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 torelant to glufosinate herbicide (*cry1Ac*, *bar*, *Zea mays* subsp. *mays* (L.) Iltis) (DBT418, OECD UI: DKB-89614-9)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, 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

(1) Information concerning donor nucleic acid

i) Composition and origins of component elements

The composition of the donor nucleic acid and the origins of component elements that were used for the development of the maize resistant to Lepidoptera and tolerant to glufosinate herbicide (*cry1Ac*, *bar, Zea mays* subsp. *mays* (L.) Iltis) (DBT418, OECD UI: DKB-89614-9) (hereinafter referred to as "this recombinant maize") are shown in Figures 1 to 3 and Tables 1 to 3.

The nucleotide sequences of component elements of the donor nucleic acid are shown in Annex 1.

ii) Functions of component elements

Functions of component elements of donor nucleic acid that were used for the development of this recombinant maize are shown in Tables 1 to 3.

[*cry1Ac* gene]

1) The *cry1Ac* gene, the target gene to confer resistance to Lepidoptera, is derived from *Bacillus thuringiensis* subsp. *kurstaki* HD-73 strain. The Cry1Ac protein which is encoded by the *cry1Ac* gene possesses an insecticidal activity against European corn borer (*Ostrinia nubilalis*), which is one of the major insect pests of the order Lepidoptera to maize cultivation in the US. *B.t.* proteins which are produced by the bacterium *B.t.* including Cry1Ac protein, bind to the specific receptors on the midgut epithelium of the target insects and form cation selective pores, which leads to the inhibition of the digestive process and results in the insecticidal activity (Reference 12; Reference 13). *B.t.* proteins do not possess enzyme activity and function independently from the metabolic system of the recipient organism. Therefore, it is considered that the Cry1Ac protein does not affect physiological/ecological characteristics of the recipient organism.

2) In order to investigate whether the Cry1Ac protein shares functionally important amino acid sequences with known allergens, the Cry1Ac protein was compared with the allergens in the database (GenPept, PIR, SwissProt). As a result, the Cry1Ac protein did not share structurally related sequences with known allergens.

[*bar* gene]

1) A crop produces ammonia during nitrogen metabolism through nitrate

reduction, amino-acid degradation, photorespiration, etc. For detoxification of ammonia produced, a glutamine synthase plays a primary role. However, the glufosinate herbicide sprayed inhibits the activity of glutamine synthase, and as a result, ammonia is accumulated in the plant body, causing the plant to die.

Phosphinothricin acetyltransferase (PAT protein), which is encoded by the *bar* gene introduced, acetylates glufosinate to be N-acetylglufosinate, and consequently inactivates inhibitory activity of glufosinate to glutamine synthase. As a result, ammonia is not accumulated in the plant body, conferring the plant tolerance to glufosinate herbicide applications.

The PAT protein shows high affinity with glufosinate. The glufosinate, which is classified into L-amino acids, does not promote acetyl transfer to various types of amino acids, has little affinity with glutamic acid which is similar in structure specifically, and does not cause transfer reaction in the plant body (Reference 14). In addition, the acetyl transfer reaction of the PAT protein to the glufosinate was not inhibited, in the excessive presence of various types of amino acids (Reference 15). Based on the above understanding, it is considered that the PAT protein possesses high substrate specificity toward glufosinate.

In order to investigate whether the PAT protein shares functionally important amino acid sequence with known allergens, the PAT protein was compared with allergens in the database (GenPept, PIR, SwissProt). As a result, the PAT protein did not share structurally related homologous sequences with any of the known allergens examined.

(2) Information concerning vector

i) Name and origin

The origin of all plasmid vectors used for the development of this recombinant maize is plasmid pUC 19 derived from *Escherichia coli* (Reference 16), etc.

ii) Properties

The total number of base pairs of plasmid vectors is 4,609 bp for pDPG165, 6,006 bp for pDPG320, and 6,309 bp for pDPG699. All of them possess *bla* gene (*amp* gene) as a selectable marker for construction vector in *E. coli*. The infectivity of these plasmid vectors is not known. The nucleotide sequences of component elements are shown in Annex 1.

(3) Method of preparing living modified organisms

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

The component elements of this plasmid vector transferred in the recipient organism are shown in Tables 1 to 3. In addition, the location and section broken by restriction enzyme of the component elements of the nucleic acid in the vector are shown in Figures 1 to 3.

