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

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Approved Type 1 Use Regulations

Names of Types of Living Modified Organisms	Maize resistant to *Lepidoptera* pests, and tolerant to glufosinate, glyphosate and aryloxyalkanoate herbicides (*cry1A.105*, modified *cry2Ab2*, modified *cry1F*, *pat*, modified *cp4 epsps*, modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034×*B.t.* Cry1F maize line 1507× NK603×DAS40278, OECD UI: MON-89Ø34-3×DAS-Ø15Ø7-1× MON-ØØ6Ø3-6×DAS-4Ø278-9) (including the progeny lines which are isolated from the maize lines, MON89034, *B.t.* Cry1F maize line 1507, NK603 and DAS40278, that contain a combination of their respective transferred genes (except those already granted an approval regarding Type I Use Regulation))
Content of Type 1 Use of Living Modified Organisms	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of Type 1 Use of Living Modified Organisms	—

Outline of the Biological Diversity Risk Assessment Report

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

1. Information concerning preparation of living modified organisms

Maize resistant to Lepidoptera pests, and tolerant to glufosinate, glyphosate and aryloxyalkanoate herbicides (*cry1A.105*, modified *cry2Ab2*, modified *cry1F*, modified *aad-1*, *pat*, modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034 × *B.t.* Cry1F maize line 1507 × NK603 × DAS40278, OECD UI: MON-89Ø34-3 × DAS-Ø15Ø7-1 × MON-ØØ6Ø3-6 × DAS-4Ø278-9) (hereinafter referred to as "this stacked maize line") is the progeny line created by interbreeding with the following four modified maize lines using the conventional crossing.

This stacked maize line will be commercialized as a F1 hybrid line (F1). Due to the genetic segregation the seeds harvested from this stacked maize line include the stacked maize line or a combination of the genes transferred in the respective parent lines of this stacked maize line.

- Maize resistant to *Lepidoptera* pests (*cry1A.105*, modified *cry2Ab2*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034, OECD UI: MON-89Ø34-3) (hereinafter referred to as "MON89034")
- Maize resistant to *Lepidoptera* pests and tolerant to glufosinate herbicide (modified *cry1F*, *pat*, *Zea mays* subsp. *mays* (L.) Iltis) (*B.t.* Cry1F maize line 1507, OECD UI: DAS-Ø15Ø7-1) (hereinafter referred to as "Cry1F line 1507")
- Maize tolerant to glyphosate herbicide (modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (NK603, OECD UI: MON-ØØ6Ø3-6) (hereinafter referred to as "NK603")
- Maize tolerant to aryloxyalkanoate herbicide (modified *aad-1*, *Zea mays* subsp. *mays* (L.) Iltis) (DAS40278, OECD UI：DAS-4Ø278-9) (hereinafter referred to as "DAS40278")

The summary of the information concerning preparation of MON89034, Cry1F line 1507, NK603, and DAS40278 is described below.

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acids and the origins of component elements used for each development of MON89034, Cry1F line 1507, NK603, and DAS40278 is shown in Tables 1-4 (p. 4-8).

Table 1. Origins and functions of the component elements of PV-ZMIR245 used for the development of MON89034

Component elements	Origin and function
T-DNA I region	
B [Note 1]-Right Border	DNA fragment containing the right border sequence of nopaline type T-DNA region derived from *Agrobacterium tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to the plant genome (Depicker *et al.*, 1982; Zambryski *et al.*, 1982).
P [Note 2]-*e35S*	Cauliflower mosaic virus (CaMV) 35SRNA (Odell *et al.*, 1985) promoter and 9 bp leader sequence, containing double enhancer regions (Kay *et al.*, 1987). Involved in the constant expression of the target genes in entire tissues of the plant body.
L [Note 3]-*Cab*	5'-terminal untranslated leader region of wheat chlorophyll a/b binding protein derived from wheat. It activates the expression of target genes (Lamppa *et al.*, 1985).
I [Note 4]-*Ract1*	Intron of the actin gene from rice (*Oryza sativa*) (McElroy *et al.*, 1991). It involves the regulation of expression of target genes.
CS [Note 5]-*cry1A.105*	Gene coding for the Cry1A.105 protein. The Cry1A.105 protein encoded by the *cry1A.105* gene, which is used to develop MON89034, is the synthetic Bt protein composed of the domains I and II of the Cry1Ab protein, the domain III of the Cry1F protein, and the C-terminal domain of the Cry1Ac protein. It is developed to enhance insecticidal activity against the target Lepidoptera pests by combination of domains of different Bt proteins.
T [Note 6]-*Hsp17*	3'-terminal untranslated region of the wheat heat shock protein 17.3. It terminates transcription and induces polyadenylation (McElwain and Spiker, 1989).
P-*FMV*	35S promoter derived from the Figwort Mosaic Virus (Rogers, 2000). It induces the constitutive expression of the target genes in all tissues of the plant body.
I-*Hsp70*	First intron of the maize heat shock protein 70 gene (Brown and Santino, 1995). It activates the expression of target genes.
TS [Note 7]-*Rbcs (Zm)*	Transit peptide of the small subunit of ribulose-1,5-bisphosphate carboxylase of maize. It contains the first intron sequence (Matsuoka *et al.*, 1987). It transfers the proteins connected with the downstream region to the plastids.
CS-modified *cry2Ab2*	Gene coding for the modified Cry2Ab2 protein derived from *Bacillus thuringiensis* (Widner and Whiteley, 1989). In order to add a restriction endonuclease cleavage site, which is used in cloning, an aspartic acid is inserted after the methionine at the N-terminal, compared to the wild-type Cry2Ab2 protein.
T-*nos*	3'-terminal untranscribed region of nopaline synthase (*nos*) gene derived from *A.tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983).
B-Left Border	DNA fragment containing the left border sequence (25 bp) derived from *A. tumefaciens*. It is the termination point of T-DNA transfer from *A. tumefaciens* to the plant genome (Barker *et al.*,1983).

