Appendix 5-2

Confirmation of safety of feed using recombinant DNA technology

Herbicides glyphosate and 4-hydroxyfe Nilpyruvate dioxygenase-inhibiting weeding Agent resistant cotton GHB811

September 18, 2018

Ministry of Agriculture, Forestry and Fisheries

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"Glyphosate herbicide and 4-hydroxyphenylpyruvate dioxygenase
Confirmation of Safety for Inhibitory Herbicide Resistant Cotton GHB811

I Introduction

Herbicide glyphosate and 4-hydroxyphenylpyruvate dioxygenase
Inhibitory Herbicide Resistant Cotton GHB811 (hereinafter referred to as “GHB811 Cotton”)
Since application for safety confirmation as genetically modified feed was made on February 21,
“Procedure for Confirming Safety of Feeds and Feed Additives Applied with Recombinant DNA Technology” (2002
The deliberation was conducted based on the Ministry of Agriculture, Forestry and Fisheries Notification No. 1780 on November 26).

II Overview of feed to be confirmed

Feed name: Herbicide glyphosate and 4-hydroxyphenylpyruvate dioxygena
Hase-resistant herbicide resistant cotton GHB811
Properties: Herbicide glyphosate and 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicide tolerance
Applicant: BASF Japan K.K.
Developer: BASF Agricultural Solutions Seed US LLC

GHB811 cotton contains herbicide glyphosate and 4-hydroxyphenylpyruvate dioxide.
Tolerance to shigenase-inhibiting herbicides (hereinafter referred to as “HPPD-inhibiting herbicides”)
2mepsps gene from corn (Zea mays) and Pseudomonas
The hppdPfW336-1Pa gene derived from the fluorescens A32 strain has been introduced.
In GHB811 cotton, 5-enolpyrubilushi expressed from the 2mepsps gene
Kimi-3-phosphate synthase (hereinafter referred to as “2mEPSPS protein”)
Because it is not affected by the herbicide glyphosate, even in the presence of glyphosate
By making it possible to synthesize aromatic amino acids, plants are made resistant to glyphosate.
Give.
In addition, 4-hydroxyphenylpyrubin expressed from the hppdPfW336-1Pa gene
Acid dioxygenase (hereinafter referred to as “HPPD W336 protein”)
In the presence of HPPD-inhibiting herbicides as they are not affected by HPPD-inhibiting herbicides
But by making it possible to synthesize homogentisic acid, plants can be HPPD-inhibited
Provides resistance to herbicides.
When GHB811 cotton and non-recombinant cotton were compared,
There were no differences, except for the nature given. For this reason, GHB811
The safety of the properties given to cotton was evaluated.
The point which becomes a problem of was not recognized. Therefore, GHB811 cotton and feed
Therefore, it was considered that there was no risk of affecting the health of the livestock consumed.
Cotton is mainly used as a raw material for feed for dairy cattle in the form of cottonseed oil residue.

III Contents of deliberation

1. Matters concerning the equivalence of existing products

(1) Matters concerning genetic material

The host used to produce GHB811 cotton is Malvaneae.

(Gossypium) Cotton (G. hirsutum L.) non-recombinant commercial variety Coker312.

GHB811 cotton contains 2mepsps gene derived from maize (Z. mays) and P.

The hppdPfW336-1Pa gene derived from the fluorescens A32 strain has been introduced.

2mepsps gene and hppdPfW336-1Pa gene were 2mEPSPS respectively.

Encodes protein and HPPD W336 protein.

GHB811 cotton produces 2mEPSPS protein and HPPD W336 protein.

Tolerant to the herbicide glyphosate and HPPD-inhibiting herbicides.

Have sex.

(2) Matter about safe breeding experience such as domestic animals

Cotton is generally cultivated as a fiber crop and is made from cotton seed from which cotton yarn has been removed.

In the form of cottonseed oil cake extracted from real oil, it is mainly used as feedstock for dairy cattle.

