Corporation obtaining approval, the name of its representative, and the address of its main office

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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Maize resistant to Lepidoptera, and tolerant to glufosinate herbicide and glyphosate herbicide (Modified *cry1Ab*, *pat*, *mEPSPS*, *Zea mays* subsp. *mays* (L.) Iltis) (Bt11×GA21, OECD UI: SYN-BTØ11-1×MON-ØØØ21-9)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

I. Information collected prior to assessing Adverse Effect on Biological Diversity

1. Information concerning preparation of living modified organisms

The parent lines of this stack line, Bt11 and GA21, were developed by Syngenta Seeds, Inc. and Monsanto Company, respectively. This stack line is given the traits to be resistant to Lepidoptera and tolerant to glufosinate herbicide due to the transferred genes in the Bt11, and to be tolerant to glyphosate herbicide due to the transferred genes in the GA21. Therefore, information on the Bt11 and the GA21 is explained individually in the following statements. To create this evaluation document, we referred to the proprietary data of Syngenta Seeds, Inc., the published international publication for patent applications (Reference 1) and the Biological Diversity Risk Assessment Report (Annex 1) prepared based on the published references for the GA21, and the Biological Diversity Risk Assessment Report for the Bt11 (Annex 2).

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the development of the Bt11 are shown in Table 1. In addition, the composition of donor nucleic acid and the origins of component elements used for the development of the GA21 are shown in Table 2.

Table 1 Origins and functions of the component elements of the donor nucleic acid used for the production of Bt11

Gene cassette resistant to Lepidoptera	
Component elements	Origin and Function
35S promoter	A promoter obtained as *Dde*I-*Dde*I fragment derived from cauliflower mosaic virus (CaMV) CM1841 strain. This promoter makes the target gene (modified *cry1Ab*) expressed in all the tissues constitutively (Reference 13).
IVS6-ADH1	Intron derived from alcohol dehydrogenase 1S (Adh1-S) gene of maize (Reference 14). Adh1-S intron was used to enhance the expression of target gene (modified *cry1Ab*) in plants (Reference 15).
Modified *cry1Ab*	A modified version of the full-length *cry1Ab* gene that encodes Cry1Ab protein of *Bacillus thuringiensis* subsp. *kurstaki* HD-1 strain, by partially deleting the C-terminal code region which is independent from the insecticidal activity of Cry1Ab protein and modifying some nucleotide sequences to enhance its expression level in plants. This modification does not change any amino acid sequences of the core protein.
NOS term	3' untranslated region of nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*, which contains a transcription terminator and a signal for polyadenylation of mRNA (Reference 16, Reference 17). This sequence terminates transcription of target gene (modified *cry1Ab*).
Gene cassettes tolerant to glufosinate herbicide	
Component elements	Origin and Function
35S promoter	A promoter obtained as *Alu*I-*Dde*I fragment derived from cauliflower mosaic virus (CaMV) Cabb-s strain. This promoter makes the target gene (*pat*) expressed in all the tissues constitutively (Reference 18).
IVS2-ADH1	An intron derived from alcohol dehydrogenase 1S (Adh1-S) gene of maize (Reference 14). Adh1-S intron was used to enhance the expression of target gene (*pat*) in plants (Reference 15).
pat	A gene that encodes the PAT protein of *Streptomyces viridochromogenes*. PAT protein, that confers glufosinate herbicide tolerance, was used as a selective marker for modified plants at the time of transferring of genes. The *pat* gene has some nucleotide sequences modified to enhance its expression level in plants. The amino acid sequence of PAT protein expressed by the modification remains unchanged (Reference 19).
NOS term	3' untranslated region of nopaline synthase (NOS) gene of *Agrobacterium tumefaciens*, which contains a transcription terminator and a signal for polyadenylation of mRNA (Reference 16, Reference 17). This sequence terminates transcription of target gene (*pat*).