Table 1. Origins and functions of the component elements of PV-ZMIR245 used for the development of MON89034 (continued)

Component elements	Origin and function
T-DNA II region	
B-Right Border	DNA fragment containing the right border sequence (24 bp) of nopaline type T-DNA region derived from *A. tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to the plant genome (Depicker *et al*., 1982; Zambryski *et al*., 1982).
T-*nos*	3'-terminal untranscribed region of nopaline synthase (*nos*) gene derived from *A.tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al*., 1983).
CS-*nptII*	Gene derived from *Escherichia coli* transposon Tn5 (Beck *et al.*, 1982). It codes for the neomycin phosphotransferase II and confers kanamycin tolerance on plants. It is used as a marker to select transgenic plants in transferring genes (Fraley *et al*.,1983).
P-*35S*	35S promoter region of the cauliflower mosaic virus (CaMV) (Odell *et al*., 1985). It induces the constitutive expression of the target genes in all tissues of the plant body.
B-Left Border	DNA fragment containing the left border sequence (25 bp) derived from *A. tumefaciens*. It is the termination point of T-DNA transfer from *A. tumefaciens* to the plant genome (Barker *et al*.,1983).
Vector backbone region	
OR [Note 8]-*ori V*	Replication origin region isolated from the broad-host range plasmid RK2. It allows autonomous replication of vectors in *A. tumefaciens* (Stalker *et al.*, 1981).
CS-*rop*	Coding sequence for suppression of primer protein to maintain the number of copies of plasmid in *E. coli* (Giza and Huang, 1989).
OR-*ori-pBR322*	Replication origin region isolated from the pBR322. It allows autonomous replication of vectors in *E.coli* (Sutcliffe, 1979).
aadA	Bacteria promoter, code region and terminator for the 3"(9)-*O*-nucleotidyltransferase, the aminoglycoside modified enzyme derived from transposon Tn7 which confers resistance to spectinomycin or streptomycin (Fling *et al.*, 1985).

[Note 1] B – Border
[Note 2] P – Promoter
[Note 3] L – Leader
[Note 4] I – Intron
[Note 5] CS – Coding sequence
[Note 6] T – Transcript termination sequence
[Note 7] TS – Targeting sequence
[Note 8] OR – Origin of Replication

Table 2. Origins and functions of the component elements of PHP8999 used for the development of Cry1F line 1507

Component elements	Origin and function
Modified *cry1F* gene expression cassette	
UBIZM1(2) Promoter	Ubiquitin constitutive promoter [1)] derived from *Z. mays* (including intron and 5'-terminal untranslated region) (Christensen *et al.*, 1992).
Modified *cry1F*	Gene coding for the modified Cry1F protein derived from *B. thuringiensis* var. *aizawai*. The nucleotide sequence has been modified to enhance the expression in plants. In the modified Cry1F protein, phenylalanine at position 604 of the amino acid sequence is replaced by leucine.
ORF25PolyA Terminator	Terminator to terminate transcription derived from *A.tumefaciens* pTi15955 (Barker *et al.*, 1983)
pat gene expression cassette	
CAMV35S Promoter	35S constitutive promoter [1)] derived from the cauliflower mosaic virus (CaMV) (Hohn *et al.*, 1982).
pat	Gene coding for phosphinothricin acetyltransferase (PAT protein) derived from *Streptomyces viridochromogenes*. The nucleotide sequence has been modified to enhance expression in plants, but the amino acid sequence has not been changed by the modification (Eckes *et al.*, 1989).
CAMV35S Terminator	35S terminator to terminate transcription derived from the cauliflower mosaic virus (Hohn *et al.*, 1982).

[1)] Constitutive promoter: a promoter to express the target genes in the entire plant body.

Table 3. Origins and functions of the component elements of PV-ZMGT32 used for the development of NK 603

Component elements	Origin and function
Modified *cp4 epsps* gene cassette (1)	
P [Note1]-*Ract1*	Promoter region of the actin 1 gene derived from rice (*O. sativa*) (McElroy *et al.*, 1990). It induces the constitutive expression of the target genes in all tissues of the plant body.
I [Note 2]-*Ract1*	Intron of the actin gene derived from rice (*O. sativa*) (McElroy *et al.*, 1991). It is involved in the regulation of expression of target genes.
TS [Note 3]-*CTP 2*	Nucleotide sequence coding for the N-terminal chloroplast transit peptide of the protein EPSPS in the *epsps* gene of thale cress, *Arabidopsis thaliana* (Klee *et al.*, 1987). It transports the target proteins from the cytoplasm to chloroplast.
CS [Note 4]-modified *cp4 epsps*	5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium* CP4 strain (Barry *et al.*, 1997; Padgette *et al.*, 1996a). In the modified CP4 EPSPS protein, the second amino acid, serine, from the N-terminal in the wild-type CP4 EPSPS protein is modified to leucine to enhance the level of expression in plants.
T [Note 5]-*nos*	3'-terminal untranslated region of the nopaline synthase (*nos*) gene derived from *A. tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983).
Modified *cp4 epsps* gene cassette (2)	
P-*e35S*	Promoter and 9 bp leader sequence of the cauliflower mosaic virus (CaMV) 35SRNA (Odell *et al.*, 1985) with a double enhancer region (Kay *et al.*, 1987). It induces the constitutive expression of the target genes in all tissues of the plant body.
I-*Hsp70*	Intron of the maize heat shock protein 70 gene. The ZmHsp70 intron is used to enhance the level of foreign gene expression in plants (Rochester *et al.*, 1986).
TS-*CTP2*	Nucleotide sequence coding for the N-terminal chloroplast transit peptide of the EPSPS protein in the *epsp* gene of thale cress, *A. thaliana(* Klee *et al.*, 1987). It transports the target proteins from the cytoplasm to the chloroplast.
CS- modified *cp4 epsps*	5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium* CP4 strain (Barry *et al.*, 1997; Padgette *et al.*, 1996a). In the modified CP4 EPSPS protein, the second amino acid, serine, from the N-terminal in the wild-type CP4 EPSPS protein is modified to leucine to enhance the level of expression in plants.
T-*nos*	3'-terminal untranslated region of the nopaline synthase (*nos*) gene derived from *A. tumefaciens* T-DNA. It terminates transcription of mRNA and induces polyadenylation (Bevan *et al.*, 1983).

Table 3. Origins and functions of the component elements of PV-ZMGT32 used for the development of NK 603 (continued)

Others (not existing in the plant body)	
Lac	Sequence consisting of a partial coding region of *lacI* (Farabaugh, 1978), the *lac* promoter (Dickson *et al.*, 1975), and a partial coding region of *lacZ*. It hydrolyzes lactose to express β-galactosidase used as a selective marker (Shuman and Silhavy, 2003).
OR [Note6]*-ori- pBR 322*	Origin of replication isolated from the pBR322. It allows autonomous replication of vectors in *E.coli* (Sutcliffe, 1979).
nptII	Sequence coding for phosphotransferase II (NPTII) derived from the *E. coli* transposon Tn5 (Beck *et al.*, 1982). It confers resistance to neomycin and kanamycin. The region contains the partial *ble* gene derived from the Tn5 (Mazodier *et al.*, 1985), is regulated by the *nptII* promoter and the β-lactamase termination sequence, and is used as a marker to select the transgenic plants in transferring genes (Fraley *et al.*, 1983).