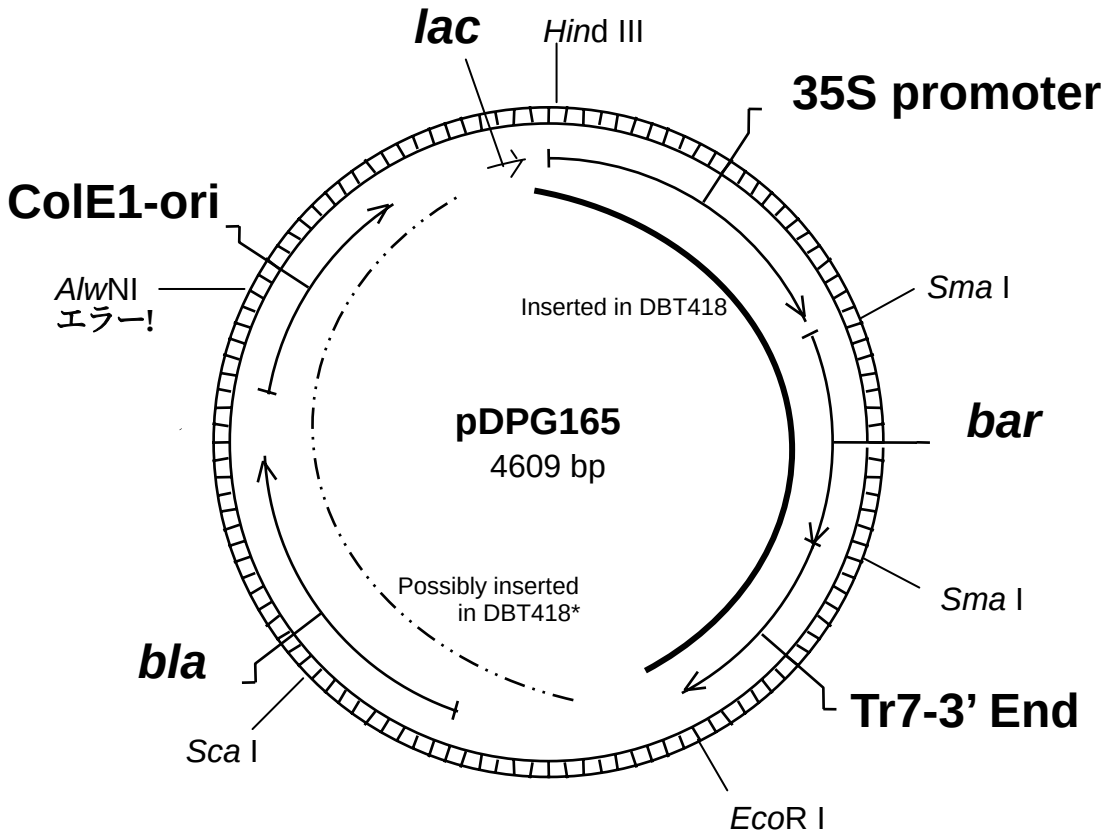

Figure 1 Plasmid vector pDPG165 [1]

* *bla* and ColE1 are common component elements of three plasmids pDPG165, pDPG320 and pDPG699 used for the development of this recombinant maize. Therefore, the original plasmid of these component elements transferred into this recombinant maize cannot be specified.

[1] All the rights pertinent to the information in the above diagram and the responsibility for the content rest upon Monsanto Japan Limited.

