMN (lb/ha)
ADL1	HCEM485 hybrid	NA	NA
	Control hybrids	NA	0.00
	Mean Difference	NA	NA
ADL2	HCEM485 hybrid	15.43	57.00
	Control hybrids	14.44	57.33
	Mean Difference	0.99	-0.33
ATL	HCEM485 hybrid	15.20	56.50
	Control hybrids	15.06	56.56
	Mean Difference	0.14	-0.06
BIR	HCEM485 hybrid	30.30	52.33
	Control hybrids	27.50	50.44
	Mean Difference	2.80	1.89
BLO	HCEM485 hybrid	11.80	53.83
	Control hybrids	12.24	53.89
	Mean Difference	-0.44	-0.06
DUR	HCEM485 hybrid	23.13	53.50
	Control hybrids	24.17	53.67
	Mean Difference	-1.03	-0.17
EDM	HCEM485 hybrid	10.70	55.17
	Control hybrids	11.10	55.50
	Mean Difference	-0.40	-0.33
FIT	HCEM485 hybrid	15.53	57.50
	Control hybrids	15.30	57.72
	Mean Difference	0.23	-0.22
LAU	HCEM485 hybrid	21.23	55.50
	Control hybrids	20.32	55.28
	Mean Difference	0.91	0.22
LEN	HCEM485 hybrid	21.83	53.00
	Control hybrids	20.10	52.72
	Mean Difference	1.73	0.28
LIN	HCEM485 hybrid	13.40	57.83
	Control hybrids	12.99	58.39
	Mean Difference	0.41	-0.56
MAR	HCEM485 hybrid	11.83	56.17
	Control hybrids	11.71	55.56
	Mean Difference	0.12	0.60
OH	HCEM485 hybrid	37.30	53.67
	Control hybrids	35.40	54.39
	Mean Difference	1.90	-0.72
SHE	HCEM485 hybrid	17.20	56.17
	Control hybrids	16.98	55.89
	Mean Difference	0.22	0.28
SMI	HCEM485 hybrid	NA	NA
	Control hybrids	NA	NA
	Mean Difference	NA	NA



**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

19. APPENDIX C: INDIVIDUAL PLOT DATA

State	Location	Code	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP	TWS
IA	Adel-1	ADL1	1	HCEM485	1	4	9.00	7.00	9.00	4.00	7.00	8.00	99.06	264.16	46.77	29	1254.0	1341.0	0.0	NA	13500.0	0.0	NA	0.0
IA	Adel-1	ADL1	1	HCEM485	2	1	9.00	6.00	9.00	5.00	7.00	9.00	91.44	256.54	37.10	23	1284.0	1368.0	0.0	NA	11500.0	0.0	NA	0.0
IA	Adel-1	ADL1	1	HCEM485	3	3	9.00	6.33	9.00	4.67	7.00	8.67	93.98	259.08	42.47	26.33	1264.00	1350.00	0.00	NA	12500.00	0.00	NA	0.00
Mean							9.00	6.58	9.00	4.58	7.00	8.58	94.40	261.26	42.44	27.41	1280.67	1353.67	0.00	NA	12500.00	0.00	NA	0.00
Standard Deviation							0.00	0.58	0.00	0.58	0.00	0.58	4.40	4.40	4.93	3.06	17.32	15.59	0.00	NA	1000.00	0.00	NA	0.00
IA	Adel-1	ADL1	4	90325056	1	1	9.00	8.00	9.00	5.00	8.00	8.00	101.60	266.70	43.55	27	1312.0	1396.0	0.0	NA	13000.0	0.0	NA	0.0
IA	Adel-1	ADL1	5	9635056	1	2	9.00	7.00	9.00	5.00	7.00	8.00	104.14	269.24	48.39	30	1341.0	1451.0	0.0	NA	11000.0	0.0	NA	0.0
IA	Adel-1	ADL1	3	92885056	1	5	9.00	7.00	9.00	5.00	7.00	9.00	106.68	279.40	48.39	30	1227.0	1312.0	0.0	NA	14000.0	0.0	NA	0.0
IA	Adel-1	ADL1	4	90325056	2	2	9.00	6.00	9.00	5.00	8.00	8.00	96.52	266.70	46.77	29	1312.0	1396.0	0.0	NA	10000.0	0.0	NA	0.0
IA	Adel-1	ADL1	3	92885056	2	3	9.00	7.00	9.00	5.00	7.00	8.00	99.06	261.62	51.61	32	1254.0	1341.0	0.0	NA	12000.0	0.0	NA	0.0
IA	Adel-1	ADL1	5	9635056	2	4	9.00	7.00	9.00	5.00	8.00	8.00	101.60	256.54	43.55	27	1312.0	1396.0	0.0	NA	14000.0	0.0	NA	0.0
IA	Adel-1	ADL1	4	90325056	3	1	9.00	5.00	9.00	5.00	8.00	8.00	96.52	269.24	54.84	34	1341.0	1451.0	0.0	NA	10000.0	0.0	NA	0.0
IA	Adel-1	ADL1	3	92885056	3	4	9.00	7.00	9.00	5.00	8.00	8.00	99.06	251.46	43.55	27	1227.0	1312.0	0.0	NA	12000.0	0.0	NA	0.0
IA	Adel-1	ADL1	4	90325056	3	5	9.00	6.67	9.00	5.00	7.67	8.11	100.47	265.01	47.67	29.56	1296.33	1389.56	0.00	NA	12111.11	0.00	NA	0.00
Mean							9.00	6.87	9.00	5.00	7.67	8.33	99.39	263.83	46.45	29.41	1296.33	1389.56	0.00	NA	12111.11	0.00	NA	0.00
Standard Deviation							0.00	0.87	0.00	0.80	0.50	0.33	3.39	8.03	3.88	2.40	47.60	56.83	0.00	NA	1536.59	0.00	NA	0.00

State	Location	Code	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP	TWS
IA	Adel-2	ADL2	1	HCEM485	1	1	9.00	8.00	9.00	5.00	7.00	8.00	96.52	256.54	41.94	26	1227.0	1341.0	0.0	183.35	14000.0	0.0	15.50	57.500
IA	Adel-2	ADL2	1	HCEM485	2	5	9.00	8.00	9.00	5.00	7.00	8.00	101.60	269.24	46.77	29	1254.0	1341.0	0.0	199.99	14000.0	0.0	15.60	56.500
IA	Adel-2	ADL2	1	HCEM485	3	5	9.00	8.33	9.00	5.00	7.00	8.00	99.91	260.77	47.85	29.67	1255.00	1350.00	0.00	201.62	16000.0	0.00	15.20	57.000
Mean							9.00	8.33	9.00	5.00	7.00	8.00	99.91	260.77	47.85	29.67	1255.00	1350.00	0.00	194.98	14666.67	0.00	15.43	57.000
Standard Deviation							0.00	0.58	0.00	0.80	0.40	0.00	2.93	7.33	6.52	4.04	28.51	15.59	0.00	10.11	1154.70	0.00	0.21	0.50
IA	Adel-2	ADL2	3	92885056	1	3	9.00	8.00	9.00	5.00	7.00	7.00	99.06	254.00	35.48	22	1227.0	1312.0	0.0	165.25	11500.0	0.0	14.30	57.500
IA	Adel-2	ADL2	4	90325056	1	4	9.00	8.00	9.00	4.00	8.00	8.00	101.60	248.92	46.77	29	1341.0	1451.0	0.0	188.23	14500.0	0.0	14.50	57.000
IA	Adel-2	ADL2	5	9635056	1	5	9.00	9.00	9.00	5.00	7.00	9.00	96.52	256.54	48.39	30	1341.0	1422.0	0.0	208.99	15000.0	0.0	14.50	57.000
IA	Adel-2	ADL2	2	9635056	2	1	9.00	8.00	9.00	5.00	8.00	8.00	106.68	271.78	54.84	34	1341.0	1451.0	0.0	210.67	16000.0	0.0	11.90	58.000
IA	Adel-2	ADL2	3	92885056	2	2	9.00	9.00	9.00	5.00	7.00	8.00	93.98	259.08	43.55	27	1227.0	1312.0	0.0	187.63	14000.0	0.0	15.10	57.000
IA	Adel-2	ADL2	4	90325056	3	2	9.00	9.00	9.00	5.00	8.00	8.00	96.52	248.92	40.32	25	1312.0	1396.0	0.0	152.73	13000.0	0.0	14.40	58.000
IA	Adel-2	ADL2	4	90325056	3	2	9.00	9.00	9.00	6.00	8.00	9.00	96.52	251.46	45.16	28	1312.0	1396.0	0.0	210.89	14500.0	0.0	15.50	58.500
IA	Adel-2	ADL2	3	92885056	3	3	9.00	9.00	9.00	5.00	8.00	8.00	91.44	256.54	53.23	33	1254.0	1341.0	0.0	217.97	16000.0	0.0	14.90	57.000
IA	Adel-2	ADL2	5	9635056	3	4	9.00	8.56	9.00	5.00	7.56	8.33	97.93	255.13	46.77	29.00	1296.33	1386.33	0.00	193.97	16000.0	0.00	14.44	56.000
Mean							9.00	8.56	9.00	5.00	7.56	8.33	97.93	255.13	46.77	29.00	1296.33	1386.33	0.00	193.97	14500.00	0.00	14.44	57.33
Standard Deviation							0.00	0.53	0.00	0.50	0.53	0.71	4.42	7.31	6.45	4.00	47.60	53.64	0.00	22.51	1520.69	0.00	1.03	0.75

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMRGR	EAGRR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRRNP	YGSMN	HAVPN	DROPP	GMSTP
IA	Atlantic	ATL	1	HCEM485	1	1	9	9	9	5	8	7	99.06	264.16	32.26	20	1245.0	NA	0.0	79.23	10500.0	0.0	15.90
IA	Atlantic	ATL	2	HCEM485	2	2	9	9	9	5	7	7	104.14	266.70	30.65	19	1273.5	NA	0.0	48.75	7500.0	0.0	15.40
IA	Atlantic	ATL	4	HCEM485	3	4	9	9	9	5	7	8	101.60	261.62	27.42	17	1245.0	NA	0.0	25.63	4500.0	0.0	14.30
Mean							9.00	9.00	9.00	5.00	7.33	7.33	101.60	264.16	30.11	18.67	1254.50	NA	0.00	26.88	7500.00	0.00	15.20
Standard Deviation							0.00	0.00	0.00	0.58	0.58	0.58	2.54	2.54	2.46	1.53	16.45	NA	0.00	26.88	3000.00	0.00	0.82
IA	Atlantic	ATL	3	9289/5056	1	3	9	9	9	4	6	6	99.06	254.00	22.58	14	1245.0	NA	0.0	16.64	3500.0	0.0	14.90
IA	Atlantic	ATL	4	9032/5056	1	4	9	9	9	5	7	7	96.52	251.46	27.42	17	1273.5	NA	0.0	67.74	8000.0	0.0	15.30
IA	Atlantic	ATL	5	963/5056	1	5	9	9	9	6	7	7	96.52	256.54	25.81	16	1326.0	NA	0.0	43.30	6000.0	0.0	14.40
IA	Atlantic	ATL	5	963/5056	2	1	9	9	9	5	7	7	106.68	271.78	25.81	16	1300.0	NA	0.0	50.22	6500.0	0.0	14.80
IA	Atlantic	ATL	4	9032/5056	2	4	9	9	9	5	8	8	96.52	248.92	32.26	20	1273.5	NA	0.0	58.18	7500.0	0.0	13.70
IA	Atlantic	ATL	3	9289/5056	2	5	9	9	9	5	7	7	91.44	259.08	35.48	22	1245.0	NA	0.0	60.82	8500.0	0.0	16.00
IA	Atlantic	ATL	3	9289/5056	3	2	9	9	9	5	8	8	93.98	251.46	14.32	14	1245.0	NA	0.0	75.84	7500.0	0.0	16.10
IA	Atlantic	ATL	4	9032/5056	3	3	9	9	9	5	7	8	99.06	259.08	22.58	14	1300.0	NA	0.0	84.97	9500.0	0.0	15.80
IA	Atlantic	ATL	5	963/5056	3	5	9	9	9	5	7	8	99.06	254.00	25.81	16	1326.0	NA	0.0	44.63	6000.0	0.0	14.50
Mean							9.00	9.00	9.00	5.00	7.11	7.33	97.65	256.26	25.81	16.00	1281.56	NA	0.00	55.82	7000.00	0.00	15.06
Standard Deviation							0.00	0.00	0.00	0.50	0.60	0.71	4.23	6.77	5.98	3.71	33.11	NA	0.00	20.22	17500.00	0.00	0.81

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMRGR	EAGRR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRRNP	YGSMN	HAVPN	DROPP	GMSTP
MN	Bred/land	BIR	1	HCEM485	1	1	9	9	9	5	8	8	106.68	251.46	50.00	31	1337.0	1386.0	0.0	174.14	16500.0	0.0	28.10
MN	Bred/land	BIR	2	HCEM485	2	2	9	9	9	5	8	6	99.06	251.46	43.55	27	1337.0	1370.5	0.0	145.40	18000.0	0.0	32.10
MN	Bred/land	BIR	4	HCEM485	3	4	9	9	9	5	8	5	91.44	251.46	41.94	26	1337.0	1355.5	0.0	113.37	13000.0	0.0	30.70
Mean							9.00	8.67	9.00	5.00	8.00	6.33	99.06	251.46	45.16	28.00	1337.00	1370.67	0.00	144.30	15833.33	0.00	30.30
Standard Deviation							0.00	0.58	0.00	0.00	0.00	1.53	7.62	0.00	2.65	2.65	0.00	15.25	0.00	30.40	2565.80	0.00	2.03
MN	Bred/land	BIR	4	9032/5056	1	2	9	5	9	5	7	4	99.06	228.60	48.39	30	1370.5	1404.5	0.0	79.73	15000.0	0.0	26.40
MN	Bred/land	BIR	3	9289/5056	1	3	9	8	9	5	8	5	91.44	236.22	45.16	28	1355.5	1386.0	0.0	93.68	15000.0	0.0	29.30
MN	Bred/land	BIR	5	963/5056	1	4	9	7	9	5	7	6	99.06	243.84	51.61	32	1337.0	1432.0	0.0	110.63	14500.0	0.0	25.30
MN	Bred/land	BIR	3	9289/5056	2	1	9	9	9	5	7	5	91.44	236.22	48.39	30	1370.5	1386.0	0.0	107.05	16000.0	0.0	30.50
MN	Bred/land	BIR	5	963/5056	2	3	9	7	9	5	7	6	99.06	243.84	48.39	30	1370.5	1432.0	0.0	81.49	14500.0	0.0	26.70
MN	Bred/land	BIR	4	9032/5056	2	5	9	9	9	5	7	7	106.68	251.46	50.00	31	1386.0	1386.0	0.0	111.77	13000.0	0.0	28.60
MN	Bred/land	BIR	3	9289/5056	3	2	9	9	9	5	7	4	99.06	236.22	38.71	24	1337.0	1370.5	0.0	53.69	12000.0	0.0	26.30
MN	Bred/land	BIR	4	9032/5056	3	3	9	9	9	5	7	4	83.82	213.36	48.39	30	1370.5	1404.5	0.0	60.16	14000.0	0.0	26.80
MN	Bred/land	BIR	5	963/5056	3	5	9	9	9	5	7	4	83.82	205.74	40.32	25	1370.5	1432.0	0.0	34.71	12500.0	0.0	27.60
Mean							9.00	7.89	9.00	5.00	7.11	5.00	94.83	232.83	46.59	28.89	1363.11	1403.72	0.00	81.44	14655.56	0.00	27.50
Standard Deviation							0.00	1.36	0.00	0.00	0.33	1.12	7.73	14.81	4.38	2.71	16.65	23.57	0.00	27.36	1309.69	0.00	1.66

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGRR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
MIN	Blomkest	BLO	1	HCEM485	1	5	9	9	9	5	8	8	114.30	274.32	62.90	39	139.5	1409.0	0.0	155.73	19500.0	0.0	11.30
MIN	Blomkest	BLO	1	HCEM485	2	5	9	9	9	5	8	6	114.30	274.32	50.00	31	132.0	1409.0	0.0	150.41	15500.0	0.0	12.20
MIN	Blomkest	BLO	1	HCEM485	3	2	9	9	9	5	8	6	121.92	266.70	64.52	40	139.0	1387.0	0.0	107.84	19500.0	0.0	11.90
Mean							9.00	9.00	9.00	5.00	8.00	6.67	116.84	271.78	59.14	36.67	1340.83	1401.67	0.00	137.99	18166.67	0.00	11.80
Standard Deviation							0.00	0.00	0.00	0.00	0.00	1.15	4.40	4.40	7.96	4.93	17.54	12.70	0.00	26.25	2309.40	0.00	0.46
MIN	Blomkest	BLO	4	9032/5056	1	2	9	9	9	5	8	3	114.30	251.46	53.23	33	1387.0	1437.5	0.0	50.38	9000.0	0.0	13.30
MIN	Blomkest	BLO	3	9289/5056	1	3	9	9	9	5	8	6	106.68	266.70	56.45	35	1359.0	1409.0	0.0	120.19	19000.0	0.0	14.20
MIN	Blomkest	BLO	5	963/5056	1	4	9	9	9	5	8	7	99.06	243.84	61.29	38	1409.0	1463.0	0.0	138.20	19500.0	0.0	11.10
MIN	Blomkest	BLO	4	9032/5056	2	1	9	9	9	5	8	7	106.68	251.46	51.61	32	1359.0	1437.5	0.0	135.54	16000.0	0.0	11.40
MIN	Blomkest	BLO	3	9289/5056	2	2	9	9	9	5	7	6	106.68	266.70	58.06	36	1324.0	1409.0	0.0	140.23	18000.0	0.0	13.30
MIN	Blomkest	BLO	5	963/5056	2	3	9	9	9	5	7	6	106.68	251.46	51.61	32	1409.0	1437.5	0.0	118.45	16000.0	0.0	11.30
MIN	Blomkest	BLO	5	963/5056	3	3	9	9	9	5	8	7	106.68	251.46	58.06	36	1387.0	1463.0	0.0	141.88	18000.0	0.0	11.40
MIN	Blomkest	BLO	3	9289/5056	3	4	9	9	9	5	8	7	114.30	266.70	66.13	41	139.5	1437.5	0.0	152.33	20500.0	0.0	12.20
MIN	Blomkest	BLO	4	9032/5056	3	5	9	9	9	5	8	6	114.30	266.70	61.29	38	1387.0	1409.0	0.0	156.45	18000.0	0.0	12.00
Mean							9.00	9.00	9.00	5.00	7.78	6.11	108.37	257.39	57.53	35.67	1373.39	1433.67	0.00	128.18	17111.11	0.00	12.24
Standard Deviation							0.00	0.00	0.00	0.00	0.44	1.27	5.08	9.16	4.91	3.04	29.78	21.23	0.00	31.78	3379.88	0.00	1.10