Other regions	
Component elements	Origin and Function
ColE1 ori	The replication origin derived from *Escherichia coli* plasmid pUC18 (Reference 20, Reference 21). Permits replication of plasmid in bacteria.
amp^R	Derived from *Escherichia coli*, it has the function to code β-lactamase and confer the tolerance to antibiotic ampicillin (Reference 21).

Table 2 Origins and functions of the component elements of the donor nucleic acid used for the production of GA21

Gene cassettes tolerant to glyphosate herbicides	
Component elements	Origin and Function
Act promoter ＋intron	A promoter derived from rice actin 1 gene inducing the initiation of transcription of target gene throughout the entire plant body, including up to the first intron region which functions to enhance the efficiency of transcription (Reference 32).
sssu＋mssu (Hereinafter referred to as "OTP")	The optimized transit peptide (OTP) sequences composed of the ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCo) gene derived chloroplast transit peptide sequence (sssu) from sunflowers and the *RuBisCo* gene derived chloroplast transit peptide sequence (mssu) from maize, functioning to transport the mEPSPS protein expressed by the target gene *mEPSPS* gene to chloroplasts, where the protein takes action (Reference 33).
mEPSPS	A gene obtained from mutation of the 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) gene of *Zea mays* (Reference 22); It encodes the 5-enol-pyruvyl-shikimate-3-phosphate synthase (mEPSPS), the activity of which is not inhibited by the glyphosate herbicide, with the 102nd amino acid threonine in the wild-type EPSPS amino acid sequence modified to isoleucine, and the 106th proline modified to serine (Reference 1).
NOS	A polyadenylation sequence of nopaline synthase (NOS) gene from *Agrobacterium tumefaciens*, terminating transcription (Reference 16).
Backbone region (Not contained in GA21)	
Component elements	Origin and Function
amp	Consists of the lac sequence, composed of partial coding sequence for lacI derived from bacteriophage M13, promoter plac and partial coding sequence for β-galactosidase or lacZ protein (Reference 21), and the β-lactamase gene (*bla*) conferring the ampicillin tolerance derived from plasmid pBR322 of *Escherichia coli* (Reference 34); Selects and maintains the *Escherichia coli* which contains the constitutive plasmid by expression of β-lactamase.
ori-puc	The replication origin region derived from the high-copy-number plasmid pUC19 of *Escherichia coli*, conferring the autonomous replication potency of plasmid in *Escherichia coli* (Reference 35).

2) Functions of component elements

(a) Functions of individual component elements of donor nucleic acid, including target gene, expression regulatory region, localization signal, and selective marker

Functions of component elements of the donor nucleic acid that was used for the development of the Bt11 are shown in Table 1. In addition, functions of component elements of the donor nucleic acid that was used for the development of the GA21 are shown in Table 2.

(b) Functions of proteins produced by the expression of target genes and selectable markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergen (except allergenicity as food)

Modified Cry1Ab protein;

The insecticidal protein (=Bt protein), isolated from the soil microorganism *Bacillus thuringiensis*, exhibits its insecticidal activity against limited species of insects. It is known that the Bt protein, when fed and digested by sensitive species of insects, becomes an active polypeptide (= core protein) through specific digestion of protein, which specifically binds to the specific receptors on the surface of midgut of insects, causing cytoclasis or cell-destruction and leading to destructed digestive tracts and death of the insects (Reference 26). This mechanism of action also holds for the Cry1Ab protein derived from *Bacillus thuringiensis* subsp. *kurstaki*. Regarding the insecticidal activity of Cry1Ab protein, detail investigational results are listed in the database operated by the Canadian Government (Reference 23), showing that it exhibits its insecticidal activity against European corn borer (*Ostrinia nubilalis*), Corn earworm (*Helicoverpa zea*), Fall armyworm (*Spodoptera frugiperda*) and other order Lepidopteran insects which are the major pest insects for cultivation of maize. On the other hand, Cry1Ab protein exhibits no or least little insecticidal activity against any insects other than the order Lepidoptera. It has been confirmed based on the homology search using the publicly available database (SWISS-PROT, FARRP, etc.) that the modified Cry1Ab protein does not share structurally related homologous sequences with any of the known allergens.

