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 Coleoptera and Lepidoptera, and tolerant to glufosinate herbicide and glyphosate herbicide (*cry34Ab1*, *cry35Ab1*, *cry1F*, *pat*, *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (59122×1507×NK603, OECD UI：DAS-59122-7×DAS-Ø15Ø7-1×MON-ØØ6Ø3-6)
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

I. Information concerning preparation of living modified organisms

The stack line 59122×1507×NK603 was developed by Pioneer Hi-Bred International, Inc (USA). The parent lines Event DAS-59122-7 and Cry1F line 1507 were developed jointly by Dow AgroSciences LLC (USA) and Pioneer Hi-Bred International, Inc (USA), and NK603 was developed by Monsanto Company (USA). The stack line 59122×1507×NK603 is a cultivar created through the conventional crossbreeding method using inbred lines Event DAS-59122-7 and 1507×NK603, which is produced through the conventional crossbreeding method using Cry1F line 1507 and NK603.

The following genes are introduced into the stack line 59122 × 1507 × NK603: the *cry34/35Ab1* gene to confer the resistance to the insects of the order Coleoptera derived from the Event DAS-59122-7, the *cry1F* gene to confer the resistance to the insects of the order Lepidoptera derived from the Cry1F line 1507, the *pat* gene to confer the tolerance to glufosinate herbicide derived from the Event DAS-59122-7 and the Cry1F line 1507, and the *cp4 epsps* gene to confer the tolerance to glyphosate herbicide derived from the NK603.

For the parent lines, Event DAS-59122-7, Cry1F line 1507 and NK603, applications have been field already for the Type I Use of Living Modified Organism, in line with Item 2 of Article 4 of the “Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms” enacted in February 2004. The Committee on Biological Diversity Risk Assessment judged that Event DAS-59122-7, Cry1F line 1507 or NK603 would not result in Adverse Effect on Biological Diversity when used as Type I Use which was described in the application for this stack line 59122×1507×NK603. Consequently, for the Event DAS-59122-7, solicitation of public comments was closed on February 21, 2005, and the Cry1F line 1507 and the NK603 gain approval on March 2, 2005 and November 22, 2004, respectively.

To create this evaluation document, we referred to summary documents of Event DAS-59122-7, Cry1F line 1507 and NK603, which are found in the web site of Japan Biosafety Clearing House (J-BCH) provided by the Ministry of the Environment, and the summary documents of NK603 disclosed in the GM database of AGBIOS (Canada). (http://www.bch.biodic.go.jp/download/lmo/public_comment/DAS59122-7ap.pdf, http://www.bch.biodic.go.jp/download/lmo/public_comment/1507ap.pdf, http://www.bch.biodic.go.jp/download/lmo/public_comment/NK603ap.pdf, http://www.agbios.com/docroot/decdocs/02-269-007.pdf.)

1. Information concerning donor nucleic acid

(1) Composition and origins of component elements

Table 1, Table 2 and Table 3 show the composition and the origins of component elements of the donor nucleic acid used to produce Event DAS-59122-7, Cry1F line 1507 and NK603, respectively.

Table 1　Composition and origins of component elements of the donor nucleic acid used for developing Event DAS-59122-7

Component elements	Size (kbp)	Origin and function
cry34Ab1 gene expression cassette		
UBIZM1(2) PRO	1.98	Ubiquitin constitutive promoter[1)] derived from *Zea mays* [including intron and 5' untranslated region（Christensen, *et al*., 1992）]
cry34Ab1	0.37	A gene that encodes Cry34Ab1 protein derived from *Bacillus thuringiensis* PS149B1 strain
PIN Ⅱ TERM	0.32	Protease inhibitor II terminator to terminate transcription derived from *Solanum tuberosum*
cry35Ab1 gene expression cassette		
TA Peroxidase PRO	1.30	Peroxidase promoter (base sequence 45-1342 of GenBank X53675) derived from *Triticum aestivum* known to express in roots
cry35Ab1	1.15	A gene that encodes Cry35Ab1 protein derived from *Bacillus thuringiensis* PS149B1 strain
PIN Ⅱ TERM	0.32	Protease inhibitor II terminator to terminate transcription derived from *Solanum tuberosum*
pat gene expression cassette		
CAMV 35S PRO	0.53	35S constitutive promoter[1)] derived from cauliflower mosaic virus (CaMV)（Hohn, *et al*., 1995）
Pat	0.55	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *Streptomyces viridochromogenes* [Optimized to activate the expression in plant body [2)] (Eckes, *et al*., 1989)]
CAMV 35S TERM	0.21	35S terminator to terminate transcription derived from cauliflower mosaic virus (CaMV)（Hohn, *et al*., 1995）

1)　Constitutive promoter: A promoter that drives the expression of target genes in all sites in plant body

2）　The produced protein has none of its amino acids modified.

Table 2 Composition and origins of component elements of the donor nucleic acid used for developing Cry1F line 1507

Component elements	Size (kbp)	Origin and function
cry1F gene expression cassette		
UBIZM1(2) PRO	1.98	Ubiquitin constitutive promoter[1)] derived from *Zea mays* [including intron and 5' untranslated region（Christensen, *et al*., 1992）]
cry1F	1.82	A gene that encodes Cry1F protein derived from *Bacillus thuringiensis* var. *aizawai*. [Optimized to activate the expression in plant body[2)]（GenBank AAA22347）]
ORF25PolyA TERM	0.72	A terminator to terminate transcription from *Agrobacterium tumefaciens* pTi5955（Berkar, *et al*., 1983）
pat gene expression cassette		
CAMV35S PRO	0.53	35S constitutive promoter[1)] derived from cauliflower mosaic virus (CaMV)（Hohn, *et al*., 1995）
Pat	0.55	A gene that encodes phosphinothricin acetyltransferase (PAT protein), derived from *Streptomyces viridochromogenes*. [Optimized to activate the expression in plant body[3)]（Eckes, *et al*., 1989）]
CAMV35S TERM	0.21	35S terminator to terminate transcription from cauliflower mosaic virus (CaMV)（Hohn, *et al*., 1995）

1) Constitutive promoter: A promoter that drives the expression of target genes in all sites in plant body.

2) In the produced protein, the residue at the second position from the C-terminal of its amino acid sequence is changed from phenylalanine to leucine.

3) The produced protein has none of its amino acids modified.

Table 3 Composition and origins of component elements of the donor nucleic acid used for developing NK603

Component elements	Size (Kpb)	Origin and function
cp4 epsps gene expression cassette (1)		
P-*ract 1*	0.9	Promoter region of actin 1 gene derived from rice. It makes target genes expressed.
ract 1 intron	0.5	Intron of rice actin gene. It makes target genes expressed by enhancing splicing.
CTP 2	0.2	N-terminal chloroplast transit peptide sequence derived from the *Arabidopsis epsps* gene. Transfers target proteins from cytoplasm to chloroplast.
cp4 epsps	1.4	5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* CP4 strain.
NOS 3'	0.3	3' untranslated region of nopaline synthase (NOS) gene from T-DNA of *Agrobacterium tumefaciens.* It terminates transcription of mRNA and induces polyadenylation.
cp4 epsps gene expression cassette (2)		
E35S	0.6	Contains 35S promoter and duplicated enhancer from cauliflower mosaic virus (CaMV). Makes target genes expressed in all the tissues constantly.
ZmHsp70 intron	0.8	Intron of heat shock protein gene from maize. ZmHsp70 intron is used to enhance the expression of foreign genes in plants.
CTP 2	0.2	N-terminal chloroplast transit peptide sequence derived from the *Arabidopsis epsps* gene. Transfers target proteins from cytoplasm to chloroplast.
cp4 epsps	1.4	5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene from *Agrobacterium* CP4 strain.
NOS 3'	0.3	3' untranslated region of nopaline synthase (NOS) gene from T-DNA of *Agrobacterium tumefaciens*. It terminates transcription of mRNA and induces polyadenylation.