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGRR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
MI	Durand	DUR	1	HCEM485	1	4	9	7	9	5	7	9	93.98	228.60	43.55	27	1326.5	1363.0	0.0	138.61	13000.0	0.0	24.00
MI	Durand	DUR	1	HCEM485	2	5	9	7	9	5	7	8	93.98	228.60	50.00	31	1343.5	1363.0	0.0	129.33	15000.0	0.0	24.80
MI	Durand	DUR	1	HCEM485	3	5	9	5	9	5	8	8	99.06	231.14	51.61	32	1343.5	1363.0	0.0	90.23	15000.0	0.0	20.60
Mean							9.00	6.33	9.00	5.00	7.33	8.33	95.67	229.45	48.39	30.00	1337.83	1363.00	0.00	119.39	14333.33	0.00	23.13
Standard Deviation							0.00	1.15	0.00	0.00	0.58	0.58	2.93	1.47	4.27	2.65	9.81	0.00	0.00	25.67	1154.70	0.00	2.23
MI	Durand	DUR	3	9289/5056	1	2	9	7	9	5	8	9	99.06	248.92	53.23	33	1343.5	1404.0	0.0	155.94	15000.0	0.0	25.60
MI	Durand	DUR	4	9032/5056	1	3	9	7	9	4	8	9	96.52	233.68	61.29	38	1363.0	1404.0	0.0	195.59	17000.0	0.0	29.40
MI	Durand	DUR	5	963/5056	1	5	9	8	9	5	8	9	91.44	223.52	62.90	39	1385.0	1419.0	0.0	115.28	18500.0	0.0	17.90
MI	Durand	DUR	4	9032/5056	2	1	9	6	9	4	7	9	96.52	228.60	43.55	27	1363.0	1404.0	0.0	125.06	13000.0	0.0	24.90
MI	Durand	DUR	3	9289/5056	2	2	9	6	9	5	7	8	91.44	248.92	61.29	38	1343.5	1385.0	0.0	146.23	17500.0	0.0	24.60
MI	Durand	DUR	5	963/5056	2	3	9	5	9	5	8	8	88.90	223.52	53.23	33	1385.0	1419.0	0.0	116.13	14500.0	0.0	21.20
MI	Durand	DUR	3	9289/5056	3	1	9	6	9	5	7	9	96.52	248.92	56.45	35	1343.5	1385.0	0.0	162.87	16500.0	0.0	24.90
MI	Durand	DUR	4	9032/5056	3	2	9	7	9	5	7	9	99.06	231.14	40.32	25	1363.0	1404.0	0.0	162.98	11500.0	0.0	27.00
MI	Durand	DUR	5	963/5056	3	4	9	7	9	6	7	8	78.74	215.90	62.90	39	1404.0	1419.0	0.0	153.06	19500.0	0.0	22.00
Mean							9.00	6.67	9.00	4.89	7.44	8.67	93.13	233.68	55.02	34.11	1365.94	1404.78	0.00	148.13	15888.89	0.00	24.17
Standard Deviation							0.00	1.00	0.00	0.60	0.53	0.50	6.48	12.51	8.36	5.18	21.54	13.19	0.00	26.02	2607.41	0.00	3.38

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGR	ERHIN	PLHIN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
AR	Edmondson	EDM	1	HCEM485	1	5	9	7	9	5	7	7	66.04	182.88	43.55	27	1950.5	2139.0	0.0	46.56	13500.0	0.0	10.40
AR	Edmondson	EDM	1	HCEM485	2	2	9	9	9	5	6	6	63.50	200.66	40.32	25	1988.0	2139.0	0.0	70.28	12000.0	0.0	10.90
AR	Edmondson	EDM	1	HCEM485	3	4	9	8	9	5	7	7	66.04	233.68	87.10	54	1950.5	2177.5	0.0	30.94	16500.0	0.0	10.80
Mean							9.00	8.00	9.00	5.00	6.67	6.67	65.19	205.74	56.99	35.33	1963.00	2151.83	0.00	49.26	14000.00	0.00	10.70
Standard Deviation							0.00	1.00	0.00	0.00	0.58	0.58	1.47	25.78	26.12	16.20	21.65	22.23	0.00	19.81	2291.29	0.00	0.26
AR	Edmondson	EDM	5	963/5056	1	1	9	8	9	6	8	8	73.66	195.58	64.52	40	2025.0	2177.5	0.0	64.39	19000.0	0.0	10.70
AR	Edmondson	EDM	3	9289/5056	1	2	9	8	9	5	7	7	53.34	177.80	56.45	35	1950.5	2139.0	0.0	55.10	17500.0	0.0	11.70
AR	Edmondson	EDM	4	9032/5056	1	3	9	8	9	5	8	8	50.80	165.10	61.29	38	1988.0	2177.5	0.0	49.21	17500.0	0.0	10.20
AR	Edmondson	EDM	5	963/5056	2	3	9	8	9	6	7	7	60.96	187.96	77.42	48	2062.5	2219.0	0.0	45.23	23000.0	0.0	11.20
AR	Edmondson	EDM	3	9289/5056	2	4	9	8	9	5	8	8	50.80	203.20	58.06	36	1950.5	2139.0	0.0	49.21	17500.0	0.0	11.60
AR	Edmondson	EDM	4	9032/5056	2	5	9	9	9	5	7	7	66.04	187.96	50.00	31	1988.0	2177.5	0.0	46.12	15000.0	0.0	10.80
AR	Edmondson	EDM	3	9289/5056	3	1	9	8	9	6	8	8	50.80	167.64	66.13	41	1914.5	2139.0	0.0	59.08	20000.0	0.0	11.90
AR	Edmondson	EDM	4	9032/5056	3	3	9	9	9	5	8	8	60.96	177.80	59.68	37	1988.0	2177.5	0.0	38.45	15000.0	0.0	11.00
AR	Edmondson	EDM	5	963/5056	3	5	9	8	9	5	7	7	81.28	177.80	53.23	33	2025.0	2219.0	0.0	56.43	20000.0	0.0	10.80
Mean							9.00	8.22	9.00	5.33	7.56	7.44	60.96	182.32	60.75	37.67	1988.00	2173.89	0.00	51.47	18277.78	0.00	11.10
Standard Deviation							0.00	0.44	0.00	0.50	0.53	0.53	11.00	12.49	8.06	5.00	45.42	31.17	0.00	7.98	2550.87	0.00	0.55

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGR	ERHIN	PLHIN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
IL	Fithian	FIT	1	HCEM485	1	5	9	6	9	5	7	7	106.68	284.48	50.00	31	1343.0	1470.5	0.0	166.54	15500.0	0.0	16.40
IL	Fithian	FIT	1	HCEM485	2	2	9	7	9	6	7	8	129.54	297.18	56.45	35	1343.0	1470.5	0.0	163.18	17000.0	0.0	15.20
IL	Fithian	FIT	1	HCEM485	3	4	9	7	9	5	7	8	111.76	292.10	51.61	32	1343.0	1440.0	3.3	165.24	15000.0	0.0	15.00
Mean							9.00	6.67	9.00	5.33	7.00	7.67	115.99	291.25	52.69	32.67	1343.00	1460.33	1.11	164.99	15833.33	0.00	15.53
Standard Deviation							0.00	0.58	0.00	0.58	0.00	0.58	12.00	6.39	3.36	2.08	0.00	17.61	1.92	1.70	1040.83	0.00	0.76
IL	Fithian	FIT	3	9289/5056	1	1	9	7	9	5	8	7	111.76	289.56	51.61	32	1343.0	1440.0	3.1	162.44	16000.0	0.0	15.10
IL	Fithian	FIT	4	9032/5056	1	2	9	7	9	5	8	7	119.38	287.02	50.00	31	1375.0	1470.5	0.0	165.00	16500.0	0.0	14.70
IL	Fithian	FIT	5	963/5056	1	4	9	7	9	5	8	8	111.76	281.94	64.52	40	1409.0	1499.5	2.5	165.39	20000.0	0.0	15.00
IL	Fithian	FIT	5	963/5056	2	1	9	7	9	6	8	8	114.30	302.26	61.29	38	1375.0	1470.5	0.0	210.02	19500.0	0.0	14.50
IL	Fithian	FIT	4	9032/5056	2	4	9	7	9	5	8	8	119.38	287.02	50.00	31	1375.0	1470.5	0.0	163.31	16500.0	0.0	16.20
IL	Fithian	FIT	3	9289/5056	2	5	9	8	9	5	8	8	116.84	297.18	59.68	37	1313.0	1409.0	0.0	179.20	19000.0	0.0	16.60
IL	Fithian	FIT	3	9289/5056	3	2	9	6	9	5	7	7	101.60	279.40	41.94	26	1375.0	1440.0	0.0	113.40	14500.0	0.0	15.30
IL	Fithian	FIT	4	9032/5056	3	3	9	6	9	5	8	8	91.44	284.48	50.00	31	1409.0	1470.5	0.0	129.59	16000.0	0.0	15.80
IL	Fithian	FIT	5	963/5056	3	5	9	7	9	6	8	9	116.84	304.80	48.39	30	1409.0	1470.5	0.0	144.63	15000.0	0.0	14.50
Mean							9.00	6.89	9.00	5.22	7.89	7.78	111.48	290.41	53.05	32.89	1375.89	1460.11	0.63	159.28	17000.00	0.00	15.50
Standard Deviation							0.00	0.60	0.00	0.44	0.33	0.67	9.28	8.98	7.23	4.48	32.27	26.25	1.25	28.01	2000.00	0.00	0.75

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGR	ERHIN	PLHIN	EMRGP	EASTCT	HUSIN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
NE	Laurel	LAU	1	HCEM485	1	3	8	9	9	5	7	8	99.06	238.76	64.52	40	1357.0	NA	0.0	201.95	16500.0	0.0	20.20
NE	Laurel	LAU	1	HCEM485	2	4	8	9	9	5	8	8	101.60	231.14	43.55	27	1384.5	NA	0.0	165.72	12500.0	0.0	21.70
NE	Laurel	LAU	1	HCEM485	3	1	8.00	9.00	9.00	5.00	7.67	8.00	96.52	230.29	51.08	31.67	1366.17	NA	0.00	161.30	12000.0	0.0	21.80
Mean							8.00	9.00	9.00	5.00	7.67	8.00	96.52	230.29	51.08	31.67	1366.17	NA	0.00	176.32	13666.67	0.00	21.23
Standard Deviation							0.00	0.00	0.00	0.00	0.58	0.00	6.72	8.92	11.67	7.23	15.88	NA	0.00	22.31	2466.44	0.00	0.90
NE	Laurel	LAU	4	9032/5056	1	1	8	9	9	5	8	7	91.44	215.90	51.61	32	1384.5	NA	0.0	136.85	13500.0	0.0	23.30
NE	Laurel	LAU	3	9289/5056	1	4	8	9	9	5	7	8	88.90	228.60	46.77	29	1357.0	NA	0.0	169.28	14500.0	0.0	19.90
NE	Laurel	LAU	5	963/5056	1	5	8	9	9	5	8	8	83.82	223.52	50.00	31	1410.5	NA	0.0	153.12	13500.0	0.0	17.70
NE	Laurel	LAU	4	9032/5056	2	1	8	9	9	5	7	7	88.90	223.52	37.10	23	1410.5	NA	0.0	126.86	9500.0	0.0	22.40
NE	Laurel	LAU	5	963/5056	2	2	8	9	9	5	7	8	93.98	233.68	50.00	31	1384.5	NA	0.0	166.36	14500.0	0.0	18.40
NE	Laurel	LAU	3	9289/5056	2	5	8	9	9	5	7	7	86.36	220.98	41.94	26	1384.5	NA	0.0	114.22	11500.0	0.0	20.70
NE	Laurel	LAU	5	963/5056	3	2	8	9	9	5	8	8	88.90	231.14	56.45	35	1410.5	NA	0.0	199.05	17000.0	0.0	18.30
NE	Laurel	LAU	4	9032/5056	3	4	8	9	9	5	7	7	88.90	215.90	54.84	34	1384.5	NA	0.0	132.76	12000.0	0.0	21.10
NE	Laurel	LAU	3	9289/5056	3	5	8	9	9	5	6	7	81.28	210.82	32.26	20	1357.0	NA	0.0	84.19	8500.0	0.0	21.10
Mean							8.00	9.00	9.00	5.00	7.22	7.44	88.05	222.67	46.77	29.00	1387.06	NA	0.00	142.52	12722.22	0.00	20.32
Standard Deviation							0.00	0.00	0.00	0.00	0.67	0.53	3.81	7.62	8.14	5.05	20.86	NA	0.00	33.85	2647.06	0.00	1.92

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGR	ERHIN	PLHIN	EMRGP	EASTCT	HUSIN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
SD	Lennox	LEN	1	HCEM485	1	5	5	9	2	5	7	9	86.36	215.90	62.90	39	1235.5	1340.5	0.0	61.38	16000.0	0.0	20.70
SD	Lennox	LEN	1	HCEM485	2	3	6	9	3	5	7	7	78.74	172.72	61.29	38	1260.5	1340.5	0.0	69.46	16000.0	0.0	22.00
SD	Lennox	LEN	1	HCEM485	3	2	6	9	3	5	7	7	81.28	190.50	54.84	34	1260.5	1340.5	0.0	77.55	16000.0	0.0	22.80
Mean							5.67	9.00	2.67	5.00	7.00	7.67	82.13	193.04	59.68	37.00	1252.17	1340.50	0.00	69.46	16000.00	0.00	21.83
Standard Deviation							0.58	0.00	0.58	0.00	0.00	1.15	3.88	21.70	4.27	2.65	14.43	0.00	8.09	0.00	0.00	1.06	
SD	Lennox	LEN	3	9289/5056	1	1	5	9	2	5	8	8	88.90	198.12	70.97	44	1235.5	1340.5	0.0	73.75	17500.0	0.0	20.10
SD	Lennox	LEN	4	9032/5056	1	2	6	9	2	4	7	8	76.20	182.88	59.68	37	1317.5	1409.5	0.0	76.25	14000.0	0.0	19.40
SD	Lennox	LEN	5	963/5056	1	4	6	9	3	5	8	9	83.82	182.88	64.52	40	1317.5	1431.0	0.0	47.70	14500.0	0.0	18.10
SD	Lennox	LEN	4	9032/5056	2	1	5	9	3	5	8	8	83.82	172.72	61.29	38	1340.5	1409.5	0.0	67.42	16500.0	0.0	19.10
SD	Lennox	LEN	3	9289/5056	2	4	5	9	2	5	8	8	83.82	190.50	61.29	38	1235.5	1317.5	0.0	50.19	13500.0	0.0	20.10
SD	Lennox	LEN	5	963/5056	2	5	5	9	3	5	7	8	78.74	175.26	64.52	40	1317.5	1409.5	0.0	66.24	15500.0	0.0	19.30
SD	Lennox	LEN	3	9289/5056	3	1	6	9	5	5	7	8	88.90	223.52	45.16	28	1235.5	1360.0	0.0	100.66	12000.0	0.0	22.40
SD	Lennox	LEN	5	963/5056	3	3	5	9	3	5	8	8	78.74	172.72	56.45	35	1340.5	1431.0	0.0	109.81	17000.0	0.0	20.30
SD	Lennox	LEN	4	9032/5056	3	5	5	9	3	5	8	8	83.82	175.26	61.29	38	1340.5	1409.5	0.0	83.59	16500.0	0.0	22.10
Mean							5.33	9.00	2.89	4.89	7.67	8.11	82.97	185.98	60.57	37.56	1297.83	1390.89	0.00	75.07	15222.22	0.00	20.10
Standard Deviation							0.50	0.00	0.93	0.33	0.50	0.33	4.40	16.50	7.04	4.36	47.80	41.05	0.00	20.74	1839.01	0.00	1.39

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGRR	ERHIN	PLHIN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
IL	Lincoln	LIN	1	HCEM485	1	4	9	8	9	5	7	8	101.60	228.60	64.52	40	1324.5	NA	0.0	130.23	20000.0	0.0	13.10
IL	Lincoln	LIN	1	HCEM485	2	1	9	9	9	5	7	8	129.54	241.30	51.61	32	1324.5	NA	0.0	105.76	16000.0	0.0	13.10
IL	Lincoln	LIN	1	HCEM485	3	3	9	8	9	5	7	7	101.60	231.14	59.68	37	1353.0	NA	0.0	125.20	19000.0	2.6	14.00
Mean							9.00	8.33	9.00	5.00	7.00	7.67	110.91	233.68	58.60	36.33	1334.00	NA	0.00	127.07	18333.33	0.88	13.40
Standard Deviation							0.00	0.58	0.00	0.00	0.00	0.58	16.13	6.72	6.52	4.04	16.45	NA	0.00	22.30	2081.67	1.52	0.52
IL	Lincoln	LIN	4	9032/5056	1	1	9	7	9	5	7	8	99.06	200.66	61.29	38	1384.0	NA	0.0	143.92	19000.0	0.0	13.10
IL	Lincoln	LIN	5	963/5056	1	2	9	7	9	5	8	8	114.30	228.60	61.29	38	1447.0	NA	0.0	154.39	19000.0	0.0	11.90
IL	Lincoln	LIN	3	9289/5056	1	5	9	9	9	5	8	8	93.98	223.52	59.68	37	1384.0	NA	0.0	139.06	18000.0	2.8	13.90
IL	Lincoln	LIN	4	9032/5056	2	2	9	7	9	5	8	8	109.22	226.06	66.13	41	1384.0	NA	0.0	155.25	21000.0	0.0	13.30
IL	Lincoln	LIN	3	9289/5056	2	3	9	9	9	5	8	6	106.68	241.30	51.61	32	1353.0	NA	0.0	116.95	16500.0	0.0	13.60
IL	Lincoln	LIN	5	963/5056	2	4	9	9	9	5	8	8	104.14	231.14	66.13	41	1414.5	NA	0.0	159.08	21000.0	0.0	13.30
IL	Lincoln	LIN	5	963/5056	3	1	9	9	9	5	8	8	111.76	218.44	59.68	37	1447.0	NA	0.0	142.60	19000.0	2.6	12.40
IL	Lincoln	LIN	3	9289/5056	3	4	9	9	9	5	7	8	109.22	228.60	66.13	41	1384.0	NA	0.0	163.65	20500.0	0.0	13.60
IL	Lincoln	LIN	4	9032/5056	3	5	9	7	9	4	9	9	111.76	213.36	59.68	37	1414.5	NA	0.0	147.60	19000.0	0.0	12.80
Mean							9.00	7.78	9.00	4.89	7.89	8.00	106.68	223.52	61.29	38.00	1401.33	NA	0.00	146.95	19222.22	0.60	12.99
Standard Deviation							0.00	0.97	0.00	0.33	0.60	0.87	6.60	11.64	4.63	2.87	31.73	NA	0.00	13.88	1460.12	1.19	0.56