[Note 1] P – Promoter
[Note 2] I – Intron
[Note 3] TS – Targeting sequence
[Note 4] CS – Coding sequence
[Note 5] T – Transcript termination sequence
[Note 6] OR – Origin of replication

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Table 4. Origins and functions of the component elements of pDAS1740 used for the development of DAS40278

Component elements	Origin and function
Modified *aad-1* cassette	
RB7 MAR	Nuclear matrix attachment region derived from tobacco (Allen *et al.*, 1996). It stabilizes the expression of the modified AAD-1 protein.
ZmUbi1	Ubiquitin promoter derived from maize and contains the exon and intron regions (Christensen *et al.*, 1992). It initiates the transcription of genes in the entire plant body.
Modified *aad-1*	Gene modified from the aryloxyalkanoate dioxygenase gene derived from a gram-negative bacillus, *Sphingobium herbicidovorans*, to have a codon appropriate for expression in plants. It expresses the modified AAD-1 protein. As for the amino-acid sequence of the modified AAD-1 protein, alanine is added in the second position to introduce a cloning site (Dow AgroSciences LLC, 2004).
ZmPer5 3'UTR	Terminator derived from maize (Dow AgroSciences LLC, 1997). It terminates gene transcription.
RB7 MAR	Nuclear matrix attachment region derived from tobacco (Allen *et al.*, 1996). It stabilizes the expression of the modified AAD-1 protein.

2) Function of component elements

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

The functions of the component elements of the donor nucleic acids used for the development of MON89034, Cry1F line 1507, NK603, and DAS40278 are shown in Tables 1-4 (p. 4-8).

The modified *aad-1* cassette transferred to DAS40278 contains a nuclear matrix attachment region, the *RB7 MAR* gene**.** A nuclear matrix attachment region is frequently found in genomic DNA sequences and thought to have the function of attaching DNA to the nuclear matrix to form the DNA loop structure. It has been reported that when the nuclear matrix attachment region is adjacent to either end of the transferred gene, the level of expression of the transferred gene increases and gene silencing (which inhibits gene expression) decreases (Allen *et al.*, 2000; Halweg *et al.*, 2005).

(b) 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 that is known to possess any allergenicity

a. Functions of proteins produced by target gene expression

-- Pest resistance protein--

The insecticidal proteins (Bt proteins) are derived from crystalline inclusion bodies which are produced by a gram-positive bacterium, *B. thuringiensis*, which is generally present in soil. It is known that Bt proteins are associated with a specific receptors in the midgut epithelium of the target insect to form cation selective pores, resulting in inhibiting the digestion process to exhibit insecticidal activity (Hofmann *et al.*, 1988; Slaney *et al.*, 1992; VanRie *et al.*, 1990). Previous studies also revealed that the Bt proteins consist of multiple domains and what function each domain has. For example, it is known that the Bt protein consists of domains I, II, and III and a C-terminal domain: the domain I forms cation selective pores to inhibit the digestion process, the domain II recognizes specific receptors, the domain III is associated with the receptors, and the C-terminal is involved in the crystal structure of the Bt protein (de Maagd *et al.*, 2001; Masson *et al.*, 2002).

< *Lepidoptera* pest resistance protein >

【Cry1A.105 Protein】

The Cry1A.105 protein, which is expressed in MON89034, is a synthetic Bt protein consisting of domains I and II of the Cry1Ab protein, domain III of the Cry1F protein, and C-terminal domain of the Cry1Ac protein. This synthetic protein was developed in order to enhance the insecticidal activity against target Lepidoptera pests by combining it with different Bt proteins.

The insecticidal spectrum of the Cry1A.105 protein was examined by dietary administration of the Cry1A.105 protein to 15 insect species, including five Lepidoptera insects. The results showed that the Cry1A.105 protein exhibits insecticidal activity against larvae of major Lepidoptera pests of maize including; corn earworm (*Helicoverpa zea*) (MacRae *et al.*, 2005), black cutworm (Tamayanaga) (*Agrotis ipsilon*) (MacRae, 2005), fall armyworm (Tsumajirokusayoto) (*Spodoptera frugiperda*) (MacRae, 2005), southwestern corn borer (*Diatraea grandiosella*) (MacRae, 2005), and European corn borer (Yoroppa awanomeiga) (*Ostrinia nubilalis*) (MacRae *et al.*, 2006a). The Cry1A.105 protein is not active against beneficial insects other than Lepidoptera insects, such as honeybees (Richards, 2006a; Richards, 2006b) and ladybird beetles (Paradise, 2006a).

Based on the above observations, it was confirmed that the Cry1A.105 protein exhibits selective insecticidal activity against only *Lepidoptera* pests, but not against other insects, like its component elements, the Cry1Ab, Cry1F, and Cry1Ac proteins, which only exhibit insecticidal activity against Lepidoptera pests.

【Modified Cry2Ab2 protein】

The insecticidal spectrum of the modified Cry2Ab2 protein, which is expressed in MON89034, was examined by dietary administration of the Cry2Ab2 protein mixed with artificial feed to 15 insect species including four Lepidoptera insects. The results showed that the modified Cry2Ab2 protein was active against the larval corn earworm (MacRae *et al.*, 2006a), fall armyworm (MacRae *et al.*, 2006b) and European corn borer (MacRae *et al.*, 2006a) out of the four major Lepidoptera pests. The modified Cry2Ab2 protein exhibited no insecticidal activity against black cutworm (MacRae *et al.*, 2006b). Moreover, it did not exhibit insecticidal activity against beneficial insects other than Lepidoptera pests, including honeybees (Maggi, 2000a; Maggi, 2000b) and ladybird beetles (Paradise, 2006b). Therefore, it was confirmed that the modified Cry2Ab2

protein exhibited selective insecticidal activity against certain *Lepidoptera* pests, but not against other insect species.

The modified Cry2Ab2 protein encoded by the modified *cry2Ab2* gene has an aspartic acid inserted after the methionine at the N-terminal, compared to the wild-type Cry2Ab2 protein, in order to add a restriction endonuclease cleavage site, which is used in cloning.

【Modified Cry1F protein】

The modified Cry1F protein, which is expressed in Cry1F line 1507, exhibits high insecticidal activity against larvae of several Lepidoptera pests, including European corn borer, fall armyworm, and beet armyworm (Shiroichimonjoto) (*Spodoptera exigua*), but the toxicity of the protein has not been observed against insects other than Lepidoptera insects, including Coleoptera, Hymenoptera, Neuroptera and Collembola, and other non-target organisms, including mammals, birds, and fish (EPA, 2005).