(2) Functions of component elements

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

Table 1, Table 2 and Table 3 show functions of individual component elements of donor nucleic acid, including target genes, expression regulation regions, localization signals, and selective markers, in Event DAS-59122-7, Cry1F line 1507 and NK603, respectively.

2) Functions of proteins produced by the expression of target gene and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergenicity

a. Cry34Ab1 protein and Cry35Ab1 protein

Cry34Ab1 protein and Cry35Ab1 protein are ones of *B.t.* protein derived from *Bacillus thuringiensis* PS149B1 strain, having an insecticidal activity against corn rootworm (*Diabrotica spp.*). Since they function in collaboration with each other, they are called binary proteins (Ellis *et al*., 2002).

It is suggested that the Cry34Ab1 protein and Cry35Ab1 protein work in concert to effect, and, like other *B.t.* proteins, destroy midgut cell membrane of target insects (Masson *et al.*, 2004). It is generally known that *B.t.* proteins have extremely high specific insecticidal activity (Shirai, 2003). In addition, Cry34Ab1 protein and Cry35Ab1 protein also show high specific insecticidal activity, and it is confirmed as a result of biological test that they exhibit the insecticidal activity against the larvae of only two kinds of the insects of the order Coleoptera, northern corn rootworm (*Diabrotica barberi*) and western corn rootworm (*Diabrotica virgifera virgifera*) (Poletika, 2003).

It has not been confirmed that the Cry34Ab1 protein and Cry35Ab1 protein share amino acid sequence homology with any of the known allergenic proteins (Song, 2003).

b. Cry1F protein

Cry1F protein is one of insecticidal crystal protein (*B.t.* protein) known as δ-endotoxin produced by *Bacillus thuringiensis* (hereinafter referred to as "*B.t.*"), a gram-positive bacterium, universally exists in soil. The Cry1F protein is derived from *Bacillus thuringiensis* var. *aizawai* and shows an insecticidal

activity against European corn borer (*Ostrinia nubilalis*).

The Cry1F protein binds to the specific receptors in the midgut cells of the target pest insects when ingested similarly as other *B.t.* proteins and forms pores in the cells, which leads to the destruction of ion channels and results in the broken midgut cells and successful insecticide activity (Schnept, *et al*., 1998). In general, *B.t.* proteins are known to have an extremely specific insecticidal activity. Likewise, the Cry1F protein shows toxicity only against Lepidoptera such as European corn borer, Fall armyworm and Beet armyworm, not against other non-target species (EPA, 2001a).

It has not been confirmed that the Cry1F protein shares amino acid sequence homology with any of the known allergenic proteins (Meyer, 1999).

c. PAT protein

PAT protein (phosphinothricin acetyltransferase) confers the tolerance to glufosinate herbicide. The glufosinate herbicide contains *L*-glufosinate, the active ingredient, which inhibits the activity of glutamine synthase that synthesizes glutamine from glutamic acid and ammonia. As a result, ammonia is accumulated in the plant body, causing the plant to die. The PAT protein acetylates and detoxifies *L*-glufosinate, thereby conferring the glufosinate tolerance to the plant body. It is reported that the PAT protein shows extremely high substrate specificity against *L*-glufosinate, and that it does not accept other *L*-amino acids or select *D*-glufosinate for substrates (OECD, 1999).

It has not been confirmed that the PAT proteins share amino acid sequence homology with any of the known allergenic proteins (Meyer, 1999).

d. CP4 EPSPS protein

CP4 EPSPS protein confers the tolerance to glyphosate herbicide. The glyphosate herbicide inhibits the activity of 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) in the shikimate pathway for aromatic amino acid biosynthesis. As a result, plants treated with glyphosate cannot synthesize amino acids essential for growth and ultimately die. The CP4 EPSPS protein is not inhibited even in the presence of glyphosate and it properly works as an enzyme in the shikimate pathway, thereby conferring the glyphosate tolerance on plants. In addition, it is suggested that the EPSPS protein is the enzyme which specifically reacts with the phosphoenolpyruvate (PEP) and shimimate-3-phosphate (S3P), and that it is not the rate-determining enzyme in

the aromatic amino acid biosynthesis pathway.

It is not reported that the CP4 EPSPS protein share amino acid sequence homology with any of the known allergenic proteins.

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

It is reported that Cry34Ab1 protein, Cry35Ab1 protein and Cry1F protein never act as enzyme in any plant body similarly as other Cry proteins. It is reported that the PAT protein exhibits high substrate specificity (OECD, 1999). On the other hand, as mentioned above, it is suggested that the EPSPS protein works as the enzyme that specifically reacts with the phosphoenolpyruvate (PEP) and shimimate-3-phosphate (S3P), and that it is not the rate-determining enzyme in the aromatic amino acid biosynthesis pathway. As a result, it is considered that EPSPS protein does not affect the metabolism of plants unintentionally.

Based on the above understanding that Cry34Ab1 protein, Cry35Ab1 protein and Cry1F protein are considered not to possess any enzyme activity, and that PAT protein and CP4 EPSPS protein possess high substrate specificities and differ from each other in the action mechanism, it is considered that the introduced genes in this stack line 59122×1507×NK603 do not affect nor interact unintentionally with the metabolic system of the recipient organism.

2. Information concerning vector

(1) Name and origin

The vector used to produce Event DAS-59122-7 is plasmid PHP17662 derived from the plasmid pSB1 of *Escherichia coli* (Figure 1, p.13). The vector used to produce Cry1F line 1507 is plasmid PHP8999 derived from the plasmid pUC19 of *Escherichia coli* (Figure 2). In addition, the vector used to produce NK603 is plasmid PV-ZMGT32 derived from the plasmid pUC119 of *Escherichia coli* (Figure 3).

(2) Properties

1) The numbers of base pairs and nucleotide sequence of vector

The number of base pairs of the vectors used for the production of Event DAS-59122-7, Cry1F line 1507 and NK603 are 50,321bp, 9,504bp and 9,308bp, respectively. The nucleotide sequences of the component elements in each vector have become clear.

2) Types of any nucleotide sequence having specific functions

The vector backboneof plasmid PHP17662, it contains antibiotic resistant marker genes (*tet* gene and *spc* gene) to select the microorganisms that contain transformed plasmid while the vector is growing in the microorganisms. On the other hand, in the region other than the donor nucleic acid of plasmids PHP8999 and PV-ZMGT32, it contains antibiotic resistant marker gene (*nptII* gene) to select the microorganisms that contain transformed plasmid. The *tet* gene confers the resistance to antibiotics, tetracycline; the *spc* gene confers the resistance to antibiotics, spectinomycin; and the *nptII* gene confers the resistance to antibiotics, kanamycin. These antibiotic resistant genes are not introduced to the recipient organism.

3) Presence or absence of infectivity of vector

None of these vectors is known to be infectious.

3. Method of preparing living modified organisms

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

As for plasmid PHP17662 used to create Event DAS-59122-7, its T-DNA region consists of the following components; [*UBIZM1(2) Promoter*]-[*cry34Ab1*]-[*PINII Terminator*]-[*TA Peroxidase Promoter*]-[*cry35Ab1*]-[*PINII Terminator*]-[*CAMV35S Promoter*]-[*pat*]- [*CAMV35S Terminator*].

On the other hand, plasmid PHP8999 was treated with the restriction enzyme *Pme*I to produce a linear DNA fragment (PHI8999A) containing the donor nucleic acid only, which was used to create Cry1F line 1507. The antibiotic-resistant marker (*nptII* gene) is present outside the donor nucleic acid region, and therefore is not introduced into the recipient organism. The linear DNA fragment (PHI8999A) consists of the following components; [*UBIZM1(2) Promoter*]-[*cry1F*]-[*ORF25PolyA Terminator*]-[*CAMV35S*

Promoter]-[*pat*]-[*CAMV35S Terminator*].

In addition, the linear DNA fragment (PV-ZMGT32L) used to create NK603 consists of two (2) *cp4 epsps* gene cassettes; [*P-ract1*]-[*ract1 intron*]-[*CTP2*]-[*cp4 epsps*]-[*NOS 3'*] and [*E35S*]-[*Zmhsp70*]-[*CTP2*]-[*cp4 epsps*]-[*NOS 3'*].