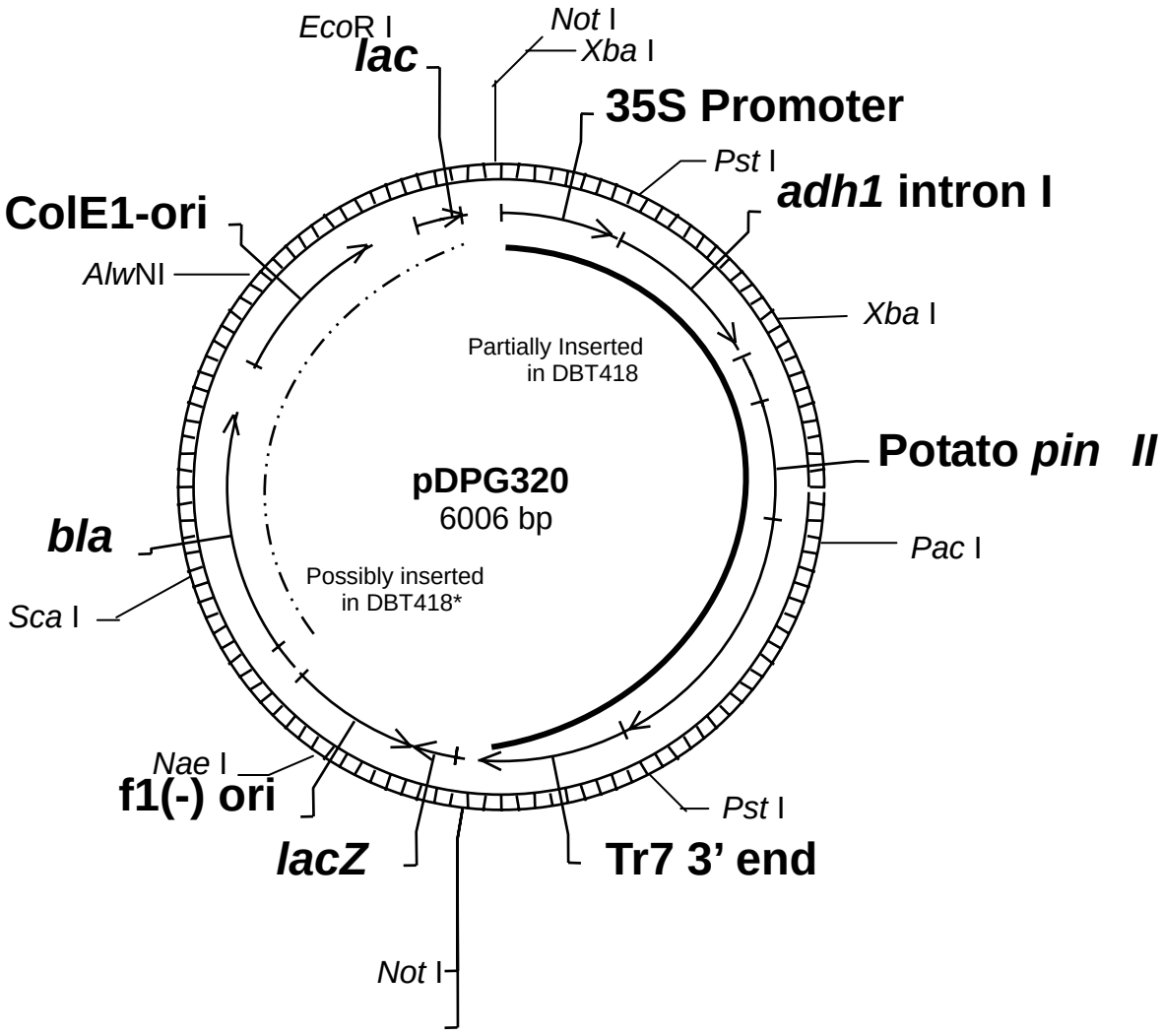

Figure 2 Plasmid vector pDPG320[2]

* *bla* and ColE1 are common component elements of three plasmids pDPG165, pDPG320 and pDPG699 used for the development of this recombinant maize. Therefore, the original plasmid of these component elements transferred into this recombinant maize cannot be specified.

[2] All the rights pertinent to the information in the above diagram and the responsibility for the content rest upon Monsanto Japan Limited.

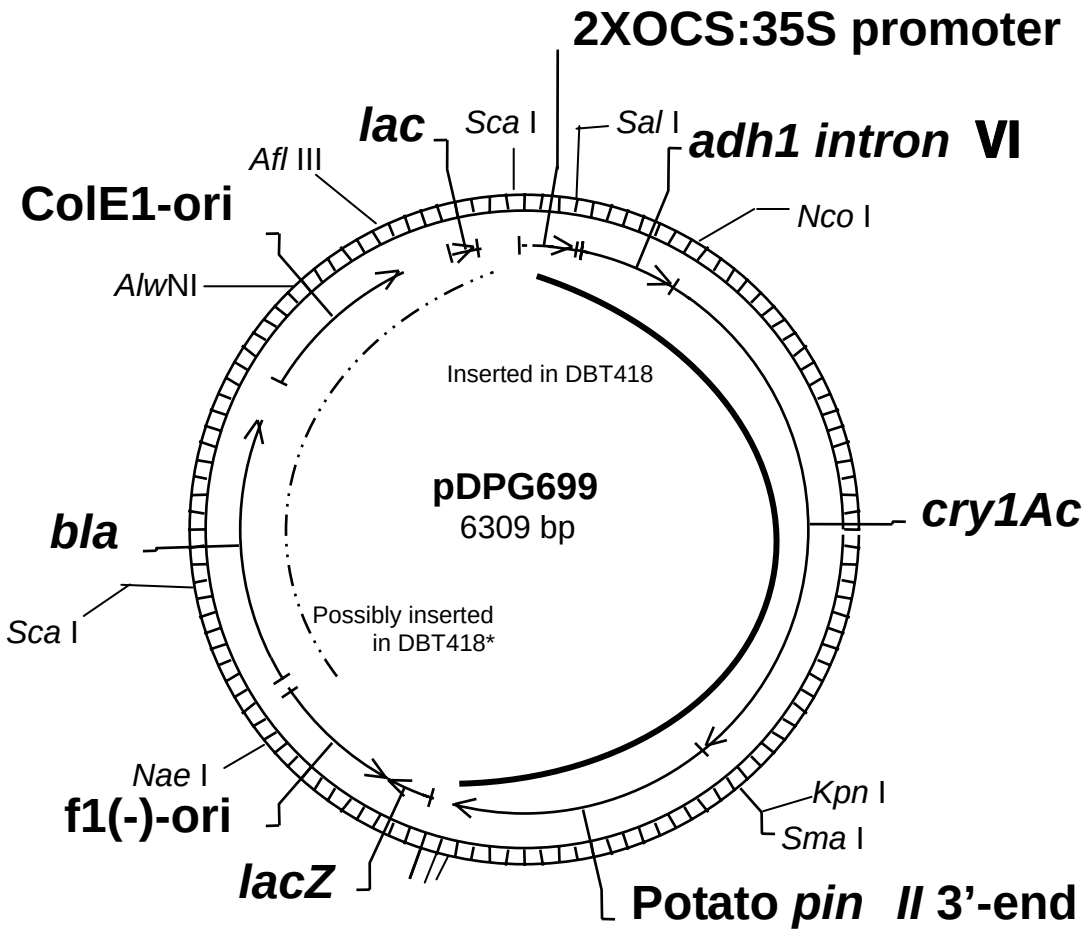

Figure 3 Plasmid vector pDPG699[3]

* *bla* and ColE1 are common component elements of three plasmids pDPG165, pDPG320 and pDPG699 used for the development of this recombinant maize. Therefore, the original plasmid of these component elements transferred into this recombinant maize cannot be specified.