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RILDR	LFCLR	EMGR	EAGRR	ERHIN	PLHIN	EMRGP	EASTCT	HUSSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP
AR	Marion	MAR	1	HCEM485	1	1	9	8	9	3	7	7	60.96	208.28	48.39	30	1387.5	1487.0	0.0	46.11	14000.0	0.0	12.70
AR	Marion	MAR	1	HCEM485	2	5	9	8	9	7	7	8	60.96	193.04	59.68	37	1422.5	1487.0	0.0	69.25	20500.0	0.0	10.40
AR	Marion	MAR	1	HCEM485	3	1	9	8	9	6	8	8	71.12	198.12	70.97	44	1387.5	1513.0	0.0	29.91	16500.0	0.0	12.40
Mean							9.00	8.00	9.00	5.33	7.33	7.67	64.35	199.81	59.68	37.00	1399.17	1495.67	0.00	48.42	17000.00	0.00	11.83
Standard Deviation							0.00	0.00	0.00	2.08	0.58	0.58	5.87	7.76	11.29	7.00	20.21	15.01	0.00	19.77	3278.72	0.00	1.25
AR	Marion	MAR	3	9289/5056	1	3	9	8	9	3	8	8	66.04	200.66	38.71	24	1387.5	1487.0	0.0	32.85	12000.0	0.0	13.40
AR	Marion	MAR	4	9032/5056	1	4	9	7	9	5	8	8	86.36	208.28	58.06	36	1422.5	1513.0	0.0	52.01	17500.0	0.0	11.00
AR	Marion	MAR	5	963/5056	1	5	9	8	9	5	8	8	71.12	213.36	48.39	30	1457.0	1542.5	0.0	74.69	14000.0	0.0	12.20
AR	Marion	MAR	3	9289/5056	2	1	9	8	9	7	7	8	66.04	203.20	48.39	30	1387.5	1487.0	0.0	28.29	13500.0	0.0	11.10
AR	Marion	MAR	4	9032/5056	2	2	NA	NA	NA	NA	NA	NA	NA	NA	61.29	30	NA	NA	NA	NA	NA	NA	NA
AR	Marion	MAR	5	963/5056	2	4	9	9	9	6	8	8	60.96	182.88	46.77	29	1487.0	1542.5	0.0	37.57	13000.0	0.0	11.70
AR	Marion	MAR	4	9032/5056	3	3	9	9	9	3	7	7	60.96	213.36	64.52	40	1387.5	1487.0	0.0	45.67	11000.0	0.0	11.00
AR	Marion	MAR	3	9289/5056	3	4	9	8	9	4	7	7	50.80	208.28	37.10	23	1387.5	1487.0	0.0	31.24	16500.0	0.0	11.30
AR	Marion	MAR	5	963/5056	3	5	9	8	9	3	8	8	66.04	208.28	38.71	24	1457.0	1572.5	0.0	62.17	12000.0	0.0	12.00
Mean							9.00	8.25	9.00	4.50	7.67	7.75	66.04	204.79	49.10	30.44	1421.69	1514.81	0.00	45.56	13687.50	0.00	11.71
Standard Deviation							0.00	0.71	0.00	1.51	0.50	0.46	10.16	9.88	10.20	6.33	40.42	33.72	0.00	16.45	2266.80	0.00	0.82

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STIKLR	RTLDR	LFCLR	EMGR	EAGR	ERHTN	PLHTN	EMRGP	EASTCT	HUSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP	TWSMN
OH	OH	OH	1	HCEM485	1	2	9	9	9	3	6	7	95.98	226.06	58.06	36	1330.0	1393.0	2.5	116.06	20000.0	0.0	39.70	53.500
OH	OH	OH	1	HCEM485	2	4	9	9	9	4	7	8	106.68	248.92	53.23	33	1330.0	1393.0	4.9	95.07	20500.0	0.0	37.40	53.500
OH	OH	OH	1	HCEM485	3	4	9	8	9	4	6	8	101.60	228.60	40.32	25	1298.0	1393.0	20.6	86.86	17000.0	0.0	35.30	54.000
Mean							9.00	8.67	9.00	3.67	6.33	7.67	100.75	234.53	50.54	31.33	1319.33	1393.00	9.32	99.33	19166.67	0.00	37.30	53.67
Standard Deviation							0.00	0.58	0.00	0.58	0.58	0.58	6.39	12.53	9.17	5.69	18.48	9.83	15.06	1892.97	0.00	1.95	0.29	
OH	OH	OH	4	9032/5056	1	1	9	9	9	2	6	7	99.06	226.06	53.23	33	1363.0	1420.5	0.0	132.65	19500.0	0.0	39.80	54.000
OH	OH	OH	3	9289/5056	1	3	9	8	9	3	7	8	86.36	231.14	43.55	27	1363.0	1393.0	0.0	102.11	14000.0	0.0	38.40	54.000
OH	OH	OH	5	963/5056	1	4	9	9	9	5	6	7	86.36	228.60	50.00	31	1393.0	1470.0	7.1	101.12	21000.0	0.0	35.30	55.000
OH	OH	OH	3	9289/5056	2	1	9	9	9	4	7	8	91.44	220.98	37.10	23	1330.0	1393.0	3.3	102.58	15000.0	0.0	36.00	55.000
OH	OH	OH	4	9032/5056	2	3	9	9	9	2	6	7	99.06	228.60	50.00	31	1393.0	1444.5	7.9	104.36	19000.0	0.0	34.60	55.500
OH	OH	OH	5	963/5056	2	5	9	8	9	5	7	8	88.90	220.98	48.39	30	1420.5	1470.0	6.5	77.05	15500.0	0.0	30.40	55.500
OH	OH	OH	3	9289/5056	3	2	9	9	9	4	6	7	101.60	233.68	45.16	28	1363.0	1393.0	3.6	106.97	14000.0	0.0	37.40	53.000
OH	OH	OH	4	9032/5056	3	3	9	8	9	3	7	8	96.52	210.82	46.77	29	1363.0	1420.5	3.2	110.65	15300.0	0.0	37.20	54.500
OH	OH	OH	5	963/5056	3	5	9	9	9	5	6	7	96.52	228.60	43.55	27	1393.0	1444.5	6.7	70.44	15000.0	0.0	29.50	54.500
Mean							9.00	8.67	9.00	3.67	6.44	7.44	93.98	225.50	46.42	28.78	1375.72	1427.67	4.25	100.88	16500.00	0.00	35.40	54.39
Standard Deviation							0.00	0.50	0.00	1.22	0.53	0.53	5.82	6.93	4.76	2.95	26.55	31.34	2.98	18.21	2610.08	0.00	3.47	0.74

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STIKLR	RTLDR	LFCLR	EMGR	EAGR	ERHTN	PLHTN	EMRGP	EASTCT	HUSN	HUPSN	BRNRP	YGSMN	HAVPN	DROPP	GMSTP	TWSMN
IN	Sheridan	SHE	1	HCEM485	1	1	9	9	9	5	8	8	99.06	200.66	33.87	21	1347.5	NA	0.0	95.42	10500.0	0.0	16.70	55.500
IN	Sheridan	SHE	4	HCEM485	2	4	9	9	9	5	7	7	104.14	208.28	46.77	29	1347.5	NA	0.0	141.78	16000.0	0.0	17.90	56.500
IN	Sheridan	SHE	1	HCEM485	3	5	9	9	9	5	8	8	96.52	198.12	30.65	19	1347.5	NA	0.0	113.82	11500.0	0.0	17.00	56.500
Mean							9.00	9.00	9.00	5.00	7.67	7.67	99.91	202.35	37.10	23.00	1347.50	NA	0.00	117.01	12666.67	0.00	17.20	56.17
Standard Deviation							0.00	0.00	0.00	0.00	0.58	0.58	3.88	5.29	8.53	5.29	0.00	NA	0.00	23.35	2929.73	0.00	0.62	0.58
IN	Sheridan	SHE	3	9289/5056	1	3	9	9	9	6	8	8	109.22	215.90	43.55	27	1347.5	NA	0.0	131.63	15000.0	0.0	17.40	56.000
IN	Sheridan	SHE	4	9032/5056	1	4	9	8	9	5	8	8	111.76	205.74	46.77	29	1376.0	NA	0.0	153.00	15500.0	0.0	16.50	56.500
IN	Sheridan	SHE	5	963/5056	1	5	9	8	9	5	9	9	99.06	182.88	43.55	27	1438.5	NA	0.0	118.83	13500.0	0.0	16.90	55.000
IN	Sheridan	SHE	3	9289/5056	2	1	9	9	9	6	7	8	99.06	182.88	43.55	27	1376.0	NA	0.0	120.59	13500.0	0.0	17.70	56.000
IN	Sheridan	SHE	4	9032/5056	2	3	9	8	9	5	8	8	99.06	210.82	50.00	31	1376.0	NA	0.0	157.40	15500.0	0.0	17.10	55.500
IN	Sheridan	SHE	5	963/5056	2	5	9	9	9	5	8	8	104.14	193.04	45.16	28	1407.5	NA	0.0	137.98	21000.0	0.0	16.50	56.500
IN	Sheridan	SHE	4	9032/5056	3	1	9	9	9	5	8	8	109.22	193.04	41.94	26	1376.0	NA	0.0	153.43	13500.0	0.0	16.80	54.500
IN	Sheridan	SHE	3	9289/5056	3	2	9	9	9	6	8	8	111.76	215.90	46.77	29	1347.5	NA	0.0	173.59	15000.0	0.0	17.50	57.000
IN	Sheridan	SHE	5	963/5056	3	3	9	8	9	6	8	8	101.60	193.04	41.94	26	1438.5	NA	0.0	144.46	12500.0	0.0	16.40	56.000
Mean							9.00	8.56	9.00	5.44	8.00	8.11	104.99	199.25	44.80	27.78	1387.06	NA	0.00	143.43	15000.00	0.00	16.98	55.89
Standard Deviation							0.00	0.53	0.00	0.53	0.50	0.33	5.54	13.14	2.65	1.64	34.15	NA	0.00	18.01	2487.47	0.00	0.48	0.78

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**Annex 1 - Agronomic analysis of maize.
Laboratory Study ID: SSF-323**

**Maize Line HCEM485
USDA Extension Petition**

State	Location	LocCode	Entry	Genotype	Range	Row	ERTLR	STKLR	RTLDR	LFCLR	EMRGR	EAGR	ERHTN	PLHTN	EMRGP	EASTCT	HUSSN	HUPSN	BRENP	YGSMN	HAVPN	DROPP	GMSTP	TWSMN
IL	Smithshire	SMI	1	HCEM485	1	1	9	NA	NA	5	7	8	109.22	246.88	30.65	19	1266.0	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	1	HCEM485	2	2	9	NA	NA	5	6	8	109.22	243.84	38.71	24	1239.0	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	1	HCEM485	3	5	9	NA	NA	5	7	8	91.44	241.30	37.10	23	1266.0	NA	NA	NA	NA	NA	NA	NA
Mean							9.00	NA	NA	5.00	6.67	8.00	103.29	243.84	35.48	22.00	1257.00	NA	NA	NA	NA	NA	NA	NA
Standard Deviation							0.00	NA	NA	0.00	0.58	0.00	10.27	2.54	4.27	2.65	15.59	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	3	9289/5056	1	3	9	NA	NA	5	6	7	106.68	236.22	12.90	8	1213.0	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	4	9032/5056	1	4	9	NA	NA	5	7	8	99.06	223.52	30.65	19	1321.5	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	5	963/5056	1	5	9	NA	NA	4	7	8	104.14	246.38	32.26	20	1349.0	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	4	9032/5056	2	1	9	NA	NA	5	7	8	114.30	238.76	24.19	15	1293.5	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	3	9289/5056	2	3	9	NA	NA	5	5	7	104.14	238.76	6.45	4	1239.0	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	5	963/5056	2	4	9	NA	NA	4	8	7	104.14	248.92	33.87	21	1321.5	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	4	9032/5056	3	2	9	NA	NA	5	7	8	109.22	238.60	19.35	12	1321.5	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	3	9289/5056	3	3	9	NA	NA	5	7	7	88.90	238.76	25.81	16	1239.0	NA	NA	NA	NA	NA	NA	NA
IL	Smithshire	SMI	5	963/5056	3	4	9	NA	NA	5	8	8	91.44	241.30	33.87	21	1349.0	NA	NA	NA	NA	NA	NA	NA
Mean							9.00	NA	NA	4.78	6.89	7.56	102.45	237.91	24.37	15.11	1294.11	NA	NA	NA	NA	NA	NA	NA
Standard Deviation							0.00	NA	NA	0.44	0.93	0.53	8.13	7.93	9.76	6.05	51.15	NA	NA	NA	NA	NA	NA	NA

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Annex 2

Morphology and viability of pollen collected from
HCEM485 maize

Laboratory Study ID: SSF-07-288

STUDY TITLE

Morphology and Viability of Pollen Collected from HCEM485 Maize

LABORATORY STUDY ID

SSF-07-288

STUDY COMPLETED ON

15 October 2007

PERFORMING LABORATORIES

**Stine Biotechnology
2501 N. Loop Drive, Suite 1612, Building 1
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SUBMITTED BY

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Adel, Iowa 50003
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Summary

In order to assess whether the presence of the mutated EPSP synthase encoding gene, the gene product, or the genetic modification process altered the pollen characteristics of HCEM485 maize, pollen morphology and viability were investigated by microscopically examining pollen grains that had been fixed and stained according to the method described by Alexander (1969).

Although the viability of HCEM485 pollen (94.6%) was statistically significantly greater than the control (84%), both values were within the range that has been reported for other reference samples of maize pollen and the observed difference was, therefore, considered small and unlikely to be of biological significance. There were no readily discernable differences in HCEM485 and control pollen morphology and no significant difference in average cell diameter was detected between HCEM485 and control pollen samples.

Based on this study there were no biologically significant differences in either pollen viability or morphology that would be indicative of an unintended effect of the genetic modification.

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Annex 2 - Morphology and viability of pollen collected
from HCEM485 maize.

Laboratory Study ID: SSF-07-288

Maize Line HCEM485

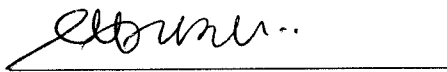
USDA Extension Petition

CBI Copy

Statement of Good Laboratory Practices

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

PRINCIPAL INVESTIGATORS:

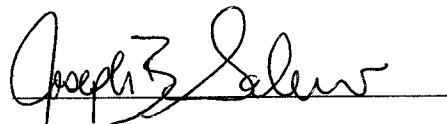


V. Sekar

02/24/2009

Date

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Feb. 24, 2009

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20. INTRODUCTION

Herbicide-tolerant maize line HCEM485 was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene (Held *et al.*, 2006). The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-resistant form of the native maize EPSP synthase. Except for the introduced mutations, the amino acid sequence of the double-mutated maize EPSPS (2mEPSPS) enzyme expressed in maize line HCEM485 is identical to the native wild-type maize EPSPS sequence reported by Gardiner *et al.* 2004. Maize line HCEM485 does not contain any heterologous DNA sequences, either coding or non-coding, from any other species.

The purpose of this study was to assess whether the presence of the mutated EPSP synthase encoding gene, the gene product, or the genetic modification process altered the pollen characteristics of HCEM485 maize. Pollen morphology and viability were investigated by microscopically examining pollen grains that had been fixed and stained according to the method described by Alexander (1969).

21. METHODS AND RESULTS

21.1 PLANT MATERIAL

The following hybrid lines were used as sources of pollen:

HCEM485 hybrid	(((HCEM485)2/9289/9032)4/5056) [trait positive]
Control hybrid	9289x5056 [trait negative]

A pedigree map showing the derivation of the HCEM485 hybrid is shown in Figure 1. The control hybrid was produced by crossing the inbred maize line 9289, which was used as one of the parents during the production of hybrid HCEM485 maize, with inbred line 5056, which was also used in creating the HCEM485 hybrid.

Five pots of each hybrid were planted and maintained in an environmentally controlled greenhouse. The greenhouse operated on a 16 hr/8 hr light/dark cycle with daytime temperatures ranging from 23–28°C and nighttime temperatures ranging from 18–22°C.

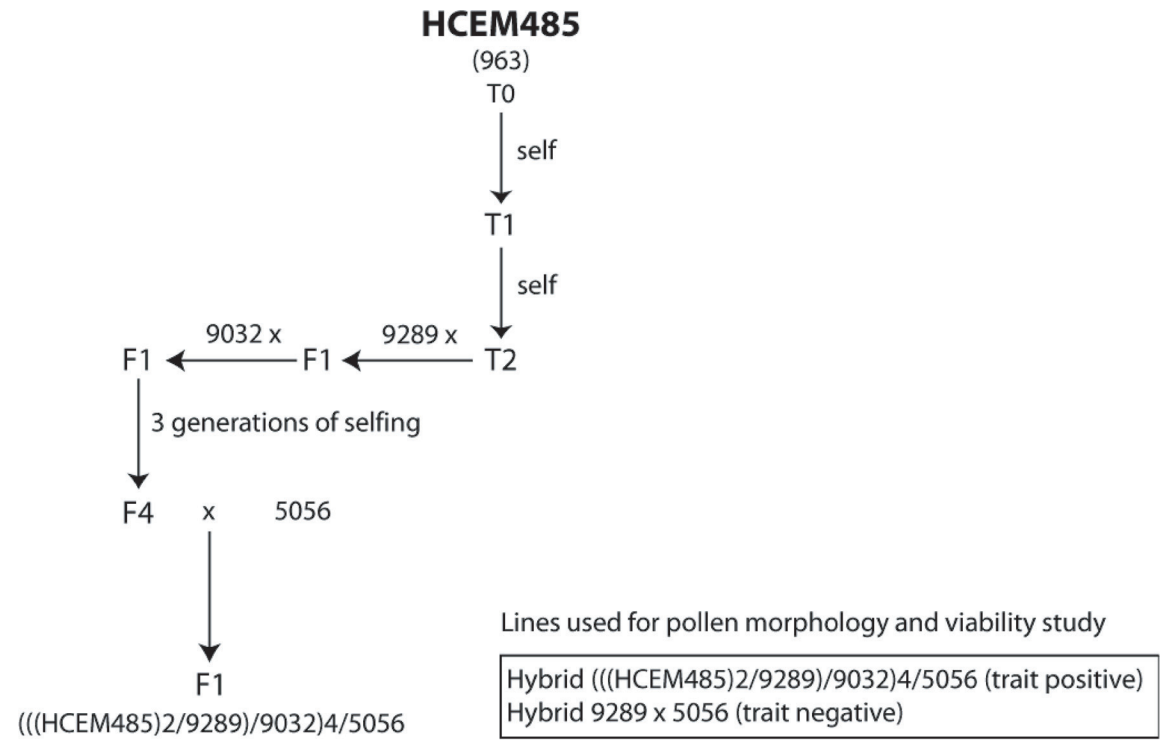


Figure 1: Pedigree map of HCEM485 hybrid

21.2 POLLEN MORPHOLOGY AND VIABILITY

Pollen was collected from each plant during anthesis (*ca.* 69–70 days after planting), stained with Alexander’s stain (Alexander, 1969), and examined by light microscopy. Viability determinations were made at 70X magnification. Percent viability was determined by examining a minimum of 100 pollen grains per sample and mean percent viability was determined for the HCEM485 and control samples. These means were compared by a t-test with significance assigned at the standard $p < 0.05$ level. Viability of HCEM485 pollen was significantly greater than the control (Table 1) but both values were within the range that has been reported for other reference samples of maize pollen (86–100%; Monsanto, 2004). The observed difference was, therefore, considered small and unlikely to be of biological significance.