(2) Method of transferring nucleic acid transferred in the recipient organism

Introduction of nucleic acid into recipient organism was based on the *Agrobacterium* method for Event DAS-59122-7, and the particle gun bombardment method for Cry1F line 1507 and NK603. For Event DAS-59122-7 and Cry1F line 1507, the nucleic acid was introduced into the Hi-II callus of the maize variety A188×B73, and for NK603, the nucleic acid was introduced into the maize variety AW×CW.

(3) Processes of rearing of living modified organisms

The parent lines, Event DAS-59122-7, Cry1F line 1507 and NK603, were bred based on the conventional F1 crossbreeding method. In addition, this stack line 59122×1507×NK603 was bred, as shown in the process of rearing in Figure 4, by Pioneer Hi-Bred International, Inc (USA) based on the conventional crossbreeding method.

In Japan, for the Event DAS-59122-7, an applications was filed for the Type I Use of Living Modified Organism, in line with Item 2 of Article 4 of the "Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms" carried into effect in February 2004, and solicitation of public comments was closed on February 21, 2005. In addition, applications were filed for approval for its safety as food with the Ministry of Health, Labour and Welfare in May 2004, and applications for approval for its safety as feed were submitted to the Ministry of Agriculture, Forestry and Fisheries in June 2004.

In addition, it was confirmed in June 2002 that the intended use of Cry1F line 1507 in any open system met the "Guidelines for the Use of Recombinant in Agriculture, Forestry, and Fisheries" (hereinafter referred to as "Guidelines"). Then, also for the Cry1F line 1507, similar as the Event DAS-59122-7, an application was filed for the Type 1 Use of Living Modified Organism, in line with Item 2 of Article 4 of the "Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms" enacted in February 2004, and then gained approval on March 2, 2005. In addition, its safeties as food and as feed were confirmed in July 2002 and in May 2002, respectively (the safety as feed was re-approved in March 2003 in accordance with the legislation of the examination system).

On the other hand, in accordance with the Guidelines, the intended use of the NK603 in any open system in Japan was approved in May 2001 as conforming to the Guidelines. Then, also for the NK603, similarly as the Event DAS-59122-7 and Cry1F line 1507, an application was filed for the Type I Use of Living Modified Organism, in line with Item 2 of Article 4 of the “Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms”, and then gained approval on November 22, 2004. In addition, its safeties as food and as feed were confirmed in March 2001 (the safety as feed was re-approved in March 2003 in accordance with the legislation of the examination system).

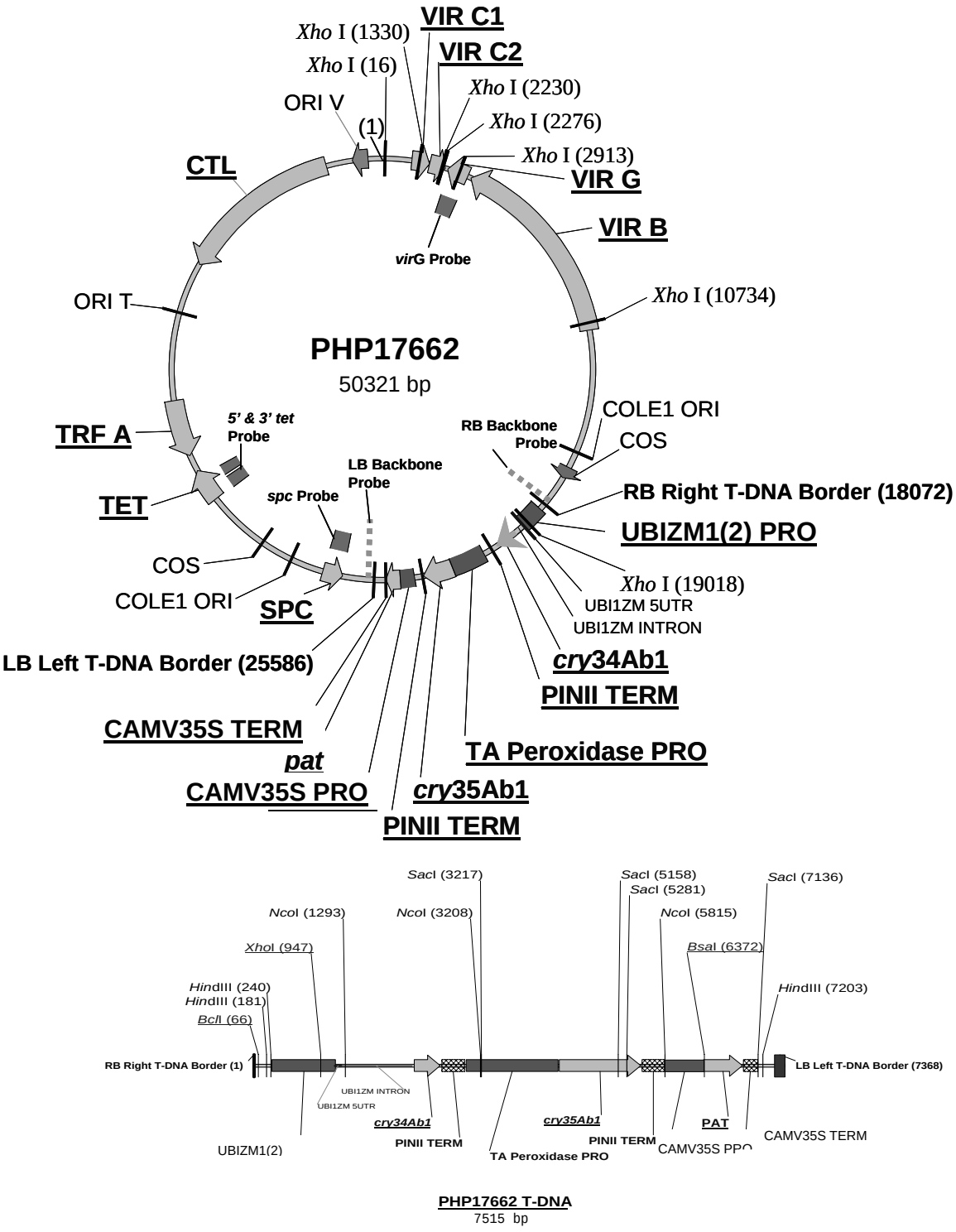

Figure 1 Compositions of plasmid PHP17662* and T-DNA region

*Vector used for the production of Event DAS-59122-7

(All the rights pertinent to the information in the diagram above and the responsibility for the contents lie with Du Pont Kabushiki Kaisha.)