(Ministry of Agriculture, Forestry and Fisheries, 2013). Cottonseed itself is used as a raw material for dairy cattle feed

Sometimes (OECD, 2004).

(3) Matters concerning feed components

Analytical values and literature values of GHB811 cotton and non-recombinant cotton are clarified.

Comparison is possible.

(4) Matters concerning differences in usage between existing and new varieties

GHB811 cotton expresses 2mEPSPS protein and HPPD W336 protein

Resistance to the herbicide glyphosate and HPPD-inhibiting herbicide

have. Except this point, GHB811 cotton is no different from non-recombinant cotton.

Period (maturity), intake of livestock, etc. (edible), intake of livestock, preparation and processing

The method is not different from non-recombinant cotton.

According to (1) to (4), in the safety evaluation of GHB811 cotton as feed,

It was judged that comparison with recombinant cotton was possible.

2. Matters concerning the purpose and method of use of recombinants

GHB811 cotton produces 2mEPSPS protein by the introduced 2mepsps gene.

Appears to be resistant to the herbicide glyphosate. In addition, the introduced hppdPfW336-1Pa gene

Expresses HPPD W336 protein and exhibits resistance to HPPD-inhibiting herbicides. to this

Since GHB811 cotton can use herbicides with different mechanisms of action,

Provide a wide range of weed control options.

3. Matters concerning the host

(1) Matters related to taxonomic position such as scientific name, variety, strain name, etc.

GHB811 cotton host is Malvaneae cotton (Gossypium) cotton

(G. hirsutum L.) non-recombinant commercial variety Coker312.

(2) Matters concerning genetic ancestry

Cotton cultivation begins in Mesoamerica and begins in the Tehuacan Valley, Mexico. The evidence of cotton cultivation around 3,500-2300 was confirmed. Cultivated today The origin of cotton is considered to be Mexico near the border of Guatemala, 18th century It spread to the southeastern United States and then spread to tropical and subtropical countries around the world. (OECD, 2008).

(3) Matters concerning the production of harmful physiologically active substances

Cotton has gossypol and cyclopropene fatty acids as harmful physiologically active substances. include.

There are two types of gossypol: free and bound, and free gossypol has stronger physiological activity. Have Gossypol is toxic to non-ruminants, birds, etc. Thus, it is known to cause loss of appetite and weight loss. Ruminants on the other hand Is able to convert free form to bound form in the rumen, so the effect of gossypol It is hard to receive. In addition, most gossypol in the processing stage of cottonseed oil residue Cotton seed oil reduces the toxicity of gossypol because it binds with protein (OECD, 2008).

Cyclopropene fatty acids (malvalic acid, sterlic acid and dihydrosterk) Phosphoric acid) is known to inhibit the metabolism of saturated fatty acids (OECD, 2008). The Clopropene fatty acid inhibits the desaturation of saturated fatty acids, thereby To cause discoloration of white and decrease in hatching rate, to cottonseed oil cake and cottonseed oil poultry feed Use is restricted (OECD, 2004).

(4) Matters concerning parasitic property and fixing property

Cotton is a seed plant, and cotton does not infest or settle in livestock.

(5) Matters concerning not being contaminated with pathogenic foreign factors such as viruses

In cotton, diseases such as withering disease caused by filamentous fungi and half body wilt disease occur. The pathogenicity of these pathogens to livestock has not been reported (OECD, 2008).

(6) Matters concerning survival and proliferation ability under experimental conditions that reflect the natural environment

Cotton is a cultivated crop and has not been reported to become weedy (OGTR, 2008).

(7) Matters concerning sexual reproductive cycle and crossability

Cotton is a perennial plant that can be shrubs, but is commercially grown as an annual plant. Ru. Cotton is basically self-fertilizing, but it is known that cross-pollination occurs in insect media. (OECD, 2008). Wild relatives that can cross with cotton are native to Japan. Not.

