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

Name Monsanto Japan Limited
Applicant Seiichiro Yamane, President
Address 4-10-10, Ginza, Chuo-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Maize tolerant to drought, resistant to Lepidoptera, and tolerant to glyphosate herbicide (modified *cspB*, *cry1A.105*, modified *cry2Ab2*, modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (MON87460 × MON89034 × NK603, OECD UI: MON-8746Ø-4 × MON-89Ø34-3 ×MON-ØØ6Ø3-6) (including the progeny lines isolated from the maize lines, MON87460, MON89034, and NK603, that contain a combination of any of the transferred genes in the individual maize lines [except those already granted an approval regarding Type 1 Use Regulation])
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal, and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

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

5 1 Information concerning preparation of living modified organisms

The crossed progeny (OECD UI: MON-8746Ø-4 × MON-89Ø34-3 × MON-ØØ6Ø3-6) (hereinafter referred to as "this stack line maize") was developed through the crossing of the following three recombinant maize lines with the use of
10 traditional crossbreeding method. The three recombinant maize lines are: drought-tolerant maize (modified *cspB*, *Zea mays* subsp. *mays* (L.) Iltis) (MON87460, OECD UI: MON-8746Ø-4) (hereinafter referred to as "MON87460"); maize resistant to Lepidoptera (*cry1A.105*, modified *cry2Ab2*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034, OECD UI: MON-89Ø34-3) (hereinafter referred to as
15 "MON89034"); and maize resistant to glyphosate herbicide (modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (NK603, OECD UI: MON-ØØ6Ø3-6) (hereinafter referred to as "NK603"). Therefore, this stack line maize possesses the characteristics of these three parent recombinant maize lines, MON87460, MON89034, and NK603. This stack line maize is commercialized as a hybrid variety
20 (F1), and the grain harvested from this stack line maize is composed of combinations of the transferred genes in the individual parent lines of this stack line maize due to the genetic segregation. The information concerning preparation of MON87460, MON89034, and NK603 is explained individually in the following sections.

25 (1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the development of the parent lines MON87460, MON89034, and NK603
30 are shown individually in Figure 1 to Figure 3 (p. 4–6) and Table 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions to Table 3 (p. 7–13).

2) Function of component elements

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

Functions of individual component elements of donor nucleic acid that were used

for the production of MON87460, MON89034, and NK603 are shown individually in Table 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions to Table 3 (p. 7–13). Detailed information of the target gene, that is, the modified cold shock protein B (modified *cspB*) gene, the *cry1A.105* gene, the modified *cry2Ab2* gene, and the modified *cp4 epsps* gene, is shown individually inTable 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functionsTable 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions to Table 3 (p.7–13).