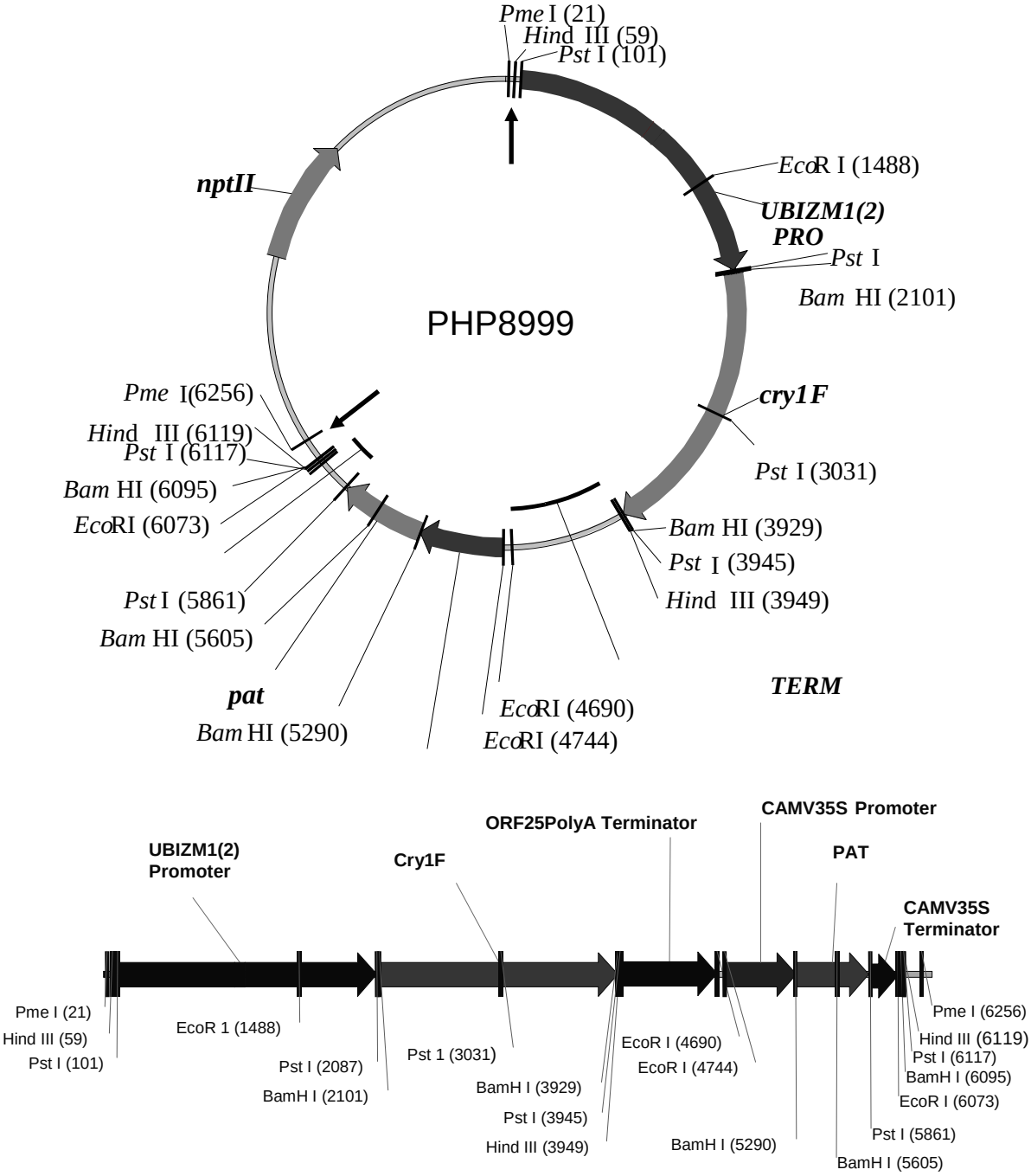

Figure 2 Compositions of plasmid PHP8999* (upper diagram) and inserted DNA region PHI8999A (lower diagram)

* Vector used for the production of Cry1F line 1507

Plasmid PHP8999 was treated with the restriction enzyme *Pme* I (cleaved at the two points indicated by arrows in the upper diagram) and the resultantly obtained linear DNA fragment PHI8999A (lower diagram) was used for introduction of genes into the recipient organism.

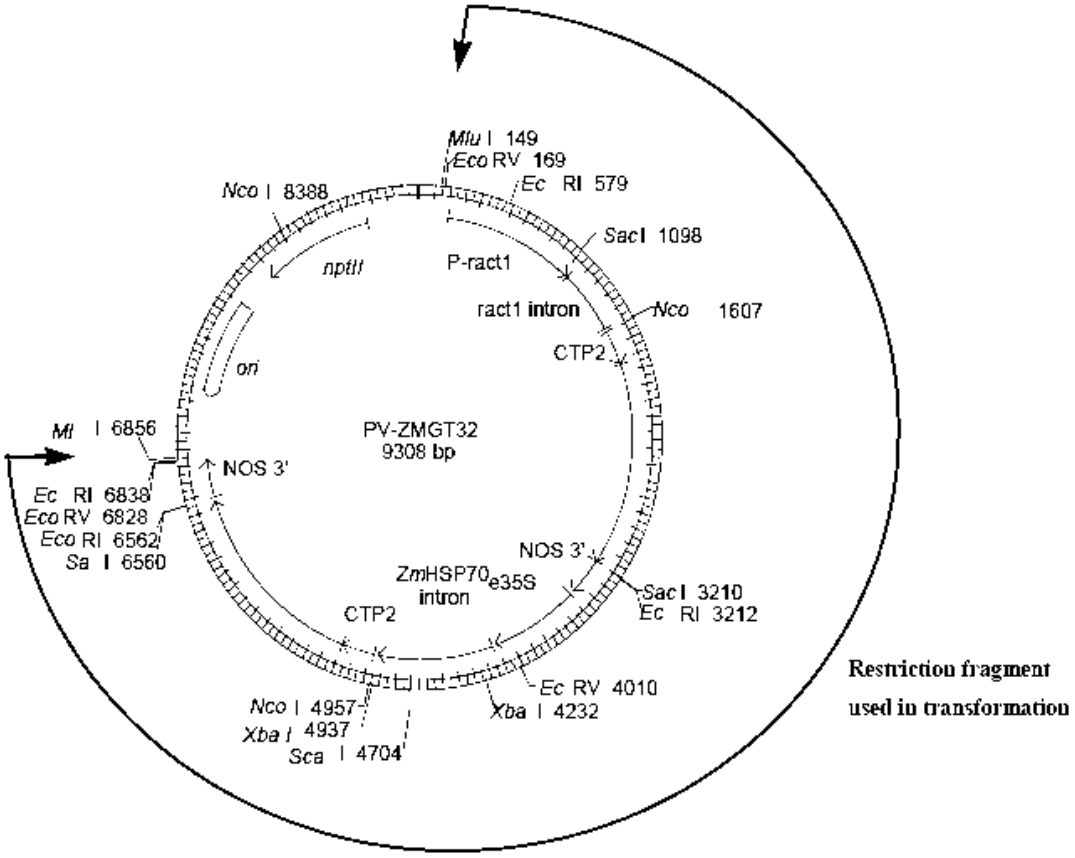

Figure 3 Compositions of plasmid PV-ZMGT32*

* Vector used for the production of NK603

Plasmid PV-ZMGT32 was treated with the restriction enzyme *Mlu*I (cleaved at the two points indicated by arrows in the upper diagram) and the resultantly obtained linear DNA fragment PV-ZMGT32L was used for introduction of genes into the recipient organism.