(8) Matter about history used for feed

Cotton is generally cultivated as a fiber crop and is made from cotton seed from which cotton yarn has been removed. In the form of cottonseed oil cake extracted from real oil, it has been mainly used as a raw material for dairy cattle feed The In Japan, it is mainly used as a raw material for mixed feed and mixed feed for dairy cattle. (Ministry of Agriculture, Forestry and Fisheries, 2018)

(9) Matter about safe use of feed

Cotton seeds contain gossypol, etc. as harmful physiologically active substances, Mainly used as a feed ingredient for dairy cows, ruminants that are not susceptible to gossypol. It is used.

(10) Matters concerning conditions that limit survival and proliferation ability

Cotton seeds have a dormancy of 2 to 3 months. Sexuality is lost or minimized. The survival and proliferation ability of cotton Limited by conditions such as temperature, humidity, and soil (OECD, 2008).

(11) Matters concerning production of harmful physiologically active substances of related species

As with cotton, G. barbadense, a related species of cotton, is Gossypol and It is known to contain cyclopropene fatty acids.

4 Matters concerning vector

(1) Matter about name and origin

The introduction plasmid pTSIH09 used for the production of GHB811 cotton is Created based on the plasmid pGSC1700 derived from Agrobacterium tumefaciens There is.

(2) Matters concerning properties

The introduction plasmid pTSIH09 has a base number of 13,099 bp. Plus for introduction The entire base sequence, restriction enzyme cleavage site, component, origin and function of mid pTSIH09 It has been clarified (References 2 and 3), and the base sequence that produces known harmful proteins is Not included.

(3) Matters concerning drug resistance

Introduction plasmid pTSIH09 confers resistance to aminoglycoside antibiotics The aadA gene (Fling et al., 1985) is included and the plasmid pTSIH09 for introduction is constructed. Used as a selectable marker during adulthood. aadA gene is transferred to plasmid pTSIH09 It is not contained in GHB811 cotton because it is located outside the T-DNA region in the GHB811. The absence of the aadA gene in GHB811 cotton It has been confirmed by network analysis.

(4) Matters concerning transmission

The plasmid pTSIH09 for introduction does not contain a sequence that enables plasmid transfer.

(5) Items related to host dependence

The characterization of all genes contained in the transfer plasmid pTSIH09

Therefore, it does not contain sequences that can be propagated in plants or livestock.

(6) Matters concerning the method of creating expression vectors

Introducing plasmid pTSIH09 based on plasmid pGSC1700, 2mepsps

By incorporating the T-DNA region containing the gene and the hppdPfW336-1Pa gene cassette,

An expression vector is being created.

(7) Matters concerning the method and position of inserting the expression vector into the host

GHB811 cotton is a 2mepsps gene expression cassette and hppdPfW336-1Pa gene.

Non-recombinant cotton with T-DNA region containing expression cassette by Agrobacterium method

Created by introducing into the species Coker312. Insertion position is plasmid for introduction

It is from the right border sequence including the T-DNA region of pTSIH09 to the left border sequence.

5. Matters concerning inserted genes

(1) Matter about donor

Name, origin and classification

The 2mepsps gene is derived from maize (Lebrun et al., 2003). Also

The hppdPfw336-1Pa gene is a gram-negative bacillus that exists in soil.

Derived from the fluorescens A32 strain (Boudec et al., 2001).

Safety matters

Maize, the donor of the 2mepsps gene, is one of the world's major cereals.

Animals and humans have a dietary experience. Also donate hppdPfW336-1Pa gene

The body, P. fluorescens, is a gram-negative bacilli commonly found in soil and water.

There are no reports of pathogenicity or allergy to humans and livestock.

(2) Matters concerning gene insertion methods

The introduced DNA is introduced into the host using the plasmid for introduction pTSIH09.