In addition, in the modified Cry1F protein, the phenylalanine at position 604 in the native amino acid sequence is substituted by leucine.

-- Herbicide tolerant proteins --

【PAT protein】

The PAT protein (phosphinothricin acetyltransferase), which is expressed in Cry1F line 1507, confers tolerance to the herbicide, glufosinate. Glufosinate inhibits glutamine synthetase, which synthesizes glutamine from glutamic acid and ammonia, and the resulting accumulation of ammonia in the plant causes plant death. The PAT protein acetylates glufosinate herbicide to transform it into nonherbicidal acetyl glufosinate, resulting in conferring the tolerance to glufosinate on the plant body.

【Modified CP4 EPSPS protein】

The modified CP4 EPSPS protein, which is expressed in NK603, exhibits tolerance to the herbicide, glyphosate. Plants treated with glyphosate die because 5-enolpyruvylshikimate 3-phosphate synthetase (enzyme number: E.C.2.5.1.19, hereinafter referred to as “EPSPS protein”) is inhibited a which inhibits the synthesis of aromatic amino acids essential to protein synthesis. The activity of the modified CP4 EPSPS protein is not inhibited even in the presence of glyphosate, and therefore the recombinant plants expressing this protein can grow by the normal synthesis of

shikimate.

Moreover, for the modified *cp4 epsps* gene, the nucleotide sequence of the wild-type *cp4 epsps* gene is modified in order to improve the level of expression in plants without changing the functional activity of the wild-type CP4 EPSPS protein. As for the amino acid sequence of the modified CP4 EPSPS protein, only serine at the second position from the N-terminal is substituted by leucine. In addition, the two modified *cp4 epsps* gene cassettes are introduced into NK603 to enhance the tolerance to glyphosate.

【Modified AAD-1 protein】

The modified AAD-1 protein expressed in DAS40278 is an enzyme, which transforms a compound into one without herbicidal activity by catalyzing the reaction of oxygen introduction to aryloxyalkanoate herbicides. For example, the modified AAD-1 protein catalyzes the reaction of oxygen introduction to 2,4-dichlorophenoxyacetic acid (2,4-D) herbicide to transform it into 2,4-dichlorophenol (2,4-DCP) , which has no herbicidal activity, and glyoxylic (Dow AgroSciences LLC, 2004).

In addition, the modified *aad-1* gene has codons modified to optimize expression in plants and in its amino acid sequence alanine is added in the second position in order to introduce a cloning site.

b. Homology to known allergen proteins

The amino acid sequences of Cry1A.105, modified Cry2Ab2, modified Cry1F, PAT, modified CP4 EPSPS, and modified AAD-1 proteins were examined for sharing the functionally important with the known allergens, using the following databases. The results showed that structurally similar sequences of those proteins were not shared with the known allergens.

AD11: Cry1A.105, modified Cry2Ab2, and modified CP4 EPSPS proteins
FARRP Allergen Database version 11: Modified Cry1F, PAT, and modified AAD-1 proteins

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

The Cry1A.105, modified Cry2Ab2 and modified Cry1F proteins are all the Bt proteins. A number of studies have been performed on the mechanism of the insecticidal activity of these Bt proteins (OECD, 2007), and so far it has not been reported that the Bt proteins have other functions. Therefore, it is believed that these Bt proteins have no

enzymatic activities and do not change the metabolic system of recipient organisms.

The PAT protein acetylates L-phosphinothricin (classified as an L-amino acids), an active ingredient of glufosinate herbicide, but does not acetylate other L-amino acids. The PAT protein has little affinity for glutamic acid, the structure of which is especially similar to L-phosphinothricin (Thompson *et al.*, 1987). Furthermore, it has been reported that even in the excessive presence of various amino acids, the transacetylation reaction of glufosinate by the PAT protein is not inhibited and PAT has extremely high substrate specificity to glufosinate (OECD, 1999). Therefore, considering their high substrate specificity, the PAT protein is not thought to change the metabolic system of recipient organisms.

The EPSPS protein, functionally identical to the modified CP4 EPSPS protein, is an enzyme protein, which catalyzes the shikimate pathway for biosynthesis of aromatic amino acids. However, it is not a rate-determining enzyme in the pathway, and therefore it has been considered that the levels of the aromatic amino acids, the end products of this pathway, do not increase even with the increased activities of the EPSPS proteins (Padgette *et al.*, 1996b; Ridley *et al.*, 2002). In addition, it has been identified that the EPSPS protein specifically reacts with the substrates, phosphoenolpyruvate (hereinafter referred to as "PEP") and shikimate-3-phosphate (hereinafter referred to as "S3P") (Gruys *et al.*, 1992). Other than those substrates, only shikimic acid, an analog of S3P, is known to react with the EPSPS protein. However, the comparison of the reaction of the EPSPS proteins with shikimate and S3P by the specificity constant (k_{cat}/K_m), which represents the degree of occurrence of reaction, showed that the reaction specificity between the EPSPS protein and shikimate is one-two millionth of that between the EPSPS protein and S3P (Gruys *et al.*, 1992), and shikimate is unlikely to react as a substrate of the EPSPS protein. Therefore it is not thought that the modified CP4 EPSPS protein changes the metabolic system of recipient organisms.

The modified AAD-1 protein is an enzyme to catalyze the reactions when oxygen is specifically introduced to the compounds with aryloxyalkanoate group, especially R-enantiomers of chiral as well as achiral compounds. Endogenous plant compounds that share structural and physiological similarities to the compounds with an aryloxyalkanoate group were examined for the activities of the modified AAD-1 protein and the impact on the metabolic pathways were discussed. As a substrate, plant hormones (indole-3-acetic acid, abscisic acid, gibberellic acid and aminocyclopropane 1-carboxylic acid) and phenylpropanoid intermediates (trans-cinnamic acid, coumaric acid, and sinapinic acid) were examined. Twenty kinds of L-amino acids were also examined.