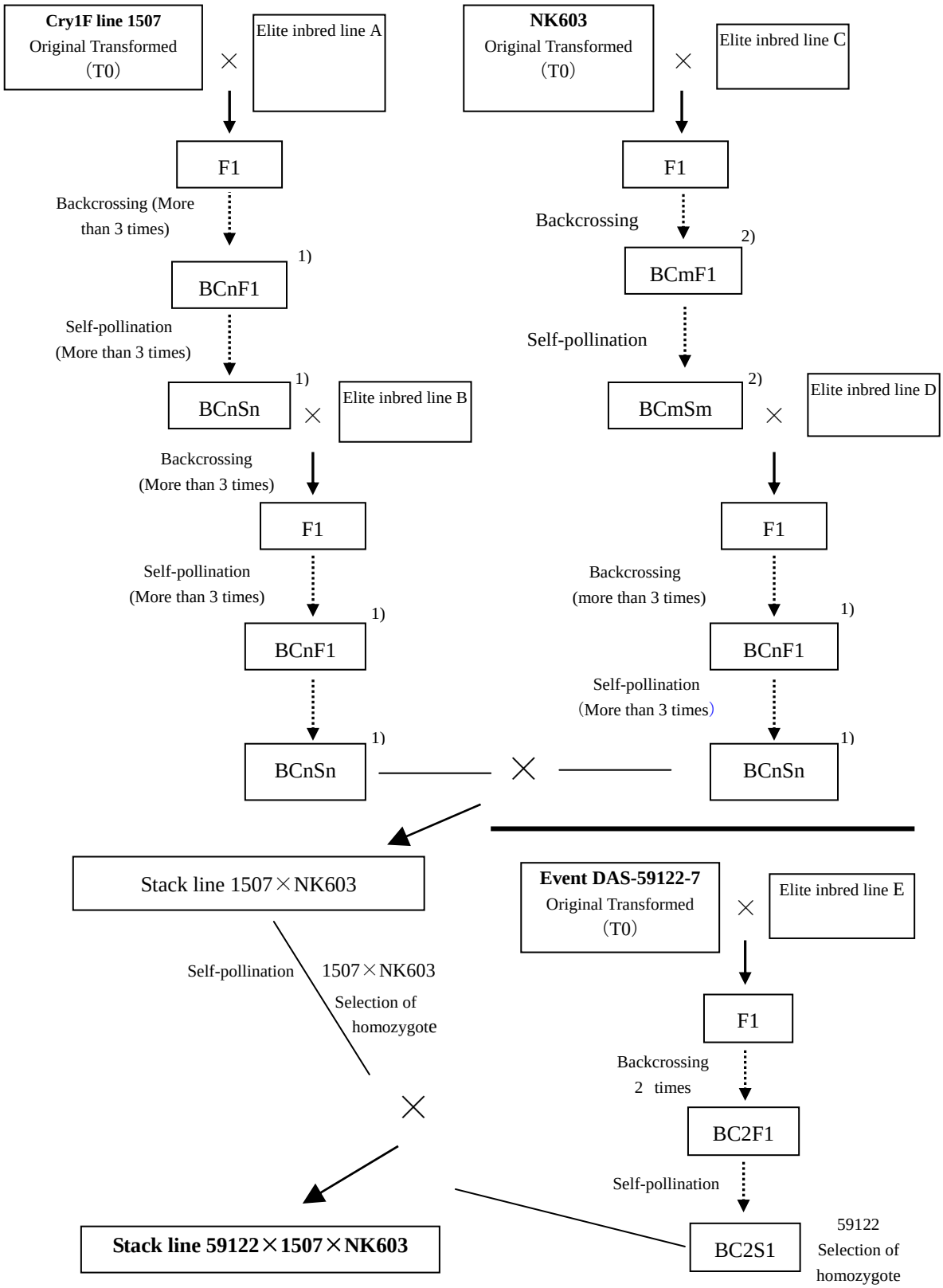

Figure 4　Outline of the process of rearing the stack line 59122×1507×NK603

1) The parameter n means n≧3, indicating that backcrossing (BC) or self-pollination (S) has been carried out more than 3 times.
2) BCmSm, provided from Monsanto Company (USA) to Pioneer Hi-Bred International, Inc (USA), is a variety crossed based on the breeding program of Monsanto Company (USA). The suffix "m" in BCmF1 and BCmSm refers to the number of times of backcrossing or self-pollination.

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

(1) Location of the copy of transferred nucleic acid

It was confirmed based on the result of Southern blotting analysis that a copy of transferred nucleic acid was introduced into the maize genome in each of Event DAS-59122-7, Cry1F line 1507 and NK603.

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

For Event DAS-59122-7, as a result of Southern blotting analysis, it was confirmed that one copy of each of *cry34Ab1* gene expression cassette and *cry35Ab1* gene expression cassette to confer the resistance to insects of the order Coleoptera including corn root worm, and *pat* gene expression cassette to confer the tolerance to glufosinate herbicide is inserted in the maize genome in the intact form. It was also confirmed that the introduced genes are all inherited stably in offspring.

For Cry1F line 1507, as a result of Southern blotting analysis, it was confirmed that one copy of each of *cry1F* gene expression cassette to confer the resistance to Lepidoptera such as corn borer and *pat* gene expression cassette to confer the tolerance to glufosinate herbicide is inserted in the maize genome in the intact form. It was also confirmed that the introduced genes are all inherited stably in offspring. In addition, as a result of sequencing of the introduced DNA, it was confirmed that the introduced DNA contained a part of the *cry1F* gene sequence in the 5'-terminal region, a part of the *pat* gene sequence in the 5'-terminal and 3'-terminal regions, and a part of *ORF25PolyA Terminator* sequence in the 3'-terminal region. However, Northern blotting analysis confirmed that these gene fragments were not transcribed into mRNA, thereby not functioning.

For NK603, as a result of Southern blotting analysis, it was confirmed that one copy of linear DNA fragment (PV-ZMGT32L, composed of two *cp4 epsps* gene expression cassettes) to confer the tolerance to glyphosate herbicide is inserted in the maize genome, and that the introduced genes are all inherited stably in offspring. It was confirmed that 217bp fragment of *P-ract1* exists in the reverse direction near the 3'-terminal of the introduced gene, though this fragment never causes any additional production of protein. In addition, it was also confirmed that the bases of *cp4 epsps* gene induced by *E35S* were changed during production of this recombinant maize and consequently one of the amino acids constituting the CP4 EPSPS protein was changed, though the function of the CP4 EPSPS protein remains unchanged.

(3) The stability of the expression among individuals and generations under natural conditions with respect to the physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acid

It was confirmed based on the results of herbicide-spraying tests and the biological test using the target insects that the obtained traits in the stack line 59122×1507×NK603 derived from Event DAS-59122-7, Cry1F line 1507 and NK603 are stably expressed. The results of the tests are described below.

Herbicide-spraying test

To identify whether the stack line 59122×1507×NK603 is comparable to the parent lines Event DAS-59122-7, Cry1F line 1507 and NK603 in possession of the tolerance to the both herbicides glufosinate and glyphosate, the spraying tests of the herbicides were conducted in March 2005 in a greenhouse at Pioneer Hi-Bred International, Inc (USA) (Appendix 1).

Fifteen seeds from each of the stack line 59122×1507×NK603; the parent lines Event DAS-59122-7, Cry1F line 1507 and NK603; and the non-recombinant maize were sowed on a tray (tray size: length×width×depth = approximately 35 cm×50 cm×10 cm). These plants were thinned out on the eighth day after the sowing, whereby ten plants were left in each tray. Three test fields were provided for spraying of glufosinate herbicide alone, glyphosate herbicide alone, and glyphosate herbicide followed by glufosinate herbicide. As listed in Table 4 (p.20), the glufosinate herbicide-spraying field was planted with the stack line 59122×1507× NK603, Event DAS-59122-7, Cry1F line 1507 and the non-recombinant maize; the glyphosate herbicide-spraying field was planted with the stack line 59122×1507×NK603, NK603 and the non-recombinant maize; and the glyphosate herbicide followed by glufosinate herbicide-spraying field was planted with the stack line 59122×1507×NK603 and the non-recombinant maize. The herbicides were sprayed in higher dosage than approved in the registration of agricultural chemicals (standard dosage), and also 16-times and 32-times higher ones than standard dosage. On the 12th day after seeding, the typical timing of herbicide spraying by farmers, glyphosate herbicide was sprayed, and on the 15th day after seeding, glufosinate herbicide was sprayed. On the 23rd day after seeding, visual inspection was carried out on the individual trays to identify the level of pesticide-induced damage (growth inhibition, color fading, spots, etc.), and evaluation was made with the score from 0% to 100% (0%＝no pesticide-induced damage, 100%＝plant death). The test was repeated three times.

As a result of the tests, no statistically significant difference was observed in the level of pesticide-induced damage due to the treatment with spraying of glufosinate herbicide and glyphosate herbicide between the stack line 59122×1507×NK603 and the parent lines, Event DAS-59122-7, Cry1F line 1507 and NK603 (Table 4). Based on the above understanding, it was confirmed that the stack line 59122 × 1507 × NK603 is comparable to the parent lines Event DAS-59122-7, Cry1F line 1507 and NK603 in possession of the tolerance to the both herbicides glufosinate and glyphosate.

In actuality, standard dosage assures sufficient herbicidal effects and thus the actual farming fields never be exposed to such 16-times or 32-times higher dosages of glyphosate herbicide or glufosinate herbicide than standard dosage as applied in the tests which were sprayed specifically for the purpose of the tests. In addition, glyphosate herbicide and glufosinate herbicide were sprayed in succession to certain group in the tests, though such use of herbicides is not approved in the registration of agricultural chemicals that was applied specifically for the purpose of the tests.