Performed by the therium method. Introduced hypocotyl of non-recombinant cotton variety Coker312

By co-culturing with Agrobacterium harboring plasmid pTSIH09

After replacement, transfer to a medium supplemented with antibiotics (ticarcillin) and glyphosate.

As a result, Agrobacterium was sterilized and transformants were selected.

Thereafter, the selected individuals were redifferentiated to obtain redifferentiated individuals (T0). Of this T0 generation

Among them, individuals who are resistant to tembotrione, one of HPPD-inhibiting herbicides, are selected.

However, the T1 generation, the progeny of these self-breeding, is further sprayed with glyphosate to show resistance Individuals were selected. GHB811 cotton is finally nurtured from individuals selected in this way did.

(3) Structure matters

Matters related to promoters

2mepsps gene expression cassette is expressed by Ph4a748 promoter. It is controlled. Ph4a748 promoter is Arabidopsis thaliana) -derived sequence containing the promoter region of histone H4 gene. Induces transcription in the vesicle (Chabouté et al., 1987).

The hppdPfW336-1Pa gene expression cassette is driven by the Pcsvmv promoter. Expression is controlled. Pcsvmv promoter is cassava vein mosaic A sequence containing a promoter region derived from a virus (Cassava Vein Mosaic Virus) And induces transcription in plant cells (Verdaguer et al., 1996).

Terminator matters

The terminator of the 2mepsps gene expression cassette is Arabidopsis (A. thaliana) H4 gene 3 'end untranslated region (Chabouté et al., 1987). Terminates the copy and induces polyadenylation.

The terminator of the hppdPfW336-1Pa gene expression cassette is the Arabidopsis thaliana 3'-terminal untranslated region of the H4 gene from (A. thaliana) (Chabouté et al., 1987) Terminates transcription and induces polyadenylation.

Matters concerning not including known harmful base sequences

The function of each component of the inserted DNA is already known and known harmful bases. Does not include sequences.

(4) Matters concerning properties

Table 1 shows the components, origin and function of the inserted DNA. 2mepsps heredity Details of the offspring and the hppdPfW336-1Pa gene are listed outside the table.

Table 1 Origin and function of inserted DNA components

Component	Origin and function
	hppdPfW336-1Pa gene expression cassette
ThistonAt Terminator	3 'non-translation of histone H4 gene from Arabidopsis thaliana Sequence containing translation region (Chabouté et al. , 1987) for polyadenylation Terminates transcription of mRNA more.
hppdPfW336-1Pa	Derived from the gene encoding HPPD of P. fluorescens A32

gene	The 336th amino acid sequence is modified. This will cause HPPD Tolerance to inhibitory herbicides is conferred (Boudec et al. , 2001). The The codon has been optimized to be suitable for expression in cotton However, this modification does not change the amino acid sequence. Of sunflower (Helianthus annuus) and maize (Zea mays) Coding of plastid peptide derived from RuBisCo small subunit gene
TPotpY-1Pa	It is synthesized based on the region (Lebrun et al. , 1996). HPPD W336 Transports protein to the plastid. Suitable for expression in cotton The codons are optimized as described above. Has not changed.
Pcsvmv promoter	Contains a region of the promoter sequence of Cassava Vein Mosaic Virus, The target gene is constitutively expressed in plants (Verdaguer et al. , 1996).
	2mepsps gene expression unit
Ph4a748 promoter	Promoting the histone H4 gene of Arabidopsis (A. thaliana) The target gene is constitutively expressed in plants. (Chabouté et al. , 1987).
Intron1 h3At	Arabidopsis thaliana (A. Thaliana of histone H3III first II gene) first A sequence containing one intron (Chaubet et al. , 1992) Increases transcriptional activity in plants in combination with lomotor.
TPotpC	Sunflower (H. annuus) and corn (Z. mays) RuBisCo small Based on the coding region of the plastid peptide derived from the subunit gene (Lebrun et al. , 1996). 2mEPSPS protein to pigment Transport to the body.
ThistonAt Terminator	3 'non-histone of the histone H4 gene from Arabidopsis thaliana A polyadenylation sequence containing a translation region (Chabouté et al. , 1987) Terminates the transcription of mRNA.
	Exoskeleton region
aadA gene	A gene containing an aminoglycoside antibiotic resistance gene derived from E. coli Column (Fling et al. , 1985).
ORIpVS1	Pseudomonas introduced for replication in A. tumefaciens Sequence containing the origin of replication of plasmid pVS1 (Hajdukiewicz et al. , 1994).
ORI ColE1	E. coli plasmid introduced for replication in E. coli Sequence containing the origin of replication from pBR322 (Bolivar et al. , 1977).