[3] All the rights pertinent to the information in the above diagram and the responsibility for the content rest upon Monsanto Japan Limited.

Table 1 Component elements of pDPG165 to be used for introduction and their origin and function[4]

Component elements	Origin and Function
bar gene expression cassette	
35S promoter	A promoter from cauliflower mosaic virus (CaMV) (Reference 17). Makes target genes expressed in all the tissues constantly.
bar	The gene derived from *Streptomyces hygroscopicus*, encodes phosphinothricin acetyltransferase (PAT) (Reference 14). The glufosinate tolerance is conferred to the plant body due to the expression of this gene.
Tr7 3' end	3' untranslated region derived from T-DNA transcript product 7 of *Agrobacterium tumefaciens* (Reference 18). It terminates transcription and induces polyadenylation.
Component elements that may be transferred*	
bla	A gene derived from *E. coli* plasmid pBR322, encoding β-lactamase (Reference 19; Reference 16). It confers the resistance to ampicillin and other penicillin to bacteria.
ColE1-ori	The replication origin derived from *E. coli* plasmid pBluescript SK[-]. Permits autonomous replication of vectors in *E. coli* (Stratagene).
Other component elements	
lac	Consists of partial coding sequence to encode *lac* repressor, *lac* promoter and partial coding sequence to encode β-galactosidase (Reference 16). Used as a selective marker in cloning experiments in *E. coli*. The *lac* gene is not expressed in this recombinant maize since it is regulated by a bacterial promoter.

* *bla* and ColE1 are common component elements of three plasmids pDPG165, pDPG320 and pDPG699 used for the development of this recombinant maize. Therefore, the original plasmid of these component elements transferred into this recombinant maize cannot be specified.

[4] All the rights pertinent to the information in the above table and the responsibility for the content rest upon Monsanto Japan Limited.

Table 2 Component elements of pDPG320 to be used for introduction and their origin and function[5]

Component elements	Origin and Function
Pin II gene expression cassette	
35S promoter	A promoter from cauliflower mosaic virus (CaMV) (Reference 17). Makes target genes expressed in all the tissues constantly.
adh1 intron I	The first intron of *adh1* gene of maize. It enhances the expression level of target genes (Reference 20).
Potato *pin II*	Consists of untranslated region derived from potato, coding region and transcription termination region of protease inhibitor II protein with intron (Reference 21). It enhances the insectidal activity of Cry1Ac protein against Lepidopteran insects (Reference 22). This gene does not express in this recombinant plant.
Tr7 3' end	3' untranslated region from T-DNA transcript product 7 of *Agrobacterium tumefaciens* (Reference 18). It terminates transcription and induces polyadenylation.
Component elements that may be transferred*	
bla	A gene derived from *E. coli* plasmid pBR322, encoding β-lactamase (Reference 19; Reference 16). It confers the resistance to ampicillin and other penicillin to bacteria.
ColE1-ori	The replication origin derived from *E. coli* plasmid pBluescript SK[-]. Permits autonomous replication of vectors in *E. coli* (Stratagene).
Other component elements	
lacZ	Partial coding sequence to encode β-galactosidase protein (LacZ protein). Used as a selective marker in cloning experiments in *E. coli*.
f1(-) ori	The replication origin of pBluescript SK[-], the phagemid derived from f1 bacteriophage. Permits autonomous replication of vectors in *E. coli*.
lac	Consists of partial coding sequence to encode *lac* repressor, *lac* promoter and partial coding sequence to encode β-galactosidase (Reference 16). Used as a selective marker in cloning experiments in *E. coli*. The *lac* gene is not expressed in this recombinant maize since it is regulated by a bacterial promoter.

* *bla* and ColE1 are common component elements of three plasmids pDPG165, pDPG320 and pDPG699 used for the development of this recombinant maize. Therefore, the original plasmid of these component elements transferred into this recombinant maize cannot be specified.

[5] All the rights pertinent to the information in the above table and the responsibility for the content rest upon Monsanto Japan Limited.