Table 4　Levels of pesticide-induced damage by spraying of the herbicides

Levels of pesticide-induced damage due to spraying of glufosinate herbicide

Tested plant	Levels of pesticide-induced damage (%)			
	No spraying	Normal dosage (0.5kg ai/ha)	16 times (8.0kg ai/ha)	32 times (16.0kg ai/ha)
Stack line 59122×1507×NK603	0.0±0.0 b	13.3±8.8 ab	13.3±3.3 ab	20.0±0.0 a
Event DAS-59122-7	0.0±0.0 b	6.7±3.3 ab	6.7±3.3 ab	10.0±0.0 ab
Cry1F line 1507	0.0±0.0 b	0.0±0.0 b	6.7±3.3 ab	13.3±3.3 ab
Non-recombinant maize	0.0±0.0 b	40.0±0.0 c		

Levels of pesticide-induced damage due to spraying of glyphosate herbicide

Tested plant	Levels of pesticide-induced damage (%)			
	No spraying	Normal dosage (1.1kg ai/ha)	16 times (17.6kg ai/ha)	32 times (35.2kg ai/ha)
Stack line 59122×1507×NK603	0.0±0.0 b	10.0±10.0 b	3.3±3.3 b	6.7±3.3 b
NK603	0.0±0.0 b	0.0±0.0 b	3.3±3.3 b	3.3±3.3 b
Non-recombinant maize	0.0±0.0 b	63.3±3.3 a		

Levels of pesticide-induced damage due to spraying of glufosinate herbicide+glyphosate herbicide

Tested plant	Levels of pesticide-induced damage (%)			
	No spraying	Normal dosage (0.5kg ai/ha, 1.1kg ai/ha)	16 times (8.0kg ai/ha, 17.6kg ai/ha)	32 times (16.0kg ai/ha, 35.2kg ai/ha)
Stack line 59122×1507×NK603	0.0±0.0 c	3.3±3.3 bc	16.7±3.3 ab	23.3±3.3 a
Non-recombinant maize	0.0±0.0 c	73.3±3.3 d		

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For each maize type, the levels of pesticide-induced damage were compared with data from a non-spraying control plot. Symbols written with damage levels indicate that damage levels showed no statistically significant differences within the same group (a/b/c/d) (Tukey's multiple test, $P<0.05$). Standard deviation is given to the right of ±. Herbicide dosage are expressed in the amount of active ingredient per hectare (kg ai/ha).

Biological test using Western corn rootworm

To identify whether the stack line 59122×1507×NK603 is comparable to the parent line Event DAS-59122-7 in possession of the resistance to Coleoptera, biological test was conducted in March 2005 in a greenhouse at Pioneer Hi-Bred International, Inc (USA) using the target insect of Western corn rootworm (Appendix 2).

The stack line 59122×1507×NK603, the parent lines Event DAS-59122-7 and Cry1F line 1507, and the non-recombinant maize were cultivated (one maize individual per pot, pot size: 24 cm in diameter, 22 cm in height, and 6.4 L in volume), and at the V4 stage

(the 4th foliage leaf stage), 100 eggs of Western corn rootworm were inoculated at the roots of each plant every other day a total of four times (400 eggs in total). Then, at the point of time when the hatched larvae grew to the pupal stage (approximately two weeks after inoculation), observation was made to identify the degree of root damage by the Western corn rootworm. The test was repeated five times in total with four maize individuals subjected to each test.

As a result, no statistically significant difference was observed in the level of damage by Western corn rootworm between the stack line 59122×1507×NK603 and Event DAS-59122-7, and then, it was also confirmed that the obtained trait of resistance to Coleoptera is stably expressed in the stack line 59122×1507×NK603 in the comparable level as in the parent line Event DAS-59122-7 (Table 5) (Appendix 2).

Table 5　Resistance to Western corn rootworm

Tested plant	Score of feeding damage at roots *
Stack line 59122×1507×NK603	0.12±0.03
Event DAS-59122-7	0.09±0.01
Cry1F line 1507	2.65±0.08
Non-recombinant maize	2.64±0.08

* Node-Injury Scale developed by Iowa State University in the USA
（0-3: 0 = no feeding damage, 3 = three or more root nodes eaten)
（http://www.ent.iastate.edu/pest/rootworm/nodeinjury/nodeinjury.html)
Standard deviation is given to the right of ±.

(All the rights pertinent to the information in the table above and the responsibility for the contents lie with Du Pont Kabushiki Kaisha.)

Biological test using European corn borer

To identify whether the stack line 59122×1507×NK603 is comparable to the parent line Cry1F line 1507 in possession of the resistance to Lepidoptera, the biological test using the target insect of European corn borer, was conducted in a growth chamber at Pioneer Hi-Bred International, Inc (USA) in March 2005 (Appendix 2).

The stack line 59122×1507×NK603, parent lines Event DAS-59122-7 and Cry1F line 1507, and the non-recombinant maize were cultivated in a greenhouse.　Leaves at the V6 stage (6th foliage leaf stage) were collected, punched into a circle about 1 cm in diameter.　Subsequently, five newly hatched European corn borer larvae were released on each sample. They were incubated at 27°C in a growth chamber for three days. Then the fertility rate of larvae and the levels of feeding damage were observed. The test was

repeated five times in total with four maize individuals to each test.

As a result, the fertility rate of European corn borer larvae and the levels of feeding damage showed no statistically significant differences between the stack line 59122×1507×NK603 and Cry1F line 1507. This result confirmed that obtained trait of resistance to Lepidoptera was expressed in the stack line 59122×1507×NK603 as stably as one of its parent lines, Cry1F line 1507 (Table 6) (Appendix 2).

Table 6　Resistance to European corn borer larvae

Tested plant	Mortarity rates of larvae (%)	Levels of feeding damage to foliage leaves (%)
Stack line 59122×1507×NK603	96±2	1±0
Event DAS-59122-7	9±3	96±2
Cry1F line 1507	94±3	1±0
Non-recombinant maize	13±3	92±4

Standard deviation is given to the right of ±.

(All the rights pertinent to the information in the table above and the responsibility for the contents lie with Du Pont Kabushiki Kaisha.)

Conclusion

Based on the above understanding, it was confirmed that the obtained traits derived from Event DAS-59122-7, Cry1F line 1507 and NK603 are stably expressed in the stack line 59122×1507×NK603.

(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

Transmission of this item is absence, because the transferred nucleic acid does not contain any sequence allowing transmission.

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

ELISA method using the polyclonal antibodies respectively for Cry1F protein, PAT protein and CP4 EPSPS protein have been developed and the analysis kits for detection of the individual proteins are commercially available. The analysis kits for detection of Cry34Ab1 protein and Cry35Ab1 protein are now under development and expected commercially available in August 2005. This stack line 59122×1507×NK603 can be detected by applying

these three methods to each grain of maize seeds.

The Cry1F protein detection kit can detect one Cry1F protein-containing grain per 600 maize grains. The PAT protein detection kit can detect one PAT protein-containing grain per 500 maize grains. The CP4 EPSPS protein detection kit can detect one CP4 EPSPS protein-containing grain per 800 maize grains. These detection kits have been all certified as reliable enough by a variety of tests.

6. Difference from the recipient organism or the taxonomic species to which the recipient organism belongs

(1) Specific contents of physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acids

1) Resistance to Coleoptera

The stack line 59122 × 1507 × NK603 produces the Cry34Ab1 protein and Cry35Ab1 protein due to the introduction of *cry34Ab1* gene and *cry35Ab1* gene derived from *B.t.* PS149B1 strain into one of its parent lines, Event DAS-59122-7. As a result, the stack line is conferred resistance to corn root worm, which causes feeding damage to maize.

2) Resistance to Lepidoptera

The stack line 59122 × 1507 × NK603 produces the Cry1F protein due to the introduction of *cry1F* gene derived from *B.t.* var. *aizawai* into one of its parent lines, Cry1F line 1507. As a result, the stack line is conferred resistance to European corn borer, which causes feeding damage to maize.

