Information Supporting a Regulatory Status Review of Maize Genetically Engineered to Produce β-Glucanase Enzyme

Agrivida, Inc. is submitting this information to support a Regulatory Status Review by the USDA Animal and Plant Health Inspection Service under 7 CFR Part 340.4

Submitted by:

James M. Ligon, Ph.D.
VP, Regulatory Affairs and Stewardship
Agrivida, Inc.
1023 Christopher Drive
Chapel Hill, NC 27517

This document was prepared by James Ligon, Agrivida, Inc.

No CBI
Agrivida, Inc. does not consider any information contained in this document to be confidential business information or to be a trade secret.

July 7, 2021

Table of Contents

Summary

Agrivida, Inc. is submitting this information to USDA APHIS to support a Regulatory Status Review of maize that has been genetically engineered to produce a glucanase enzyme. The glucanase producing maize developed by Agrivida, Inc. contains transgenes that produce two proteins novel to maize, β-glucanase and phosphomannose isomerase. Glucanases are ubiquitous in nature and are produced by many microbes and plants. Glucanases are a class of enzymes that depolymerize 1,3 and 1,4-β-D-glucan polysaccharides that are present in the cell walls of cereals and other plants. The primary enzymatic activity of the glucanase enzyme engineered into maize is endo-1,4-β-glucanase but it also exhibits lesser levels of other carbohydrase type activities, including endocellulase, exocellulase, and endo-mannanase. Glucanases are commonly added to the diets of monogastric animals fed diets high in soluble non-starch polysaccharides (NSP) to decrease the viscosity of the digesta in the digestive tract. Diets high in NSPs often become very viscous in the intestinal tract leading to reduced digestibility of the diet and disturbances of the digestive tract. The gene encoding the glucanase enzyme (referred to as AC1 Glucanase) in maize was derived from a gene isolated from an environmental DNA library that is identical to the Cel5A glucanase gene of *Thermotoga maritima* (NCBI accession Q9X273) (Nelson *et al*., 1999). The gene sequence was modified by Gene Site Saturation Mutagenesis (GSSM) to improve its thermostability. The resulting AC1 Glucanase differs from the Cel5A glucanase by 12 amino acids. Maize engineered to contain the glucanase gene was generated by *Agrobacterium*-mediated transformation into immature maize embryo tissue as described by Negrotto *et al*. (2000) using a gene cassette containing the optimized AC1 Glucanase gene and the *manA* gene encoding the phosphomannose isomerase (PMI) enzyme. Expression of the AC1 Glucanase gene is directed by the rice glutelin promoter (Qu *et al*., 2008), a seed-specific promoter, such that the AC1 Glucanase is produced primarily in the grain of *Z. mays*. The *manA* gene encoding PMI was included in the transformation cassette as a plant selectable marker. The *manA* gene is expressed by the promoter from the *Z. mays* ubiquitin 1 gene that provides expression in all maize tissues.

Multiple lines of evidence indicate the presence of a single T-DNA insertion from the transformation plasmid pAG4588. The nucleotide sequence of the insertion, including the flanking genomic maize DNA, was determined and demonstrates that the T-DNA is located on maize chromosome 6.

Agrivida has conducted a food and feed safety assessment of the AC1 Glucanase and concluded that this protein does not present any safety concerns related to consumption of maize containing the AC1 Glucanase by humans or animals. Agrivida, Inc. has completed an Early Food Safety Evaluation of the AC1 Glucanase with the U.S. Food and Drug Administration (NPC No. 00018; Agrivida, 2017) and a detailed assessment of human and animal safety of the AC1 Glucanase protein has been provided to the FDA as part of a food and feed safety and nutritional

assessment for maize expressing the glucanase gene (BNF 181). The safety and efficacy of the AC1 Glucanase as a feed additive in poultry has been reviewed by the FDA Center for Veterinary Medicine who had no questions concerning Agrivida Inc.'s conclusion that the AC1 Glucanase is GRAS for this purpose (CVM, 2020; AGRN 31). The safety of the PMI protein has been extensively characterized (Privalle *et al.*, 2006) and this selectable marker has been widely used in maize and other crop species that have been approved for food use by regulatory authorities in the United States and other countries.

Cereal grains are broadly classified into two major categories, viscous and non-viscous cereals depending on their content of soluble non-starch polysaccharides (NSPs). Rye, barley, oats, and wheat contain considerable amounts of soluble NSP and are classified as viscous grains, whereas corn, sorghum, millet and rice contain reduced amounts of soluble NSP and are considered to be non-viscous cereals. The content of viscous NSP in feeds produced from these grains may result in high digesta viscosity in the gastrointestinal (GI) tracts of monogastric animals resulting in reduced nutrient digestibility and availability, negative impacts on the gut microbiome and other negative effects (Burnett, 1966; Choct and Annison, 1992; Bedford and Classen, 1992; Danicke *et al*., 1999). Since the 1980's glucanase and other enzymes that degrade soluble NSP have been added to the feed of monogastric animals to decrease digesta viscosity and improve the performance of animals fed diets based on grains with a high soluble NSP content (Hesselman and Åman, 1986; Campbell *et al*., 1989; Broz and Frigg, 1986; Newman and Newman, 1987). A large number of enzymes categorized as NSPase are approved for use in animal feed to hydrolyze soluble NSPs and improve the digestion of nutrients in feeds based on grains high in soluble NSP content. These include glucanase as well as galactosidase, mannanase, pectinase, and xylanase. β-glucans are a primary soluble NSP in grains and are present at levels of 0.2-0.7% in wheat and 1.9-5.4% in barley (Havrlentová and Kraic, 2006). β-glucan is a glucose polymer containing a mixture of β-1-3 and β-1-4 linkages that make its physicochemical properties different from cellulose that is a straight-chain glucose polymer with only β-1-4 linkages. Four types of endo-acting glucanases, classified according to the type of glycosidic linkage they cleave, are capable of depolymerizing (1,3)-(1,4)-β-D-glucan: endo-(1,3)-(1,4)-β-glucanases, endo-1,3(4)-β-glucanases, endo-1,4-β-glucanases, and to a lesser extent, endo-1,3-β-glucanases (McCarthy *et al*. 2003).

Although glucanases have been widely used in feeds based on grains high in soluble NSPs, their utility in corn-soybean meal based diets has also been demonstrated. NSPs in corn-soybean meal based diets have been shown to decrease the digestibility of nutrients by restricting access of digestive enzymes such as amylase and proteases to nutrients intertwined in fibrous cellular matrices (Cowieson, 2005). In addition, legume (e.g., soybean) NSPs are more complex in structure than those of cereals, containing a mixture of colloidal polysaccharides (galacturonans, galactan and arabinans). Accordingly, the addition of pectinase to a corn-soybean meal diet has been shown to significantly increase the metabolizable energy (ME)

value of the diet. This improvement in the ME coincided with increased digestibility of galactose-rich polysaccharides (Kocher *et al*., 2002).

Agrivida, Inc. is developing animal feed enzyme products that are produced in maize grain. Genes encoding the enzymes, under the regulation of monocot-derived seed-specific promoters, are transformed into maize. The enzyme products produced in this manner will be marketed under the trade name of GraINzyme®. One of the GraINzyme® products under development by Agrivida, Inc. is a glucanase feed enzyme whose primary activity is endo-1,4-β-glucanase.