Table 3 Component elements of pDPG699 to be used for introduction and their origin and function[6]

Component elements	Origin and Function
cry1Ac gene expression cassette	
2xOCS:35S promoter	It is a chimera promoter partially linking two OCS elements derived from *Agrobacterium tumefaciens* and a 35S promoter derived from cauliflower mosaic virus (CaMV) (Reference 23; Reference 17). Makes target genes expressed in all the tissues constantly.
adh1 intron VI	The 6th intron of *adh1* gene of maize. It enhances the expression level of target genes (Reference 20).
cry1Ac	A gene that encodes Cry1Ac protein of *Bacillus thuringiensis* subsp. *krustaki* HD-73 strain in the soil. The detail of its function was described in p. 2.
Potato *pin II* 3' end	3'-terminal region of protease inhibitor II gene derived from potato (Reference 21). It terminates transcription.
Component elements that may be transferred*	
bla	A gene derived from *E. coli* plasmid pBR322, encoding β-lactamase (Reference 19; Reference 16). It confers the resistance to ampicillin and other penicillin to bacteria.
ColE1-ori	The replication origin derived from *E. coli*. Plasmid pBluescript SK[-]. Permits autonomous replication of vectors in *E. coli* (Stratagene).
Other component elements	
lacZ	Partial coding sequence to encode β-galactosidase protein (LacZ protein). Used as a selective marker in cloning experiments in *E. coli*.
f1(-) ori	The replication origin of pBluescript SK[-], the phagemid derived from f1 bacteriophage. Permits autonomous replication of vectors in *E. coli*.
lac	Consists of partial coding sequence to encode *lac* repressor, *lac* promoter and partial coding sequence to encode β-galactosidase (Reference 16). Used as a selective marker in cloning experiments in *E. coli*. The *lac* gene is not expressed in this recombinant maize since it is regulated by a bacterial promoter.

* *bla* and ColE1 are common component elements of three plasmids pDPG165, pDPG320 and pDPG699 used for the development of this recombinant maize. Therefore, the original plasmid of these component elements transferred into this recombinant maize cannot be specified.

[6] All the rights pertinent to the information in the above table and the responsibility for the content rest upon Monsanto Japan Limited.

ii) Method of transferring nucleic acid transferred in the recipient organism

The mixture of plasmid vectors pDPG165, pDPG320 and pDPG699 was introduced by particle gun bombardment (into the regeneration line derived from embryo culture callus that is classified into dent type) [Confidential: Not made available or disclosed to unauthorized persons].

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iii) Processes of rearing of living modified organisms

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

The callus to which the three plasmids on the above were introduced [Confidential: Not made available or disclosed to unauthorized persons] was grown on a tissue culture media containing glufosinate, and then the recombinant plant was selected. From the selected callus, the regenerated plant was obtained and the expression of the Cry1Ac protein was analyzed.

(b) Presence or absence of remaining *Agrobacterium* in case of using *Agrobacterium* method for transferring nucleic acid

Plasmids were introduced in this recombinant maize by particle gun bombardment, so confirmation of remaining *Agrobacterium* was not carried out.

(c) 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

The DBT418 (R_0) was selected from individuals which were regenerated on a tissue culture media containing glufosinate. Then, it was crossed with the conventional variety [Confidential], and the generation [Confidential] was produced. A sibling line was developed by back-crossing of the above [Confidential] or crossing the above [Confidential] with the conventional variety. The progeny developed by back-crossing of the sibling line and self-propagation repeatedly, and the first cross which was developed by crossing with the conventional variety were provided for isolated field tests and/or gene analysis (see Figure 4 for the details of the generation used for tests). The selection in the processes of rearing was conducted by bioassay of Cry1Ac protein and glufosinate tolerance.

The following shows the approvals received from organizations in Japan.

December, 1997: 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 (used for processing and feed) was certified by the Ministry of Agriculture, Forestry and Fisheries. (The compatibility of

the guideline to the posterity, DBT418-DK566 was certified.)

June, 1999: 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 (used for processing and feed) 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", safety of use for food was approved by the Ministry of Health, Labor and Welfare (Ministry of Health and Welfare, at that time).

September, 2000: The safety of use of the cultivar for feed was approved in accordance with "Guideline for the safety evaluation of feed derived from recombinant-DNA plants, 6-(2)" by the Ministry of Agriculture, Forestry and Fisheries.

March, 2001: The safety of use of the cultivar for food, in accordance with "Safety Evaluation Criteria for Food and Additives derived from Recombinant-DNA Techniques" was certified by the Ministry of Health, Labour and Welfare.

March, 2003: The safety of the use of the cultivar as feed, following "Procedure to Check the Safety of Feed and Additives Produced by Recombinant-DNA Techniques" was certified by the Ministry of Agriculture, Forestry and Fisheries.

Confidential: Not made available or disclosed to unauthorized person

Figure 4 The process of rearing the maize DBT418 resistant to Lepidoptera and tolerant to glufosinate herbicide

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

i) Location of copies of the transferred nucleic acids

Regarding the traits caused by the transferred gene of DBT418 in multiple generations, the segregation ratio showed conformity with one dominant locus as described in Mendel's law. Therefore, it is judged that the transferred gene exists at one site on the chromosome (Annex 4).