Cell morphology and the diameter of stained pollen samples from five HCEM485 and five control plants were examined at 80X magnification. Morphology was assessed by a visual examination of all cells in the field of view and pollen diameter was measured on 12 cells per sample. Mean diameter was computed for the HCEM485 and control samples. These means were compared by a t-test with significance assigned at the customary $p < 0.05$ level. There were no readily discernable differences in HCEM485 and control pollen morphology (Figure 2) and no significant difference in average cell diameter was detected between HCEM485 and control pollen samples (Table 1).

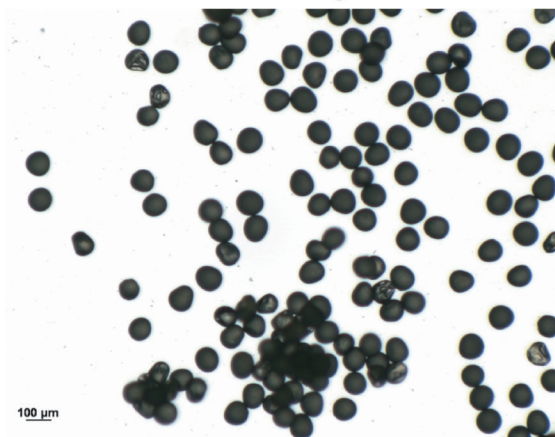
Table 1: Pollen viability and diameter measurements.

Genotype	Mean Pollen Viability \pm SD (%) ^a	Mean Pollen Diameter \pm SD (μ m)
HCEM485	94.6 \pm 2.9	105.5 \pm 6.5
Control (9289x5056)	86.0 \pm 4.2	104.8 \pm 5.2
p-value	<i>0.007*</i>	0.527
N	5	60

a. Mean percent pollen viability and mean diameter measurements are presented with their respective standard deviations (SD). The HCEM485 and control means were compared by a t-test.

* = indicates that the difference between the HCEM485 and control hybrid was statistically significant at $p < 0.05$.

Control 9289 x 5056 Plant (80X Magnification)



HCEM485 Plant (80X Magnification)

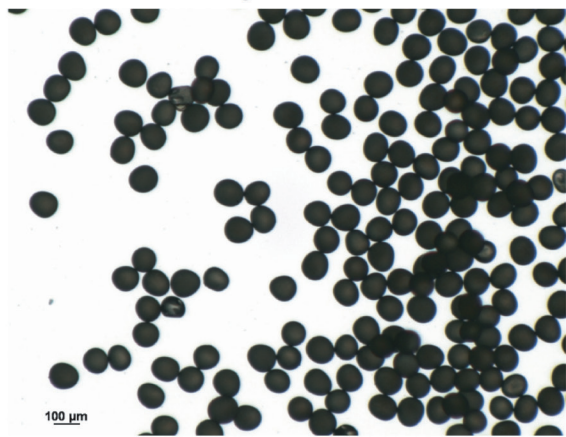


Figure 2: Morphology of pollen from HCEM485 and control hybrids. Representative photomicrographs of control (left) and HCEM485 (right) pollen samples. Pollen samples were stained with Alexander's stain and examined under light microscopy (80X magnification). The scale bar representing 100 μ m is indicated in each photomicrograph.

22. CONCLUSIONS

Based on this study there were no biologically significant differences in either pollen viability or morphology that would be indicative of an unintended effect of the genetic modification.

23. RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Stine Seed Farm, Inc., 22555 Laredo Trail, Adel, Iowa 50003.

24. REFERENCES

- Alexander, M.P. (1969). Differential staining of aborted and non-aborted pollen. *Stain Technology* **41**: 117–122.
- Gardiner, J., Schroeder, S., Polacco, M.L., Sanchez-Villeda, H., Fang, Z., Morgante, M., Landewe, T., Fengler, K., Useche, F., Hanafey, M., Tingey, S., Chou, H., Wing, R., Soderlund, C. and Coe, E.H. (2004). Anchoring 9,371 maize expressed sequence tagged unigenes to the bacterial artificial chromosome contig map by two-dimensional overgo hybridization. *Plant Physiology* **134**: 1317–1326.
- Held, B.M., Wilson, H.M., Dykema, P.E., Lewnau, C.J. and Eby, J.C. (2006). Glyphosate resistant plants. US Patent No. 7,045,684.
- Monsanto (2004). Petition for the determination of nonregulated status for MON 88017 corn. Monsanto Petition # 04-CR-108U (CBI-Deleted).

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25. APPENDIX 1: POLLEN MEASUREMENTS AND STATISTICAL ANALYSIS.

Pollen Morphology Raw Data

Genotype	Diameter (µm)	Genotype	Diameter (µm)
HCEM485	106.68	9289x5056	106.33
HCEM485	100.52	9289x5056	105.42
HCEM485	104.00	9289x5056	103.88
HCEM485	108.19	9289x5056	104.71
HCEM485	109.61	9289x5056	106.05
HCEM485	108.54	9289x5056	110.23
HCEM485	111.81	9289x5056	114.29
HCEM485	110.88	9289x5056	115.61
HCEM485	114.13	9289x5056	107.30
HCEM485	111.26	9289x5056	104.35
HCEM485	110.97	9289x5056	100.61
HCEM485	115.61	9289x5056	107.30
HCEM485	116.13	9289x5056	101.96
HCEM485	109.79	9289x5056	103.87
HCEM485	107.45	9289x5056	97.44
HCEM485	111.49	9289x5056	92.78
HCEM485	112.02	9289x5056	103.99
HCEM485	105.38	9289x5056	99.98
HCEM485	102.49	9289x5056	100.76
HCEM485	105.51	9289x5056	94.75
HCEM485	106.74	9289x5056	99.17
HCEM485	101.25	9289x5056	100.76
HCEM485	110.16	9289x5056	102.49
HCEM485	94.88	9289x5056	105.03
HCEM485	106.40	9289x5056	112.33
HCEM485	108.56	9289x5056	99.32
HCEM485	98.54	9289x5056	111.58
HCEM485	102.08	9289x5056	103.44
HCEM485	105.95	9289x5056	113.55
HCEM485	110.97	9289x5056	109.75
HCEM485	110.23	9289x5056	97.61
HCEM485	110.92	9289x5056	105.43
HCEM485	114.88	9289x5056	98.80
HCEM485	105.42	9289x5056	106.41
HCEM485	118.89	9289x5056	110.97
HCEM485	112.65	9289x5056	105.72
HCEM485	92.49	9289x5056	106.23
HCEM485	107.84	9289x5056	107.42
HCEM485	101.78	9289x5056	100.53
HCEM485	106.41	9289x5056	111.27
HCEM485	99.80	9289x5056	100.49
HCEM485	103.13	9289x5056	98.53
HCEM485	103.67	9289x5056	114.59
HCEM485	106.78	9289x5056	105.78
HCEM485	109.46	9289x5056	108.19
HCEM485	100.52	9289x5056	100.01
HCEM485	109.79	9289x5056	99.03
HCEM485	103.06	9289x5056	96.88
HCEM485	102.31	9289x5056	113.70
HCEM485	91.85	9289x5056	107.68
HCEM485	85.92	9289x5056	102.31
HCEM485	93.17	9289x5056	105.78
HCEM485	100.95	9289x5056	110.06
HCEM485	104.69	9289x5056	108.13
HCEM485	99.77	9289x5056	103.44
HCEM485	106.53	9289x5056	104.17
HCEM485	96.26	9289x5056	99.45
HCEM485	101.68	9289x5056	105.43
HCEM485	104.00	9289x5056	106.19
HCEM485	98.16	9289x5056	110.97

Summary Statistics

Variable	Observations	Minimum	Maximum	Mean	Std. deviation
HCEM485 Pollen Diameter (µm)	60	85.92	118.89	105.52	6.45
9289x5056 Pollen Diameter (µm)	60	92.78	115.61	104.84	5.20

Anderson-Darling test (HCEM485 Diameter (µm)):

A ²	0.430
p-value	0.299
Alpha	0.05

Test interpretation:

H0: The sample follows a Normal distribution.

Ha: The sample does not follow a Normal distribution.

As the computed p-value is greater than the significance level alpha=0.05, one should accept the null hypothesis H0.

The risk to reject the null hypothesis H0 while it is true is 29.90%.

Anderson-Darling test (9289x5056 Diameter (µm)):

A ²	0.292
p-value	0.593
Alpha	0.05

Test interpretation:

H0: The sample follows a Normal distribution.

Ha: The sample does not follow a Normal distribution.

As the computed p-value is greater than the significance level alpha=0.05, one should accept the null hypothesis H0.

The risk to reject the null hypothesis H0 while it is true is 59.34%.

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means: -1.44025876 to 2.79925876

Difference	0.6795
t (Observed value)	0.6348
t (Critical value)	1.9803
DF	118
p-value (Two-tailed)	0.5268
Alpha	0.05

Test interpretation:

H0: The difference between the means is not significantly different from 0.

Ha: The difference between the means is significantly different from 0.

As the computed p-value is greater than the significance level alpha=0.05, one should accept the null hypothesis H0.

The risk to reject the null hypothesis H0 while it is true is 52.68%.

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26. APPENDIX 2: POLLEN VIABILITY AND STATISTICAL ANALYSIS.

Pollen Viability Analysis

Genotype	Sample	Viability	Genotype	Sample	Viability
9289x5056	5	0.90	HCEM485	1	0.90
9289x5056	11	0.90	HCEM485	2	0.95
9289x5056	23	0.85	HCEM485	3	0.98
9289x5056	27	0.85	HCEM485	7	0.95
9289x5056	30	0.80	HCEM485	22	0.95

Summary Statistics

Variable	Observations	Minimum	Maximum	Mean	Std. deviation
9289x5056 Viability	5	0.800	0.900	0.860	0.042
HCEM485 Viability	5	0.900	0.980	0.946	0.029

t-test for two independent samples / Two-tailed test:

95% confidence interval on the difference between the means: -0.139604283 to -0.0323957

Difference	-0.086
t (Observed value)	-3.786
t (Critical value)	2.360
DF	7.098
p-value (Two-tailed)	0.007
alpha	0.05

The number of degrees of freedom is approximated by the Welch-Satterthwaite formula.
The critical t is estimated using the Cochran-Cox approximation.

Test interpretation:

H0: The difference between the means is not significantly different from 0.

Ha: The difference between the means is significantly different from 0.

The risk to reject the null hypothesis H0 while it is true is lower than 0.67%.

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Annex 3

Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA

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STUDY TITLE

**Compositional Analysis of Grain and Forage Derived from HCEM485
Hybrid Maize Grown During 2007 in the USA**

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Summary

Key nutritional components in forage and grain from HCEM485 hybrid maize plants were measured and compared to forage and grain samples from conventionally bred control hybrids. HCEM485 maize produces a form of the maize 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme that was specifically modified through site-directed mutagenesis to confer tolerance to glyphosate-containing herbicides.

Small field trials of the HCEM485 and control hybrids were conducted in the United States during the 2007 growing season at locations selected to be representative of the range of environmental conditions under which maize is typically grown. Forage samples were collected from two locations and grain samples were collected from four locations and analyzed for up to 87 components in grain and 8 components in forage. Data for each quantifiable analyte were subjected to an analysis of variance across all locations with genotype and location as independent factors. Average values for each analyte were compared to data for forage and grain composition published in both the International Life Sciences Institute (ILSI) crop composition database (ILSI, 2006) and the Organization for Economic Co-operation and Development consensus document on new maize varieties (OECD, 2002) to assess whether any observed variation was within the natural range for cultivated maize forage and grain.

Forage from HCEM485 and control hybrids was analyzed for proximates (including ADF and NDF), calcium and phosphorus. There was a small, but statistically significant, increase in protein (ca. 6.8%) and decrease in fat (ca. -13%) content between HCEM485 and control samples, and calcium content was slightly elevated (ca. 13%) in HCEM485 samples. Average values for all analytes tested in forage, including those where statistically significant differences were observed, were within the ranges of natural variation as reported in the literature.

Grain from HCEM485 and control hybrids was analyzed for proximates (including starch, ADF, NDF, and TDF), minerals, vitamins, amino acids, fatty acids, antinutrients and secondary metabolites, and phytosterols. Of the 65 analyte comparisons that were suitable for statistical analysis, 45 showed no statistically significant difference. Where there were statistically significant differences, the magnitudes of the differences were generally small (ranging up to 18%) and in every case, average values determined for both HCEM485 and control samples were within the ranges of natural variation as reported in the literature.


Overall, no consistent patterns emerged to suggest that biologically significant changes in composition of the grain or forage had occurred as an unintended consequence of the genetic modification resulting in maize line HCEM485. The conclusion based on these data was that grain and forage from HCEM485 maize were substantially equivalent in composition to both the control hybrids included in this study and to other commercial maize hybrids.

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Statement of Good Laboratory Practices

This study was not conducted in compliance with Good Laboratory Practice Standards (40 CFR 160, Federal Register, 1989) pursuant to the Federal Insecticide, Fungicide and Rodenticide Act, and subsequent revisions. However, the study was conducted according to accepted scientific methods, and the raw data and study records have been retained.

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27. INTRODUCTION

This study was conducted to measure and compare important nutritional, antinutritional and secondary metabolites of forage and grain from a HCEM485 maize (field corn) hybrid and conventional control hybrids as part of a comparative safety assessment.

Maize line HCEM485 was developed by Stine Seed Farm to incorporate the trait of tolerance to glyphosate herbicides. The line was produced by introducing a 6.0 kb maize genomic fragment, originally isolated from a bacterial artificial chromosome (BAC) library derived from the maize inbred line B73, containing a modified form of the endogenous maize EPSPS encoding gene (Held *et al.*, 2006). The only DNA sequences introduced into maize line HCEM485 were those derived from maize following the introduction of two point-mutations resulting in the expression of a glyphosate-resistant form of the native maize EPSP synthase. Except for the introduced mutations, the amino acid sequence of the double-mutated maize EPSPS (2mEPSPS) enzyme expressed in maize line HCEM485 is identical to the native wild-type maize EPSPS sequence reported by Gardiner *et al.* 2004.

Small field trials of the HCEM485 and control hybrids were conducted in the United States during the 2007 growing season at locations selected to be representative of the range of environmental conditions under which maize is typically grown. Forage samples were collected from two locations and grain samples were collected from four locations and analyzed for the various nutritive components identified in Table 3. Data for each quantifiable analyte were subjected to an analysis of variance across all locations with genotype and location as independent factors. Average values for each analyte were compared to data for forage and grain composition published in both the International Life Sciences Institute (ILSI) crop composition database (ILSI, 2006) and the Organization for Economic Co-operation and Development consensus document on new maize varieties (OECD, 2002) to assess whether any observed variation was within the natural range for cultivated maize forage and grain.

28. MATERIALS AND METHODS

28.1 PLANT MATERIAL

During 2007, hybrid maize plants were grown, according to local agronomic practices, at 15 locations in the USA representing the agricultural regions where the hybrid varieties would typically be cultivated. Samples of maize grain from HCEM485 and control hybrids were harvested from four locations and forage samples were obtained from two of these locations (Table 1).

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Table 1: Field trial locations used for grain and forage sampling.

Location Code	Sample Type	City and State	Planting Date	Harvest Date
L1	Grain, Forage	Adel, IA	5 June 2007	17 October 2007
L2	Grain, Forage	Fithian, IL	7 June 2007	15 October 2007
L3	Grain	Blomkest, MN	5 June 2007	26 October 2007
L4	Grain	Spencerville, OH	12 June 2007	12 October 2007

All trials were conducted under USDA-APHIS notification number: 07-046-110n.

At each location, a single HCEM485 hybrid and three control hybrids were grown in a randomized complete block design, with three replicates for each genotype. The breeding pedigree of the HCEM485 and control hybrids is illustrated in Figure 1. The control hybrids were produced by crossing the inbred lines Stine 963, 9289 or 9032, each of which were used as parental lines in the breeding of HCEM485, with inbred line 5056, which was also used in creating the HCEM485 hybrid. Samples of maize grain or forage from each of the individual control hybrids were combined into a composite ‘control hybrid’ sample for each replicated plot.

The HCEM485 and control hybrids were all grown according to local agronomic practices. Prior to anthesis, silks were bagged to ensure self-pollination. Grain and forage samples from the HCEM485 hybrid were obtained from plants treated with glyphosate herbicide (3–4 leaf stage; Roundup WeatherMAX; 32 oz/acre) at the usual commercial application rate.

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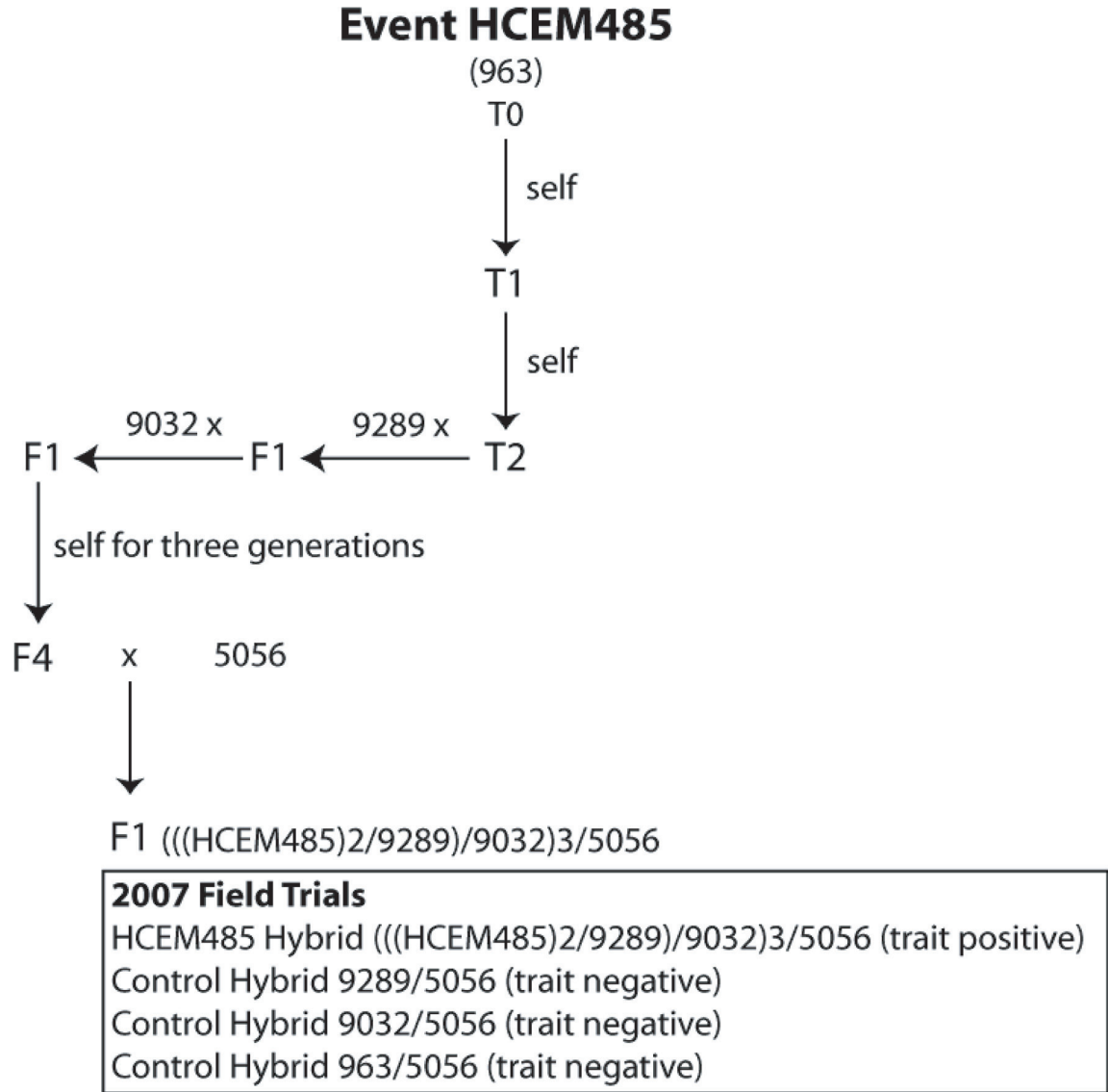


Figure 1: Pedigree chart of HCEM485 seed used in 2007 field trials.