Based on the information and data contained in this document, Agrivida, Inc. requests that USDA APHIS conduct a Regulatory Status Review for maize expressing the glucanase gene under the new SECURE Rule (7 CFR Part 340.4; Federal Register, 2020).

1. Description of *Zea mays* L. (Maize), the Comparator Plant

Zea is a genus of the family Graminae (Poaceae), commonly known as the grass family. Maize (*Zea mays* L.) is a tall, monecious annual grass with overlapping sheaths and broad conspicuously distichous blades. Plants have staminate spikelets in long spike-like racemes that form large spreading terminal panicles (tassels) and pistillate inflorescences in the leaf axils, in which the spikelets occur in 8 to 16 rows, approximately 30 cm long, on a thickened, almost woody axis (cob). The whole structure (ear) is enclosed in numerous large foliaceous bracts and long styles (silks) protrude from the tip of the ear as a mass of silky threads (Hitchcock and Chase, 1971). Pollen is produced entirely in the staminate inflorescence and eggs, entirely in the pistillate inflorescence. Maize is wind-pollinated and both self and cross-pollination are usually possible. Shed pollen usually remains viable for 10 to 30 minutes, but can remain viable for longer durations under favorable conditions (Coe et al., 1988). Cultivated maize is presumed to have been derived from teosinte (*Z. mexicana*) and is thought to have been introduced into the old world in the sixteenth century. Maize is cultivated worldwide and represents a staple food for a significant proportion of the world's population. No significant native toxins are reported to be associated with the genus *Zea* (International Food Biotechnology Council, 1990).

1. Genotype of Maize Expressing the Glucanase Gene

A. Summary

Data from Southern analyses demonstrated that a single copy of the T-DNA from the transformation construct pAG4588 is inserted into the genome of the transformed maize. The nucleotide sequence of the T-DNA and the flanking maize genomic DNA of the T-DNA insertion was determined and revealed that the insertion is in maize chromosome 6. The following is a summary of data and information relevant to the genetic characterization of the T-DNA insertion of the glucanase maize and this information was previously submitted to the FDA/CVM in Agrivida, Inc.'s GRAS notice (Agrivida, 2019; AGRN 31) for the use of AC1 Glucanase produced by maize in poultry feed.

B. Origin of the Gene Encoding the AC1 Glucanase

The gene encoding the AC1 Glucanase enzyme was derived from a gene isolated from an environmental DNA library. The isolated gene encoded an enzyme that is identical to the Cel5A cellulase from *Thermotoga maritima* (NCBI accession Q9X273) (Nelson *et al*., 1999). This gene was modified by Gene Site Saturation Mutagensis (GSSM) resulting in 12 amino acids changes in order to improve the thermostability of the AC1 Glucanase. The modified gene encodes a glucanase enzyme that is a 37.7 kDa protein with 96% identity to the Cel5A glucanase of *T. maritima*. Agrivida, Inc. has compared the Cel5A and AC1 Glucanase enzymes and has demonstrated that the enzyme kinetics of these two glucanases is nearly identical (Table 1). Expression of the AC1 gene is directed by a monocot-derived seed-specific promoter, such that the GraINzyme® AC1 Glucanase is produced only in the grain of *Z. mays*. The nucleotide coding sequence and deduced amino acid sequence of the AC1 Glucanase gene are shown in Figure 1.

Since only the coding sequence of the AC1 Glucanase gene and no other DNA derived from the original host is included in the the transformation construct of plasmid pAG4588, the identity of the source organism or its safety profile is not relevant to a discussion of the safety of the AC1 Glucanase protein.

Table 1. Comparison of the enzyme kinetic properties of the Cel5A glucanase of *T. maritima* and the AC1 Glucanase produced in maize.

	Vmax µmoles/min/mg	Kcat min^{-1}	Km mg/mL
Cel5A	36.5	1365	0.38
AC1	31.2	1167	0.22

Figure 1. Comparison of the deduced amino acid sequences of the AC1 Glucanase and the *Thermotoga maritima* Cel5A glucanase. Amino acid differences in the AC1 Glucanase relative to the Cel5A glucanase are shaded in green. Identical amino acid residues in the two proteins are indicated in the third row as asterisks (*), conservative replacements as colons (:), and non-conservative replacements as spaces. The maize Z27 γ-zein signal peptide that directs the protein to the endoplasmic reticulum at the N-terminus of the protein and the endoplasmic retention signal peptide at the C-terminus are underlined. The Cel5A active site residues (Mahadevan *et al.*, 2008) are indicated with red shading.

```
                                                                        60
AC1     MRVLLVALALLALAASATSGVDPFERNKILGRGINIGNALEAPNEGDWGVVIKDEFFDII
CEL5A                       GVDPFERNKILGRGINIGNALEAPNEGDWGVVIKDEFFDII
                            ****************************************

                                                                       120
AC1     KEAGFSHVRIPIRWSTHAQAFPPYKIEPSFFKRVDEVINGALKRGLAVVINIHHYEELMN
CEL5A   KEAGFSHVRIPIRWSTHAYAFPPYKIMDRFFKRVDEVINGALKRGLAVVINIHHYEELMN
        ****************** *******   ******************************

                                                                       180
AC1     DPEEHKERFLALWKQIADRYKDYPETLFFEILNEPHGNLTPEKWNELLEEALKVIRSIDK
CEL5A   DPEEHKERFLALWKQIADRYKDYPETLFFEILNEPHGNLTPEKWNELLEEALKVIRSIDK
        ************************************************************

                                                                       240
AC1     KHTVIIGTAEWGGISALEKLRVPKWEKNAIVTIHYYNPFEFTHQGAEWVPGSEKWLGRKW
CEL5A   KHTIIIGTAEWGGISALEKLSVPKWEKNSIVTIHYYNPFEFTHQGAEWVEGSEKWLGRKW
        ***:*************** *******:******************** **********

                                                                       300
AC1     GSPDDQKHLIEEFNFIEEWSKKNKRPIYIGEFGAYRKADLESRIKWTSFVVREAEKRGWS
CEL5A   GSPDDQKHLIEEFNFIEEWSKKNKRPIYIGEFGAYRKADLESRIKWTSFVVREMEKRRWS
        ************************************************** *** **

                                                              341
AC1     WAYWEFCSGFGVYDPLRKQWNKDLLEALIGGDSIESEKDEL
CEL5A   WAYWEFCSGFGVYDTLRKTWNKDLLEALIGGDSIE
        ************** *** ***************
```