For the 20 L-amino acids, no reactions were observed at a concentration of 1 μM modified AAD-1 protein. Among the plant hormones and phenylpropanoid intermediates treated with 1 μM modified AAD-1 protein, abscisic acid, gibberellic acid, trans-cinnamic acid, and coumaric acid showed slight reactions. Treatment with 5 μM modified AAD-1 protein produced a slight reaction with aminocyclopropane 1-carboxylic acid and at 10 μM modified AAD-1 protein, a slight reaction was observed for indole-3-acetic acid. Since no correlation was observed between the concentrations of the modified AAD-1 protein and enzyme activity, oxides were then measured by Fourier transform mass spectrometry (FT/MS). The results showed that oxides of indole-3-acetic acid and trans-cinnamic acid were detected by the treatment with the modified AAD-1 protein at 10 μM. However, reaction rates were very slow and the parameters, *Km* and *Vmax*, of the Michaelis-Menten equation could not be obtained. Since oxides were detected only when highly sensitive Fourier transform mass spectrometry was performed on the compounds treated with high levels of the modified AAD-1 protein and since reaction rates were extremely slow, the oxidative reactions observed are unlikely to affect the metabolic pathways of plants (Cicchillo *et al.*, 2010).

In addition, since no compounds of the aryloxyalkanoate group have been yet identified in plant bodies, the modified AAD-1 protein is not thought to change any other metabolic pathways in plant bodies.

(2) Information concerning vectors

1) Name and origin

The plasmid vectors used for the development of the parent lines are as follows.

MON89034: PV-ZMIR245 constructed from the vector pBR322 derived from *E. coli*
Cry1F line 1507: PHP8999 constructed from the vector pUC19 derived from *E. coli*
NK603: PV-ZMGT32 constructed from the vector pUC119 derived from *E. coli*
DAS40278: pDAS1740 constructed from the plasmid pUC19 derived from *E. coli*

2) Properties

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

The numbers of base pairs in the plasmid vectors used for the development of parent lines are as follows.

MON89034: PV-ZMIR245; 17,600 bp
Cry1F line 1507: PHP8999; 9,504 bp

NK603: PV-ZMGT32; 9,308 bp
DAS40278: pDAS1740; 8,512 bp, linear DNA used for transfection; 6,236 bp

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

The antibiotic resistant genes used as selective markers are as follows. None of these antibiotic resistant genes have been transferred in the recipient organism.

MON89034: *aadA* gene conferring the resistance to spectinomycin and streptomycin and *nptII* gene conferring the resistance to kanamycin
Cry1F line 1507: *nptII* gene conferring the resistance to kanamycin
NK603: *nptII* gene conferring the resistance to kanamycin
DAS40278: ap^r gene conferring the resistance to ampicillin

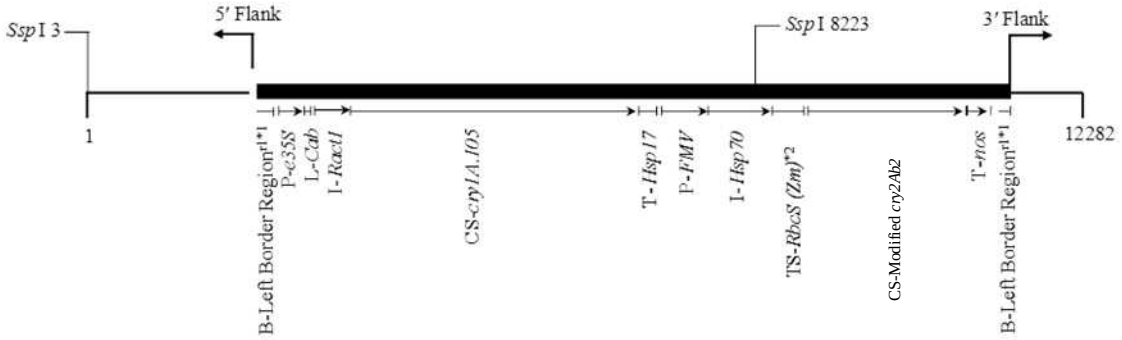
(c) Presence or absence of infectious characteristics of vector and, if present, the information concerning the host range

The infectivity of PV-ZMIR245, PHP8999, PV-ZMGT32, and pDAS1740 is not known.

(3) Method of preparing living modified organisms

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

The structure diagrams of the entire nucleic acid transferred to the MON89034, Cry1F line 1507, NK603, and DAS40278 are shown in Figure 1 to 4 (p. 15-16).



[*1]: r1 means that the B-Left Border Region in the MON89034 is shorter than that before the transfection.

[*2]: TS-R*bcS (Zm)* was considered as "TS-*SSU-CTP*" in the Biological Diversity Risk Assessment Report of MON89034.

Figure 1. Structure diagram of the entire nucleic acid transferred to MON89034

The angled arrows in the structure diagram show the 5'- and 3'-terminal regions of the transferred genes and the subsequent adjacent endogenous sequences of maize. The positions of the component elements and sites cleaved by restriction enzymes are shown as estimated approximate positions.

(All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

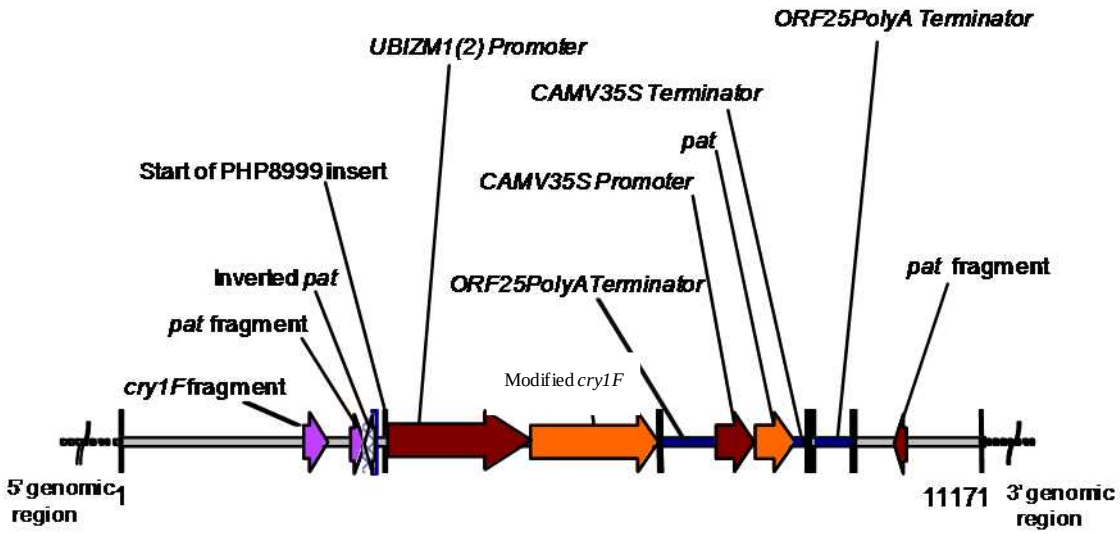


Figure 2. Structure diagram of the entire nucleic acid transferred to Cry1F line 1507