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28.2 FORAGE SAMPLING AND PROCESSING

The entire above ground portion from five plants of each hybrid was harvested at approximately the kernel dough stage (R4), the stage at which silage would typically be prepared, from each of the three replicated plots at each location. For each genotype, plants were pooled to create a composite sample for each plot, then ground with a chipper-shredder and a sub-sample from each composite sample was stored frozen in a freezer set to maintain -20°C. Prior to shipment for analysis, samples from each of the three control hybrids were further combined into a single composite sample for each replicated plot from each location. All samples were shipped on dry ice to EPL Bio-Analytical Services, Niantic, IL, for analysis.

28.3 GRAIN SAMPLING AND PROCESSING

Ears were harvested after physiological maturity (R6) and then mechanically dried to approximately 10–13% moisture content. Each grain sample represented grain shelled from ears collected from 15 plants of each hybrid growing in each of the three replicated plots at each location. Samples were ground and stored frozen in a freezer set to maintain -20°C. Prior to analysis, grain samples from each of the three control hybrids were further combined into a single composite sample for each replicated plot from each location. All samples were shipped on dry ice to EPL Bio-Analytical Services, Niantic, IL, for analysis.

28.4 COMPOSITIONAL ANALYSIS

Selection of analytes for measurement in forage and grain (Table 2) was based on recommendations contained in the OECD Task Force for the Safety of Novel Foods and Feeds consensus document for new maize varieties (OECD, 2002). Forage was analyzed for proximates and the minerals calcium and phosphorus. Grain was analyzed for major constituents (proximates, including starch, ADF, NDF, and TDF), minerals, amino acids, fatty acids, vitamins, selected antinutrients and secondary metabolites, and phytosterols. With the exception of grain TDF, starch, chromium, and selenium, which were analyzed by Eurofins Scientific Laboratories, all compositional analyses were performed by EPL Bio-Analytical Services according to methods published and approved by AOAC, or other industry standard analytical methods.

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Table 2: Analytes measured in maize forage and grain samples.

Forage Analytes	Grain Analytes
Acid detergent fiber (ADF)	Acid detergent fiber (ADF)
Neutral detergent fiber (NDF)	Amino acid composition
Minerals:	Fatty acids (complete fatty acid profile)
Calcium	Ferulic and p-coumaric acids
Phosphorus	Folic acid (Vitamin B9)
Proximates:	Furfural
Ash	Iositol
Fat	Neutral detergent fiber (NDF)
Moisture	Minerals:
Protein	Calcium
Carbohydrates (CHO)	Chromium
	Copper
	Iron
	Magnesium
	Manganese
	Phosphorus
	Potassium
	Sodium
	Selenium
	Zinc
	Phytic acid
	Phytosterols:
	Cholesterol
	Campesterol
	Stigmasterol
	β-Sitosterol
	Stigmastanol
	Total phytosterols
	Proximates:
	Ash
	Fat
	Moisture
	Protein
	Carbohydrates (CHO)
	Raffinose
	Starch
	Total dietary fiber (TDF)
	Trypsin inhibitor
	Vitamin A (β-carotene) [including β-cryptoxanthine]
	Vitamin B1 (thiamine)
	Vitamin B2 (riboflavin)
	Vitamin B3 (niacin)
	Vitamin B6 (pyridoxine)
	Vitamin E (α-tocopherol) [including β-, γ-, δ- and total tocopherols]

28.5 STATISTICAL ANALYSIS

For each analyte, data were subjected to an analysis of variance across locations using the generalized linear model:

$$Y_{ij} = U + T_i + L_j + LT_{ij} + e_{ij}$$

where Y_{ij} is the observed response for genotype i at location j , U is the overall mean, T_i is the treatment (HCEM485 vs. control genotypes) effect, L_j is the location effect, LT_{ij} is the location

x treatment (genotype) interaction effect and e_{ij} is the residual error. For each variate, the statistical significance of the genotype effect (*i.e.*, HCEM485 vs. control hybrids) was determined using a standard F-test. An F-test probability of < 0.05 indicates that the difference between the genotypes was statistically significant with 95% confidence. An F-test was also used to assess the significance of the location x genotype interaction – a significant outcome (F-test probability < 0.05) indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations may not be meaningful.

The analyte composition tables for forage and grain include the overall averages for each analyte across locations in both the HCEM485 and control hybrids and the computed 95% confidence interval (95% CI) for each mean value. Also included are the F-test probabilities for both the genotype comparisons and the location by genotype interactions. F-test probabilities that were statistically significant ($p < 0.05$) are indicated in italics with asterisks.

Moisture levels in grain were not subject to statistical analysis of variance since the moisture analysis was performed on grain that had been mechanically dried, thus altering the original moisture content of the harvested grain. Mechanical drying after harvest is a standard agronomic practice for improving storage conditions of maize grain.

29. RESULTS AND DISCUSSION

29.1 PROXIMATES

Analysis of the major constituents of maize, or proximates, was used to determine the nutritional properties of maize grain and forage from different hybrids. The major constituents of maize grain and forage are carbohydrates, protein, fat and ash. Fiber is the predominant form of carbohydrate present in forage and starch is the major carbohydrate in maize grain. Fiber is measured by the neutral detergent fiber method (NDF), which measures the insoluble fiber: lignin, cellulose and hemicellulose. Total dietary fiber (TDF) consists of the insoluble and soluble fiber (pectin). The soluble fiber fraction in maize is negligible, so the NDF value in maize grain is comparable to that of TDF. The acid detergent fiber (ADF) method solubilizes hemicellulose, measuring only cellulose and lignin (Watson, 1987).

Comparison of the proximate composition of the HCEM485 grain and the negative control grain samples is shown in Table 3. No statistically significant differences were observed for protein, fat, carbohydrates, ADF, NDF, ash, starch or carbohydrate. A statistically significant difference was observed for TDF, however, the magnitude of the difference was small (*ca.* 6.4%). The average values for all proximates measured in grain were within the ranges reported in the literature.

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Table 3: Proximate composition of grain from HCEM485 and control hybrids.

Samples		Moisture (%FW)	Protein (%DW)	Fat (%DW)	ADF (%DW)	NDF (%DW)
HCEM485	Mean	12.06	10.14	4.36	4.28	13.56
	95%CI	(11.7-12.4)	(9.0-11.2)	(4.2-4.6)	(4.2-4.4)	(13.1-14.1)
Control hybrids	Mean	12.05	10.23	4.52	4.58	13.60
	95%CI	(11.7-12.4)	(9.2-11.3)	(4.2-4.8)	(4.3-4.8)	(13.2-14.0)
Mean Difference (%)		0.08%	-0.91%	-3.42%	-6.57%	-0.27%
F-test probability for genotype			0.453	0.256	0.075	0.891
F-test genotype x location			0.434	0.259	0.391	0.596
Literature Values						
GA21†	Mean	14.60	9.90	3.50	3.90	11.40
ILSI (2006)	Mean	11.30	10.30	3.555	4.05	11.23
	Range	6.1-40.5	6.15-17.26	1.74-5.82	1.82-11.34	5.59-22.64
OECD (2002)	Range	7.0-23.0	6.0-12.7	3.1-5.8	3.0-4.3	8.3-11.9
Samples			TDF (%DW)	Ash (%DW)	Starch (%DW)	CHO (%DW)
HCEM485	Mean		11.29	1.36	60.64	84.14
	95%CI		(10.7-11.9)	(1.3-1.4)	(59.6-61.7)	(83.1-85.2)
Control hybrids	Mean		10.61	1.39	60.12	83.86
	95%CI		(10.3-11.0)	(1.4-1.4)	(59.6-60.6)	(82.7-85.0)
Mean Difference (%)			6.40%	-1.91%	0.87%	0.34%
F-test probability for genotype			0.044*	0.232	0.271	0.225
F-test genotype x location			0.201	0.851	0.008**	0.598
Literature Values						
GA21†	Mean		ND	1.30	ND	85.20
ILSI (2006)	Mean		16.43	1.439	57.7	84.6
	Range		8.85-35.31	0.616-6.282	26.5-73.8	77.4-89.5
OECD (2002)	Range		11.1	1.1-3.9		82.2-82.9

* = indicates that the difference between the genotypes means was statistically significant at (p < 0.05).

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

All values expressed as percent dry weight, except for moisture. Moisture levels in grain not subject to analysis of variance as grain was mechanically dried after harvest.

95%CI = computed 95% confidence interval around the mean value.

CHO = carbohydrate; ADF = acid detergent; NDF = neutral detergent fiber; TDF = total dietary fiber.

ND = Not determined.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.

Comparison of the proximate composition of the HCEM485 forage and the negative control forage is shown in Table 4. No statistically significant differences were found in five (moisture, ash, carbohydrates, ADF, and NDF) of the seven analytes tested. The only statistically significant differences observed were a higher (*ca.* 6.8%) mean protein content, which was not consistent across locations, and a lower (*ca.* 13.2%) level of total fat in the HCEM485 hybrid samples than in the control samples. The average values for all proximates in forage, including protein and fat, were within the ranges reported in the literature.

Table 4: Proximate composition of forage from HCEM485 and control hybrids.

Samples		Moisture (%FW)	Protein (%DW)	Fat (%DW)	ADF (%DW)	NDF (%DW)	Ash (%DW)	CHO (%DW)
HCEM485	Mean	71.41	9.36	3.14	28.29	48.80	4.19	83.31
	95%CI	(70.4-72.4)	(8.5-10.3)	(2.9-3.3)	(22.7-33.9)	(43.0-54.6)	(4.1-4.3)	(82.4-84.2)
Control hybrids	Mean	70.60	8.77	3.61	26.95	46.89	4.00	83.62
	95%CI	(69.7-71.5)	(8.4-9.1)	(3.4-3.9)	(23.7-30.1)	(42.2-51.3)	(3.9-4.2)	(83.0-84.3)
Mean Difference (%)		1.14%	6.81%	-13.17%	4.99%	4.06%	4.73%	-0.37%
F-test probability for genotype		0.271	0.033*	0.024*	0.742	0.604	0.091	0.598
F-test genotype x location		0.925	0.002**	0.908	0.365	0.317	0.665	0.063
Literature Values								
ILSI (2006)	Mean	70.20	7.78	2.04	27.00	41.51	4.63	85.60
	Range	49.1-81.3	3.14-11.57	0.296-4.570	16.13-47.39	20.29-63.71	1.527-9.638	76.4-92.1
OECD (2002)	Range	62.0-78.0	4.7-9.2	1.5-3.2	25.6-34.0	40.0-48.2	2.9-5.7	

* = indicates that the difference between the genotypes means was statistically significant at (p < 0.05).

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

All values expressed as percent dry weight, except for moisture, which is expressed as percent fresh weight.

CHO = carbohydrate; ADF = acid detergent fiber; NDF = neutral detergent fiber.

29.2 MINERALS

Several mineral ions are recognized as essential plant nutrients and are required by the plant in significant quantities. These macronutrients include calcium, phosphorous, potassium and sodium. The micronutrient minerals, iron, copper and zinc are incorporated in plant tissues in only trace amounts. Maize is an important source of selenium in animal feed (Watson, 1987), and this analyte was also included in the analyses of grain.

Comparison of the mineral composition of the HCEM485 grain and the negative control grain is shown in Table 5. No statistically significant differences were observed for levels of iron, magnesium, manganese, phosphorus, sodium or zinc. Small but statistically significant differences were noted for calcium, copper, and potassium. For selenium, values that were below the limit of quantification (<LOQ) were distributed equally between the HCEM485 hybrid and control hybrids, where 5 out of 12 total values for each set of samples were <LOQ. Analytes with values <LOQ were not suitable for statistical analysis but quantifiable levels of selenium in the HCEM485 samples (ranging from 0.11–0.21 mg/kg dry weight) were all within ranges reported in the literature. Levels of chromium in HCEM485 and control samples were all <LOQ. For all minerals that were statistically analyzed, including those that showed statistically significant differences, average values were within the ranges reported in the literature.

Table 5: Mineral composition of grain from HCEM485 and control hybrids.

Samples		Concentration (ppm dry weight)				
		Ca	Cu	Fe	Mg	Mn
HCEM485	Mean	35.40	1.17	25.15	1314.27	6.13
	95%CI	(33.5-37.3)	(1.10-1.24)	(22.9-27.4)	(1255-1373)	(5.62-6.63)
Control hybrids	Mean	37.51	1.34	25.94	1293.83	5.92
	95%CI	(35.9-39.1)	(1.26-1.43)	(23.5-28.4)	(1255-1332)	(5.33-6.51)
Mean Difference (%)		-5.61%	-13.01%	-3.04%	1.58%	3.51%
F-test probability for genotype		0.015*	<0.001*	0.235	0.437	0.166
F-test genotype x location		0.872	0.556	0.555	0.478	0.050
Literature Values						
GA21†	Mean	30.0	ND	ND	ND	ND
ILSI (2006)	Mean	46.4	1.75	21.81	1193.80	6.18
	Range	12.7-208.4	0.73-18.5	10.42-49.07	594-1940	1.69-14.3
OECD (2002)	Range	30-1000	0.9-10	1-100	820-10000	
Samples		P	K	Na	Se	Zn
HCEM485	Mean	3208.79	3739.93	1.44	<LOQ-0.21	19.82
	95%CI	(3088-3330)	(3637-3843)	(1.04-1.83)		(18.4-21.2)
Control hybrids	Mean	3148.27	3600.90	2.25	<LOQ-0.20	20.50
	95%CI	(3047-3249)	(3485-3716)	(1.36-3.15)		(19.2-21.8)
Mean Difference (%)		1.92%	3.86%	-36.15%		-3.34%
F-test probability for genotype		0.336	0.014*	0.199		0.153
F-test genotype x location		0.381	0.138	0.380		0.718
Literature Values						
GA21†	Mean	2900	ND	ND	ND	ND
ILSI (2006)	Mean	3273.5	3842	31.75	0.20	21.6
	Range	1470-5330	1810-6030	0.17-731.54	0.05-0.75	6.5-37.2
OECD (2002)	Range	2340-7500	3200-7200	0-1500	0.01-1.0	12-30

* = indicates that the difference between the genotypes means was statistically significant at p < 0.05.

Where some of the sample values were less than the limit of qualification (<LOQ), statistical comparison was not possible, so only the range is shown. Values for chromium in all samples tested were <LOQ.

95%CI = computed 95% confidence interval around the mean value.

Ca=calcium; Cu=copper; Fe=iron; Mg=magnesium; Mn=manganese; P=phosphorous; K=potassium; Na=sodium; Se=selenium; Zn=zinc.

ND = Not determined.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.

Comparison of the calcium and phosphorus composition of the HCEM485 forage and the control forage samples is shown in Table 6. Only calcium was statistically significantly higher (ca. 13%) in HCEM485 samples than control samples, and mean levels of both calcium and phosphorus were within the ranges reported in the literature.

Table 6: Mineral composition of forage from HCEM485 and control hybrids.

Samples		Concentration (mg/kg dry weight)	
		Ca	P
HCEM485	Mean	1829.2	2167.7
	95%CI	(1642-2017)	(1935-2401)
Control hybrids	Mean	1617.3	2106.1
	95%CI	(1498-1736)	(1876-2336)
Mean Difference (%)		13.10%	2.93%
F-test probability for genotype		0.006*	0.202
F-test genotype x location		0.065	0.626
Literature Values			
ILSI (2006)	Mean	2028.6	2066.1
	Range	713.9-5767.9	936.2-3704.1
OECD (2002)	Range	1500-3100	2000-2700

* = indicates that the difference between the genotypes means was statistically significant at $p < 0.05$.
Ca=calcium, P=phosphorous.

29.3 VITAMINS

Although animal feed formulations are usually supplemented with additional vitamins to achieve nutritional balance, maize contains two fat-soluble vitamins, vitamin-A (β -carotene) and vitamin E, and most of the water-soluble vitamins. Vitamin A occurs in two forms in nature. Its true form, retinol, is present in foods of animal origin such as fish oils and liver. Provitamin A, in the form of the carotenoids β -carotene and cryptoxanthin are found in plants and converted in the body to vitamin A. Vitamin E (tocopherol) occurs in a variety of vegetable, nut, and oilseed crops, and of the various structural isomers (alpha-, beta-, delta- and gamma-tocopherol), α -tocopherol is the most biologically important as a natural antioxidant. Alpha-tocopherol is the only form of vitamin E that is actively maintained in the human body, and has the greatest nutritional significance (Linus Pauling Institute, 2004). The water-soluble vitamins B1 (thiamine) and B6 (pyridoxine) are present in maize grain at quantities sufficient to be important in animal rations (Watson, 1987).

Comparison of the vitamin analysis of grain is shown in Table 7. Statistically significant differences between HCEM485 and control sample means were observed for levels of tocopherols, thiamine (B1), pyridoxine (B6) and folic acid (B9). The magnitudes of these differences were small, ranging from *ca.* 7–18%, and in some cases (*e.g.*, B6, B9, and α -tocopherol), the differences were not consistent across growing locations. Levels of β -cryptoxanthine and riboflavin (B2) were below the limit of quantification in all samples. For all of the quantifiable analytes, the mean values were within the ranges reported in the literature, including those where significant differences were observed between samples from HCEM485 and control hybrids.

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Table 7: Vitamin analysis of grain from HCEM485 and control hybrids.