C. Origin and Mechanism of Action of the PMI Gene

The gene encoding phosphomannose isomerase (PMI) that is included in the T-DNA of the transformation construct of plasmid pAG4588 is derived from the native *manA* gene of *Escherichia coli* (GenBank Accession Number M15380). The PMI enzyme catalyzes the reversible isomerization of mannose-6-phosphate to fructose-6-phosphate (Goldsworthy and Street, 1965) and its expression in maize results in the ability of tissues to grow on artificial growth media using mannose as a carbon source (Negrotto *et al*., 2000). The PMI gene is expressed from the promoter of the *Zea mays* polyubiquitin gene, containing the first intron (GenBank Accession Number S94464.1). This promoter provides constitutive expression in monocots (Christensen *et al*., 1992). The same ubiquitin promoter/PMI gene combination has been used as a selectable marker gene in several other maize varieties that are widely cultivated in the U.S., including maize events 5307 (10-336-01p) and MIR604 maize with resistance to corn rootworm (04-362-01p), lepidoptera-resistant MIR162 (07-253-01p), and α-amylase expressing event 3272 (05-280-01p), all products of Syngenta Seeds. While the expression of the *manA* gene and production of PMI is critical for the growth and selection of transformed cells on artificial growth media containing mannose as a carbon source, it does not result in an observable phenotype in maize plants growing in the environment since mannose is not a major sugar in the metabolism or physiology of maize. This is supported by observations in field studies that compared the growth, development, and phenotypes of the glucanase engineered maize compared to conventional maize as well as a lack of an observable phenotype in the above mentioned commercial maize lines that express the *manA* gene. The PMI gene/maize host combination is included on the list of plant/trait/mechanisms of action that are not considered to be regulated by the USDA (2021).

D. Characteristics of the AC1 Gene Expression Construct

A transformation gene cassette containing the gene encoding the AC1 Glucanase under the control of the *Oryzae sativa*-derived glutelin-1 gene promoter including intron 9 from the maize PEP carboxylase gene as an enhancer of transcription, and CaMV 35S polyadenylation signal was constructed in plasmid pAG4588 (Figure 2). The genetic elements of plasmid pAG4588 that was used to transform maize are described in Table 2 and presented in Figure 2. This plasmid was transformed by *Agrobacterium*-mediated transformation into immature maize embryo tissue as described by Negrotto *et al*. (2000) and transformants were selected based on the presence of the plant selectable marker phosphomannose isomerase (PMI) gene (*manA*) on the transformed DNA fragment. Maize plants containing the AC1 Glucanase gene were cultivated and produced grain accumulating 150 to 300 units of endoglucanase activity per gram of grain.

In addition to containing the AC1 Glucanase coding sequence, the AC1 Glucanase gene of pAG4588 also contains coding sequences of the maize Z27 γ-zein signal peptide at the amino-terminus of the protein, which directs the protein to the

endoplasmic reticulum, and the endoplasmic retention signal peptide, with the amino acid sequence SEKDEL, at the C-terminus. The expressed protein is transported across the membrane of the endoplasmic reticulum where the γ-zein signal peptide at the N-terminus is cleaved from the protein, resulting in the mature AC1 Glucanase protein that consists of 322 amino acids. Agrivida, Inc. has conducted N-terminal amino acid sequencing of AC1 and verified post-translational processing of the γ-zein signal peptide.

Figure 2. Genetic map of pAG4588, the plant transformation vector containing the AC1 Glucanase gene. The genetic elements of this vector are described in Table 2. Locations of the cleavage sites for restriction enzymes *Bam*HI, *Eco*RI, *Sph*I and *Hin*dIII are shown

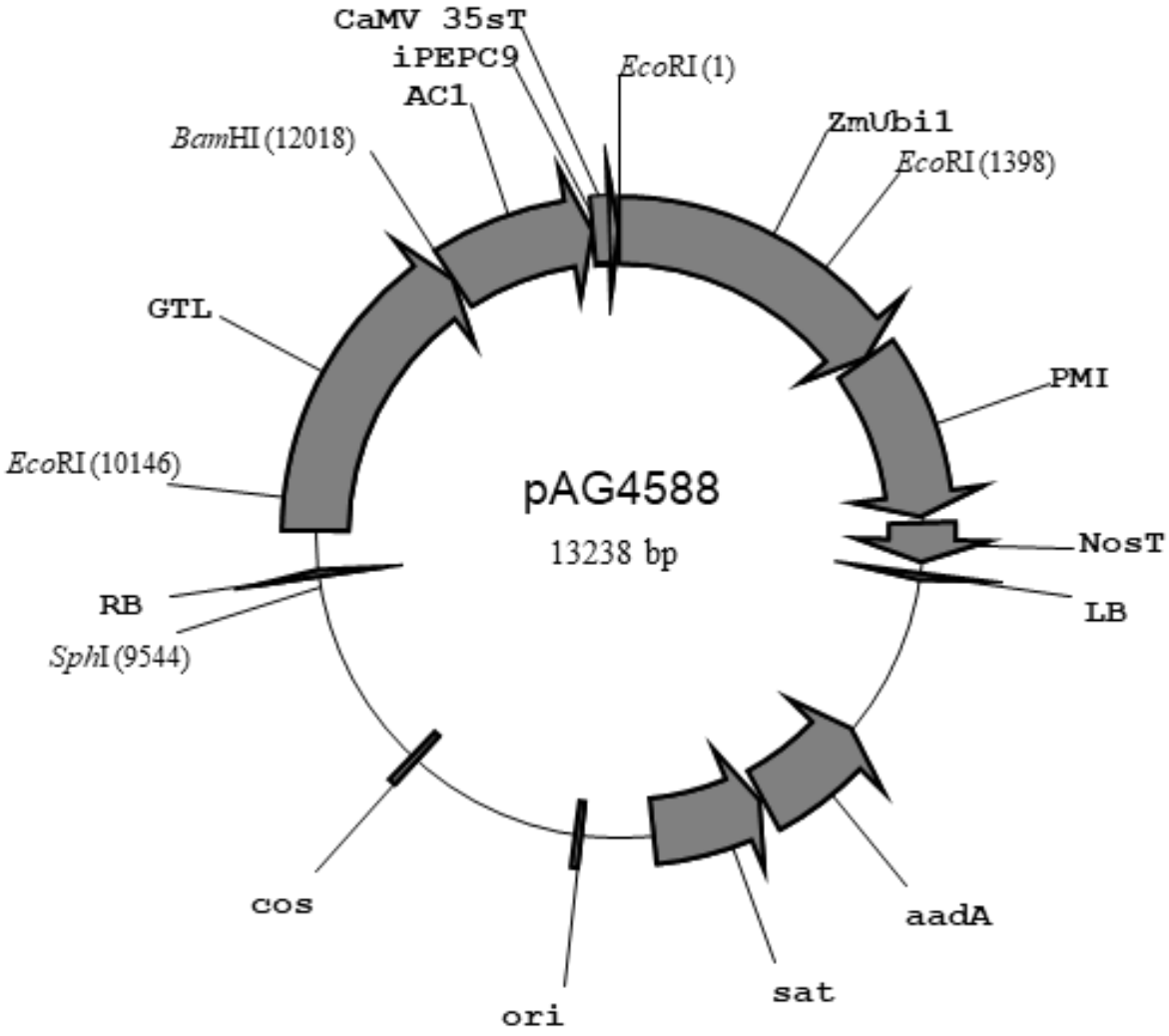

Table 2. Description of the genetic elements in the 13,238 bp vector plasmid pAG4588 containing the AC1 Glucanase and *manA* (PMI) genes.