PAT protein;

The glufosinate herbicide inhibits glutamine synthase in plants and then it causes plants to die due to the accumulated ammonia in the cells. However, the expression of PAT protein acetylates and inactivates the glufosinate, which releases the glutamine synthase from inhibition. Consequently, the plants, which express the PAT protein, exhibit the tolerance to glufosinate herbicides and thus they have been used as a selection marker for the Bt11. It has been confirmed based on the homology search using the publicly available database

(SWISS-PROT, FARRP, etc.) that the PAT protein does not share structurally related homologous sequences with any of the known allergens.

mEPSPS protein;

The glyphosate herbicide is a nonselective herbicide acting on stems and leaves, which inhibits the activity of 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS), one of the enzymes in the shikimate pathway for aromatic amino acid biosynthesis, and interrupts the aromatic amino acid biosynthesis, thereby causing plants to die (Reference 36). The mEPSPS protein encoded by the *mEPSPS* gene exhibits the EPSPS activity even in the presence of glyphosate herbicide, and enables the aromatic amino acid biosynthesis in place of plant-intrinsic EPSPS and confers the tolerance to glyphosate herbicide. It has been confirmed based on the homology search using the publicly available database (SWISS-PROT, FARRP, etc.) that the mEPSPS protein does not share structurally related homologous sequences with any of the known allergens.

(c) Contents of any change caused to the metabolic system of recipient organism

There is no report that the modified Cry1Ab protein possesses any enzyme activity. The PAT protein possesses very high substrate specificity against *L*-phosphinothricin (active ingredient of glufosinate herbicide) and dimethyl-phosphinothricin, and there is no report on any other protein or amino acid than these which would become the substrate of PAT protein (Reference 29). The mEPSPS protein is one of the enzymes that catalyze the shikimate pathway (Reference 30), and it is reported to react specifically with phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Reference 31).

Based on the above understanding, it is considered very unlikely that these proteins affect the metabolic system of the recipient organism, but they would function independently in the recipient organism.

(2) Information concerning vectors

1) Name and origin

The plasmid pZO1502 used for the production of the Bt11 was constructed based on the pUC18 derived from *Escherichia coli*. In addition, the plasmid pDPG434 used for the production of the GA21 is derived from plasmids including pUC19 from *Escherichia coli* (Reference 37).

2) Properties

(a) The numbers of base pairs and nucleotide sequence of vector

The total number of base pairs of the plasmid pZO1502 used for the production of the Bt11 is 7,240bp. In addition, the total number of base pairs of the plasmid pDPG434 used for the production of the GA21 is 6,128bp (Reference 1).

(b) Presence or absence of nucleotide sequence having specific functions, and the functions

The plasmid pZO1502 used for the production of the Bt11 and the plasmid pDPG434 used for the production of the GA21 contain the *amp* gene which is used as a selective marker for bacteria and thus, the plasmids possess the resistance to ampicillin, though the antibiotic resistant marker gene is not transferred in the Bt11 and the GA21.

(c) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present

There is no report that the plasmid pZO1502 used for the production of the Bt11 and the plasmid pDPG434 used for the production of the GA21 contain any sequence showing infectivitiy.

(3) Method of preparing living modified organisms

1) Structure of the entire nucleic acid transferred in the recipient organism

The nucleic acid transferred to the recipient organism of the Bt11 refers to the segment where the plasmid pZO1502 is cleaved by the restriction enzyme *Not*I and the amp^R gene is deleted. In addition, for transferring the nucleic acid to the recipient organism of the GA21, the DNA fragment composed of only the herbicide resistant gene cassette (Act promoter + intron/OTP/ *mEPSPS*/NOS) obtained by cleaving the plasmid pDPG434 by the restriction enzyme *Not*I is used (Reference 1).

2) Method of transferring nucleic acid transferred to the recipient organism

To transfer the nucleic acid to the recipient organism for creating the Bt11, the electroporation method was used. In addition, to transfer the nucleic acid to the recipient organism for creating the GA21, the particle gun bombardment was used (Reference 1).