Samples		Concentration (mg/100g dry weight)					
		A	B1	B3	B5	B6	B9
HCEM485	Mean	<LOQ-1.382	0.264	2.007	0.530	0.795	0.070
	95%CI		(0.25-0.28)	(1.66-2.36)	(0.53-0.54)	(0.73-0.86)	(0.06-0.08)
Control hybrids	Mean	<LOQ-1.377	0.301	1.899	0.537	0.856	0.083
	95%CI		(0.28-0.32)	(1.55-2.24)	(0.53-0.55)	(0.77-0.94)	(0.07-0.09)
Mean Difference (%)			-12.23%	5.67%	-1.29%	-7.14%	-15.25%
F-test genotype			0.002*	0.235	0.106	0.002*	0.018*
F-test genotype x location			0.417	0.022**	0.062	0.004**	0.015**
Literature Values							
ILSI (2006)	Mean	0.684	0.530	2.376		0.644	0.0651
	Range	0.019-4.68	0.126-4.00	1.04-4.69		0.368-1.13	0.015-0.146
OECD (2002)			0.23-0.86	0.93-7.0		0.46-0.96	
Samples		Tocopherols (mg/100g dry weight)					
		alpha	beta	gamma	delta	total	
HCEM485	Mean	1.336	0.112	3.260	0.135	4.843	
	95%CI	(1.23-1.44)	(0.11-0.12)	(2.72-3.80)	(0.11-0.16)	(4.37-5.32)	
Control hybrids	Mean	1.543	0.119	3.724	0.165	5.551	
	95%CI	(1.40-1.68)	(0.12-0.12)	(3.22-4.23)	(0.14-0.19)	(5.14-5.96)	
Mean Difference (%)		-13.38%	-5.90%	-12.46%	-18.19%	-12.75%	
F-test genotype		<0.001*	0.001*	0.001*	<0.001*	<0.001*	
F-test genotype x location		0.028**	0.251	0.789	0.565	0.628	
Literature Values							
ILSI (2006)	Mean	1.03	0.701	2.948	0.206	4.040	
	Range	0.15-6.87	0.058-2.28	0.646-6.1	0.038-1.61	0.869-13.3	

* = indicates that the difference between the genotypes means was statistically significant at the 95% confidence level.

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

Vitamin A is reported as β-carotene. Identity of B vitamins is as follows: B1=thiamine; B2=riboflavin; B3=niacin; B5=pantothenic acid; B6=pyridoxine; B9=folic acid.

95%CI = computed 95% confidence interval around the mean value.

Where some of the sample values were less than the limit of quantification (<LOQ) statistical comparison was not possible, so only the range is shown. Values for riboflavin (B2) and β-cryptoxanthine were <LOQ for all samples and not included in this analysis.

29.4 AMINO ACIDS

The quality of protein produced by different maize hybrids can be determined by measuring the content of different amino acids. Eighteen amino acids commonly found in maize are considered to be important for compositional analysis (EuropaBio, 2003). Levels of methionine and cysteine are important for formulation of animal feed, as are lysine and tryptophan, which cannot be produced by non-ruminant animals such as swine and poultry and are present at low concentrations in maize.

Comparison of the amino acid composition of HCEM485 grain and the control grain is shown in Table 8. The only significant difference was in mean methionine content between HCEM485 and control samples, however, this difference was not consistent across all growing locations.

Average levels of all amino acids, including methionine, were within the ranges reported in the literature.

Table 8: Amino acid composition of grain from HCEM485 and control hybrids.

		Concentration (mg/g dry weight)								
Samples		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
HCEM485	Mean	7.10	3.40	4.63	21.47	9.55	3.70	7.92	2.22	4.97
	95%CI	(6.31-7.90)	(3.10-3.70)	(4.10-5.16)	(18.6-24.3)	(8.36-10.7)	(3.43-3.98)	(6.91-8.92)	(2.00-2.44)	(4.43-5.51)
Control hybrids	Mean	7.13	3.41	4.74	21.87	9.73	3.72	8.11	2.31	5.00
	95%CI	(6.41-7.85)	(3.13-3.69)	(4.21-5.27)	(19.2-24.5)	(8.63-10.8)	(3.45-4.00)	(7.13-9.09))	(2.05-2.56)	(4.50-5.50)
Mean Difference (%)		-0.32%	-0.41%	-2.24%	-1.82%	-1.85%	-0.63%	-2.41%	-3.78%	-0.58%
F-test genotype		0.836	0.742	0.200	0.319	0.267	0.720	0.146	0.477	0.684
F-test genotype x location		0.454	0.711	0.594	0.522	0.676	0.614	0.529	0.384	0.590
Literature Values										
GA21†	Mean	6.60	3.80	5.40	19.40	8.80	3.70	7.70	2.10	4.50
ILSI (2006)	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
OECD (2002)	Range	4.8-8.5	2.7-5.8	3.5-9.1	12.5-25.8	6.3-13.6	2.6-4.9	5.6-10.4	0.8-3.2	2.1-8.5
Samples		Met	He	Leu	Tyr	Phe	Lys	His	Arg	Trp
HCEM485	Mean	2.49	3.38	12.68	1.55	4.62	3.15	2.89	3.79	0.74
	95%CI	(2.26-2.72)	(2.98-3.79)	(10.88-14.47)	(1.41-1.68)	(4.04-5.20)	(2.85-3.46)	(2.62-3.15)	(3.42-4.16)	(0.68-0.79)
Control hybrids	Mean	2.36	3.46	13.07	1.48	4.74	3.14	2.88	3.83	0.73
	95%CI	(2.18-2.54)	3.08-3.84	(11.39-14.75)	(1.37-1.59)	(4.21-5.28)	(2.84-3.44)	(2.66-3.10)	(3.48-4.17)	(0.68-0.78)
Mean Difference (%)		5.30%	-2.08%	-3.01%	4.64%	-2.57%	0.28%	0.20%	-0.92%	1.31%
F-test genotype		0.011*	0.175	0.102	0.140	0.192	0.892	0.890	0.672	0.358
F-test genotype x location		0.013**	0.673	0.710	0.584	0.802	0.387	0.426	0.572	0.597
Literature Values										
GA21†	Mean	2.00	3.50	13.20	4.00	5.10	2.80	7.70	4.00	0.60
ILSI (2006)	Mean	2.09	3.68	13.41	3.36	5.25	3.15	2.96	4.33	0.63
	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
OECD (2002)	Range	1.0-4.6	2.2-7.1	7.9-24.1	1.2-7.9	2.9-6.4	1.5-3.8	0.5-5.5	2.2-6.4	0.4-1.3

* = indicates that the difference between the genotypes means was statistically significant at (p < 0.05).
 ** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.
 95%CI = computed 95% confidence interval around the mean value.
 Asp=aspartic acid; Thr=threonine; Ser=serine; Glu=glutamic acid; Pro=proline; Gly=glycine; Ala=alanine; Cys=cysteine; Val=valine;
 Met=methionine; Ile=isoleucine; Leu=leucine; Tyr=tyrosine; Phe=phenylalanine; His=histidine; Lys=lysine; Arg=arginine; Trp=tryptophan.
 † ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.

29.5 FATTY ACIDS

Five fatty acids account for nearly 98 percent of the total fatty acids in maize grain (ILSI, 2006), with the most abundant being linoleic (C18:2 Δ9,12; 57.6%) and oleic (C18:1 Δ9; 26.0%) acids. Less abundant, but occurring at measurable levels are palmitic (C16:0; 11.03%), stearic (C18:0; 1.8%) and α-linolenic (C18:3 Δ9,12,15; 1.13%) acids.

The desaturation of oleic acid to form linoleic acid, and its subsequent desaturation to form α -linolenic acid, occurs only in plants, hence both linoleic and α -linolenic acids are essential fatty acids for mammals. For this reason, it was desirable to measure for any unintended changes in the levels of linoleic and α -linolenic acids, and their key precursors, palmitic, stearic and oleic acids, in grain from HCEM485.

Other polyunsaturated and longer chain polyunsaturated fatty acids, such as γ -linolenic (C18:3 Δ 6,9,12), eicosatrienoic (C20:3 Δ 8,11,14) and arachidonic (C20:4 Δ 5,8,11,14) acids can all be synthesized by mammals from dietary sources of α -linolenic and linoleic acid. Hence, small changes in the levels of these trace fatty acids in HCEM485-derived grain would have little or no biological significance to either humans or animals consuming HCEM485 grain products. The synthesis of palmitoleic (C16:1 Δ 9) and saturated fatty acids with chain lengths greater than 18 (e.g., C20:0, C22:0, C24:0), can be accomplished in mammals through *de novo* fatty acid synthesis without dietary requirements for palmitic and stearic acids, respectively.

The complete fatty acid profile of maize grain from HCEM485 and control hybrids was determined and the results are summarized in Table 9. The concentrations of the following fatty acids were below the limit of quantification in one or more samples and not included in the analysis: caprylic (C8:0); capric (C10:0); lauric (C12:0); myristic (C14:0); myristoleic (C14:1); pentadecanoic (C15:0); pentadecenoic (C15:1); palmitoleic (C16:1); heptadecanoic (C17:0); heptadecenoic (C17:1); gamma-linolenic (C18:3); eicosadienoic (C20:2); arachidonic (C20:4); eicosatrienoic (C20:3); behenic (C22:0); and erucic (C22:1). Statistically significant differences observed for quantifiable fatty acids were for palmitic (C16:0), stearic (C18:0), oleic (C18:1), linolenic (C18:3) and eicosenoic (C20:1), however, the magnitude of these differences was small, ranging between *ca.* 1% and 4.4%. Average levels of all quantifiable fatty acids, including those where significant differences were observed, were within the ranges reported in the literature.

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Table 9: Fatty acid composition of grain from HCEM485 and control hybrids.

Samples	Amount (% total fatty acids)								
	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Arachidic (C20:0)	Eicosenoic (C20:1)	Lignoceric (C24:0)	
HCEM485	Mean	9.79	1.87	24.85	61.35	1.06	0.43	0.25	0.22
	95%CI	(9.7-9.9)	(1.8-1.9)	(24.2-25.5)	(60.5-62.2)	(1.03-1.10)	(0.43-0.44)	(0.25-0.26)	(0.22-0.23)
Control hybrids	Mean	9.69	1.79	25.42	60.87	1.09	0.43	0.26	0.23
	95%CI	(9.5-9.8)	(1.7-1.9)	(25.0-25.8)	(60.3-61.5)	(1.06-1.12)	(0.4-0.4)	(0.26-0.27)	(0.22-0.24)
Mean Difference (%)		1.06%	4.38%	-2.22%	0.80%	-2.40%	0.61%	-3.31%	-2.87%
F-test genotype		0.017*	0.001*	0.01*	0.079	0.008	0.488	0.01*	0.267
F-test genotype x location		0.292	0.145	0.046**	0.124	0.537	0.685	0.111	0.052
Literature Values									
GA21†	Mean	9.90	1.80	27.1	59.1	1.1	0.40	0.30	ND
ILSI (2006)	Mean	11.5	1.82	25.8	57.6	1.2	0.41	0.3	0.17
	Range	7.94-20.71	1.02-3.40	17.4-40.2	36.2-66.5	0.57-2.25	0.28-0.97	0.17-1.92	0.140-0.230

* = indicates that the difference between the genotypes means was statistically significant at p < 0.05.

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

The concentrations of the following fatty acids were below the limit of quantification (<LOQ) in one or more samples and were not subject to statistical analysis: caprylic (C8:0); capric (C10:0); lauric (C12:0); myristic (C14:0); myristoleic (C14:1); pentadecanoic (C15:0); pentadecenoic (C15:1); palmitoleic (C16:1); heptadecanoic (C17:0); heptadecenoic (C17:1); gamma-linolenic (C18:3); eicosadienoic (C20:2); eicosatrienoic (C20:3); arachidonic (C20:4); behenic (C22:0) and erucic (C22:1).

ND = Not determined.

† ANZFA (2000). Values for GA21 samples from plants treated with glyphosate.

29.6 SECONDARY METABOLITES AND ANTINUTRIENTS

Secondary metabolites are defined as those natural products which do not function directly in the primary biochemical activities that support growth, development and reproduction of the organism in which they occur (EuropaBio, 2003). One class of secondary metabolites, antinutrients, is responsible for deleterious effects related to the absorption of nutrients and micronutrients from foods (Shahidi, 1997). There are generally no recognized antinutrients in maize at levels considered to be harmful, but for the purposes of safety assessment OECD recommends testing for the following secondary metabolites in maize: ferulic acid, p-coumaric acid, furfural, inositol, phytic acid, raffinose and trypsin inhibitor. These secondary metabolites and antinutrients were analyzed in grain samples from HCEM485 and control hybrids (Table 10).

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Table 10: Secondary metabolites and antinutrients in grain from HCEM485 and control hybrids.

Samples	Concentration (mg/100g)						
	Ferulic acid	ρ -Coumaric acid	Inositol	Phytic acid	Raffinose	Trypsin inhibitor (TIU/mg)	
HCEM485	Mean	222.82	16.52	12.00	800.57	207.73	4.31
	95%CI	(208.7-237.0)	(14.0-19.1)	(10.8-13.2)	(749.9-851.2)	(184.3-231.1)	(4.2-4.4)
Control hybrids	Mean	219.81	16.85	13.85	782.17	205.32	4.17
	95%CI	(212.9-226.7)	(14.9-18.8)	(13.2-14.5)	(747.8-816.5)	(174.0-236.6)	(4.0-4.3)
Mean Difference (%)		1.37%	-1.95%	-13.36%	2.35%	1.17%	3.38%
F-test genotype		0.679	0.598	0.001*	0.533	0.731	0.011*
F-test genotype x location		0.775	0.644	0.139	0.295	0.419	0.012**
Literature Values							
ILSI (2006)	Mean	220.1	21.8	133.2	745	132	2.73
	Range	29.2-388.6	5.34-57.6	8.9-376.5	111-1570	20-320	1.09-7.18
OECD (2002)		20-300	3-30		450-1000	210-310	

* = indicates that the difference between the genotypes means was statistically significant at $p < 0.05$.

** = indicates that the effect of genotype was not consistent across all locations, in which case the comparison of genotypes averaged across locations is questionable.

95%CI = computed 95% confidence interval around the mean value.

Levels of furfural in all samples were <LOQ and were not included in this analysis.

Phenolic acids — may have beneficial health effects because of their anti-oxidant properties. Ferulic acid and ρ -coumaric acid are weak anti-oxidants. *In vitro* tests are equivocal as to whether ferulic acid enhances or inhibits the effects of mutagenic substances (Sasaki *et al.*, 1989; Stich, 1992). Ferulic acid and ρ -coumaric acid are found in vegetables, fruit and cereals. They are also used as flavoring in foods, as supplements and in traditional Chinese herbal medicine. Daily intake of phenolic acids by humans is estimated to be 0.2–5.2 mg/day (Clifford, 1999; Radtke *et al.*, 1998).

There were no significant differences in mean ferulic acid or ρ -coumaric acid between grain samples from HCEM485 and control hybrids (Table 10).

Furfural — is a heterocyclic aldehyde which occurs in several vegetables, fruits and cereals. It is used as a pesticide, but also in foodstuff as flavoring. Furfural is generally recognized as safe (GRAS) under conditions of intended use as a flavor ingredient. Field maize generally contains < 0.01 ppm (< 0.001 mg/100g) furfural (Adams *et al.*, 1997). Furfural was below the lower limit of quantification in all grain samples.

Phytic acid — (myo-inositol 1,2,3,4,5,6-hexakis[dihydrogenphosphate]) is considered to be an antinutrient due to its ability to bind minerals, proteins and starch at physiological pH (Rickard and Thompson, 1997). Phytic acid is present in maize germ and binds 60–75 percent of phosphorous in the form of phytate (NRC, 1998), decreasing the bioavailability of phosphorous in maize for non-ruminant animals. Phytic acid levels in maize grain vary from 0.45–1.0 percent of dry matter (Watson, 1982).

There was no significant difference in mean phytic acid level between grain samples from HCEM485 or control hybrids, although average inositol levels were significantly lower (*ca.*

-13%) in HCEM485 grain samples (Table 10). In both cases, the average values were well within the ranges reported in the literature for these two analytes.

Alpha-galactosides — of sucrose, including raffinose, are widely distributed in higher plants (Naczki *et al.*, 1997). Due to the absence of alpha-galactosidase activity in human and animal mucosa, raffinose cannot be broken down by enzymes in the gastrointestinal tract and is considered an antinutrient, although it is not toxic. No statistically significant differences were detected in raffinose levels between the HCEM485 and control grain samples and all values were within ranges reported in the literature (Table 10).

Protease inhibitors — are found in abundance in raw cereals and legumes, especially soybeans. Trypsin inhibitors in soybean give rise to inactivation and loss of trypsin in the small intestine, triggering the induction of excess trypsin in the pancreas at the expense of sulfur-containing amino acids (Shahidi, 1997). Maize contains low levels of trypsin and chymotrypsin inhibitors, neither of which is considered nutritionally significant (White and Pollak, 1995). A small, but statistically significant increase (*ca.* 3.4%) in mean trypsin inhibitor activity was observed from HCEM485 grain samples compared with control samples (Table 10), but this difference was not consistent across all growing locations and levels of trypsin inhibitor for all samples were within the range reported in the literature.

29.7 PHYTOSTEROLS

Phytosterols are cholesterol-like molecules found in all plant foods, with the highest concentrations occurring in vegetable oils. They are absorbed only in trace amounts but have the beneficial effect of inhibiting the absorption of dietary cholesterol (Ostlund, 2002). Phytosterols are not endogenously synthesized in the body but are derived solely from the diet (Rao and Koratkar, 1997).

There were no significant differences in mean levels of cholesterol, campesterol, stigmasterol, β -sitosterol, stigmastanol, or total phytosterols between grain samples from HCEM485 or control hybrids (Table 11).

Table 11: Phytosterol composition of grain from HCEM485 and control hybrids.

Samples	Concentration (mg/100g)						
	Cholesterol	Campesterol	Stigmasterol	β -sitosterol	Stigmastanol	Total	
HCEM485	Mean	0.232	9.376	2.961	54.412	10.879	77.860
	95%CI	(0.21-0.26)	(8.5-10.2)	(2.7-3.2)	(53.1-55.7)	(10.3-11.4)	(76.1-79.6)
Control hybrids	Mean	0.234	9.508	3.099	55.692	10.739	79.273
	95%CI	(0.23-0.24)	(8.7-10.3)	(2.9-3.3)	(54.0-57.4)	(10.11-11.4)	(77.2-81.3)
Mean Difference (%)		-1.11%	-1.39%	-4.44%	-2.30%	1.30%	-1.78%
F-test genotype		0.870	0.591	0.134	0.170	0.513	0.307
F-test genotype x location		0.598	0.470	0.424	0.544	0.761	0.575
Literature Values							
Souci <i>et al.</i> (1994)			32	21	120		

95%CI = computed 95% confidence interval around the mean value.