Gene Element	Description	Size (kb)	Donor Organism	Function	Reference
ZmUbi1	Promoter plus first intron from the maize ubiquitin 1 gene (NCBI accession number S94464.1)	2	*Zea mays*	Transcriptional Promoter	Quail, *et al*., 1996
PMI	Phosphomannose isomerase (NCBI accession number M15380)	1.1	*E. coli*	Selectable marker	Negrotto *et al*., 2000
NosT	Nopaline synthase terminator (similar to NCBI accession number AJ237588.1)	0.3	*Agrobacterium tumefaciens*	Transcriptional terminator/poly-adenylation signal	Depicker *et al*., 1982
LB	Left Border (NCBI accession number J01825)	<0.1	*Agrobacterium tumefaciens*	T-DNA boundary	Zambryski *et al*., 1982
aadA	aminoglycoside-adenyltransferase (NCBI accession number X03043)	0.8	*E. coli*	Bacterial selectable marker	Fling *et al*., 1985
sat	streptothricin acetyltransferase (similar to NCBI accession number LT898487.1)	0.8	*E. coli*	Bacterial selectable marker	Horinouchi *et al.*, 1987
ori	Origin of replication in *E. coli* (similar to NCBI accession number V00268)	0.8	*E. coli*	Plasmid origin of replication	Itoh and Tomizawa, 1978
cos	cos site from bacteriophage lambda (NCBI accession number KT232076.1)	<0.1	*E. coli bacteriophage lambda*	Site of recombination into disarmed Ti plasmid	Collins and Hohn, 1978
RB	Right Border (NCBI accession number J01826)	<0.1	*Agrobacterium tumefaciens*	T-DNA boundary	Wang *et al*., 1984
GTL	Rice glutelin 1 promoter (similar to NCBI accession number EU264103.1)	2	*Oryza sativa*	Transcriptional Promoter	Qu *et al*., 2008
AC1	β-glucanase coding sequence (similar to NCBI accession Q9X273) with maize Z27 γ-zein N-terminal signal sequence (similar to NCBI accession number AB086264.1) and C-terminal SEKDEL retention sequence to target protein accumulation to the endoplasmic reticulum	1	*Thermotoga maritima*	Gene of interest; enables digestion of beta glucans	This document
iPEPC9	Intron 9 from the maize PEP carboxylase gene (from NCBI accession number X15239.1)	0.2	*Zea mays*	Enhancer of expression	Tuttle, 2007
CaMV 35 sT	CaMV 35S polyadenylation signal (from NCBI accession number NC_001497.2)	0.2	*Zea mays*	Transcriptional terminator/ poly-adenylation signal	Tuttle, 2007

3. Sequence of the AC1 Gene T-DNA and the Flanking Maize Genome

The complete nucleotide sequence of the T-DNA insertion and flanking maize genomic DNA in the maize transformed with the glucanase gene was determined. Comparison of this sequence with the sequence of the T-DNA in the transforming plasmid pAG4588 determined that, with the exception of a partial left border repeat of 14 nucleotides, the coding sequences of the AC1 Glucanase and *manA* genes in the T-DNA were not altered during the transformation process and are identical to the sequences of these genes in pAG4588. The genetic map and complete DNA sequence of the T-DNA locus incuding the flanking maize genomic DNA are presented in Figures 3 and 4, respectively.

Figure 3. Diagram of the T-DNA locus of the AC1 Glucanase gene insertion in the maize genome. Cleavage sites for key restriction enzymes are shown. The labels of other genetic elements are as listed in Table 2.

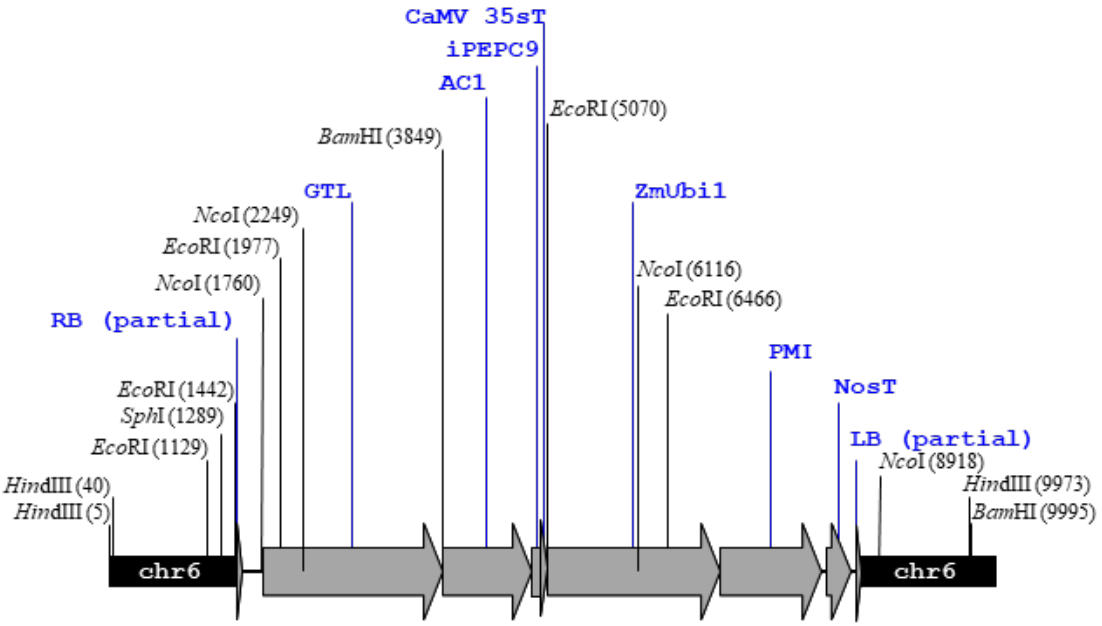

Figure 4. The Annotated Nucleotide Sequence of the Glucanase Gene T-DNA Locus in Maize

A. Annotation of the locus sequence**.** Abbreviations are as listed in Table 2.

Nucleotide Position		
Start	**End**	**Genetic Element**
1	1478	Maize chromosomal DNA ("chr6")
1479	1481	RB (partial)
1777	3847	GTL promoter
3857	4879	AC1 Glucanase gene with N-terminal Z27 gamma zein signal sequence and C-terminal SEKDEL
4889	4996	iPEPC9
4999	5068	CaMV 35sT
5075	7065	ZmUbi1 promoter
7081	8256	PMI
8305	8580	NosT
8651	8664	LB (partial)
8665	10261	Maize chromosomal DNA ("chr6")