3) Processes of rearing of living modified organisms

(a) Mode of selecting the cells containing the transferred nucleic acid

Regarding the Bt11, transformed cells were selected on the medium containing glufosinate. Regarding the GA21, transformed cells were selected on the medium containing glyphosate herbicide (Reference 1).

(b) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line with which the state of existence of replication products of transferred nucleic acid was confirmed, the line subjected to isolated field tests; and the line used for collection of other necessary information for assessment of Adverse Effect on Biological Diversity

This stack line was developed by cross-breeding between the Bt11, maize resistant to Lepidoptera and tolerant to glufosinate herbicide, and the GA21, maize tolerant to glyphosate herbicide.

The status of approval and application for approval of the Bt11 and the GA21 in Japan are the following.

Bt11:

May, 1996:	Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being utilized in a simulated environment (isolated field tests for importing) was certified by the Ministry of Agriculture, Forestry and Fisheries.
September, 1996:	Based on the “Guideline for the conduct of Food Safety Assessment of Food and Additives derived from Recombinant-DNA Plants, Chapter 4”, the safety of use for food was approved by the Ministry of Health, Labour and Welfare.
September, 1996:	Based on the “Guideline for the safety evaluation of feed derived from recombinant-DNA plants, 6-(2)” by the Ministry of Agriculture, Forestry and Fisheries, safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries.
October, 1996:	Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being imported to Japan was certified by the Ministry of Agriculture, Forestry and Fisheries.
March, 2001:	The safety for use as food in accordance with the "Guideline for the safety evaluation of food and additives derived from recombinant-DNA techniques" was approved by the Ministry of Health, Labour and Welfare.
May, 2001:	Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being utilized in a simulated environment (isolated field tests for cultivation) was certified by the Ministry of Agriculture, Forestry and Fisheries.
June, 2002:	Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being cultivated was certified by the Ministry of Agriculture, Forestry and Fisheries.
March, 2003:	Based on the “Procedure to Check the Safety of Feed and Additives Produced by Recombinant-DNA Techniques”, the safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries.

April, 2007: Type I Use (Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them) in accordance with the "Law concerning the conservation and sustainable use of biological diversity through regulations on the use of living modified organisms" was approved by the Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment.

GA21:

May, 1998: Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being utilized in a simulated environment was certified by the Ministry of Agriculture, Forestry and Fisheries.

December, 1998: Based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being imported to Japan and cultivated in Japan was certified by the Ministry of Agriculture, Forestry and Fisheries.

November, 1999: Based on the “Guideline for the conduct of Food Safety Assessment of Food and Additives derived from Recombinant-DNA Plants, Chapter 4”, the safety of use for food was approved by the Ministry of Health, Labour and Welfare.

December, 1999: Based on the “Guideline for the safety evaluation of feed derived from recombinant-DNA plants, 6-(2)” by the Ministry of Agriculture, Forestry and Fisheries, safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries.

March, 2003: The approval was obtained, in accordance with “Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA Techniques” from the Ministry of Health, Labour and Welfare.

March, 2003: Based on the “Procedure to Check the Safety of Feed and Additives Produced by Recombinant-DNA Techniques”, the safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries.

November, 2005: Type I Use (Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them) in accordance with the "Law concerning the conservation and sustainable use of biological diversity through regulations on the use of living modified organisms" was approved by the Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment.

(4) State of existence of nucleic acid transferred in cells and stability of expression of traits caused by the nucleic acid

1) Place where the replication product of transferred nucleic acid exists (on the chromosome, in the cell organelle, or in the protoplasm)

In the Bt11, it was confirmed based on the segregation analysis and sequence analysis that the transferred genes are present on the chromosome. In addition, in the GA21, it was confirmed based on the Southern blotting analysis and segregation analysis that the transferred genes are present on the chromosome.