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

This recombinant maize includes two copies of the *cry1Ac* gene cassette, one copy of the *bar* gene expression cassette, three copies of the *bla* gene, four incomplete copies of the *bar* gene, two incomplete copies of the *pinII* gene, three copies of the ColE1 region, etc. as transferred genes. The *bar* gene exists between two *cry1Ac* genes. The details of the physical relationship of each gene cassette are shown in Figure 5, the genetic map of transferred gene. This recombinant maize is developed using three plasmids, and these genes are confirmed to exist at one site on the genome by Southern blotting analysis, PCR analysis and sequence analysis.

The complete *pinII* gene was not introduced to this recombinant maize, but the fragment *pinII* gene was confirmed. Therefore, Western blotting analysis was conducted for the PinII protein, and as a result, the PinII protein was not detected from this recombinant maize (Figure 4 of Annex 2).

In addition, the *bla* gene used as a selective marker during plasmid building in *E. coli* was introduced, and the promoter of this gene functions in bacteria. In practice, it was confirmed that as a result of Western blotting analysis. There was no β-lactamase, a product of *bla* gene, detected in this recombinant maize (Figure 5 of Annex 2).

It was demonstrated by Southern blotting analysis for the multiple generations (generations marked with * in Figure 4) that the transferred genes are stably inherited in posterity (Figure 6 of Annex 2). In addition, the selection in the processes of rearing was conducted by bioassay of Cry1Ac protein and glufosinate tolerance. As a result, it was confirmed during the process of selection that resistance to Lepidopteran insects and tolerance to glufosinate herbicide are stably expressed in multiple generations.

iii) Nearby or separate location of multiple copies, if present, on the chromosome

There are multiple copies of the *cry1Ac* gene, the *bla* gene and the ColE1 in this recombinant maize. As a result of analysis of cross posterity and inserted genes, it was found that these copies exist at one site in the genome.

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

It was demonstrated as a result of ELISA method on the multiple generations that the transferred genes of this recombinant maize are stably inherited (Figure 4, Annex 2). In addition, it was also confirmed during the process of selection that the resistance to Lepidopteran insects and tolerance to glufosinate herbicide were expressed stably in the multiple generations.

v) 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

It is confirmed that the all replication origins of plasmids pDPG165, pDPG320 and pDPG699 are ColE1, therefore the region of recipient organism which allows autonomous replication is limited to gram-negative bacteria such as *E. coli* and *A. tumefaciens*. Consequently there is no possibility that the plasmids might be transmitted to any wild animals and wild plants under the natural environment.

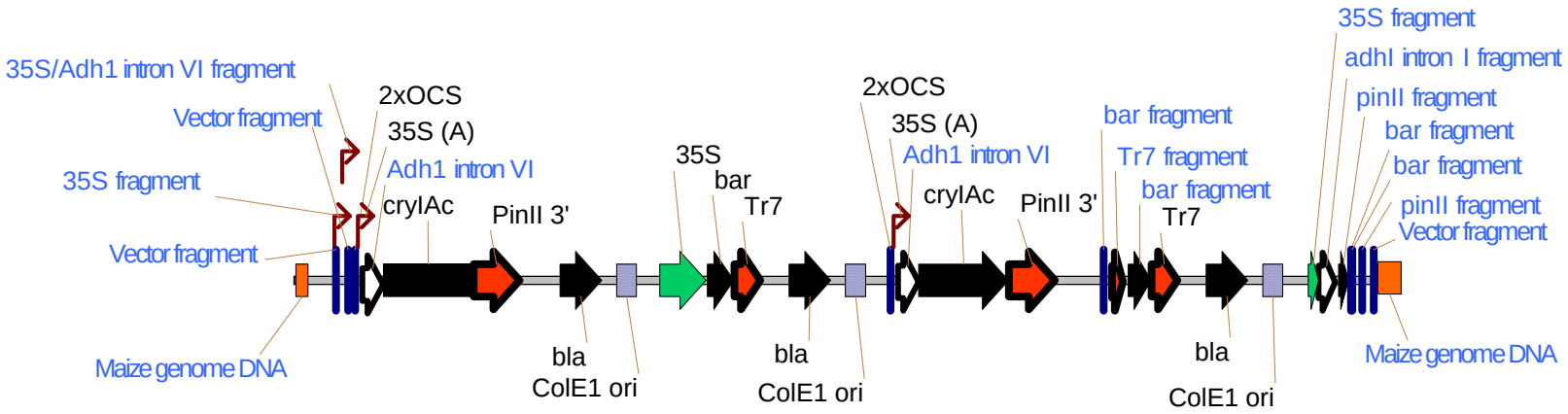

Figure 5 Genetic map of transferred genes in DBT418 line (22.7kb)[7]

[7] All the rights pertinent to the information in the above diagram and the responsibility for the content rest upon Monsanto Japan Limited.

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

For the detection and identification of this recombinant maize, the inserted genes and the nearby regions of the plant genome are used as primers. This method makes it possible to specifically detect DBT418 (Annex 5).