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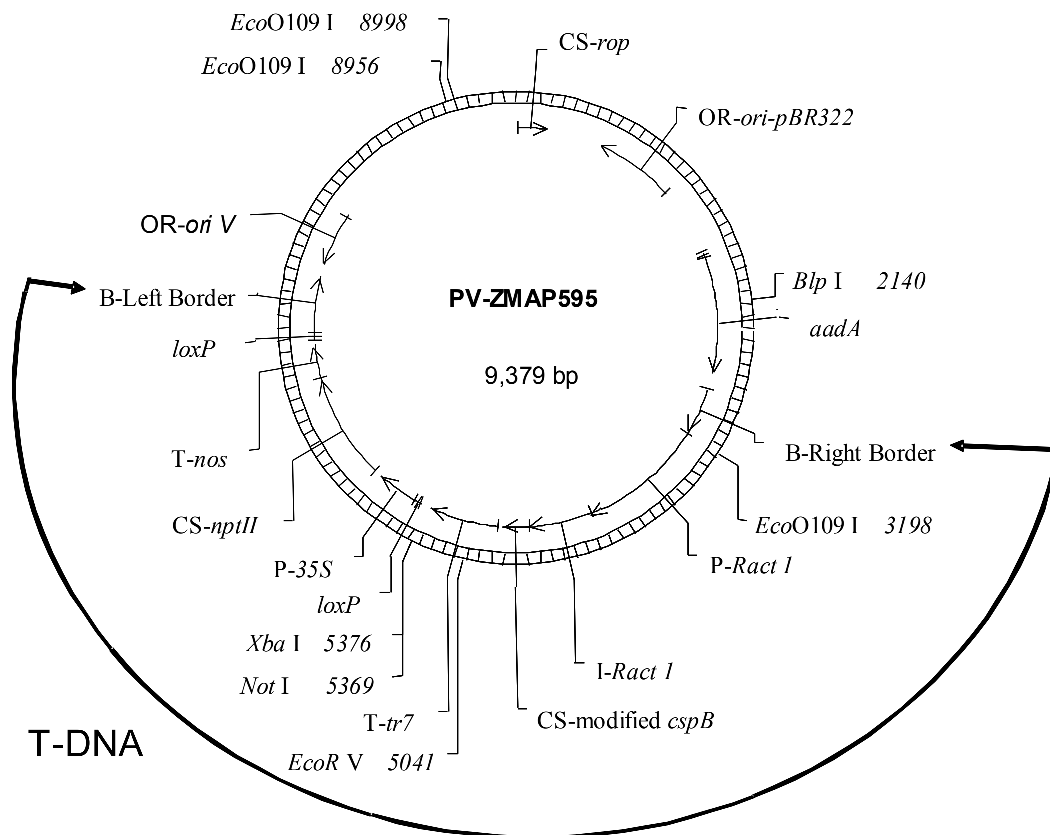

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Figure 1 Map of the plasmid PV-ZMAP595 used for the development of MON87460[1]