2) The number of copies of replication products of transferred nucleic acid and stability of its inheritance through multiple generations

In the Bt11, it was confirmed that one copy of transferred genes is present on the chromosome and also that the transferred genes are inherited stably in multiple generations. In addition, in the GA21, it was confirmed that the transferred genes are present on the chromosome at one site, which is composed of six (6) consecutive regions derived from the herbicide tolerant gene cassette (Act promoter + intron/OTP/*mEPSPS*/NOS) fragments, and also that the transferred genes are stably inherited in multiple generations.

3) Inter-individual or inter-generational expression stability under a natural environment with respect to the characteristics referred to specifically in (6)-1)

The stability of expression of resistance to Lepidoptera and tolerance to glufosinate herbicide in the Bt11 was confirmed based on the ELISA method and bioassay. In addition, the stability of expression of tolerance to glyphosate herbicide in the GA21 was confirmed based on the bioassay.

In order to investigate the stability of expression in this stack line of the resistance to Lepidoptera and the tolerance to glufosinate herbicide derived from the Bt11 and of the tolerance to glyphosate herbicide derived from the GA21, European corn borer resistance test, glufosinate herbicide spraying test and glyphosate herbicide spraying test were carried out using this stack line, the parent line Bt11 or GA21, and the non-recombinant control maize.

As a result of the European corn borer resistance test, no significant difference was observed in insect damage to leaves and insecticidal activity between this stack line and the Bt11 (Table 3). In addition, as a result of the glufosinate herbicide and glyphosate herbicide spraying tests, no significant difference was observed between this stack line and the parent lines in the severity of herbicide injury due to the different dosages of herbicide (1x, 4x and 8x) (Table 4 and Table 5).

Based on the above results, it has been confirmed that this stack line is equivalent to the patent lines Bt11 and GA21 in the resistance to Lepidoptera, and tolerance to glufosinate herbicide and glyphosate herbicide, and also that the traits given are stably expressed similarly as in the Bt11 and the GA21.

Table 3 Levels of resistance to Lepidoptera in this stack line (Measured in the greenhouse of Syngenta Seeds, Inc. in 2004)

Evaluation item*[1]	Bt11×GA21		Bt11		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
First generation test: Severity of insect damage to leaves*[2]	1.0 b [*3]	0	1.0 b	0	7.6 a	0.6
Second generation test: Number of surviving larvae/plant	0.0 b	0	0.0 b	0	6.9 a	3.0
Second generation test: Length of trace of eaten cob (cm)	0.0 b	0	0.0 b	0	3.4 a	2.5
Second generation test: Length of eaten ear (cm)	0.0 b	0	0.0 b	0	16.8 a	5.6
Second generation test: Length of trace of eaten stem (cm)	0.0 b	0	0.0 b	0	26.9 a	7.6

*1： Evaluation was conducted in the growing period (First generation test) and the maturation period (Second generation test) of maize since European corn borer (*Ostrinia nubilalis* Hübner), the major target insect pest in maize cultivation in the US, could appear consecutively in two generations.

*2： Severity of insect damage to leaves was evaluated based on the following 9-step scales (Reference 24).

1 No feeding damage, or traces of minor insect damage (limited to 2 to 3 small spots)

2 Traces of feeding damage are all found 2 mm or less in size, and the number of damaged leaves is limited to one or two.

3 Small penetrated traces are observed on three or more leaves.

4-8 Depending on the degree of expansion of damaged area (4=extension of damaged trace of 1.3 cm or less, 8=about a half of all the leaves found damaged)

9 Leaves are found seriously damaged, and the damage virtually extends to leaf vein.

*3： Evaluation items were individually subjected to statistical treatment, and for each evaluation item, the same alphabetical letters indicate that there is no significant difference between the relevant mean values (P=0.05).