B. Nucleotide sequence of the T-DNA insertion locus including maize genomic flanking DNA. Maize genomic DNA sequence is presented in lower case letters while the sequence of the T-DNA insert is presented in upper case letters.

```
>Nucleotide sequence of the T-DNA insertion locus including maize genomic
flanking DNA
tccaagcttgacaatgaggagatggcgctcgtcatcaaaagcttccgccaaatcctcaaa
caaaggaggggaaagactacaagtcccgctccaagaaggtttgctacaagtgtggtaag
cccggtcattttattgctaaatgtcctatatctagtgacagtgaccgaggtgacgacaag
aaggggaggcgaaaggagaagaaaagatactacaagaagaagggcggcgatgcccatgtt
tgtcgggaatgggactccaacgaaagctcaagcgactcctccgacgacgaggacgccgcc
aacatcgccgtcaccaagggactcctcttccccaacgtcggccacaagtgcctcatggca
aaggacggcaaaaagaaggttaaatctaaatcctctactaaatatgaatcctctagtgat
gacaatgctagtgatgaggaagataatttgcgttccctttttgccaaccttaacatagct
caaaaggaaaaattaaatgaattggttagtgctattcatgaaaaggatgacctttttggat
tcccaagaggattgtctaattaaagaaaacaaaaaacatgttaaggttaaaaaggcttat
gccctagaagtagaaaaatgtgaaaaattatctagtgagctaagcacttgccgtgaaatg
attgacaaccttagacatgaaaatgatagtttaaatgctaaggttgattctcatgtttgt
aatgtttcaatttccactcctagagataataatgatgatttgcttgctaggattgaagaa
ttgaacatttctcttgctagccttagattagagaatgaaaatttgattgctaaggcccaa
gattttgatgtttgcaaagatacaattaccgatcttagagataagaatgatatacttcgt
gctaagattgttgaacttacaccacaaccttctatgccttctgtgacattaacattacgt
cacaaacaatagtatttttgtcataccttacatgttggtgacgtgattgtgacgaaaatc
acatcgtcacagaaggtgcgtgttaaatggtgtactatgacgaataacaaaaaaacgtca
taatagtttatgacgcaaactacaaacgtcactaatctatgacactcgaattcgtcacta
attatgtctaaatacgtcacaattcatgtagtcgtgccttgccacgtggctgattacgtg
gcgagatgacatggcagttgacgtggcaggtgatgtggcgaaaatgttgtgacgagttca
ttcgtcacagatgttatgacgtggcatgccacatggcagatgatgtggcaaaattatgtg
acaaaaatatttgtcataaatatcaatgaggtggcaatatatgtgtgacgaaatttttca
tcacaaagtacgatgacgttgcaatatatttatgacgaattgttcatcataaggcgtgat
gaattcatagcgtcatggaatattatgaaatcacatgcTCAAACACTGATAGTTTAAACT
GAAGGCGGGAAACGACAACCTGATCATGAGCGGAGAATTAAGGGAGTCACGTTATGACCC
```

CCGCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAACTGACAGAACCGCAACGTTGAA
GGAGCCACTCAGCCTAAGCGGCCGCATTGGACTTAATTAAGTGAGGCCGGCCAAGCGTCG
ATTTAAATGTACCACATGGCGCGCCAACTATCATGCGATCGCTTCATGTCTAACTCGAGT
TACTGGTACGTACCAAATCCATGGAATCAAGGTACCTCCATGCTGTCCTACTACTTGCTT
CATCCCCTTCTACATTTTGTTCTGGTTTTTGGCCTGCATTTCGGATCATGATGTATGTGA
TTTCCAATCTGCTGCAATATGAATGGAGACTCTGTGCTAACCATCAACAACATGAAATGC
TTATGAGGCCTTTGCTGAGCAGCCAATCTTGCCTGTGTTTATGTCTTCACAGGCCGAATT
CCTCTGTTTTGTTTTTCACCCTCAATATTTGGAAACATTTATCTAGGTTGTTTGTGTCCA
GGCCTATAAATCATACATGATGTTGTCGTATTGGATGTGAATGTGGTGGCGTGTTCAGTG
CCTTGGATTTGAGTTTGATGAGAGTTGCTTCTGGGTCACCACTCACCATTATCGATGCTC
CTCTTCAGCATAAGGTAAAAGTCTTCCCTGTTTACGTTATTTTACCCACTATGGTTGCTT
GGGTTGGTTTTTTCCTGATTGCTTATGCCATGGAAAGTCATTTGATATGTTGAACTTGAA
TTAACTGTAGAATTGTATACATGTTCCATTTGTGTTGTACTTCCTTCTTTTCTATTAGTA
GCCTCAGATGAGTGTGAAAAAAACAGATTATATAACTTGCCCTATAAATCATTTGAAAAA
AATATTGTACAGTGAGAAATTGATATATAGTGAATTTTTAAGAGCATGTTTTCCTAAAGA
AGTATATATTTTCTATGTACAAAGGCCATTGAAGTAATTGTAGATACAGGATAATGTAGA
CTTTTTGGACTTACACTGCTACCTTTAAGTAACAATCATGAGCAATAGTGTTGCAATGAT
ATTTAGGCTGCATTCGTTTACTCTCTTGATTTCCATGAGCACGCTTCCCAAACTGTTAAA
CTCTGTGTTTTTTGCCAAAAAAAAATGCATAGGAAAGTTGCTTTTAAAAAATCATATCAA
TCCATTTTTTAAGTTATAGCTAATACTTAATTAATCATGCGCTAATAAGTCACTCTGTTT
TTCGTACTAGAGAGATTGTTTTGAACCAGCACTCAAGAACACAGCCTTAACCCAGCCAAA
TAATGCTACAACCTACCAGTCCACACCTCTTGTAAAGCATTTGTTGCATGGAAAAGCTAA
GATGACAGCAACCTGTTCAGGAAAACAACTGACAAGGTCATAGGGAGAGGGAGCTTTTGG
AAAGGTGCCGTGCAGTTCAAACAATTAGTTAGCAGTAGGGTGTTGGTTTTTGCTCACAGC
AATAAGAAGTTAATCATGGTGTAGGCAACCCAAATAAAACACCAAAATATGCACAAGGCA
GTTTGTTGTATTCTGTAGTACAGACAAAACTAAAAGTAATGAAAGAAGATGTGGTGTTAG
AAAAGGAAACAATATCATGAGTAATGTGTGGGCATTATGGGACCACGAAATAAAAAGAAC
ATTTTGATGAGTCGTGTATCCTCGATGAGCCTCAAAAGTTCTCTCACCCCGGATAAGAAA
CCCTTAAGCAATGTGCAAAGTTTGCATTCTCCACTGACATAATGCAAAATAAGATATCAT
CGATGACATAGCAACTCATGCATCATATCATGCCTCTCTCAACCTATTCATTCCTACTCA
TCTACATAAGTATCTTCAGCTAAATGTTAGAACATAAACCCATAAGTCACGTTTGATGAG
TATTAGGCGTGACACATGACAAATCACAGACTCAAGCAAGATAAAGCAAAATGATGTGTA
CATAAAACTCCAGAGCTATATGTCATATTGCAAAAAGAGGAGAGCTTATAAGACAAGGCA
TGACTCACAAAAATTCATTTGCCTTTCGTGTCAAAAAGAGGAGGGCTTTACATTATCCAT
GTCATATTGCAAAAGAAAGAGAGAAAGAACAACACAATGCTGCGTCAATTATACATATCT
GTATGTCCATCATTATTCATCCACCTTTCGTGTACCACACTTCATATATCATGAGTCACT
TCATGTCTGGACATTAACAAACTCTATCTTAACATTTAGATGCAAGAGCCTTTATCTCAC
TATAAATGCACGATGATTTCTCATTGTTTCTCACAAAAAGCATTCAGTTCATTAGTCCTA
CAACAACGGATCCACCATGAGGGTGTTGCTCGTTGCCCTCGCTCTCCTGGCTCTCGCTGC
GAGCGCCACCAGCGGCGTGGACCCGTTCGAGAGGAACAAGATCCTGGGCAGGGGCATCAA
CATCGGCAACGCCCTGGAGGCCCCGAACGAGGGCGACTGGGGCGTGGTGATCAAGGACGA
GTTCTTCGACATCATCAAGGAGGCCGGCTTCAGCCACGTGAGAATCCCGATCAGGTGGAG
CACCCACGCCCAGGCCTTCCCGCCGTACAAGATCGAGCCGAGCTTCTTCAAGAGGGTGGA
CGAGGTGATCAACGGCGCCCTGAAGAGGGGCCTGGCCGTGGTGATCAACATCCACCACTA
CGAGGAGCTGATGAACGACCCGGAGGAGCACAAGGAGAGGTTCCTGGCCCTGTGGAAGCA
GATCGCCGACAGGTACAAGGACTACCCGGAGACCCTGTTCTTCGAGATCCTGAACGAGCC
GCACGGCAACCTGACCCCGGAGAAGTGGAACGAGCTGCTGGAGGAGGCCCTGAAGGTGAT
CAGGAGCATCGACAAGAAGCACACCGTGATCATCGGCACCGCCGAGTGGGGCGGCATCAG
CGCCCTGGAGAAGCTGAGGGTGCCGAAGTGGGAGAAGAACGCCATCGTGACCATCCACTA
CTACAACCCGTTCGAGTTCACCCACCAGGGCGCCGAGTGGGTGCCGGGCAGCGAGAAGTG
GCTGGGCAGGAAGTGGGGCAGCCCGGACGACCAGAAGCACCTGATCGAGGAGTTCAACTT
CATCGAGGAGTGGAGCAAGAAGAACAAGAGGCCGATCTACATCGGCGAGTTCGGCGCCTA
CAGGAAGGCCGACCTGGAGAGCAGGATCAAGTGGACCAGCTTCGTGGTGAGGGAGGCCGA
GAAGAGGGGCTGGAGCTGGGCCTACTGGGAGTTCTGCAGCGGCTTCGGCGTGTACGACCC
GCTGAGGAAGCAGTGGAACAAGGACCTGCTGGAGGCCCTGATCGGCGGCGACAGCATCGA
GAGCGAGAAGGACGAGCTGTGACCTAGGCTGCACAAAGTGGAGTAGTCAGTCATCGATCA
GGAACCAGACACCAGACTTTTATTCATACAGTGAAGTGAAGTGAAGTGCAGTGCAGTGAG
TTGCTGGTTTTTGTACAACTTAGTATGTATTTGTATTTGTAAAATACTTCTATCAATAAA
ATTTCTAATTCCTAAAACCAAAATCCAGGAATTCCTGCAGTGCAGCGTGACCCGGTCGTG
CCCCTCTCTAGAGATAATGAGCATTGCATGTCTAAGTTATAAAAAATTACCACATATTTT
TTTTGTCACACTTGTTTGAAGTGCAGTTTATCTATCTTTATACATATATTTAAACTTTAC
TCTACGAATAATATAATCTATAGTACTACAATAATATCAGTGTTTTAGAGAATCATATAA
ATGAACAGTTAGACATGGTCTAAAGGACAATTGAGTATTTTGACAACAGGACTCTACAGT