○ Function of 2mepsps gene

2mEPSPS expressed from 2mepsps gene introduced into cotton

290 The protein confers resistance to the herbicide glyphosate on plants. EPSPS

White matter is an aromatic amino acid (tyrosine, phenylalanine and tomato in plants).
Is one of the enzymes acting in the shikimate pathway for the biosynthesis of
5-enol from sufoenol pyruvate (PEP) and shikimate 3-phosphate (S3P)
It catalyzes the reaction that produces pyruvic shikimate 3-phosphate (EPSP). Herbicide
Rephosate inhibits the activity of endogenous EPSPS proteins in plants and produces aromatic amino acids.
By killing acid synthesis, the plant is killed (Steinrücken and Amrhein, 1980;
Lebrun et al., 2003). On the other hand, 2mEPSPS gene encoded by 2mepsps gene
White matter exhibits EPSPS activity even in the presence of the herbicide glyphosate (Lebrun et al.,
2003) because of the endogenous EPSPS whose activity was inhibited by the herbicide glyphosate.
Herbicides on plants by enabling the synthesis of aromatic amino acids instead of protein
Provides resistance to glyphosate.

○ Function of hppdPfW336-1Pa gene
HPPD protein produced from the hppd gene is an enzyme in the tyrosine metabolic pathway.
Homogeneous from 4-hydroxyphenylpyruvic acid (4-HPP) and oxygen
It catalyzes the reaction that produces ntidine acid (HGA). HPPD-inhibiting herbicides are
Diketonitrile (DKN), a gift product, binds to the active site of HPPD protein
In order to inhibit this reaction, an HPPD-inhibiting herbicide is applied.
Plants are important for photosynthesis and antioxidant systems.
Production of HGA, which is a precursor of sulfa (DellaPenna et al., 2006)
Plants sprayed with HPPD-inhibiting herbicides die.
On the other hand, HPPD W336 protein produced from the hppdPfW336-1Pa gene is
A mutation is introduced into one amino acid in the amino acid sequence of HPPD protein
Thus, while binding to 4-HPP is possible, the binding affinity with DKN decreases, and DKN
Plants can be metabolized without being inhibited by HPPD.
Shows tolerance.

(5) Purity matters
The nature of the gene contained in the plasmid pTSIH09 for introduction has been clarified.
As a result of nucleotide sequence analysis, it is confirmed that the insertion region does not contain any other genes.
Confirmed (Reference 3).

(6) Matters concerning the number of copies
GHB811 Number of inserted sites, copy number, and outer skeleton sequence of genes introduced into cotton
As a result of Southern blot analysis to confirm the presence or absence, introduction into GHB811 cotton
One copy of the T-DNA region of the plasmid pTSIH09 is introduced into one place in the genome.
And that there is no outer skeleton sequence of the plasmid pTSIH09 for introduction.
(Refs. 6, 7).
In addition, in order to determine the entire base sequence of the inserted DNA and its neighboring sequences,
The nucleotide sequence analysis of the neighboring region was performed. In addition, the base sequence of the transgene is
It was confirmed that it was identical to the corresponding nucleotide sequence in Sumid pTSIH09. as a result,

The inserted nucleotide sequence in GHB811 cotton is identical to the nucleotide sequence of each component of the T-DNA region.
It was confirmed (Reference 8).