3) Tolerance to glufosinate herbicide

The stack line 59122 × 1507 × NK603 is given the tolerance to glufosinate herbicide by the function of *pat* gene derived from *Streptomyces viridochromogenes* introduced into Event DAS-59122-7 and Cry1F line 1507. The PAT protein produced by the expression of *pat* gene acetylates the glufosinate herbicide and transforms it to nontoxic acetylglufosinate, thereby conferring on the plant body the tolerance to glufosinate.

4) Tolerance to glyphosate herbicide

The stack line 59122×1507×NK603 is given the tolerance to glyphosate herbicide by the function of *cp4 epsps* gene derived from *Agrobacterium* CP4 strain introduced into NK603. The CP4 EPSPS protein produced by the expression of *cp4 epsps* gene is not inhibited even in the presence of glyphosate and it properly works as an enzyme in the shikimate pathway, thereby conferring the glyphosate tolerance on plant body.

(2) Presence or absence of difference between recombinant plant and the species to which recipient organism belongs, and the degree of difference, if any

The stack line 59122×1507×NK603 is a cultivar created through the conventional crossbreeding method using inbred lines Event DAS-59122-7 and 1507×NK603, which is produced through the conventional crossbreeding method using Cry1F line 1507 and NK603. Thus, it obtained traits derived from Event DAS-59122-7, Cry1F line 1507 and NK603. Based on the findings that the Cry34Ab1 protein, Cry35Ab1 protein and Cry1F protein expressed in this stack line are considered not to possess any enzyme activity similarly as the other Cry proteins, and that both PAT protein and CP4 EPSPS protein possess high substrate specificities and differ from each other in the action mechanism, it is considered that these proteins congerring to the traits in this stack line will not interact with each other. In fact, as mentioned in 4-I. "(3) The stability of the expression among individuals and generations under natural conditions with respect to the physiological or ecological characteristics that were accompanied by the expression of copies of transferred nucleic acid", it was confirmed as a result of herbicide-spraying test and biological test that the traits inherited from the both parent lines were stably expressed in the stack line 59122×1507×NK603.

Therefore, the difference between this stack line and the taxonomic species of maize to which the recipient organism belongs was described with the use of the results of individual examinations for the various characteristics conducted for Event DAS-59122-7, Cry1F line 1507 and NK603. The individual examinations were conducted in isolated fields in Japan in 2003 for Event DAS-59122-7, in 2001 for Cry1F line 1507, and in 2000 for NK603.

1) Morphological and growth characteristics

For the morphological and growth characteristics of Event DAS-59122-7, evaluation was conducted regarding germination rate, uniformity of germination, time of tasseling, time of silking, time of flower initiation, time of flower completion, flowering period, maturation time, plant type, tiller number, number of

productive ears, grain color and grain shape, culm length, height of ear, ear length, ear diameter, fresh weight of above-ground part, and shape of flower organ. As a result, in all evaluation items except culm length, no difference was observed between the Event DAS-59122-7 and the non-recombinant maize. For the culm length, the significant difference was observed only in one of the two recombinant varieties tested (Event DAS-59122-7: 192.0 cm, non-recombinant: 212.3 cm), though no difference was observed in the other variety.

For the morphological and growth characteristics of Cry1F line 1507, evaluation was also conducted regarding germination rate, the uniformity of germination, time of tasseling, time of silking, time of flower initiation, time of flower completion, flowering period, maturation time, plant type, the number of tillers, the total number of ears, the number of effective ears, grain color and grain shape, culm length, ear height, ear length, ear diameter and the fresh weight of the above-ground part. As a result, no statistically significant difference was observed between the Cry1F line 1507 and the non-recombinant maize in any of the examination items except germination rate and ear diameter. One of the two recombinant plants tested showed statistically significant differences in germination rate and ear diameter (germination rate in Cry1F line 1507: 96.7%; germination rate in non-recombinant: 92.8%) (ear diameter in Cry1F line 1507: 4.60 cm; ear diameter in non-recombinant: 4.32 cm), while the other did not.

For the morphological and growth characteristics of NK603, evaluation was also conducted regarding uniformity of germination, germination rate, time of tasseling, time of silking, culm length, plant shape or plant type, tiller number, height of ear, maturation time, number of ears, and weight of plant at harvesting time. As a result, in all evaluation items, no statistically significant difference was observed between the NK603 and the non-recombinant control maize.

Consequently, it is considered that there is no difference in morphological and growth characteristics between this stack line 59122×1507×NK603 and the taxonomic species of maize to which the recipient organism belongs.

As a result of germination test conducted in the USA on this stack in practice, the germination rate of this stack line was 91%, which was almost equivalent to 96% in the case of Event DAS-59122-7, 98% in Cry1F line 1507, and 98% in NK603. In addition, the germination rates measured in the test are all found higher than 90% which is the standard germination rate determined by OECD (NEBRASKA OECD GUIDELINES, 1995).

2) Cold-tolerance at the early stage of growth

Cold tolerance of the seedlings of Event DAS-59122-7 was evaluated. As a result, all the plant bodies tested withered and died when the minimum temperature dropped down to 1.5℃, and no difference was observed in the sensitivity to low temperatures between Event DAS-59122-7 and the non-recombinant maize.

Cold tolerance of the seedlings of Cry1F line 1507 was also evaluated. All the samples faded and wilted after about three weeks after placing them in a growth chamber [set to every 12 hour cycle of 12-14°C (when the light is on) and 2°C (when the light is off)]. The degree of wilting showed no differences between Cry1F line 1507 and the non-recombinant maize.

Cold tolerance of the seedlings of NK603 was also evaluated. As a result, almost all the plant bodies tested withered and died on the 14th day after exposure to the low temperature (ambient temperature of 4℃), and no difference was observed between NK603 and the non-recombinant control maize.

Consequently, regarding cold tolerance of this stack line 59122×1507×NK603, it is considered that there is no difference between this stack maize and the taxonomic species of maize to which the recipient organism belongs.

3) Wintering ability and summer survival of the matured plant

It is well known that maize is a summer type annual plant, and after ripening it usually dies out in winter and it can overwinter. In addition, after harvesting, it does not regrow, propagate vegetatively, or produce seeds. Based on the above, overwintering tests for the matured plant of Event DAS-59122-7, Cry1F line 1507 and NK603 were not carried out. However, in fact, it was confirmed that, in the USA field where cultivation tests of Event DAS-59122-7 and Cry1F line 1507 were conducted in the previous year, there was no plant body observed in the following year which remained alive through the winter. It was also observed at the end of the isolated field test for NK603 that plant bodies started withering after ripening.

Consequently, regarding wintering ability of this stack line 59122×1507×NK603, it is considered that there is no difference between this stack line maize and the taxonomic species of maize to which the recipient organism belongs.

4) Fertility and size of the pollen

To examine the fertility (maturity) and its size of Event DAS-59122-7, pollens were stained with potassium iodine solution and observed under a microscope. As a result, regarding the fertility and its size of the pollens, no difference was observed in the evaluation items between the Event DAS-59122-7 and the non-recombinant control maize.

Also for Cry1F line 1507 and NK603, in the fertility and its size of pollens, no difference was observed among Cry1F line 1507, NK603 and the non-recombinant control maize as a result of examination of the stained pollens under a microscope.

Consequently, regarding fertility and size of the pollens, it is considered that there is no difference between this stack line 59122×1507×NK603 and the taxonomic species of maize to which the recipient organism belongs.

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

The row number per ear, grain number per row and 100-kernel weight and other characteristics were examined for Event DAS-59122-7 during the isolated field test as the characteristics referring to the production of seeds. As a result, no difference was observed between Event DAS-59122-7 and the non-recombinant maize in all of the characteristics examined. In addition, for both Event DAS-59122-7 and the non-recombinant maize, no dormancy of the seeds was observed. Also in the germination rate and shedding habit of second generation hybrid (F2) seeds, no difference was observed between Event DAS-59122-7 and the non-recombinant maize.