TTTATCTTTTTAGTGTGCATGTGTTCTCCTTTTTTTTTGCAAATAGCTTCACCTATATAA
TACTTCATCCATTTTATTAGTACATCCATTTAGGGTTTAGGGTTAATGGTTTTTATAGAC
TAATTTTTTTAGTACATCTATTTTATTCTATTTTAGCCTCTAAATTAAGAAAACTAAAAC
TCTATTTTAGTTTTTTTATTTAATAATTTAGATATAAAATAGAATAAAATAAAGTGACTA
AAAATTAAACAAATACCCTTTAAGAAATTAAAAAAACTAAGGAAACATTTTTCTTGTTTC
GAGTAGATAATGCCAGCCTGTTAAACGCCGTCGACGAGTCTAACGGACACCAACCAGCGA
ACCAGCAGCGTCGCGTCGGGCCAAGCGAAGCAGACGGCACGGCATCTCTGTCGCTGCCTC
TGGACCCCTCTCGAGAGTTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATCCAGAA
ATTGCGTGGCGGAGCGGCAGACGTGAGCCGGCACGGCAGGCGGCCTCCTCCTCCTCTCAC
GGCACGGCAGCTACGGGGGATTCCTTTCCCACCGCTCCTTCGCTTTCCCTTCCTCGCCCG
CCGTAATAAATAGACACCCCCTCCACACCCTCTTTCCCCAACCTCGTGTTGTTCGGAGCG
CACACACACACAACCAGATCTCCCCCAAATCCACCCGTCGGCACCTCCGCTTCAAGGTAC
GCCGCTCGTCCTCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGTTCCGGTCCATGG
TTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGATCCGTGTTTGTGTTAGAT
CCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACGTCAGACACGTTCTGATTGCTA
ACTTGCCAGTGTTTCTCTTTGGGGAATCCTGGGATGGCTCTAGCCGTTCCGCAGACGGGA
TCGATTTCATGATTTTTTTTGTTTCGTTGCATAGGGTTTGGTTTGCCCTTTTCCTTTATT
TCAATATATGCCGTGCACTTGTTTGTCGGGTCATCTTTTCATGCTTTTTTTTGTCTTGGT
TGTGATGATGTGGTCTGGTTGGGCGGTCGTTCTAGATCGGAGTAGAATTCTGTTTCAAAC
TACCTGGTGGATTTATTAATTTTGGATCTGTATGTGTGTGCCATACATATTCATAGTTAC
GAATTGAAGATGATGGATGGAAATATCGATCTAGGATAGGTATACATGTTGATGCGGGTT
TTACTGATGCATATACAGAGATGCTTTTTGTTCGCTTGGTTGTGATGATGTGGTGTGGTT
GGGCGGTCGTTCATTCGTTCTAGATCGGAGTAGAATACTGTTTCAAACTACCTGGTGTAT
TTATTAATTTTGGAACTGTATGTGTGTGTCATACATCTTCATAGTTACGAGTTTAAGATG
GATGGAAATATCGATCTAGGATAGGTATACATGTTGATGTGGGTTTTACTGATGCATATA
CATGATGGCATATGCAGCATCTATTCATATGCTCTAACCTTGAGTACCTATCTATTATAA
TAAACAAGTATGTTTTATAATTATTTTGATCTTGATATACTTGGATGATGGCATATGCAG
CAGCTATATGTGGATTTTTTTAGCCCTGCCTTCATACGCTATTTATTTGCTTGGTACTGT
TTCTTTTGTCGATGCTCACCCTGTTGTTTGGTGTTACTTCTGCAGATCCAGATCTAAACC
ATGCAGAAACTCATTAACTCAGTGCAAAACTATGCCTGGGGCAGCAAAACGGCGTTGACT
GAACTTTATGGTATGGAAAATCCGTCCAGCCAGCCGATGGCCGAGCTGTGGATGGGCGCA
CATCCGAAAAGCAGTTCACGAGTGCAGAATGCCGCCGGAGATATCGTTTCACTGCGTGAT
GTGATTGAGAGTGATAAATCGACTCTGCTCGGAGAGGCCGTTGCCAAACGCTTTGGCGAA
CTGCCTTTCCTGTTCAAAGTATTATGCGCAGCACAGCCACTCTCCATTCAGGTTCATCCA
AACAAACACAATTCTGAAATCGGTTTTGCCAAAGAAAATGCCGCAGGTATCCCGATGGAT
GCCGCCGAGCGTAACTATAAAGATCCTAACCACAAGCCGGAGCTGGTTTTTGCGCTGACG
CCTTTCCTTGCGATGAACGCGTTTCGTGAATTTTCCGAGATTGTCTCCCTACTCCAGCCG
GTCGCAGGTGCACATCCGGCGATTGCTCACTTTTTACAACAGCCTGATGCCGAACGTTTA
AGCGAACTGTTCGCCAGCCTGTTGAATATGCAGGGTGAAGAAAAATCCCGCGCGCTGGCG
ATTTTAAAATCGGCCCTCGATAGCCAGCAGGGTGAACCGTGGCAAACGATTCGTTTAATT
TCTGAATTTTACCCGGAAGACAGCGGTCTGTTCTCCCCGCTATTGCTGAATGTGGTGAAA
TTGAACCCTGGCGAAGCGATGTTCCTGTTCGCTGAAACACCGCACGCTTACCTGCAAGGC
GTGGCGCTGGAAGTGATGGCAAACTCCGATAACGTGCTGCGTGCGGGTCTGACGCCTAAA
TACATTGATATTCCGGAACTGGTTGCCAATGTGAAATTCGAAGCCAAACCGGCTAACCAG
TTGTTGACCCAGCCGGTGAAACAAGGTGCAGAACTGGACTTCCCGATTCCAGTGGATGAT
TTTGCCTTCTCGCTGCATGACCTTAGTGATAAAGAAACCACCATTAGCCAGCAGAGTGCC
GCCATTTTGTTCTGCGTCGAAGGCGATGCAACGTTGTGGAAAGGTTCTCAGCAGTTACAG
CTTAAACCGGGTGAATCAGCGTTTATTGCCGCCAACGAATCACCGGTGACTGTCAAAGGC
CACGGCCGTTTAGCGCGTGTTTACAACAAGCTGTAAGAGCTTACTGAAAAAATTAACATC
TCTTGCTAAGCTGGGAGCTCTAGATCCCCGAATTTCCCCGATCGTTCAAACATTTGGCAA
TAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAATTTCTG
TTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGACGTTATTTATGAGATGG
GTTTTTATGATTAGAGTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAG
CGCGCAAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCGGGAATTG
GCGAGCTCGAATTAATTCAGTACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAA
GCGTCAATTTGTTTACACCACAATtcatacaagagatacttttataacttcaaatcatat
gattgtagatcatgtaaattaaaatacaaaggaaatcctataacttcaacccaaagtatg
aaagtttaaacatattccaaatttataaaacctgaatccagaactaatataacatgatac
tatagcaaccttaagatgggggcgcttggcttcgattgaagctcaacagttgatggagga
gactgttattttcttccatcgatctcttcatattgtccatggtttcctttgaggcctgtg
cctgagcctttaaggtttctacttcttcccgaagtccatctttttctagcctctcagact
ccaattgattctgaagttcttgaacctttgcagaaacagttgttgtggagcttttcttgg
agttatcttgtagaccaacattatgtagaaataaactagaaccccctagcacttcagaaa