Table 4 Resistance to glufosinate herbicide in this stack line (Measured in the greenhouse of Syngenta Seeds, Inc. in 2006)

Concentration of herbicide sprayed*[1]	Levels of herbicide injury (%) *[2]					
	Bt11×GA21		Bt11		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
No spraying	0 h *[3]	0.0	0 h	0.0	0 h	0.0
1×	0.3 h	0.2	0.3 h	0.4	97.5 a	2.5
4×	4.3 g	0.5	4.2 g	0.4	100 a	0.0
8×	14.7 f	1.0	13.8 f	0.6	99.4 a	1.2

*1： Individual maize samples cultivated in a greenhouse (3rd to 4th leaf stage, 12 days after sowing) were sprayed with herbicide containing the glufosinate as an active ingredient at recommended dosage (1×) and 4-time higher (4×) and 8-time higher (8×) dosages than the recommended dosage and then observed for levels of herbicide injury 14 days after herbicide spraying (10 plants, 3 repeats).

*2： Levels of herbicide injury were evaluated by visual inspection based on the scale from 0% (intact) to 100% (complete death).

*3： The same alphabetical letters indicate that there is no significant difference between the relevant mean values (P=0.05).

Table 5 Resistance to glyphosate herbicide in this stack line (Measured in the greenhouse of Syngenta Seeds, Inc. in 2006)

Concentration of herbicide sprayed*[1]	Levels of herbicide injury (%) *[2]					
	Bt11×GA21		GA21		Non-recombinant control maize	
	Mean value	Standard deviation	Mean value	Standard deviation	Mean value	Standard deviation
No spraying	0 h *[3]	0.0	0 h	0.0	0 h	0.0
1×	0 h	0.0	0 h	0.0	100 a	0.0
4×	27.9 d	0.4	28.0 d	2.0	100 a	0.0
8×	36.9 bc	2.2	38.2 b	3.3	100 a	0.0

*1： Individual maize samples cultivated in a greenhouse (2nd to 3rd leaf stage, 9 days after sowing) were sprayed with herbicide containing the glyphosate as an active ingredient at a recommended dosage (1×) and 4-time higher (4×) and 8-time higher (8×) dosages than the recommended dosage and then, observed for levels of herbicide injury 17 days after herbicide spraying (10 plants, 3 repeats).

*2： Levels of herbicide injury were evaluated by visual inspection based on the scale from 0% (intact) to 100% (complete death).

*3： The same alphabetical letters indicate that there is no significant difference between the relevant mean values (P=0.05).

4) Presence or absence, and if present, degree of transmission of nucleic acid transferred through virus infection and/or other routes to wild animals and wild plants

The transferred nucleic acid in the Bt11 and the GA21 does not contain any sequence allowing transmission. Therefore, it is considered unlikely that the nucleic acid transferred to the both plants could be transmitted to any other wild animals and wild plants.

(5) Methods of detection and identification of living modified organisms and their sensitivity and reliability

For specific detection of the lines Bt11 and GA21, a method based on the qualitative PCR analysis is available from the European Commission. Based on this method, the detection sensitivity was found 0.1% for the both lines, the Bt11 in terms of the ratio of concentration of genome DNA and the GA21 in terms of the ratio of weight between GA21 and the control kernel (Reference 27, Reference 28).

In order to detect and identify this stack line, one seed or plant body needs to be examined by the two methods mentioned above, and this stack line can be confirmed when the results of the both analyses are found positive.

(6) Difference from the recipient organism or the species to which the recipient organism belongs

1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of replication products of transferred nucleic acid

This stack line is given the traits to be resistant to Lepidoptera and tolerant to glufosinate herbicide due to the modified Cry1Ab protein and the PAT protein respectively which are derived from the transferred genes in the Bt11, and also to be tolerant to glyphosate herbicide due to the mEPSPS protein derived from the transferred genes in the GA21.

2) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified agricultural products and the taxonomic species to which the recipient organism belongs, and the degree of difference, if present

This stack line is given the Lepidoptera resistance derived from the Bt11 and the glyphosate herbicide tolerance derived from the GA21, though these traits have been confirmed not to be significantly different from those in the parent lines Bt11 and GA21. In addition, the modified Cry1Ab protein and the PAT protein derived from the Bt11 and the mEPSPS protein derived from the GA21 are considered to function independently from the characteristics of the individual proteins and then, it is considered very unlikely that these proteins would interact with each other in this stack line and could affect the metabolic pathway of the recipient organism.