The row number per ear, grain number per row and 100-kernel weight were examined for Cry1F line 1507. As a result, no statistically significant difference was observed in any of the examination items between Cry1F line 1507 and the non-recombinant maize. Also, both seeds (F2) collected from Cry1F line 1507 and the non-recombinant maize showed a high germination rate and no differences between them. Maize is a cultivation variety, and its dormancy is not known. Also, the germination rate of Cry1F line 1507 was as high as that of the non-recombinant maize. Therefore, it was judged that Cry1F line 1507 possesses no dormancy.

Also for NK603, the row number per ear, grain number per row and 100-kernel weight and other characteristics were examined. As a result, no difference was observed between the NK603 and the non-recombinant maize in any of the characteristics examined except in 100-kernel weight. Regarding 100-kernel weight,

significant difference was observed between one sample of the two recombinant maize NK603 samples examined, and the difference of the average weight of those was very small. In addition, regarding the germination rate of collected seeds (F2), no difference was observed between the NK603 and the non-recombinant maize and then no dormancy of the seeds was observed.

The ears of all Event DAS-59122-7, Cry1F line 1507 and NK603 were covered with bracts at the time of harvesting and thus, shedding habit was not observed in the natural condition similarly as their non-recombinant control maize samples.

Based on the above understanding, regarding the production of the seed and other characteristics of this stack line 59122×1507×NK603, it is considered that there is no difference between this stack maize and the taxonomic species of maize to which the recipient organism belongs.

6) Crossability

In Japan, the wild relatives (teosinte) that can be crossed with the recipient organism, maize in natural environment has not been reported.

7) Productivity of harmful substances

It is not known that maize secretes any harmful substances that could have adverse effects on the surrounding plants and/or microorganisms in soil. Also it is not known that maize produces any allelochemicals after dying that could affect other plants.

In the Event DAS-59122-7, Cry34Ab1 protein, Cry35Ab1 protein and PAT protein are newly produced due to the introduction of *cry34Ab1* gene, *cry35Ab1* gene and *pat* gene. In the Cry1F line 1507, Cry1F protein and PAT protein are newly produced due to the introduction of *cry1F* gene and *pat* gene. In the NK603, CP4 EPSPS protein is newly produced due to the introduction of *cp4 epsps* gene. However, it is suggested that Cry34Ab1 protein, Cry35Ab1 protein and Cry1F protein do not work as enzyme in plant body similarly as other Cry proteins in *B.t.*. In addition, it is reported that PAT protein possesses extremely high substrate specificity (OECD, 1999). It is also known that CP4 EPSPS protein possesses high substrate specificity similarly as PAT protein. Consequently, it is considered that these proteins could not affect the metabolic pathway of the maize of recipient organism and produce any unexpected harmful substances.

For the Event DAS-59122-7, analysis on the weeds growing in the areas for isolated field test for composition of species (DCA score), total number of individuals and dry weight, and soil microflora tests and plow-in test in the specific screen-house test were performed. In addition, soil microflora tests were additionally conducted in the test field (soil condition of volcanic ashes) in the State of Hawaii in the USA. Based on the above results, it was confirmed that there was no difference between the Event DAS-59122-7 and the non-recombinant control maize in the productivity of any unexpected harmful substances.

For the Cry1F line 1507, succeeding crop test, soil microflora test and plow-in test were performed in the isolated field test. In the USA, moreover, the productivity of harmful substances was evaluated by the sandwich method using the roots, leaves and stems of Cry1F line 1507 and the non-recombinant maize. Also, their effects on succeeding crops were visually observed in 46 field tests. As a result, no difference was observed between Cry1F line 1507 and the non-recombinant control maize in the productivity of any unexpected harmful substances.

For the NK603, succeeding crop test, soil microflora test and plow-in test were performed in the isolated field test. As a result, it was confirmed that there was no difference between the recombinant maize examined and the non-recombinant control maize in all evaluation items.

Consequently, regarding the productivity of any unexpected harmful substances, it is considered that there is no difference between the stack line 59122×1507×NK603 and the taxonomic species of maize to which the recipient organism belongs.

2. 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 applied 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 conventional crossbreeding method of maize resistant to Coleoptera and tolerant to glufosinate herbicide (DAS-59122-7), maize resistant to Lepidoptera and torelant to glufosinate herbicide (DAS-01507-1), and maize tolerant to glyphosate herbicide (MON-00603-6). These parent lines were individually judged at the Committee for Review on the Biological Diversity Risk Assessment as causing no Adverse Effect on Biological Diversity when applied for the Type I Use same as the stack line maize.

It is reported that the Cry34Ab1 protein and Cry35Ab1 protein (hereafter referred to as “Cry34Ab1/Cry35Ab1 protein” since the two proteins work jointly as a binary protein to exhibit insecticidal activity) which are encoded by the (*cry34Ab1* and *cry35Ab1*) genes resistant to Coleoptera derived from DAS-59122-7, and the Cry1F protein which is encoded by the (*cry1F*) gene resistant to Lepidoptera derived from DAS-01507-1 have different insecticidal spectrum and do not possess enzyme activity. It is also reported that the PAT protein which is encoded by the (*pat*) gene tolerant to glufosinate derived from DAS-59122-7 and DAS-01507-1, and the CP4 EPSPS protein which is encoded by the (*cp4 epsps*) gene tolerant to glyphosate derived from MON-00603-6 possess high substrate specificities. Therefore, it is considered that the characteristics conferred by the genes; *pat*, *cry34Ab1*, *cry35Ab1, cry1F* and *cp4 epsps* do not interact with each other.

It was confirmed based on the various herbicide-spraying tests that the tolerance to glufosinate herbicide and glyphosate herbicide are properly expressed in this stack line maize. In addition, it was confirmed based on the biological tests using Western corn rootworm (*Diabrotica virgifera virgifera*) and European corn borer (*Ostrinia nubilalis*) respectively that the resistance to Coleoptera and the resistance to Lepidoptera are properly expressed in this stack line maize.

Based on the above understanding, it is considered that there is no specific change in the characteristics in this stack line maize except it possesses the same characteristics as the parent lines do.

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

(1) Competitiveness

This stack line maize possesses the Coleoptera resistance and glufosinate herbicide tolerance derived from DAS-59122-7, the Lepidoptera resistance and glufosinate herbicide toralance derived from DAS-01507-1, and the glyphosate herbicide tolerance derived from MON-00603-6. However, it is considered that the insect damages by Coleoptera and/or Lepidoptera are not the major cause to make the maize difficult to grow in the natural environment in Japan, and also that the glufosinate and glyphosate tolerances do not exert pressure for selection under a natural environment. Consequently, it is considered that these characteristics do not increase the competitiveness and thus this stack line maize is not predominant over the parent lines in the competitiveness. Based on the above understanding, it is 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 possesses the productivity of Cry34Ab1/Cry35Ab1 proteins derived from DAS-59122-7, the productivity of Cry1F protein derived from DAS-01507-1, the productivity of PAT protein derived from DAS-59122-7 and DAS-01507-1, and the productivity of CP4 EPSPS protein derived from MON-00603-6. It is confirmed that the Cry1F protein possesses the incecticidal activity against insects of the order Lepidoptera, and the Cry34Ab1/Cry35Ab1 proteins possess the insecticidal activity against insects of the order Coleoptera, though each of the insecticidal spectrum of these proteins has extremely high specificity, and there is unlikely possibility to interact between them. In addition, it is confirmed that PAT protein and CP4 EPSPS protein are not harmful substances to animals and plants. Thus, it is considered that the productivity of harmful substances of this stack line maize would not become higher than that of parent lines even if this stack line maize contains these proteins. Based on the above understanding, it is judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to the productivity of harmful substances is valid.

(3) Crossability

In Japan, the growth of wild relatives (teosinte) that can be crossed with maize in natural environment has not been reported.

Based on the above understanding, no wild species can be specified as having some effects, it is judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to crossability 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 stack line 59122×1507×NK603 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 valid.

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