caacttccataggagacttctgttgttgtccatcctcaactggtgttgtcaccattgctt
ccatgtcaacctattataaatattatttgttagtataacatagtcgtacttgtaattttt
cagaacaaacataaaagcaaacgagtactcacaattgctttctttacattttcagaaaaa
cctttcttcttgctgcaatgcatatccttgaaaagatctattgcactcggttcagcatcc
ttatatttctcttgtttctaaagtgagaaaatgaatctggtgagaatagagaccttgaca
cataatgtacaagtcggatttataaatttcaatatgacatatattgaagattgaacattt
gttaaaagatttacaatacattttagatatggacaatactaaatgggttgaatgaaataa
tatttctagtagatatgagcagtgctaattcatcatctagaaagataaaagacagagatg
gcagcaggctgctttctctacatcttggcattgggctgctttcccagcagtagtagttgc
aatgcagccacaatgaaaagaggaagcaaactccaataaataaactagaagacatatttt
gtaccgttatcaaaatggtctataatttacaacatagaattgtggatatattgactgaaa
gctagtcaaacttctctgaaggcctaggaaacagctctgacatccaatgatcattttctt
actaataaattaggggacaactttatgagtaaatagagatgttaaaactgaatttactgg
tagcattaaacaatcaaagatttgtcaactatgcagttcagtaaccaatgatgattttct
taccagtaaataagcttttgcaatatatgattgggatcctgtgcattgttgatactgtac
ttttcacgattaagtttattcttcatacaactatcctgtacgttaaatcaacatgttag
aaataatatgtacaataagtagtgtgaaatagtatagcagttttcaaagtctacattagt
taattattgcattggttttctctaggtgctactgtatttgatgtttttagttatgttgca
gaatctaaactatacatactatttattgggtgcattcaccaaggatggtgcaaaagctag
a

4. References

Agrivida, Inc. (2017). Early food safety assessment for the AC1 Glucanase. New Protein Consultation (NPC) 000018; https://www.accessdata.fda.gov/scripts/fdcc/?set=NPC

Agrivida (2019). FDA "No Questions" letter regarding the GRAS notice for the AC1 Glucanase (GRASN 031), Current Animal Food GRAS Notices Inventory, available at: https://www.fda.gov/downloads/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/UCM581398.pdf

Agrivida (2020). GraINzyme® Glucanase: A thermotolerant β-glucanase feed enzyme expressed in Zea mays. GRASN # 031, Current Animal Food GRAS Notices Inventory, available at: https://www.fda.gov/downloads/AnimalVeterinary/Products/AnimalFoodFeeds/GenerallyRecognizedasSafeGRASNotifications/UCM581398.pdf

Bedford, M.R. and H.L. Classen (1992). Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. Journal of Nutrition **122**: 560-569. Abstract available at: https://www.ncbi.nlm.nih.gov/pubmed/1542013.

Broz, J. and M. Frigg (1986). Effects of beta-glucanase on the feeding value of broiler diets based on barley or oats. Arch. Geflügelkunde **50**:41-47.

Burnett, G.S. (1966). Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. British Poultry Science **7**: 55-75. Available at: https://doi.org/10.1080/00071666608415606

Campbell, G. L., B.G. Rossnagel, H.L. Classen, and P.A. Thacker (1989). Genotypic and environmental differences in extract viscosity of barley and their relationship to its nutritive value for broiler chickens. Anim. Feed Sci. Technol. **26**: 22t-230. Abstract available at: https://www.sciencedirect.com/science/article/pii/0377840189900369

Choct, M. and G. Annison (1992). Anti-nutritive effect of wheat pentosans in broiler chickens: roles of viscosity and gut microflora. British Poultry Science **33**:821-834. Available at: https://doi.org/10.1080/00071669208417524.