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

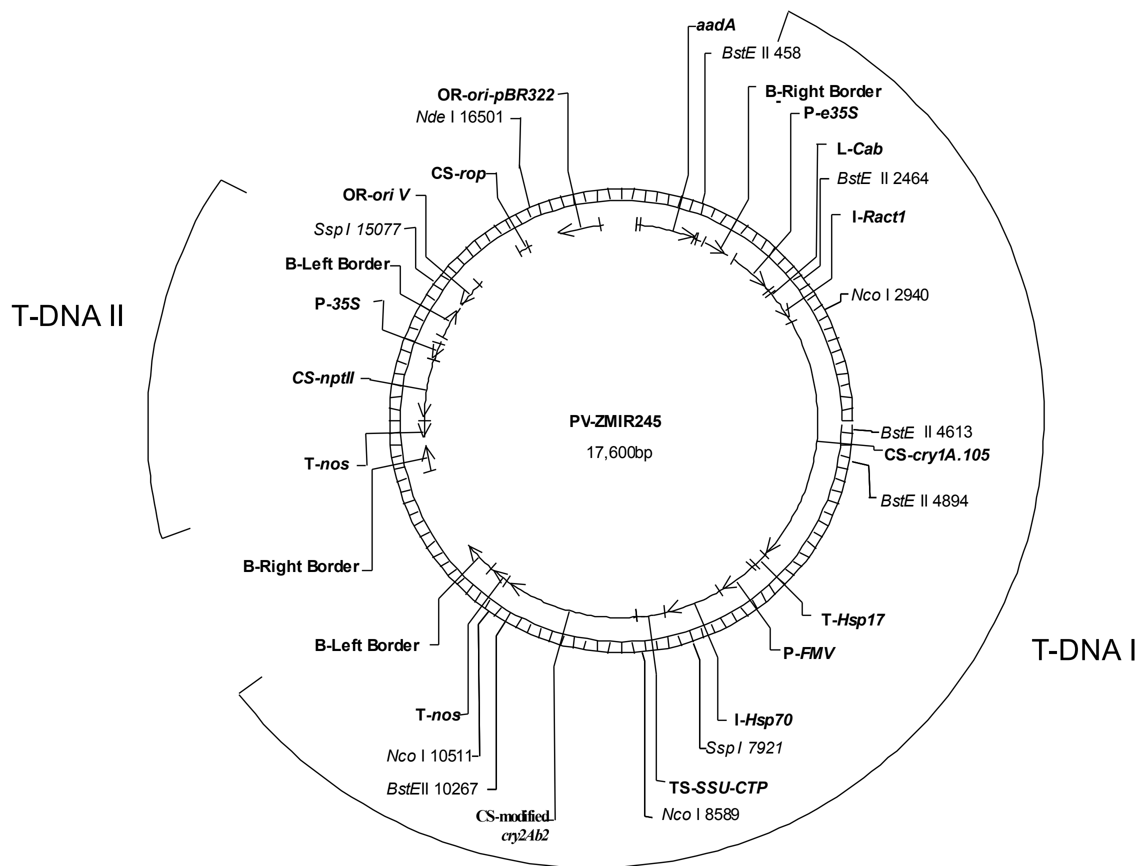

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Figure 2 Map of the plasmid PV-ZMIR245 used for the development of MON89034[2]
In the process of the rearing of MON89034, those individuals were selected that contain the T-DNA I region shown above but do not contain the T-DNA II region.

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

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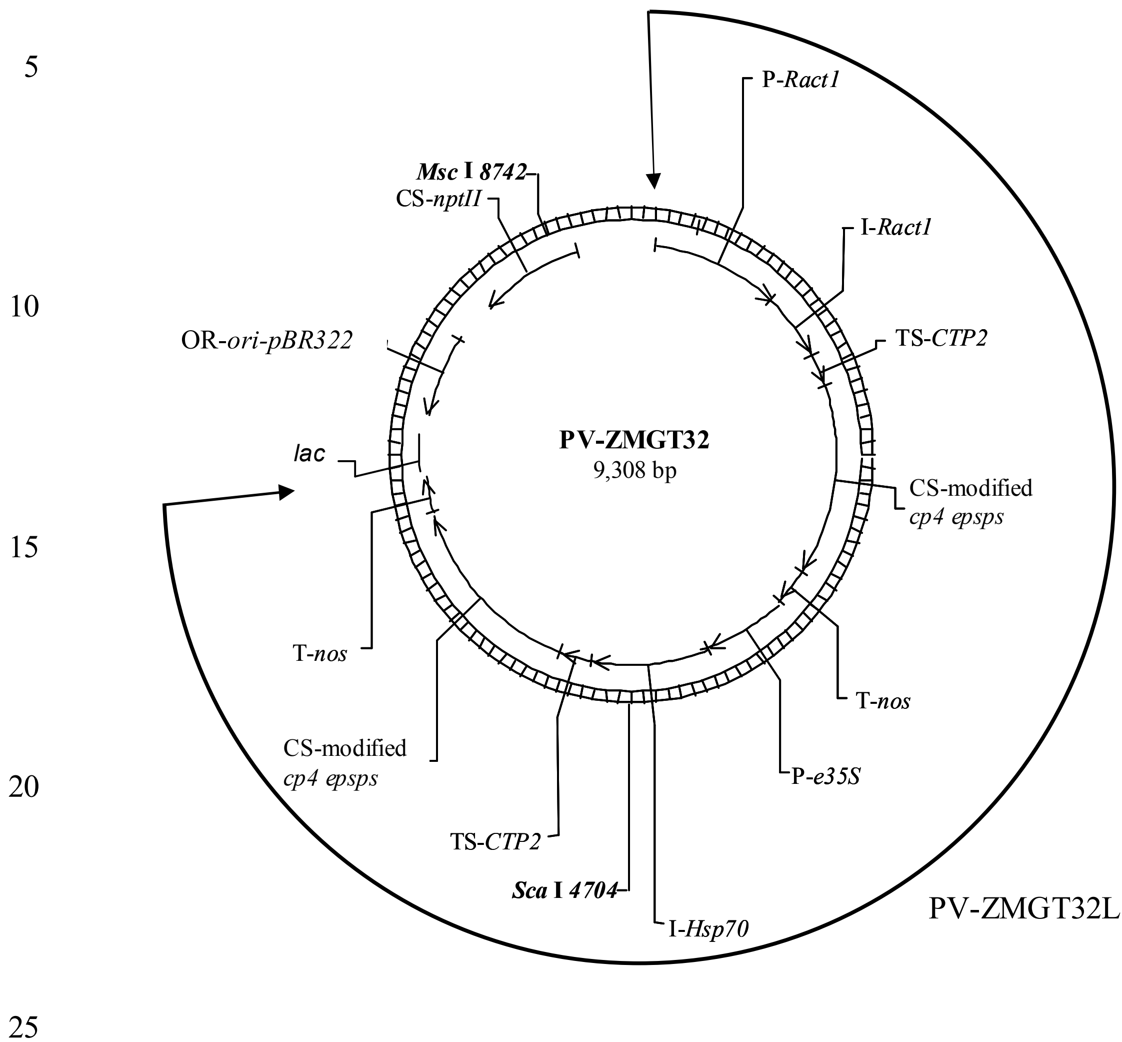