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

i) This recombinant maize is given tolerance to glufosinate herbicide with the constitutive expression of the PAT protein, which is encoded by the *bar* gene, in various regions of the plant body (Annex 2). In practice, the non-recombinant control maize died due to the influence of glufosinate herbicide, while this recombinant maize grew normally (Annex 2). In addition, expression of the Cry1Ac protein which is encoded by the *cry1Ac* gene (Annex 2) confers an insecticidal activity against European corn borer (*Ostrinia nubilalis*), which is one of the major insect pests of the order Lepidoptera to maize cultivation. Insect damage caused by European corn borer (*Ostrinia nubilalis*) in this recombinant maize decreased compared to the non-recombinant control maize (Annex 2). The expression level of the Cry1Ac protein in pollen was below the detection limit (6.7ng/g dry wt.) (Annex 4).

ii) [8] Isolated fields tests were carried out in the National Institute for Agro-Environmental Sciences (NIAES) in 1998 using the self-propagation line [Confidential] of this recombinant maize [Confidential] (Figure 4 [4]; [Confidential]), and the self-propagation line of the non-recombinant control maize, which is genetically-equivalent with the line [Confidential], for comparison. The differences between this recombinant maize and the non-recombinant control maize were examined based on the results of the tests conducted in 1998 (Annex 2). In addition, a comprehensive examination was performed using the results of the isolated field tests conducted in the National Institute for Agro-Environmental Sciences (NIAES) in 1997 (Annex 3) and the field experiments at 20 sites in the US and Canada conducted from 1993 to 1996 (Annex 2).

(a) Morphological and growth characteristics

For this recombinant maize and the non-recombinant control maize, evaluation was conducted regarding germination rate, uniformity of germination, time of tasseling, time of silking, culm length (cm), plant shape or plant type, tiller number, height of ear (cm), maturation time, number of productive ears, ear diameter (cm), row number per ear, grain number per row, grain color, 100-kernel weight (g), grain shape (dent type, flint type) and fresh weight at harvesting time (Annex 2).

[8] All the rights pertinent to the information in the paragraphs (a) through (g) following this section and the responsibility for the content rest upon Monsanto Japan Limited.

As a result, statistically significant differences were not observed between this recombinant maize and the non-recombinant control maize in any of the items except height of ear, ear diameter, ear length and 100-kernel weight (Annex 2). The average value of height of ear in which statistically significant difference was observed, was 84.0 cm for this recombinant maize, and 96.0 cm for the non-recombinant control maize (Annex 2). The average value of ear diameter in which statistically significant difference was also observed, was 3.59 cm for this recombinant maize, and 3.86 cm for the non-recombinant control maize (Annex 2). The average value of ear length was 14.89 cm for this recombinant maize, and 13.99 cm for the non-recombinant control maize (Annex 2). In addition, the average value of 100-kernel weight was 27.57 g for this recombinant maize, and 24.55 g for the non-recombinant control maize (Annex 2).

However, as a result of isolated field tests carried out in the National Institute for Agro-Environmental Sciences (NIAES) in 1997, statistically significant difference was not observed between this recombinant maize and the non-recombinant control maize in any of the characteristics including height of ear, ear diameter, ear length and 100-kernel weight (Annex 3). In addition, height of ear was examined in the field experiments at 20 sites in the US and Canada conducted from 1993 to 1996, and as a result, statistically significant difference was not observed between this recombinant maize and the non-recombinant control maize (Annex 2).

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

Cold-tolerance test at the early stage of growth was not conducted in the isolated field tests. However, in the field experiments conducted at 20 sites in the US and Canada from 1993 to 1996, and during the period of commercial cultivation in the US from 1997 to 1999, it was not reported that this recombinant maize, which grew up to seedlings after having dropped in the fields during harvesting time, could overwinter and survive into the beginning of following spring.

(c) Wintering ability and summer survival of the matured plant

Maize is a summer type annual plant, and after ripening it usually dies out in winter, and it does not regrow and propagate vegetatively or produce seeds. It was observed from field trials that dying started after ripening. Based on the above, an overwintering test for the matured plant of this recombinant maize was not carried out.

(d) Fertility and size of the pollen

Fertility and size of the pollen are not examined in the isolated field test.

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

As described in I-(6)-(a), ear length, ear diameter, row number per ear, grain number per row and 100-kernel weight of this recombinant maize obtained by sibling-crossing were examined. As a result, statistically significant difference was not observed between this recombinant maize and the non-recombinant control maize in any of the items except ear diameter, ear length and 100-kernel weight (Annex 2). The average value of ear diameter, in which statistically significant difference was observed, was 3.59 cm for this recombinant maize and 3.86 cm for the non-recombinant control maize (Annex 2). The average value of ear length was 14.89 cm for this recombinant maize and 13.99 cm for the non-recombinant control maize (Annex 2). In addition, the average value of 100-kernel weight in which statistically significant difference was also observed was 27.57 g for this recombinant maize and 24.55 g for the non-recombinant control maize (Annex 2).