Based on the above understanding, regarding the physiological or ecological difference between this stack line and the taxonomic species of maize to which the

recipient organism belongs, evaluation was conducted on the parent lines Bt11 and GA21 based on the isolated field tests conducted in Japan (Annex 1, Annex 2).

(a) Morphological and growth characteristics

For the morphological and growth characteristics, comparison was made between the Bt11 and the non-recombinant control maize regarding the uniformity of germination, time of tasseling, time of silking, time of flower initiation, time of flower completion, flowering period, maturation time, plant shape, tiller number, total number of ears, number of productive ears, ear grain color, ear grain shape, culm length, height of ear, ear length, ear diameter, row number per ear, grain number per row, 100-kernel weight, and fresh weight after harvesting. As a result, in all characteristics evaluated, no significant difference nor difference was observed between the Bt11 and the non-recombinant control maize. In addition, comparison was conducted between the GA21 and the non-recombinant control maize regarding the uniformity of germination, germination rate, time of tasseling, time of silking, culm length, height of ear, plant shape, maturation time, fresh plant weight at harvesting time, tiller number, number of ears, ear length, ear diameter, row number per ear, grain number per row, 100-kernel weight, ear grain shape, and ear grain color. As a result, in all characteristics evaluated, no significant difference nor difference was observed between the GA21 and the non-recombinant control maize.

(b) Cold-tolerance and heat-tolerance at the early stage of growth

The Bt11 and the GA21 withered otherwise died similarly as the non-recombinant control maize due to the cold treatment at the early stage of growth.

(c) Wintering ability of the matured plant

Maize is a summer type annual plant, and after ripening the matured plant body usually withers and dies out. Maize does not contain any tissue or organ other than seeds, which can regenerate the plant body, and it is considered to fail to survive when exposed to sub-zero temperatures for 6 to 8 hours, though depending on maize growing stage and cultivation environment (Reference 3).

It was actually observed in the isolated field tests that the Bt11 and the GA21 died after maturation similarly as the non-recombinant control maize.

(d) Fertility and size of the pollen

As a result of the observation under a microscope with pollen stained with a neutral red solution, no difference was observed in the fertility (maturity of the pollen due to staining), shape and size of the pollen between the Bt11 and the non-recombinant control maize. In addition, as a result of the observation with pollen stained with an acetocarmine solution, no difference was observed in the fertility (maturity of the pollen due to staining), shape and size of the pollen between the GA21 and the non-recombinant control maize.

(e) Production, shedding habit, dormancy and germination rate of the seed

Regarding seed production, no significant difference was observed between the Bt11 and the non-recombinant control maize in the ear length, ear diameter, row number per ear, grain number per row, and 100-kernel weight. In addition, no significant difference was observed between the GA21 and the non-recombinant control maize in the production of the seed.

Regarding shedding habit of the seed, the seeds of maize never shed spontaneously, since they adhere to ears and the ears are covered with husk (Reference 3). Also in the Bt11 and the GA21, similarly as the non-recombinant control maize, the ears were found covered with husk at harvest time.

The germination rate was found equivalent for both the sowing seeds and harvested seeds from the Bt11, the GA21 and the non-recombinant control maize. Dormancy has not been examined, though the possibility is considered low that the dormancy of the Bt11 and the GA21 is significantly different from that of the non-recombinant control maize, since no difference was observed in the germination rate of sowing seeds sown under different temperature conditions and harvested seeds between the parent lines and the non-recombinant control maize.

(f) Crossability

Crossability test was not performed for the parent lines Bt11 and GA21 since there is no report that any wild relatives that can be crossed with maize are growing voluntarily in Japan.

(g) Productivity of harmful substances

A plow-in test, a succeeding crop test and a soil microflora test were carried out for the Bt11 and the GA21, and as a result, they indicated no significant differences between the Bt11 and the GA21, and the non-recombinant control maize in all items.