Figure 3 Map of the plasmid PV-ZMGT32 used for the development of NK603[3]

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

Table 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions[4]

Component elements	Origin and function
Plasmid backbone region	
Intervening Sequence	Sequence used for DNA cloning
CS [Note 1]-*rop*	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *Escherichia coli* (Giza and Huang, 1989).
Intervening Sequence	Sequence used for DNA cloning
OR [Note 2]-*ori-pBR322*	The replication origin region isolated from pBR322. This confers autonomous replication ability in *E. coli* (Sutcliffe, 1979).
Intervening Sequence	Sequence used for DNA cloning
aadA	Bacterial promoter, coding sequence, and terminator for the 3"(9)-O-nucleotidyltransferase, the aminoglycoside modified enzyme, derived from transposon Tn7. Confers resistance to spectinomycin or streptomycin (Fling et al., 1985). (GenBank accession X03043)
Intervening Sequence	Sequence used for DNA cloning
T-DNA region	
B [Note 3]-Right Border	A 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 plant genome (Depicker et al., 1982; Zambryski et al., 1982).
Intervening Sequence	Sequence used for DNA cloning
P [Note 4]-*Ract1*	Promoter and leader sequence of actin gene, derived from *Oryza sativa* (rice) (McElroy et al., 1990). Constantly induces transcription of the target gene in the entire tissue of plant.
I [Note 5]-*Ract1*	Intron of actin gene, derived from *O. sativa* (rice) (McElroy et al., 1991). Activates the expression of the target gene.
Intervening Sequence	Sequence used for DNA cloning

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

Table 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions (continued)

T-DNA region (continued)	
CS- modified *cspB*	Gene encoding modified cold shock protein B (modified CSPB) derived from *Bacillus subtilis* (Willimsky et al., 1992). Detail is shown in I-2-(1)-2)-(b).
Intervening Sequence	Sequence used for DNA cloning
T [Note 6]-*tr7*	3' untranslated region of transcription 7 gene derived from *A. tumefaciens*. It induces polyadenylation (Dhaese et al., 1983).
Intervening Sequence	Sequence used for DNA cloning
loxP [Note 7]	Recombination site of bacteriophage P1. It functions in pairs. When Cre recombinase (DNA recombination enzyme) recognizes the two *lox P* sites, it removes the DNA region existing between them (Russell et al., 1992).
Intervening Sequence	Sequence used for DNA cloning
P-*35S*	35S promoter region of cauliflower mosaic virus (CaMV) (Odell et al., 1985). Involved in the constant expression of the target gene in the entire tissue of plant body.
Intervening Sequence	Sequence used for DNA cloning
CS-*nptII*	A gene derived from *E. coli* transposon Tn5 (Beck et al., 1982). Encodes neomycin phosphotransferase type II and confers resistance to neomycin and kanamycin on plants. Used as a marker to select the transgenic plant during the gene transfer (Fraley et al., 1983).
Intervening Sequence	Sequence used for DNA cloning
T-*nos*	3' untranslated region of nopaline synthase (nos) derived from *A. tumefaciens* T-DNA. Terminates transcription of mRNA and induces polyadenylation (Bevan et al., 1983).
Intervening Sequence	Sequence used for DNA cloning
loxP	Recombination site of bacteriophage P1. It functions in pairs. When Cre recombinase (DNA recombination enzyme) recognizes the two *lox P* sites, it removes the DNA region existing between them (Russell et al., 1992).