In addition, in GHB811 cotton, the inserted DNA disrupts a known endogenous gene.
To check for DNA bases in the control non-recombinant cotton.
As a result of comparison with the sequence, cotton genome at the insertion site of GHB811 cotton transgene
A 13 bp deletion was found in the endogenous sequence (Reference 9). However, the neighborhood array
As a result of BLASTn analysis, there was no disruption of known endogenous gene by introduction of T-DNA region.
I thought.

(7) Matters concerning stability

GHB811 Duplicated 2mepsps gene and hppdPfW336-1Pa gene introduced into cotton
DNA from five generations of GHB811 cotton to confirm stability over several generations
Southern blot analysis was performed using 2mepsps gene and
It was confirmed that the hppdPfW336-1Pa gene was stably inherited over multiple generations.
(Ref. 23).

(8) Matter about expression site, expression time and expression level

Expression levels of 2mepsps protein and HPPD W336 protein in GHB811 cotton
Was measured by ELISA analysis. Trials from 3 different fields in the US
GHB811 cotton leaves, roots, flower buds, drills, hairy seeds, and plants were sampled.
2mepsps protein and HPPD from all tissue samples tested
The expression of W336 protein was confirmed (Reference 11).

(9) Matters concerning the safety of antibiotic resistance marker genes

Transfer plasmid pTSIH09 confers resistance to aminoglycoside antibiotics
The aadA gene is contained outside the T-DNA region, but the aadA gene is present in GHB811 cotton.
The absence of a gene has been confirmed by Southern blot analysis.
(Reference Material 6).

(10) Presence / absence of foreign open reading frames and the possibility of transcription and expression
Matters concerning

GHB811 Open the cotton transgene and the border region between sequences near both ends.
Reading frame (ORF) search was performed. Presence of ORFs consisting of 3 amino acids
From the stop codon (TGA, TAG, TAA) for the six reading frames.
As a result of searching with don, 126 ORFs were confirmed. About them, known toxicity
Use FASTA search algorithm to check for homology with proteins, etc.
And homology with the database (NCBI non-redundant database, version2016.0906)
As a result of the search, no significant homology was found (Reference 10).

6. Matters concerning recombinants

(1) Matters concerning properties newly acquired by recombinant DNA manipulation

GHB811 cotton has 2mepsps gene and hppdPfW336-1Pa gene introduced.

2mEPSPS protein is expressed in herbicide glyphosate,

HPPD W336 Has resistance to HPPD-inhibiting herbicides due to protein expression

One. Except for these points, GHB811 cotton is non-recombinant cotton and its morphology and growth characteristics.

There is no difference in gender, and the method of use as feed is not changed.

(2) Matter related to toxicity of gene products

GHB811 Cotton 2mEPSPS protein and HPPD W336 protein are known

In order to confirm the homology with the toxin protein etc.

redundant protein database, version2016_1006 as well as Bayer toxin

database, version 17.1) for protein amino acid sequences registered in

A homology search was performed using BLOSUM50. As a result, 2mEPSPS protein and

There is no homology between HPPD W336 protein and known toxin proteins

(References 4, 5).

(3) Matters concerning susceptibility of gene products to physicochemical treatment

2mEPSPS protein and HPPD W336 protein expressed in GHB811 cotton

In order to investigate the sensitivity of physicochemical treatment to

I did it. The actual treatment was performed using 2mEPSPS protein expressed in E. coli and

HPPD W336 protein has been tested, and these proteins derived from E. coli

Proteins expressed in GHB811 cotton have molecular weight, peptide analysis, immunoreactivity,

Equivalence was confirmed by N-terminal sequence determination, enzyme activity and glycoprotein staining analysis.

(Reference material 12,13,14).