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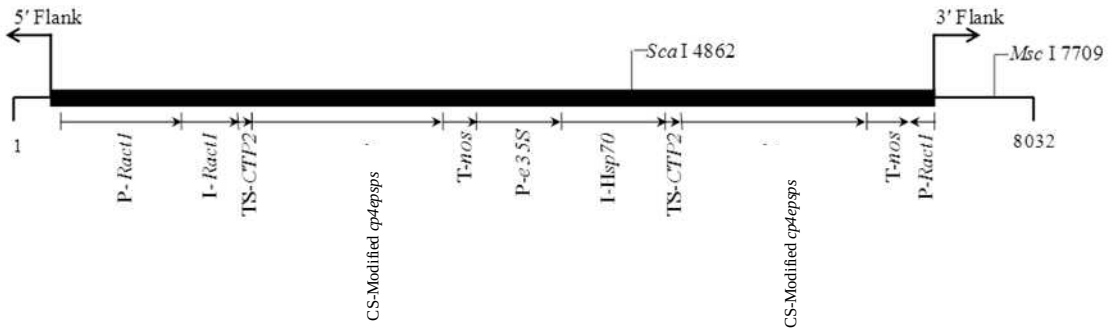

Figure 3. Structure diagram of the entire nucleic acid transferred to NK603

The angled arrows in the structure diagram show the 5'- and 3'-terminal regions of the transferred genes and the subsequent adjacent endogenous sequences of maize. The positions of the component elements and sites cleaved by restriction enzymes are shown as estimated approximate positions.

(All the rights pertinent to the information in this figure and the responsibility for the content remain with Dow Chemical Japan Ltd.)

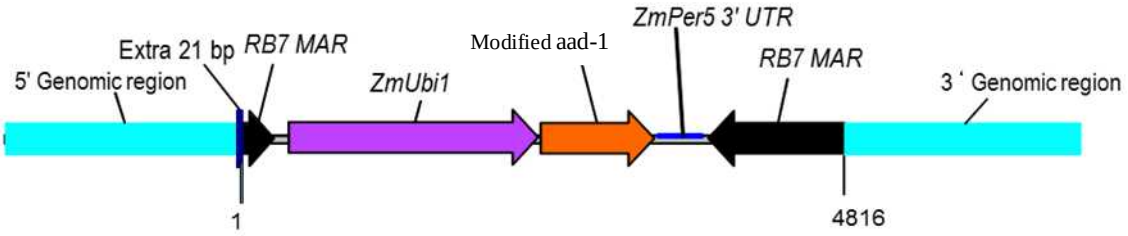

Figure 4. Structure diagram of the entire nucleic acid transferred to DAS40278

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

The transferring nucleic acids to the recipient organism was performed using the following method.

MON89034: Agrobacterium method
Cry1F line 1507: Particle gun method
NK603: Particle gun method
DAS40278: Whisker method[1]

3) Process of rearing of living modified organisms

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

The transformed cells were selected in the media added with the following:
MON89034: Paromomycin
Cry1F line 1507: Glufosinate
NK603: Glyphosate
DAS40278: Haloxyfop

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

For MON89034, *Agrobacterium* was removed by adding carbenicillin to the medium. It was confirmed that there are no residual *Agrobacterium* in MON89034 in the evaluation of the parent lines, in which MON89034 was transferred to the medium without carbenicillin and then there was no colonization of *Agrobacterium* in that medium. The nucleic acids were transferred to the recipient organisms of Cry1F line 1507 and NK603 and to the recipient organism of DAS40278 using the particle gun method and the whisker method, respectively. The agrobacterium method was not used.

[1] The embryonic suspension was obtained by liquid culture of the callus from immature embryos of Hi-II, the recipient maize. The linear DNA cut from the pDAS1740 with the restriction enzyme, *Fsp I*, and needle-like silicon carbide whisker fibers were added to the embryonic suspension and stirred, and then the silicon carbide whisker fibers created holes in the cells, which resulted in the transfer of the linear DNA to the recipient organism (Thompson *et al*., 1995).

(c) Process of rearing and pedigree trees of the following lines: cells to which the nucleic acid was transferred; the line in 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 Effects on Biological Diversity

For MON89034, R0 (regenerated plants) plants were bred with a conventional maize line LH172 to obtain LH172BC0F_1 plants. Based on the PCR method, LH172BC0F_1 plants were subsequently selected for the presence of the T-DNAI region and the absence of the T-DNAII region, and those containing the T-DNAII region were discarded.

Further selection was then performed for the selected individuals, based on the analysis of the transferred genes and the expression levels of the Cry1A.105 and modified Cry2Ab2 proteins. Subsequently, tests in the artificial climate chamber and the greenhouse were then performed and the actual resistance to pest insects and agronomic characters (morphological and growth characteristics, yield and productivity, pest insect sensitivity, etc.) were then examined in outdoor fields. MON89034 was selected upon comprehensive evaluation of these results.

For Cry1F line 1507, PCR analysis was done of leaf samples from the regenerated plant to transferred gene presence or absence. ELISA analysis was used to confirm expression of modified Cry1F protein. Insect resistance was then determined by assay on larval of European corn borer. T0 (regenerated plants) which demonstrated insect resistance were crossed with the same breeding lines to obtain seeds. Finally, Cry1F line 1507 was selected in a comprehensive manner based on the resistance to European corn borer and agronomic characters examined in outdoor field trials.

NK603 was crossed with commercial cultivars and various other cultivars of yellow dent corn and in 1997 started to be evaluated for line selection. From 1997 to 1999, morphological and growth characteristics were examined in a total of 103 fields. In addition, the modified CP4 EPSPS protein expression and transferred genes were analyzed, and finally, an excellent line was selected.

As for DAS40278, production of the modified AAD-1 protein was confirmed by spraying quizalofop, an aryloxyalkanoate herbicide to the regenerated plants (T0 generation). DAS40278 was selected based on comprehensive manner by evaluation of transgene analysis, confirmation of protein expression, the herbicide tolerance, and the agronomic characteristics in outdoor field trials conducted in US and Canada.

The status of application and approval of MON89034, Cry1F line 1507, NK603, DAS40278, and this stacked maize line in Japan is described below (Table 5. p. 21).