Table 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions (continued)

T-DNA region (continued)	
Intervening Sequence	Sequence used for DNA cloning
B-Left Border	A 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 plant genome (Barker et al., 1983).
Plasmid backbone region	
Intervening Sequence	Sequence used for DNA cloning
OR-*ori V*	The replication origin region isolated from the broad-host range plasmid RK2. Permits autonomous replication of vector in *Agrobacterium* (Stalker et al., 1981).
Intervening Sequence	Sequence used for DNA cloning

Note [1]CS – Coding Sequence
5 Note [2]OR – Origin of Replication
Note [3]B – Border
Note [4]P – Promoter
Note [5]I – Intron
Note [6]T – 3' nontranslated transcriptional termination sequence and polyadenylation signal sequences.
10 Note [7]*loxP* – The *nptII* gene is used as a marker to select transformants of the MON87460 strain. At the time of the MON87460 strain development, the European Food Safety Authority (EFSA, a safety evaluation authority for genetically modified crops in the EU) and other organizations were promoting the development and use of new selection methods to replace antibiotic resistance markers. In this regard, MON87460 was designed to remove *nptII* gene cassette by using *loxP*
15 modified sites that are recognized by Cre recombinase. Later, EFSA announced that the risk of *nptII* gene in genetically modified crops affecting human and livestock health is very low (EFSA, 2004), and therefore the *nptII* gene cassette was not removed from the MON87460 strain.

Table 2 Component elements of plasmid PV-ZMIR245 that were used for the production of MON89034, and their origins and functions[5]

Component elements	Origin and function
T-DNA I region	
B [Note 1]-Right Border	A 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 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 9bp leader sequence, containing double enhancer regions (Kay et al., 1987). Involved in the constant expression of the target gene in the entire tissue of plant body.
L [Note 3]-*Cab*	5'-terminal untranslated leader region of wheat chlorophyll a/b binding protein. Activates the expression of target gene (Lamppa et al., 1985).
I [Note 4]-*Ract1*	Rice (*O. sativa*) actin gene intron (McElroy et al., 1991). Activates the expression of the target gene.
CS [Note 5]-*cry1A.105*	A gene that encodes the Cry1A.105 protein. Detail is shown in I-2-(1)-2)-(b).
T [Note 6]-*Hsp17*	3' untranslated region of wheat heat shock protein 17.3. Terminates transcription and induces polyadenylation (McElwain and Spiker, 1989).
P-*FMV*	35S promoter derived from Figwort Mosaic Virus (Rogers, 2000). Involved in the constant expression of the target gene in the entire tissue of plant body.
I-*Hsp70*	First intron of maize heat shock protein 70 gene (Brown and Santino, 1995). Activates the expression of the target gene.
TS [Note 7]-*SSU-CTP*	Transit peptide of small subunit of ribulose 1,5-carboxylase diphosphate of maize, including the first intron sequence (Matsuoka et al., 1987). Transfers downstream-connected protein to plastid.
CS- modified *cry2Ab2*	A gene that encodes the modified Cry2Ab2 protein derived from *Bacillus thuringiensis* (Widner and Whitely, 1989). In order to add a restriction enzyme cutting site for cloning purposes, a single aspartic acid is transferred after the methionine at the N-terminal compared to the wild-type Cry2Ab2 protein.
T-*nos*	3' untranslated region of nopaline synthase (nos) derived from *A. tumefaciens* T-DNA. Terminates transcription of mRNA and induces polyadenylation (Bevan et al., 1983).
B-Left Border	A 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 plant genome (Barker et al., 1983).