II. Review by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity

A review was made by persons with specialized knowledge and experience concerning Adverse Effect on Biological Diversity (called Experts) for possible Adverse Effect on Biological Diversity caused by the use in accordance with the Type 1 Use Regulation for Living Modified Organism based on the "Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms." Results of the review are listed below.

This stack line maize was produced by crossing maize resistant to Lepidoptera and tolerant to glufosinate herbicide (Bt11) and maize tolerant to glyphosate herbicide (GA21). The Committee on Assessment of Adverse Effect on Biological Diversity judged that each of these parent lines would not result in Adverse Effect on Biological Diversity when used in line with Type I Use described in the application for this stack line.

The modified Cry1Ab protein encoded by the modified *cry1Ab* gene (Lepidoptera resistant gene) derived from the Bt11 possesses the insecticidal activity against the insects of the order Lepidoptera but it is considered not to have any enzyme activity. In addition, the PAT protein (phosphinothricin acetyltransferase) encoded by the *pat* gene (glufosinate tolerant gene) derived from the Bt11 is the enzyme that possesses high substrate specificity. On the other hand, the mEPSPS protein (5-enol-pyruvyl-shikimate-3-phosphate synthase) encoded by the *mEPSPS* gene (glyphosate herbicide tolerant gene) derived from the GA21 is also the enzyme that possesses high substrate specificity. It is therefore considered unlikely that traits conferred by the modified *cry1Ab* gene, *pat* gene and *mEPSPS* gene would interact with each other.

It has been confirmed that this stack line maize expresses resistance to Lepidoptera and tolerance to glufosinate and glyphosate herbicides, by European corn borer resistance test and herbicide spraying tests, respectively.

Based on the above understanding, it is considered unlikely that notable changes in traits have occurred in this stack line maize, except for the traits it received from both the parent lines.

1. Item-by-item assessment of Adverse Effect on Biological Diversity

(1) Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis) has been long used in Japan, though there is no report that it has become self-seeding in a natural environment in Japan. This stack line maize is given traits to be resistant to Lepidoptera due to the modified Cry1Ab protein encoded by the modified *cry1Ab* gene from the Bt11 and also to be tolerant to herbicides glufosinate and glyphosate, due to the PAT protein encoded by the *pat* gene from the Bt11 and the mEPSPS protein encoded by the *mEPSPS* gene from the GA21, respectively. However, it is not generally considered that the insect damage by Lepidopteran insects is the major cause making the difficult to grow, and the herbicides glufosinate and glyphosate are sprayed and exert pressure for selection in the natural environment in Japan. Therefore, it is unlikely that these characteristics enhance the competitiveness of this recombinant maize and therefore this stack line maize would be more competitive than its parent lines.

Based on the above understanding, it was judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to competitiveness is valid.

(2) Productivity of harmful substances

This stack line maize has both the modified Cry1Ab protein and PAT protein productivity derived from the Bt11 and the mEPSPS protein productivity derived from the GA21. The modified Cry1Ab protein possesses the insecticidal activity against the insects of order Lepidoptera. However, the PAT protein and the mEPSPS protein confer tolerance to glufosinate herbicide and tolerance to glyphosate herbicide, respectively, though they are confirmed not to be harmful substances to animals and plants. In addition, it is considered unlikely that the modified Cry1Ab protein, the PAT protein and the mEPSPS protein would interact with each other. As a result, even though this stack line maize contains these proteins in conjunction, it is unlikely that the productivity of harmful substances will be greater in this stack line maize than its parent lines

Therefore, the conclusion that the use of this stack line maize poses no risk of Adverse Effect on Biological Diversity that is attributable to the production of harmful substances, which was made by the applicant, is valid

(3) Crossability

In the Japanese natural environment, there are no wild plants which can cross with maize. Therefore, it was judged that there are no specific wild plants that are possibly affected by this recombinant maize, and that the use of such maize poses no risk of Adverse Effect on Biological Diversity that is attributable to crossability. It was judged that the conclusion above made by applicant is valid.

2. Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this recombinant maize in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is reasonable.

Bibliography

Not made available or disclosed to unauthorized person