2mEPSPS protein

A Acid treatment with artificial gastric juice and enzyme (pepsin) treatment

2) Digestibility of mEPSPS protein in artificial gastric juice

As a result of examination by Estan blot analysis, the test in artificial gastric juice was started 30 seconds or less.

(Reference 15).

(B) Alkaline treatment with artificial intestinal fluid and enzyme (pancreatin) treatment

2) Digestibility of mEPSPS protein in artificial intestinal fluid

As a result of examination by Estan blot analysis, the test in artificial gastric juice was started 30 seconds or less.

(Reference Material 16).

C) Heat treatment

The sensitivity of 2mEPSPS protein to heat treatment was examined by ELISA analysis.

As a result, when the temperature is 75 ° C or higher, the immune response is lost by heat treatment for 30 minutes or longer.

(Reference 17).

HPPD W336 Protein

A Acid treatment with artificial gastric juice and enzyme (pepsin) treatment

Regarding the digestibility of HPPD W336 protein in artificial gastric juice, SDS-PAGE and As a result of Western blot analysis, the test in artificial gastric juice started 30 seconds (Reference Material 19).

(B) Alkaline treatment with artificial intestinal fluid and enzyme (pancreatin) treatment

Regarding the digestibility of HPPD W336 protein in artificial intestinal fluid, SDS-PAGE and As a result of Western blot analysis, the test in artificial gastric juice started 30 seconds (Reference Material 20).

C) Heat treatment

Examination of heat treatment sensitivity of HPPD W336 protein by ELISA analysis

As a result, under 75 ° C or higher, the immune response was lost by heat treatment for 30 minutes or longer. (Reference Material 21).

(4) Matters concerning the influence of gene products on metabolic pathways

2mEPSPS protein

EPSPS protein is Shikimi, which is involved in the biosynthesis of aromatic amino acids in plants. One of the enzymes acting in the acid pathway, phosphoenolpyruvate (PEP) and shikimate 3- Reaction to produce 5-enolpyruvylshikimate 3-phosphate (EPSP) from phosphoric acid (S3P) Catalyzed). EPSPS protein is known to react specifically with PEP and S3P. (OECD, 1999a). In addition, the reaction with EPSPS protein is caused by aromatic amino acid production. It is considered not a rate-limiting step in synthesis (Weiss and Edwards, 1980; Herrmann and Somerville, 1983; Herrmann, 1995a, b; Heldt, 2000; Buchanan, 2005), Even if EPSPS protein activity increases, aromatic amino acids that are end products of this pathway Has not been reported to increase (Padgette *et al.* , 1996; Ridley *et al.* , 2002). Regarding GHB811 cotton that actually expresses 2mEPSPS protein, 2014, 2015 Annually, we conducted cultivation tests in a total of 8 fields in the United States to analyze the constituents in seeds. As a result, there was a statistically significant difference in aromatic amino acids from the non-recombinant control cotton. Was not recognized.

Based on the above, 2mEPSPS protein expression in GHB811 cotton It is considered that there will be no impact on the channel of thanks.

HPPD W336 Protein

HPPD W336 protein is 4-hydroxyphenyl in the tyrosine metabolic pathway An enzyme that catalyzes the reaction of pyruvate (4-HPP) to homogentisic acid (HGA) It is. Tyrosine metabolism is tightly controlled, and increased tyrosine levels are related to HPPD One of the reasons for the toxicity of inhibitory herbicides (Prisbylla et al., 1993; Pallett et al., 1998), non-recombinant with GHB811 cotton for tyrosine content