Table 5. The status of application and approval of MON89034, Cry1F line 1507, NK603, DAS40278 and this stacked maize line in Japan

	Food[1)]	Feed[2)]	Environment[3)]
MON89034	November 2007 Confirmed safety	October, 2007 Confirmed safety	January, 2008 Approved for Type I Use Regulation
Cry1F line 1507	July, 2002 Confirmed safety	March, 2003 Confirmed safety	March, 2005 Approved for Type I Use Regulation
NK603	March, 2001 Confirmed safety	March, 2003 Confirmed safety	November, 2004 Approved for Type I Use Regulation
DAS40278	May, 2012 Confirmed safety	June, 2010 Applied for safety confirmation	August, 2011 Published public comments results for Type I Use Regulation
This stacked maize line	2012 Scheduled for application	2012 Scheduled for notification	May, 2012 Applied

[1)] Food Sanitation Act

[2)] Act on Safety Assurance and Quality Improvement of Feeds

[3)] Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms

【Process of rearing of this stacked maize line】

This stacked maize line was developed from MON89034, Cry1F line 1507, NK603, and DAS40278 by crossing (Figure 5, p. 21).

Confidential information: not disclosed to unauthorized persons

Figure 5. Process of rearing of this stacked maize line

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

(a) Place where the replication product of transferred nucleic acid exists

It was confirmed that the transferred genes in MON89034, Cry1F line 1507, NK603, and DAS40278 existed on the chromosome.

(b) The number of copies of replication products of transferred nucleic acid and stability of its inheritance across multiple generations

As a result of Southern blot analysis on the parent lines, it was confirmed that one copy of respective target genes existed at a site on the chromosomes for MON89034 (Rice *et al.*, 2006), Cry1F line 1507 (In-house report 1), NK603 (Deng *et al.*, 1999), and DAS40278 (In-house report 2) by the evaluation of the parent lines. In the evaluation of the parent lines, Southern blott analysis on multiple generations also showed that the transferred genes were stably inherited to subsequent generations.

The results of the analysis of the nucleotide sequences of the transferred genes in MON89034 showed that the 5'-terminal region of P-*e35S*, which controls expression of the *cry1A.105* gene, and the neighboring right border region were substituted by the left border region within the T-DNA II region and the 5'-terminal region of P-*35S* controlling the expression of the *nptII* gene due to homologous recombination. However, the homologous recombination did not occur in the protein coding regions and normal Cry1A.105 protein expression was confirmed in individual tissues as to the Cry1A.105 protein coding region which was, the nearest open reading frame. Therefore, it was concluded that a new open reading frame was not generated by this homologous recombination.

The results of the analysis of the nucleotide sequences of nucleic acid transferred to Cry1F line 1507 showed that a part of the modified *cry1F* gene sequence, a part of the *pat* gene sequence, and a part of the *ORF25PolyA Terminator* sequence were contained in the 5'-terminal region, the 5'- and 3'-terminal regions, and the 3'-terminal region of the nucleic acid transferred, respectively. However, Northern blot analysis confirmed that transcription of these gene fragments into mRNA did not occur and that the gene fragments were not functional (In-house report 3).

In NK603, Southern blot analysis and the nucleotide sequence analysis of the 3'-terminal revealed that a 217 bp fragment of P-*Ract1* existed in the reverse

direction near the 3'-terminal of the transferred gene. As for the 217 bp fragment of P-*Ract1* near the 3'-terminal of the transferred gene in NK603, the strand-specific RT-PCR revealed a transcription product, which was thought to initiate from either the P-*Ract1* or the P-*e35S* of the transferred gene and to read through the NOS 3' terminator. However, since only the modified CP4 EPSPS protein was detected in NK603, it was thought that a stop codon upstream of the terminator was preserved in the read through transcription product (It was concluded that this reading through did not affect the safety evaluation and therefore it was approved for the Type I Use Regulation (provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them) by the Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment of Japan in November 2004, based on the Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms). In addition, in the transferred gene of NK603, the bases at positions 456 and 641 from the 5'-terminal of the coding region in the modified *cp4 epsps* gene induced by the P-*e35S* were changed from thymine (T) to cytosine (C), compared to the bases in the plasmid for expression in plants. It was revealed that the change of the base at position 456 was not associated with the change of the amino acid. However, in the modified CP4 EPSPS protein expressed by P-*e35S*, the change of the base at position 641 caused the amino acid change, leucine to proline, at position 214 from the N-terminal in the original CP4 EPSPS protein (this protein is hereinafter referred to as "L214P protein"). Regarding L214P protein, it is concluded that the structures and functions of the L214P protein and the modified CP4 EPSPS protein are comparable, because 1) proline at position 214 from the N-terminal was not one of the seven amino acids essential for activity of the EPSPS protein family, 2) this change of the amino acid did not affect the active site and the three-dimensional structure of the EPSPS protein, and 3) enzyme activity and immunoreactivity of the L214P and modified CP4 EPSPS proteins are comparable (Astwood *et al.*, 2001). The comparison of the database, in order to evaluate whether the L214P protein shared functionally important amino acid sequences with known contact allergens, revealed that the L214P protein did not share structurally similar sequences with the known contact allergens. It was concluded that the changes of the bases were observed in multiple generations and stably inherited to subsequent generations.

(c) The position relationship in the case of multiple copies existing in a chromosome

This item is not applicable because there is only one copy each of MON89034, Cry1F line 1507, NK603, and DAS40278.

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

The stability of expression of the parent lines was identified by the evaluation of the parent lines as follows.
MON89034: Confirming the expression of protein by western blotting analysis (Hartmann *et al.*, 2006).
Cry1F line 1507: Confirming the expression of protein by the ELISA (In-house report 4), the bioassay using *Lepidoptera* pests and the glufosinate herbicide-spraying test (Isolated field test report 1)
NK603: Confirming the expression of protein in multiple generations by the glyphosate-herbicide spraying test during the growth.
DAS40278: Confirming the expression of protein by the ELISA

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

The sequences of the nucleic acids transferred to MON89034, Cry1F line 1507, NK603, and DAS40278 do not contain any sequences that allow gene transmission. Therefore, it is unlikely that these genes would be transmitted through virus infection and/or any other routes to wild animals and wild plants.

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

In order to detect and identify MON89034, the DNA sequences of the transferred genes and the nearby regions of the plant genomes are used as primers. As a result, MON89034 is specifically detectable (Rice *et al.*, 2006).

For the detection and identification of Cry1F line 1507, the real-time quantitative PCR using the DNA sequences of the transferred genes and the nearby regions of the plant genomes as primers has been developed (JRC, 2005).

In order to detect and identify NK603, the DNA sequences of the transferred genes and the nearby regions of the plant genomes are used as primers. As a result, NK603 is specifically detectable (Cavato *et al.*, 2001).