Christensen, A.H., R.A. Sharrock, and P.H. Quail (1992). Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol. Biol. **18:**675-689.

Coe, E.H. Jr., Nueffer, M.G., and D.A. Hoisington. (1988). The genetics of maize. In: G.F Sprague and J.W. Dudley (Eds.), Corn and corn improvement (Agronomy Monographs No. 18; pp. 81-236). Madison, WI: American Society of Agronomy.

Collins, J. and B. Hohn (1978). Cosmids: a type of plasmid gene-cloning vector that is packageable in vitro in bacteriophage lambda heads. PNAS USA **75**:4242–4246.

Cowieson, A.J. (2005). Factors that affect the nutritional value of maize for broilers. Animal Feed Science and Technology **119**: 293-305. Available at: https://www.sciencedirect.com/science/article/pii/S0377840105000027

CVM (2020). CVM/FDA response letter to GRAS notice filed for the AC1 Glucanase; GRASn 31; available at: https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notification-program/current-animal-food-gras-notices-inventory

Dänicke, S., G. Dusel, H. Jeroch and H. Kluge (1999). Factors affecting efficiency of NSP-degrading enzymes in rations for pigs and poultry. Agrobiological Research **52**:1-24.

Depicker A, Stachel S, Dhaese P, Zambryski P, Goodman HM. (1982). Nopaline synthase: transcript mapping and DNA sequence. J Mol Appl Genet. 1:561-73. Abstract available at: https://www.ncbi.nlm.nih.gov/pubmed/7153689.

Federal Register (2020). The SECURE Rule. Vol. 85, No. 96; pp. 29790-29838; May 18, 2020.

Fling, M.E., Kopf, J., Richards, C. (1985). Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3'(9)-O-nucleotidyltransferase. Nucleic Acids Res. **13**:7095-7106.

Goldsworthy, A. and H.E. Street (1965). The carbohydrate nutrition of tomato roots VIII. The mechanism of the inhibition by D-mannose of the respiration of excised roots. Ann. Bot. **29**:45-58.

Havrlentová, M. and J. Kraic (2006). Content of β-D-glucan in cereal grains. J. Food Nutrition Res. 45(3):97-103.

Hesselman, K. and P. Åman (1986). The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low-or high viscosity. Animal Feed Science and Technology **15**: 83–93. Abstract available at: https://www.sciencedirect.com/science/article/pii/0377840189901193.

Hitchcock, A.S. and Chase, A. (1971). Manual of the grasses of the United States (Vol. 2. pp. 790-796), New York: Dover Publications.

Horinouchi, S., K. Furuya, M. Nishiyama, H. Suzuki, and T. Beppu (1987). Nucleotide sequence of the streptothricin acetyltransferase gene from *Streptomyces lavendulae* and its expression in heterologous hosts. J. Bacteriol. 169:129-137.

International Food Biotechnology Council. (1990). Biotechnologies and food: assuring the safety of foods produced by genetic modification. Reg. Tox. Pharma. 12, S1-S196.

Itoh, T. and J. Tomizawa (1978). Initiation of replication of plasmid ColE1 DNA by RNA polymerase, ribonuclease H and DNA polymerase I. Cold Spring Harbor Symposium on Quantitative Biology. 43, 409-418.

Kocher, A., M. Choct, M.D. Porter, and J. Broz (2002). Effects of feed enzymes on nutritive value of soybean meal fed to broilers. British Poultry Science **43**:54-63. Available at: https://doi.org/10.1080/00071660120109890.

Mahadevan, S.A., S.G. Wi, D.-S. Lee, and H.-J. Bae (2008). Site-directed mutagenesis and CBM engineering of Cel5A (*Thermotoga maritime*). FEMS Microbiol Lett **287**: 205 – 211. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1574-6968.2008.01324.x.

McCarthy, T., O. Hanniffy, A.V. Savage, and M.G. Tuohy (2003). Catalytic properties and mode of action of three endo-β-glucanases from Talaromyces emersonii on soluble β-1,4- and β-1,3;1,4-linked glucans. International J. Biol. Macromolecules **33**: 141–148. Abstract available at: https://www.sciencedirect.com/science/article/pii/S0141813003000801?via=ihub.

Miles, J.S. and J.R. Guest (1984). Nucleotide sequence and transcriptional start point of the phosphomannose isomerase gene (*manA*) of *Escherichia coli.* Gene **32**:41-48.

Negrotto, D., Jolley, M., Beer, S., Wenck, A.R., Hansen, G. (2000). The use of phosphomannose-isomerase as a selectable marker to recover transgenic maize plants (Zea mays L.) via Agrobacterium transformation. Plant Cell Rep. 19, 798-803.

Nelson, K.E., R.A. Clayton, S.R. Gill, M.L. Gwinn, *et al*. (1999). Evidence for lateral gene transfer between Archaea and Bacteria from genome sequence of *Thermotoga maritima*. Nature **399**:323-329. Abstract available at: https://www.nature.com/articles/20601.

Newman, R.K. and C.W. Newman (1987). Beta-glucanase effect on the performance of broiler chicks fed covered and hulless barley isotypes having normal and waxy starch. Nutrition Reports International **36**:693- 699.

Privalle, L., M. Wright, J. Reed, G. Hansen, J. Dawson, E. M. Dunder, Y.Chang, M. L.

Powell, and M. Meghji (2006). Phosphomannose Isomerase, A Novel Selectable Plant Selection System: Mode of Action and Safety Assessment. Proceedings of the 6th International Symposium on The Biosafety of Genetically Modified Organisms, Saskatoon, Canada.

Qu, L.Q., Y.P. Xing, W.X. Liu, X.P. Xu and Y.R. Song (2008). Expression pattern and activity of six glutelin gene promoters in transgenic rice. J. Exper. Botany **59:**2417–2424.

Quail, P.H., A.H. Christensen, H.P. Hershey, R.A. Sharrock and T.D. Sullivan (1996). Plant ubiquitin promoters system. U.S. Patent No. 5,510,474. USPTO. Available at: http://patft.uspto.gov/netahtml/PTO/search-bool.html.

Tuttle, A. (2007). Petition for the Determination of Nonregulated Status Maize Event 3272. United States Department of Agriculture, Animal and Plant Health Inspection Service. Available at: https://www.aphis.usda.gov/brs/aphisdocs/05_28001p.pdf.

USDA (2021). Plant-Trait-Mechanism of Action (MOA) combinations that have been determined by APHIS not to require regulation under 7 CFR part 340. Available at: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/permits-notifications-petitions/confirmations/moa/moa-table.

Wang, K., Herrera-Estrella L., Van Montagu, M., Zambryski, P. (1984). Right 25 bp terminus sequence of the nopaline T-DNA is essential for and determines direction of DNA transfer from Agrobacterium to the plant genome. Cell 38, 455-462.

Zambryski, P., Depicker, A., Kruger, K., Goodman, H.M. (1982). Tumor induction by Agrobacterium tumefaciens: analysis of the boundaries of T-DNA. J. Mol. Appl. Genet. 1, 361-370.