30. CONCLUSIONS

Levels of key nutrients, minerals, antinutrients, and secondary metabolites were determined in samples of maize grain and forage derived from HCEM485 and control hybrids collected from up to four field trial locations in 2007. For most analytes, there were no statistically significant differences and in cases where statistically significant differences were observed, the magnitudes of the differences were small and in every case, mean values determined for both HCEM485 and control samples were within the ranges of natural variation as reported in the literature. Overall, no consistent patterns emerged to suggest that biologically significant changes in composition of the grain or forage had occurred as an unintended consequence of the genetic modification resulting in maize line HCEM485. The conclusion based on these data was that grain and forage from HCEM485 maize were substantially equivalent in composition to both the control hybrids included in this study and to other commercial maize hybrids.

31. CONTRIBUTING SCIENTISTS

The analytical work reported herein was conducted by EPL Bio-Analytical Services, Niantic, IL, and Eurofins Scientific Laboratories, Des Moines, IA. Contributing scientists and staff from Stine Biotechnology (SB) and Stine Seed Farm (SSF) include:

- B. Held, V. Sekar (SB)
- J. Behn, J. Mason, K. Muir (SSF)

Approved by: _____ Date _____
Joseph B. Saluri
Vice President
Stine Seed Farm, Inc.

32. RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Stine Seed Farm, Inc., 22555 Laredo Trail, Adel, Iowa 50003.

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**Annex 3 - Compositional analysis of grain and forage derived from HCEM485 hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098**

**Maize Line HCEM485
USDA Extension Petition**

34. APPENDIX A: RAW DATA.

Compositional Data - Forage Proximates

Location	Sample	Rep	Moisture	Protein	Fat	ADF	NDF	Ash	CHO
Adel	HCEM485	4	69.17	8.32	3.51	22.89	40.04	3.99	84.17
Adel	HCEM485	5	72.01	7.64	3.29	14.95	40.52	4.18	84.90
Adel	HCEM485	6	70.62	8.29	2.63	27.77	41.95	4.35	84.73
Fithian	HCEM485	4	71.15	10.15	3.11	36.19	53.01	4.22	82.51
Fithian	HCEM485	4	71.37	10.26	3.28	31.69	53.69	4.32	82.14
Fithian	HCEM485	5	71.90	10.30	2.98	28.20	52.48	4.09	82.63
Fithian	HCEM485	6	73.63	10.58	3.15	36.36	59.90	4.21	82.06
Mean			71.41	9.36	3.14	28.29	48.80	4.19	83.31
SD			1.37	1.23	0.28	7.60	7.86	0.12	1.25
95% CI Lower Bound			70.39	8.46	2.93	22.66	42.98	4.10	82.38
95% CI Upper Bound			72.42	10.27	3.34	33.92	54.62	4.29	84.23
Adel	Control hybrids	4	68.75	8.26	3.14	20.14	38.13	3.97	84.62
Adel	Control hybrids	5	70.49	8.85	4.14	22.99	44.02	4.15	82.86
Adel	Control hybrids	5	70.20	8.80	3.87	23.18	48.96	4.00	83.33
Adel	Control hybrids	6	70.38	8.13	3.24	26.75	37.02	3.65	84.98
Fithian	Control hybrids	4	69.76	8.28	3.39	30.55	47.46	3.72	84.62
Fithian	Control hybrids	5	73.04	9.58	3.56	33.80	55.69	4.23	82.62
Fithian	Control hybrids	6	71.07	8.93	3.97	27.64	52.00	4.08	83.02
Fithian	Control hybrids	6	71.13	9.30	3.59	30.53	51.87	4.24	82.87
Mean			70.60	8.77	3.61	26.95	46.89	4.00	83.62
SD			1.24	0.52	0.36	4.62	6.71	0.22	0.96
95% CI Lower Bound			69.74	8.41	3.37	23.75	42.24	3.85	82.95
95% CI Upper Bound			71.47	9.13	3.86	30.15	51.55	4.16	84.28

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**Annex 3 - Compositional analysis of grain and forage derived from HCEM485 hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098**

**Maize Line HCEM485
USDA Extension Petition**

Compositional Data - Forage Minerals

Location	Sample	Rep	Ca	P
Adel	HCEM485	4	1575.10	2198.67
Adel	HCEM485	5	1476.32	2662.74
Adel	HCEM485	6	1650.96	2518.99
Fithian	HCEM485	4	2031.25	1892.36
Fithian	HCEM485	4	2076.23	1888.67
Fithian	HCEM485	5	1948.40	2104.09
Fithian	HCEM485	6	2045.95	1908.61
Mean			1829.17	2167.73
SD			252.95	314.71
95% CI Lower Bound			1641.79	1934.59
95% CI Upper Bound			2016.56	2400.88
Adel	Control hybrids	4	1558.91	2342.36
Adel	Control hybrids	5	1582.36	2365.08
Adel	Control hybrids	5	1462.37	2451.25
Adel	Control hybrids	6	1407.35	2438.56
Fithian	Control hybrids	4	1541.72	1673.99
Fithian	Control hybrids	5	1936.96	2025.00
Fithian	Control hybrids	6	1682.38	1725.52
Fithian	Control hybrids	6	1766.37	1826.98
Mean			1617.30	2106.09
SD			171.99	331.36
95% CI Lower Bound			1498.12	1876.47
95% CI Upper Bound			1736.49	2335.71

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**Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098**

**Maize Line HCEM485
USDA Extension Petition**

Compositional Data - Grain Proximates

Location	Sample	Rep	Moisture	Protein	Fat	ADF	NDF	TDF	Ash	Starch	CHO
Adel	HCEM485	4	11.45	11.60	4.95	4.29	12.63	11.18	1.40	58.20	82.05
Adel	HCEM485	5	11.58	11.67	4.49	4.18	13.34	11.08	1.38	59.10	82.46
Adel	HCEM485	6	11.34	11.40	4.48	4.04	12.58	10.15	1.38	60.80	82.73
Fithian	HCEM485	4	11.84	11.07	3.98	4.42	14.61	10.78	1.38	60.40	83.57
Fithian	HCEM485	5	11.66	11.37	4.09	4.50	14.47	10.64	1.35	58.50	83.19
Fithian	HCEM485	6	11.16	11.02	4.19	4.02	11.91	10.47	1.25	59.90	83.53
Blomkest	HCEM485	4	12.15	10.69	3.81	4.47	13.98	14.00	1.35	58.30	84.15
Blomkest	HCEM485	5	12.38	10.85	4.20	4.28	13.54	12.55	1.44	61.30	83.51
Blomkest	HCEM485	6	12.10	11.10	4.05	4.63	14.90	10.58	1.37	62.10	83.48
Spencerville	HCEM485	4	13.19	6.47	4.44	4.19	13.85	11.63	1.22	63.60	87.87
Spencerville	HCEM485	5	13.12	7.64	4.82	4.42	13.78	11.28	1.42	62.40	86.13
Spencerville	HCEM485	6	12.75	6.75	4.83	3.92	13.16	11.12	1.37	63.10	87.05
Mean			12.06	10.14	4.36	4.28	13.56	11.29	1.36	60.64	84.14
SD			0.68	1.96	0.37	0.22	0.89	1.06	0.06	1.89	1.86
95% CI Lower Bound			11.67	9.03	4.15	4.16	13.06	10.69	1.32	59.57	83.09
95% CI Upper Bound			12.45	11.24	4.57	4.40	14.07	11.89	1.40	61.71	85.19
Adel	Control hybrids	4	11.70	11.50	5.09	4.40	12.90	10.20	1.40	61.10	82.00
Adel	Control hybrids	5	11.80	11.80	5.31	4.79	13.10	11.23	1.44	60.00	81.50
Adel	Control hybrids	6	11.70	11.60	5.08	4.49	12.90	10.41	1.40	59.90	81.90
Fithian	Control hybrids	4	11.50	11.50	4.83	5.18	13.00	9.61	1.32	60.30	82.30
Fithian	Control hybrids	5	11.20	11.00	4.58	4.41	13.10	10.81	1.31	60.00	83.10
Fithian	Control hybrids	6	11.10	11.40	3.80	4.22	13.10	10.01	1.34	62.00	83.50
Blomkest	Control hybrids	4	12.20	10.50	3.78	4.57	15.00	9.80	1.41	60.90	84.30
Blomkest	Control hybrids	5	12.50	10.70	3.96	4.24	14.20	10.97	1.46	58.60	83.90
Blomkest	Control hybrids	6	12.10	10.80	4.10	4.18	14.00	10.70	1.44	60.20	83.60
Spencerville	Control hybrids	4	12.90	7.08	3.90	3.96	14.00	11.72	1.31	59.60	87.70
Spencerville	Control hybrids	5	12.90	7.71	4.91	4.94	14.20	11.02	1.44	59.70	85.90
Spencerville	Control hybrids	6	13.00	7.16	4.84	5.59	13.50	10.81	1.37	59.10	86.60
Mean			12.05	10.23	4.52	4.58	13.60	10.61	1.39	60.12	83.86
SD			0.66	1.81	0.57	0.47	0.69	0.62	0.06	0.91	1.97
95% CI Lower Bound			11.68	9.21	4.19	4.32	13.21	10.25	1.36	59.60	82.74
95% CI Upper Bound			12.42	11.25	4.84	4.85	13.99	10.96	1.42	60.63	84.97

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**Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
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**Maize Line HCEM485
USDA Extension Petition**

Compositional Data - Grain Amino Acids

Location	Sample	Rep	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
Adel	HCEM485	4	7.87	3.82	5.35	24.89	11.26	4.23	9.11	2.54	5.62
Adel	HCEM485	5	8.00	3.73	5.23	24.85	11.11	4.11	9.13	2.61	5.65
Adel	HCEM485	6	8.03	3.74	5.26	25.10	11.17	4.08	9.20	2.59	5.67
Fithian	HCEM485	4	7.90	3.64	5.08	23.89	10.51	3.78	8.75	2.14	5.33
Fithian	HCEM485	5	8.11	3.71	5.14	23.67	10.52	4.03	8.73	2.34	5.52
Fithian	HCEM485	6	7.70	3.69	5.23	24.46	10.59	4.01	8.88	2.31	5.45
Blomkest	HCEM485	4	7.59	3.54	4.87	23.01	10.05	3.72	8.49	2.34	5.31
Blomkest	HCEM485	5	7.99	3.64	5.15	24.29	10.50	3.85	8.92	2.39	5.45
Blomkest	HCEM485	6	7.61	3.65	4.97	23.75	10.52	3.77	8.74	2.49	5.44
Spencerville	HCEM485	4	4.46	2.37	2.90	11.65	5.50	2.76	4.46	1.86	3.10
Spencerville	HCEM485	5	5.41	2.73	3.36	15.25	6.78	3.12	5.64	1.61	3.73
Spencerville	HCEM485	6	4.57	2.50	3.03	12.80	6.13	2.95	4.93	1.43	3.37
Mean			7.10	3.40	4.63	21.47	9.55	3.70	7.92	2.22	4.97
SD			1.41	0.53	0.94	5.06	2.11	0.49	1.78	0.39	0.96
95% CI Lower Bound			6.31	3.10	4.10	18.60	8.36	3.43	6.91	2.00	4.43
95% CI Upper Bound			7.90	3.70	5.16	24.33	10.74	3.98	8.92	2.44	5.51

Location	Sample	Rep	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
Adel	HCEM485	4	2.81	3.90	14.93	1.92	5.50	3.47	3.33	4.62	0.82
Adel	HCEM485	5	2.81	3.85	14.63	1.78	5.22	3.63	3.15	4.30	0.78
Adel	HCEM485	6	2.94	3.88	14.87	1.63	5.30	3.60	3.17	4.31	0.86
Fithian	HCEM485	4	2.58	3.70	14.41	1.71	5.22	3.17	3.11	3.89	0.78
Fithian	HCEM485	5	2.63	3.76	14.22	1.80	5.39	3.47	3.22	4.33	0.82
Fithian	HCEM485	6	2.78	3.73	14.25	1.56	5.03	3.47	3.07	4.13	0.80
Blomkest	HCEM485	4	2.41	3.62	13.77	1.54	4.87	3.35	3.01	3.80	0.71
Blomkest	HCEM485	5	2.63	3.73	14.22	1.49	4.92	3.52	3.07	3.88	0.78
Blomkest	HCEM485	6	2.65	3.77	14.38	1.48	5.13	3.24	3.09	3.81	0.75
Spencerville	HCEM485	4	1.75	1.99	6.55	1.13	2.62	2.19	1.98	2.58	0.56
Spencerville	HCEM485	5	2.01	2.46	8.49	1.30	3.28	2.58	2.30	3.05	0.60
Spencerville	HCEM485	6	1.81	2.21	7.41	1.22	2.99	2.14	2.12	2.80	0.59
Mean			2.49	3.38	12.68	1.55	4.62	3.15	2.89	3.79	0.74
SD			0.41	0.71	3.17	0.24	1.03	0.54	0.47	0.65	0.10
95% CI Lower Bound			2.26	2.98	10.88	1.41	4.04	2.85	2.62	3.42	0.68
95% CI Upper Bound			2.72	3.79	14.47	1.68	5.20	3.46	3.15	4.16	0.79

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**Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
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**Maize Line HCEM485
USDA Extension Petition**

Compositional Data - Grain Amino Acids

Location	Sample	Rep	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val
Adel	Control hybrids	4	7.85	3.70	5.25	24.74	11.07	4.02	9.14	2.48	5.56
Adel	Control hybrids	5	8.12	3.81	5.40	25.81	11.40	4.02	9.55	2.29	5.71
Adel	Control hybrids	6	7.91	3.76	5.38	24.95	11.10	4.10	9.24	2.24	5.56
Fithian	Control hybrids	4	8.18	3.86	5.80	25.80	11.44	4.23	9.66	2.14	5.71
Fithian	Control hybrids	5	7.88	3.59	5.29	23.86	10.24	3.98	8.94	2.38	5.28
Fithian	Control hybrids	6	7.96	3.66	5.11	24.46	10.68	4.01	9.00	3.27	5.53
Blomkest	Control hybrids	4	7.17	3.53	4.84	22.31	10.19	3.67	8.35	2.20	5.22
Blomkest	Control hybrids	5	7.85	3.73	5.18	24.46	10.79	4.09	8.99	2.94	5.58
Blomkest	Control hybrids	6	7.36	3.44	4.83	22.90	10.07	3.61	8.50	2.22	5.13
Spencerville	Control hybrids	4	4.72	2.55	3.10	13.61	6.38	2.86	5.10	2.17	3.45
Spencerville	Control hybrids	5	5.47	2.74	3.46	15.17	7.01	3.12	5.59	1.69	3.75
Spencerville	Control hybrids	6	5.07	2.57	3.19	14.30	6.42	2.96	5.27	1.68	3.52
Mean			7.13	3.41	4.74	21.87	9.73	3.72	8.11	2.31	5.00
SD			1.27	0.49	0.94	4.65	1.94	0.48	1.73	0.45	0.88
95% CI Lower Bound			6.41	3.13	4.21	19.24	8.63	3.45	7.13	2.05	4.50
95% CI Upper Bound			7.85	3.69	5.27	24.50	10.83	4.00	9.09	2.56	5.50

Location	Sample	Rep	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Trp
Adel	Control hybrids	4	2.60	3.85	14.92	1.64	5.42	3.45	3.16	4.22	0.77
Adel	Control hybrids	5	2.47	3.98	15.61	1.72	5.48	3.44	3.10	4.19	0.79
Adel	Control hybrids	6	2.69	3.91	15.10	1.65	5.43	3.39	3.17	4.33	0.80
Fithian	Control hybrids	4	2.55	4.05	15.69	1.71	5.58	3.42	3.22	4.25	0.82
Fithian	Control hybrids	5	2.64	3.70	13.93	1.45	4.96	3.61	2.97	4.11	0.80
Fithian	Control hybrids	6	2.84	3.81	14.38	1.45	5.14	3.62	3.08	4.30	0.77
Blomkest	Control hybrids	4	2.23	3.65	14.04	1.58	5.13	3.12	3.05	3.83	0.75
Blomkest	Control hybrids	5	2.26	3.83	14.56	1.55	5.25	3.59	3.11	4.35	0.71
Blomkest	Control hybrids	6	2.28	3.60	13.83	1.35	4.86	3.17	2.95	3.70	0.75
Spencerville	Control hybrids	4	1.92	2.27	7.90	1.13	3.09	2.15	2.15	2.66	0.58
Spencerville	Control hybrids	5	1.95	2.50	8.86	1.33	3.49	2.36	2.39	3.07	0.61
Spencerville	Control hybrids	6	1.90	2.33	8.02	1.17	3.09	2.40	2.20	2.91	0.58
Mean			2.36	3.46	13.07	1.48	4.74	3.14	2.88	3.83	0.73
SD			0.32	0.67	2.97	0.20	0.94	0.53	0.39	0.61	0.09
95% CI Lower Bound			2.18	3.08	11.39	1.37	4.21	2.84	2.66	3.48	0.68
95% CI Upper Bound			2.54	3.84	14.75	1.59	5.28	3.44	3.10	4.17	0.78

**Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
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Compositional Data - Grain Fatty Acids

Location	Sample	Rep	palmitic	stearic	oleic	linoleic	linolenic	arachidonic	eicosenoic	lignocenic
Adel	HCEM485	4	10.049	1.937	25.362	60.507	1.028	0.438	0.244	0.232
Adel	HCEM485	5	9.942	1.923	26.218	59.726	1.082	0.437	0.236	0.228
Adel	HCEM485	6	9.844	1.890	25.839	60.248	1.051	0.446	0.246	0.233
Fithian	HCEM485	4	9.618	1.844	23.762	62.845	1.015	0.435	0.274	0.207
Fithian	HCEM485	5	9.617	1.880	24.267	62.213	1.023	0.437	0.264	0.205
Fithian	HCEM485	6	9.847	2.075	25.197	60.497	1.090	0.439	0.250	0.248
Blomkest	HCEM485	4	9.461	1.721	23.334	63.549	0.996	0.438	0.274	0.227
Blomkest	HCEM485	5	9.496	1.761	23.090	63.547	1.001	0.416	0.259	0.223
Blomkest	HCEM485	6	9.499	1.782	23.919	62.718	1.016	0.421	0.252	0.217
Spencerville	HCEM485	4	10.110	1.883	26.196	59.632	1.170	0.449	0.248	0.224
Spencerville	HCEM485	5	9.845	1.851	25.105	61.096	1.127	0.417	0.251	0.229
Spencerville	HCEM485	6	10.152	1.915	25.933	59.675	1.171	0.436	0.246	0.224
Mean			9.790	1.872	24.852	61.354	1.064	0.434	0.254	0.225
SD			0.247	0.093	1.131	1.526	0.063	0.011	0.012	0.011
95% CI Lower Bound			9.650	1.819	24.212	60.491	1.028	0.428	0.247	0.218
95% CI Upper Bound			9.930	1.924	25.492	62.218	1.100	0.440	0.260	0.231
Adel	Control hybrids	4	9.933	1.798	26.105	59.926	1.090	0.434	0.267	0.233
Adel	Control hybrids	5	9.959	1.796	25.236	60.646	1.088	0.438	0.254	0.228
Adel	Control hybrids	6	9.833	1.779	25.335	60.814	1.079	0.440	0.259	0.248
Fithian	Control hybrids	4	9.694	1.916	26.132	59.834	1.080	0.439	0.264	0.274
Fithian	Control hybrids	5	9.641	1.957	25.754	60.417	1.082	0.443	0.261	0.241
Fithian	Control hybrids	6	9.692	1.931	25.089	61.029	1.099	0.440	0.272	0.227
Blomkest	Control hybrids	4	9.260	1.634	24.447	62.566	1.027	0.429	0.262	0.200
Blomkest	Control hybrids	5	9.288	1.659	24.760	62.195	1.027	0.409	0.262	0.210
Blomkest	Control hybrids	6	9.302	1.635	24.327	62.676	1.021	0.409	0.258	0.194
Spencerville	Control hybrids	4	9.937	1.808	25.694	60.274	1.153	0.444	0.268	0.256
Spencerville	Control hybrids	5	9.779	1.792	26.077	60.111	1.156	0.425	0.263	0.231
Spencerville	Control hybrids	6	9.934	1.815	26.038	59.932	1.186	0.427	0.257	0.234
Mean			9.688	1.793	25.416	60.868	1.091	0.431	0.262	0.232
SD			0.266	0.109	0.654	1.042	0.053	0.012	0.005	0.023
95% CI Lower Bound			9.537	1.732	25.046	60.279	1.061	0.425	0.259	0.219
95% CI Upper Bound			9.838	1.855	25.786	61.458	1.120	0.438	0.265	0.244