As a result of isolated field tests carried out in the National Institute for Agro-Environmental Sciences (NIAES) in 1997, statistically significant difference was not observed between this recombinant maize and the non-recombinant control maize lines in ear diameter and 100-kernel weight (Annex 3).

Regarding shedding habit of the seed, shedding habit was not observed in the natural condition, because the ears of this recombinant maize and the non-recombinant control maize were covered with husks at the time of harvesting.

Examination for the germination rate of the harvested seeds was not conducted in the isolated field test. However, in the field experiments conducted in 20 sites in the US and Canada from 1993 to 1996, and during the period of commercial cultivation in the US from 1997 to 1999, it was not reported that there is a significant difference for germination rate of the seed between this recombinant plant and conventional variety. Therefore, it was considered that dormancy and germination rate of the seed is at the same level for this recombinant maize and the non-recombinant control maize.

(f) Crossability

Crossability test was not performed for this recombinant maize, because no wild relatives that can be crossed grow in Japan.

(g) Productivity of harmful substances

Succeeding crop tests and soil microflora tests were performed for this recombinant maize and the non-recombinant control maize. Statistically significant difference was not observed in any of the items (Annex 2). In addition, as part of the isolated field tests carried out in 1997, weed vegetation surveys were conducted in the cultivation zones for this recombinant maize and the non-recombinant control maize. No difference was observed between this recombinant maize and the non-recombinant control maize (Annex 3). Furthermore, as part of the isolated field tests carried out in

1997, succeeding crop tests and soil microflora tests were performed for this recombinant maize and the non-recombinant control maize Statistically significant differences were not observed in any of the items (Annex 3). Following the harvesting of this recombinant maize in field trials in the U.S. in 1999, remaining plant material was plowed into the field, then soybean and wheat were cultivated in the same field in the next year. No inhibited growth was reported.

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

A review was made by experts with specialized knowledge and experience concerning Adverse Effect on Biological Diversity 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.

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

(1) Competitiveness

Maize (*Zea mays* subsp. *mays* (L.) Iltis), to which the recipient organism belongs, has been used, including for cultivation, etc., in Japan, though there is no report that it has become volunteer in Japan. This recombinant maize is given a trait to be resistant to Lepidoptera due to the Cry1Ac protein in which the transferred *cry1Ac* gene is expressed. However, it is not generally considered that the insect damage by lepidoptera is the major cause making the maize difficulet to grow in the natural environment. Consequently, it is considered that this trait does not increase the competitiveness and thus this recombinant maize does not become dominant under a natural environment. In addition, this recombinant maize is given a trait to be tolerant to glufosinate herbicide due to the PAT protein in which the transferred *bar* gene is expressed. However, it is not generally considered that glufosinate exerts pressure for selection under a natural environment; therefore it is unlikely that these characteristics cause this recombinant maize to be dominant in competitiveness and affect wild animals and wild plants.

In addition, as a result of examination in the isolated field in Japan, significant differences were found between this recombinant maize and non-recombinant maize with regard to height of ear, ear diameter, ear length and 100-kernel weight. However, it is difficult for maize to become volunteer under a natural environment, and it is considered unlikely that this significant difference can cause this recombinant maize to be predominant over the non-recombinant maize in competitiveness. In the examination in 1997, there is no significant difference attributable to competitiveness for characteristics, including the characteristics in which significant differences were found in the examination in 1998.

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

Regarding maize, to which the recipient organism belongs, there is no report that it produces harmful substances to affect wild animals and wild plants.

This recombinant maize produces the Cry1Ac protein, which possesses an insecticidal activity against lepidopteran insects, but this protein does not have enzyme activity and functions independently from the metabolic system of the recipient organism. Therefore it is considered not to affect the metabolic system of the recipient organism. In addition, this recombinant maize possesses a trait to produce PAT protein that inactivates glufosinate herbicide and this protein is not reported as harmful to wild animals and wild plants. In addition, PAT protein possesses high substrate specificity and thus it is considered not to affect the metabolic system of the recipient organism.

In Japan, the productivity of harmful substances of this recombinant maize (including secretion from roots to affect the other plants, and secretion from roots to affect microorganisms in soil) has been investigated in the isolated fields, and there is no significant difference found between this recombinant maize and non-recombinant maize. In the fields in the U.S. and Canada, following the harvesting of this recombinant maize, plant material of this recombinant maize was plowed into the field, and soybean, etc. was cultivated in the same field in the next year. No inhibited growth was reported. Therefore, it is considered extremely low that productivity of harmful substances of the above-ground part might affect the other wild animals and wild plants.

If this recombinant maize grows to seedling after having been dropped, etc. under a natural environment, it may affect lepidopteran insects nearby. However, the expression level of the Cry1Ac protein in pollens of this recombinant maize was under the detection limit of the ELISA method, and therefore the likelihood that population of lepidopteran insects are affected by pollen of this recombinant maize is extremely low.

Based on the above understanding, there are no wild plants or wild animals that are possible to affected by this recombinant maize, and it was judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to 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 have been identified as being negatively impacted, and it was 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 recombinant maize in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above is valid.

Reference

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