In order to detect and identify DAS40278, the PCR using the DNA sequences of the transferred genes and the nearby regions of the plant genomes as primers has been developed (Dow AgroSciences LLC, 2009).

In order to detect and identify this stacked maize line, the above-mentioned methods must be applied to each grain of maize seeds.

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

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

This stacked maize line contains the following traits derived from individual parent lines.

MON89034: Resistance to the Lepidoptera pests due to Cry1A.105 protein and modified Cry2Ab2 protein derived from the transferred genes

Cry1F line 1507: Resistance to Lepidoptera pests due to modified Cry1F protein and tolerance to glufosinate due to PAT protein. The proteins are derived from the transferred genes

NK603: Tolerance to glyphosate due to modified CP4 EPSPS protein derived from the transferred genes

DAS40278: Tolerance to aryloxyalkanoate herbicides due to modified AAD-1 protein derived from the transferred genes

The possibility of the functional interaction of these proteins was examined in terms of proteins with resistance to pest insects and herbicides.

Functional interaction among proteins with resistance to pest insects

The Cry1A.105, modified Cry2Ab2 and modified Cry1F proteins exhibit insecticidal activity against Lepidoptera pests. Because the structures of the regions involved in specificity of insecticidal activity of these proteins are not changed in his stack maize line, it is not thought that insecticidal activity will not be affected. In addition, it is possible that the insecticidal activity of this stack maize line may increase additively, but it is difficult to think that a synergistic or antagonistic action would result from an interaction among the proteins in the stack.

Functional interaction among of proteins with tolerance to herbicides

The PAT, modified CP4 EPSPS, and modified AAD-1 proteins have high substrate specificity, and therefore, are not thought to change the metabolic system of recipient

organism. Each protein substrate is different and each involves an independent metabolic pathway. Therefore, it is difficult to think that these proteins will interact to produce an unexpected metabolite.

Functional interaction between proteins with resistance to pest insects and proteins with tolerance to herbicides

Since the proteins with resistance to pest insects and proteins with tolerance to herbicides have different functions, it is difficult to think that both types of proteins interact each other.

Based on the above, it is unlikely that the expressed proteins derived from respective parent lines interact with one another in this stacked maize line.

Therefore, differences in physiological and ecological properties between this stacked maize line and a maize line, the species of the taxonomy to which the recipient organism belongs were evaluated, based on the results of the individual examination of the parent lines, MON89034, Cry1F line 1507, NK603, and DAS40278.

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

The Biological Diversity Risk Assessment Report of respective parent lines was completed and confirmed that the following physiological or ecological properties were not different between the respective parent lines and their controls, non-recombinant maize. As for the information on the physiological or ecological properties, see the website of the Japan Biosafety Clearing House[2].

[2][MON89034、Cry1F line 1507、NK603]
1. In the website, https://ch.biodic.go.jp/bch/OpenSearch.do, input “Maize” in the box of “Name of Organism” and then select “Search/View.”
2. Select “Name of Organism-Maize” of the appropriate line.
3. Select “Attached documents.”
4. Select “Document 1.”

[DAS40278]
1. In the website, http://www.bch.biodic.go.jp/bch_3_1.html, select “Agriculture, Forestry and Fisheries-2011.”
2. Select “Outline of the application form-PDF” of the 47th appropriate line.

a Morphological and growth characteristics

b Cold-resistance and heat-resistance at the early stage of growth

c Wintering ability and summer survival of the mature plant

d Fertility and size of the pollen

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

f Crossability

g Productivity of harmful substances

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

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

(1) **Item-by-item assessment of Adverse Effects on Biological Diversity**

Maize resistant to Lepidoptera pests, and tolerant to glufosinate, glyphosate and aryloxyalkanoate herbicides (including the progeny lines which are isolated from the maize lines, MON89034, *B.t.* Cry1F maize line 1507, NK603 and DAS40278, that contain a combination of their respective transferred genes (except those already granted an approval regarding Type I Use Regulation)) (hereinafter referred to as "this stacked maize line") was developed with the following lines by crossing.

(a) Maize resistant to Lepidoptera pests, to which the *cry1A.105* gene coding the Cry1A.105 protein and the modified *cry2Ab2* gene coding the modified Cry2Ab2 protein were transferred (MON89034),

(b) Maize resistant to Lepidoptera pests, and tolerant to glufosinate herbicide, to which the modified *cry1F* gene coding the modified Cry1F protein and the *pat* gene coding the PAT protein (phosphinothricin acetyltransferase) were transferred (*B.t.* Cry1F maize line 1507),

(c) Maize tolerant to glyphosate herbicide, to which the modified *cp4 epsps* gene coding the modified CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase) was transferred (NK603), and

(d) Maize tolerant to aryloxyalkanoate herbicide, to which the modified *aad-1* gene coding the modified AAD-1 protein (aryloxyalkanoate dioxygenase) was transferred (DAS40278).

It was not thought that the respective Bt proteins (Cry1A.105, modified Cry2Ab2, and modified Cry1F proteins) derived from the genes transferred to this stacked line interact with one another to change the specificity of the insecticidal activity in these proteins, because the specificity regions of the individual proteins were not changed. In addition, the substrates and actions of the PAT, modified CP4 EPSPS, and modified AAD-1 proteins are different, their involved metabolic pathways are independent, and there has

been no report that Bt proteins exhibit enzyme activity. Therefore, it was concluded that these proteins did not interact to change the metabolic system of the recipient organism to produce an unexpected metabolite in this stacked line.

Based on the above, it was unlikely that these proteins derived from respective parent lines functionally interact with one another in the plant body of this stacked maize line, and therefore it was concluded that there were no trait changes to be evaluated, except having the traits which the parent line had.

The examination of the respective evaluation items has already been completed*. Based on the results of the examination, the conclusion described in the Biological Diversity Risk Assessment Report that the use of the respective parent lines in accordance with the Type I Use Regulation causes no Adverse Effect on Biological Diversity in Japan has been judged to be reasonable.

a. Competitiveness
b. Productivity of harmful substances
c. Crossability

* The results of the evaluation of the respective parent lines are available as described below.

[MON89034, Cry1F line 1507, MON88017, NK603, DAS40278]

1. In the website, https://ch.biodic.go.jp/bch/OpenSearch.do, input “Maize” in the box of “Name of Organism” and then select “Search/View.”
2. Select “Name of Organism-Maize” of the appropriate line.
3. Select “Attached documents.”
4. Select “Document 2.”

(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 stacked maize line, in accordance with the Type 1 Use Regulation, causes Adverse Effects on Biological Diversity in Japan. It was judged that the conclusion above made by the applicant is reasonable.

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