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**Maize Line HCEM485
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Compositional Data - Grain Vitamins

		Tocopherols											
Location	Sample	Rep	A	B1	B3	B5	B6	B9	alpha	beta	gamma	delta	total
Adel	HCEM485	4	<LOQ	0.298	1.332	0.526	0.957	0.065	1.514	0.104	2.060	0.092	3.770
Adel	HCEM485	5	<LOQ	0.276	1.549	0.518	0.942	0.063	1.515	0.105	2.116	0.093	3.829
Adel	HCEM485	6	<LOQ	0.232	1.520	0.525	0.941	0.069	1.718	0.106	2.160	0.092	4.077
Fithian	HCEM485	4	<LOQ	0.256	1.549	0.541	0.774	0.071	1.374	0.114	3.305	0.130	4.924
Fithian	HCEM485	5	<LOQ	0.266	2.025	0.541	0.747	0.078	1.411	0.107	3.007	0.117	4.641
Fithian	HCEM485	6	0.560	0.289	1.676	0.531	0.825	0.070	1.342	0.116	2.525	0.106	4.090
Blomkest	HCEM485	4	0.919	0.250	2.157	0.525	0.776	0.093	1.238	0.106	3.388	0.134	4.866
Blomkest	HCEM485	5	<LOQ	0.260	1.840	0.548	0.796	0.065	1.282	0.109	3.409	0.140	4.941
Blomkest	HCEM485	6	0.825	0.291	1.606	0.522	0.854	0.092	1.311	0.116	3.488	0.137	5.051
Spencerville	HCEM485	4	0.609	0.258	3.270	0.528	0.555	0.050	1.008	0.119	4.164	0.182	5.473
Spencerville	HCEM485	5	1.382	0.259	2.881	0.531	0.715	0.069	1.213	0.125	4.896	0.205	6.438
Spencerville	HCEM485	6	0.588	0.234	2.675	0.528	0.654	0.058	1.112	0.113	4.602	0.193	6.020
Mean			0.814	0.264	2.007	0.530	0.795	0.070	1.336	0.112	3.260	0.135	4.843
SD			0.313	0.021	0.621	0.009	0.121	0.013	0.191	0.007	0.951	0.039	0.840
95% CI Lower Bound			0.636	0.252	1.655	0.525	0.726	0.063	1.228	0.108	2.722	0.113	4.368
95% CI Upper Bound			0.991	0.276	2.358	0.535	0.863	0.077	1.445	0.115	3.798	0.157	5.319
Adel	Control hybrids	4	<LOQ	0.348	1.028	0.531	0.943	0.070	1.681	0.117	2.595	0.113	4.506
Adel	Control hybrids	5	1.027	0.310	1.045	0.514	0.891	0.048	1.807	0.117	2.565	0.110	4.599
Adel	Control hybrids	6	0.655	0.284	1.097	0.531	0.968	0.076	1.806	0.116	2.729	0.111	4.763
Fithian	Control hybrids	4	1.377	0.379	1.685	0.512	0.978	0.070	1.652	0.117	3.209	0.135	5.114
Fithian	Control hybrids	5	0.652	0.307	1.741	0.532	1.004	0.075	1.753	0.119	3.721	0.158	5.752
Fithian	Control hybrids	6	1.351	0.310	1.823	0.539	1.018	0.070	1.800	0.119	3.186	0.136	5.241
Blomkest	Control hybrids	4	0.641	0.293	2.448	0.541	0.846	0.095	1.475	0.121	3.688	0.166	5.450
Blomkest	Control hybrids	5	<LOQ	0.291	2.164	0.549	0.835	0.079	1.469	0.118	4.049	0.184	5.820
Blomkest	Control hybrids	6	<LOQ	0.271	2.048	0.544	0.871	0.106	1.527	0.120	4.386	0.184	6.217
Spencerville	Control hybrids	4	0.401	0.281	2.797	0.564	0.616	0.113	1.107	0.116	4.508	0.192	5.923
Spencerville	Control hybrids	5	0.439	0.270	2.267	0.553	0.670	0.112	1.207	0.130	4.994	0.236	6.567
Spencerville	Control hybrids	6	0.664	0.268	2.645	0.536	0.629	0.081	1.231	0.115	5.059	0.255	6.660
Mean			0.801	0.301	1.899	0.537	0.856	0.083	1.543	0.119	3.724	0.165	5.551
SD			0.365	0.033	0.611	0.015	0.144	0.020	0.251	0.004	0.890	0.048	0.731
95% CI Lower Bound			0.594	0.282	1.554	0.529	0.774	0.072	1.401	0.116	3.221	0.138	5.137
95% CI Upper Bound			1.007	0.320	2.244	0.546	0.937	0.094	1.685	0.121	4.228	0.192	5.964

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**Maize Line HCEM485
USDA Extension Petition**

**Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098**

Compositional Data - Grain Metabolites

Location	Sample	Rep	Ferulic	P-coumaric	Inositol	Phytic	Raffinose	TI
Adel	HCEM485	4	172.36	10.58	10.70	878.86	248.42	4.19
Adel	HCEM485	5	245.09	16.19	8.93	886.14	206.65	4.22
Adel	HCEM485	6	229.44	14.98	8.77	782.14	204.41	4.09
Fithian	HCEM485	4	227.53	15.56	11.40	981.13	238.00	4.20
Fithian	HCEM485	5	213.97	14.76	12.80	782.61	229.66	4.31
Fithian	HCEM485	6	195.08	13.98	14.80	757.70	263.41	4.24
Blomkest	HCEM485	4	214.74	14.14	13.90	822.83	222.36	4.52
Blomkest	HCEM485	5	201.01	12.90	11.90	799.85	195.27	4.52
Blomkest	HCEM485	6	234.25	14.68	10.20	818.32	187.61	4.55
Spencerville	HCEM485	4	254.59	24.95	13.50	746.09	111.27	4.36
Spencerville	HCEM485	5	228.56	21.09	14.70	722.87	178.04	4.30
Spencerville	HCEM485	6	257.29	24.44	12.40	628.26	<LOQ	4.17
Mean			222.82	16.52	12.00	800.57	207.73	4.31
SD			24.97	4.52	2.07	89.47	41.38	0.15
95% CI Lower Bound			208.70	13.96	10.83	749.94	184.32	4.22
95% CI Upper Bound			236.95	19.08	13.17	851.19	231.15	4.39
Adel	Control hybrids	4	224.32	15.05	12.50	791.56	196.11	3.75
Adel	Control hybrids	5	222.11	14.41	13.60	918.15	219.15	3.99
Adel	Control hybrids	6	226.22	15.50	12.30	722.18	221.89	3.89
Fithian	Control hybrids	4	201.93	14.64	15.40	712.78	290.08	4.12
Fithian	Control hybrids	5	208.66	16.27	15.60	779.42	276.49	4.17
Fithian	Control hybrids	6	212.78	15.72	14.70	771.42	260.94	4.08
Blomkest	Control hybrids	4	213.34	14.50	13.90	746.69	213.38	3.96
Blomkest	Control hybrids	5	214.78	14.10	14.10	877.12	190.86	4.20
Blomkest	Control hybrids	6	210.61	14.52	13.30	748.04	188.91	4.52
Spencerville	Control hybrids	4	248.22	24.06	12.90	742.45	94.48	4.43
Spencerville	Control hybrids	5	226.63	21.07	14.60	800.55	156.22	4.45
Spencerville	Control hybrids	6	228.16	22.35	13.30	775.66	155.37	4.44
Mean			219.81	16.85	13.85	782.17	205.32	4.17
SD			12.19	3.52	1.07	60.69	55.29	0.25
95% CI Lower Bound			212.91	14.86	13.24	747.83	174.04	4.02
95% CI Upper Bound			226.71	18.84	14.46	816.51	236.61	4.31

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**Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098**

**Maize Line HCEM485
USDA Extension Petition**

Compositional Data - Grain Minerals

Location	Sample	Rep	Ca	Cu	Fe	Mg	Mn	P	K	Na	Se	Zn
Adel	HCEM485	4	35.85	1.02	31.35	1335.36	7.54	3208.82	3459.15	2.33	<LOQ	22.27
Adel	HCEM485	5	32.25	1.14	28.37	1374.22	7.01	3335.59	3548.55	1.00	0.17	21.20
Adel	HCEM485	6	32.32	1.06	28.99	1405.70	7.13	3379.74	3641.37	0.87	0.18	21.31
Fithian	HCEM485	4	36.33	1.33	25.18	1349.78	6.06	3249.92	3794.28	0.81	<LOQ	20.39
Fithian	HCEM485	5	35.60	1.25	25.74	1322.32	6.16	3156.67	3708.48	0.99	0.12	21.09
Fithian	HCEM485	6	34.89	1.46	30.89	1390.21	7.23	3240.30	3654.91	0.63	<LOQ	24.80
Blomkest	HCEM485	4	36.18	1.22	21.65	1233.19	5.23	3033.52	3598.19	1.96	0.16	16.93
Blomkest	HCEM485	5	31.44	1.15	23.36	1402.39	5.22	3456.42	4053.53	1.23	0.21	17.51
Blomkest	HCEM485	6	30.56	1.09	24.12	1432.35	5.53	3536.04	4048.22	2.59	0.14	17.14
Spencerville	HCEM485	4	40.48	1.08	21.33	1091.31	5.40	2807.25	3731.60	1.68	<LOQ	17.99
Spencerville	HCEM485	5	38.29	1.13	21.32	1261.44	5.99	3192.01	3830.62	2.30	<LOQ	19.33
Spencerville	HCEM485	6	40.64	1.08	19.55	1172.95	5.04	2909.18	3810.29	0.87	0.11	17.82
Mean			35.40	1.17	25.15	1314.27	6.13	3208.79	3739.93	1.44		19.82
SD			3.33	0.13	3.98	104.55	0.89	213.44	181.68	0.70		2.44
95% CI Lower Bound			33.52	1.10	22.90	1255.11	5.62	3088.02	3637.14	1.04		18.44
95% CI Upper Bound			37.29	1.24	27.40	1373.42	6.63	3329.55	3842.73	1.83		21.19
Adel	Control hybrids	4	36.25	1.24	30.59	1348.82	6.94	3319.36	3549.51	<LOQ	0.12	21.13
Adel	Control hybrids	5	37.36	1.26	30.04	1326.80	6.65	3216.86	3417.15	<LOQ	0.10	21.88
Adel	Control hybrids	6	36.21	1.21	29.29	1366.99	6.78	3379.01	3504.19	0.65	0.14	21.60
Fithian	Control hybrids	4	40.22	1.57	30.39	1287.46	7.08	3012.34	3314.92	4.80	<LOQ	23.88
Fithian	Control hybrids	5	37.31	1.53	30.40	1295.85	7.00	3007.42	3457.12	1.36	<LOQ	23.77
Fithian	Control hybrids	6	35.12	1.53	28.04	1261.06	6.85	2928.56	3325.18	<LOQ	<LOQ	22.37
Blomkest	Control hybrids	4	37.21	1.44	23.67	1321.70	5.14	3252.32	3870.13	<LOQ	0.17	19.02
Blomkest	Control hybrids	5	34.41	1.39	23.81	1333.95	5.08	3252.01	3781.93	3.36	0.20	18.27
Blomkest	Control hybrids	6	32.50	1.30	23.38	1327.89	5.23	3309.05	3717.03	1.11	0.13	16.90
Spencerville	Control hybrids	4	41.13	1.12	19.76	1148.60	5.23	2917.71	3672.58	4.03	<LOQ	17.87
Spencerville	Control hybrids	5	41.67	1.27	22.28	1330.91	4.68	3280.25	3924.07	0.96	0.10	20.03
Spencerville	Control hybrids	6	40.69	1.26	19.66	1175.89	4.39	2904.35	3677.03	1.75	<LOQ	19.26
Mean			37.51	1.34	25.94	1293.83	5.92	3148.27	3600.90	2.25		20.50
SD			2.89	0.15	4.28	67.76	1.04	178.71	204.13	1.58		2.30
95% CI Lower Bound			35.87	1.26	23.52	1255.49	5.33	3047.15	3485.41	1.36		19.20
95% CI Upper Bound			39.14	1.43	28.36	1332.17	6.51	3249.39	3716.40	3.15		21.80

Annex 3 - Compositional analysis of grain and forage derived from
HCEM485 hybrid maize grown during 2007 in the USA.
Report Number: SSF-08-098

Compositional Data - Grain Phytosterols

Location	Sample	Rep	cholesterol	campesterol	stigmasterol	B-sifosterol	stigmastanol	total
Adel	HCEM485	4	0.23	8.08	2.38	55.18	10.97	76.83
Adel	HCEM485	5	0.22	7.53	2.31	55.22	11.11	76.39
Adel	HCEM485	6	0.22	8.04	2.60	56.23	11.60	78.69
Fithian	HCEM485	4	0.22	8.97	2.77	55.18	11.70	78.85
Fithian	HCEM485	5	0.22	8.81	2.74	54.52	11.62	77.91
Fithian	HCEM485	6	0.22	8.12	2.76	54.47	10.99	76.55
Blomkest	HCEM485	4	0.21	9.84	3.32	56.41	11.79	81.58
Blomkest	HCEM485	5	0.22	9.69	3.51	54.90	11.39	79.70
Blomkest	HCEM485	6	0.22	9.30	3.06	52.99	10.73	76.29
Spencerville	HCEM485	4	0.21	11.15	3.30	50.08	9.69	74.44
Spencerville	HCEM485	5	0.21	10.29	3.35	50.30	8.64	72.79
Spencerville	HCEM485	6	0.38	12.70	3.44	57.48	10.32	84.31
Mean			0.23	9.38	2.96	54.41	10.88	77.86
SD			0.05	1.49	0.42	2.27	0.94	3.09
95% CI Lower Bound			0.21	8.54	2.72	53.13	10.35	76.11
95% CI Upper Bound			0.26	10.22	3.20	55.69	11.41	79.61
Adel	Control hybrids	4	0.23	8.62	3.17	57.86	11.05	80.93
Adel	Control hybrids	5	0.23	8.14	2.63	56.56	11.30	78.85
Adel	Control hybrids	6	0.24	8.26	2.67	58.66	11.57	81.41
Fithian	Control hybrids	4	0.24	8.40	2.95	57.00	11.47	80.06
Fithian	Control hybrids	5	0.24	8.45	2.97	56.34	11.05	79.05
Fithian	Control hybrids	6	0.23	8.11	2.62	56.10	11.12	78.18
Blomkest	Control hybrids	4	0.23	10.09	3.54	56.18	10.94	80.97
Blomkest	Control hybrids	5	0.23	9.61	3.09	55.47	10.98	79.38
Blomkest	Control hybrids	6	0.24	10.87	3.40	59.27	12.16	85.95
Spencerville	Control hybrids	4	0.21	10.51	3.16	47.77	8.49	70.14
Spencerville	Control hybrids	5	0.27	11.27	3.24	52.96	9.38	77.12
Spencerville	Control hybrids	6	0.22	11.77	3.75	54.13	9.35	79.22
Mean			0.23	9.51	3.10	55.69	10.74	79.27
SD			0.01	1.35	0.36	3.05	1.08	3.63
95% CI Lower Bound			0.23	8.75	2.90	53.97	10.13	77.22
95% CI Upper Bound			0.24	10.27	3.30	57.42	11.35	81.33



STINE SEED FARM, INC.

22555 Laredo Trail
Adel, Iowa 50003-4570

515-677-2605 • TOLL FREE 800-362-2510

FAX (TELECOPIER) 515-677-2716

September 14, 2011

Rebecca L. Stankiewicz Gabel, Ph.D.
Senior Environmental Protection Specialist,
USDA, APHIS, BRS
Unit 147
4700 River Road
Riverdale, MD 20737
301-734-5603

**Re: Release of Confidential Business Information in Petition Extension of Determination of
Nonregulated Status for HCEM485 – application No. 09-063-01p a1**

Dear Dr. Stankiewicz Gabel,

As a confirmation of recent electronic communication with Dr. Robert Potter, this letter advises that the material noted as Confidential Business Information in the petition is now regarded as non-confidential. As such, the full petition may be released with the package for public comment and any material may be included in the Environmental Assessment.

Should you have any questions or require further information regarding this request, please contact me at (515) 677-2605.

Yours sincerely,

Harry H. Stine

President, Stine Seed Farm, Inc.

From: (515) 677-2605
Tiffany Dunbar
STINE SEED COMPANY
22555 Laredo Trail

Origin ID: SARA



J11201104290225

Adel, IA 50003

Ship Date: 15SEP11
ActWgt: 1.0 LB
CAD: 1335377/INET3180

Delivery Address Bar Code



SHIP TO: (301) 734-5603

BILL SENDER

Rebecca L. Stankiewicz Gabel
USDA-APHIS-BRS
4700 RIVER RD UNIT 147

RIVERDALE, MD 20737

Ref #
Invoice #
PO #
Dept #

MON - 19 SEP A2

** 2DAY **

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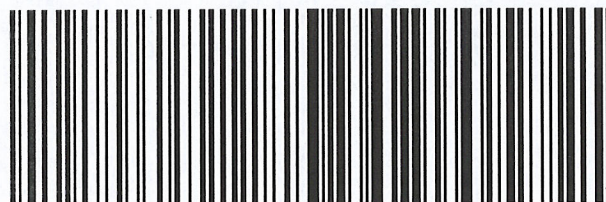
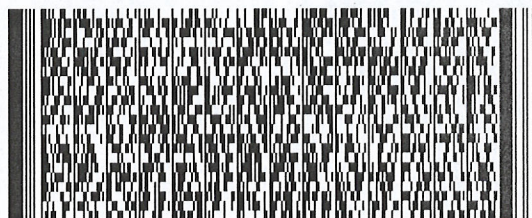
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