No statistically significant difference from cotton, other tyrosine precursors
There is no impact on the route.
In addition, even if HPPD protein is overexpressed alone, the total tocopherol level is almost the same.
It has been reported not to change (Tsegaye et al., 2002; Falk et
al., 2003; Raclaru et al., 2006; Farré et al., 2012). Also in GHB811 cotton,
HPPD W336 Protein expression increases the total amount of HPPD protein, but HGA
No increase in content was observed (Reference Material 28), downstream tocopherol content
Was also within the range of literature values.
As a substrate for HPPD protein, in addition to 4-HPP, phenylpyruvic acid (PP),
3,4-dihydroxyphenylpyruvic acid (3,4-dHPP), α-ketoisocaproic acid
(KIC) and 4-oxo-5-thiahexanoic acid (KMTB) are four kinds of compounds
(Lindstedt et al., 1977, Reference 25). This
As a result of comparison of the reaction activity between these 4 substances and HPPD W336 protein,
Compared with HPPD W336 protein, the reaction activity is extremely low.
In the presence of 4-HPP, these four substances are used as HPPD W336 protein substrates.
It was thought not to be done.
Therefore, with the introduction of the hppdPfW336-1Pa gene, HPPD W336 protein
Increased HPPD activity due to expression does not affect the tyrosine catabolism pathway,
It was considered unlikely to affect the main metabolic pathways.

(5) Matters concerning differences from the host

To assess component equivalence with GHB811 cotton and control non-recombinant cotton,
GHB811 cotton and control non-recombinant cotton grown in eight fields in the United States
For seeds other than organic matter, general ingredients, inorganic salts, vitamins, harmful
Bioactive substances, amino acids and fatty acids were analyzed (Reference Material 14). Also,
At the same time, seven non-recombinant commercial varieties were planted at the same time in these eight fields.
Cultivated and analyzed in the same way.
For herbicide application to GHB811 cotton, glyphosate (approximately 1.06-1.22 kg)
ai / ha), and the herbicide isoxaflutole, one of the HPPD-inhibiting herbicides
(About 100.3-115.2 g ai / ha) was sprayed.
As a result, the various components of GHB811 cotton are either non-recombinant control cotton or conventional
It was confirmed that it is the same level as cotton. In addition, harmful physiological activity contained in cotton
The substances gossypol (total gossypol and free gossypol) and cyclopro
Pen fatty acids (malvalic acid, sterlic acid and dihydrosteric acid)
It was also confirmed that it is equivalent to conventional cotton.

(6) Matters concerning survival and proliferation ability in the outside world

As a result of field tests conducted in the United States so far, survival of GHB811 cotton in the outside world
It has been confirmed that the growth ability is not different from that of non-recombinant cotton.

(7) Matters concerning limitations on survival and proliferation ability

As a result of field tests conducted so far in the United States, the survival and proliferation capacity of GHB811 cotton It has been confirmed that it is not different from non-recombinant cotton.

(8) Matters concerning the inactivation method

GHB811 cotton can be used for physical control (plowing) and chemical control (dispersion of sensitive herbicides). Inactivated by the conventional method of dying non-recombinant cotton.

(9) Matters concerning approvals in foreign countries

In April 2017, the US Food and Drug Administration (FDA) confirmed the safety of food and feed. Applying for approval.

Also, in August 2017, the Health Canada The Nada Food Inspection Agency (CFIA) has applied for safety confirmation as an environment and feed.

(10) Matter about production, breeding and cultivation method

GHB811 cotton is grown using the herbicide glyphosate and HPPD-inhibiting herbicide. Be able to use these herbicides to control weeds. Except for, it is the same as non-recombinant cotton.

(11) Matters related to seed production and management

GHB811 Cotton seed production and management method is different from non-recombinant cotton Absent.

7 Where knowledge about feed safety has not been obtained from the materials listed in 2-6

In the case of the following tests, matters related to the required test results

Not applicable.

IV deliberation results

Herbicide glyphosate and 4-hydroxyphenylpyruvate dioxygenase inhibitor type Regarding herbicide-tolerant cotton GHB811, “Recombinant Feed for Recombinant DNA Technology and Feed Additives” As a result of deliberation based on the procedure for confirming integrity, the safety of livestock consumed as feed It was judged that there was no problem.

V References and references

References

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