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

Table 2 Component elements of plasmid PV-ZMIR245 that were used for the production of MON89034, and their origins and functions (continued)

Component elements	Origin and function
T-DNA II region	
B-Right Border	A DNA fragment containing the right border sequence (24 bp) of nopaline type T-DNA, derived from *Agrobacterium tumefaciens*. The right border sequence is used as the initiation point of T-DNA transfer from *A. tumefaciens* to plant genome (Depicker et al., 1982; Zambryski et al., 1982).
T-*nos*	3' untranslated region of nopaline synthase (nos) gene derived from *A. tumefaciens* T-DNA. Terminates transcription of mRNA and induces polyadenylation (Bevan et al., 1983).
CS-*nptII*	A gene derived from *E. coli* transposon Tn5 (Beck et al., 1982). Encodes neomycin phosphotransferase type II and confers resistance to kanamycin on plants. Used as a marker to select the transgenic plant during the gene transfer (Fraley et al., 1983).
P-*35S*	35S promoter region of cauliflower mosaic virus (CaMV) (Odell et al., 1985). Involved in the constant expression of the target gene in the entire tissue of plant body.
B-Left Border	A 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 plant genome (Barker et al., 1983).
Plasmid backbone region	
OR [Note 8]-*ori V*	The replication origin region isolated from the broad-host range plasmid RK2. Permits autonomous replication of vector 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*	The replication origin region isolated from pBR322. Permits autonomous replication of vector in *E. coli* (Sutcliffe, 1979).
aadA	Bacterial promoter, coding sequence, and terminator for the 3"(9)-O-nucleotidyltransferase, the aminoglycoside modified enzyme, derived from transposon Tn7. Confers resistance to spectinomycin or streptomycin (Fling et al., 1985).

[Note 1] B – border
5 [Note 2] P – promoter
[Note 3] L – leader
[Note 4] I – intron
[Note 5] CS – coding sequence
[Note 6] T – transcript termination sequence
10 [Note 7] TS – targeting sequence
[Note 8] OR – Origin of Replication

Table 3 Component elements of the plasmid PV-ZMGT32L that were used for the production of NK603, and their origins and functions[6]

Component elements	Origin and function
Modified *cp4 epsps* gene cassette (a)	
P [Note 1]-*Ract1*	Promoter region of actin 1 gene derived from rice (*O. sativa*). It makes target genes expressed (McElroy et al., 1990). Involved in the constant expression of the target gene in the entire tissue of plant body.
I [Note 2]-*Ract1*	Rice (*O. sativa*) actin gene intron (McElroy et al., 1991). Activates the expression of the target gene.
TS [Note 3]-*CTP 2*	A sequence encoding the N-terminal chloroplast transit peptide of EPSPS protein in the *Arabidopsis thaliana epsps* gene (Klee et al., 1987). Transfers target proteins from cytoplasm to chloroplast.
CS [Note 4]- modified *cp4 epsps*	5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene derived from *Agrobacterium* CP4 strain (Padgette et al., 1996; Barry et al., 1997). To enhance the expression in plants, the second amino acid from the N-terminal in the wild-type CP4 EPSPS protein is modified to leucine, instead of serine.
T [Note 5]-*nos*	3' untranslated region of nopaline synthase (nos) derived from *A. tumefaciens* T-DNA. Terminates transcription of mRNA and induces polyadenylation (Bevan et al., 1983).
Modified *cp4 epsps* gene cassette (b)	
P-*e35S*	Cauliflower mosaic virus (CaMV) 35SRNA (Odell et al., 1985) promoter and 9bp leader sequence, containing double enhancer regions (Kay et al., 1987). Involved in the constant expression of the target gene in the entire tissue of plant body.
I-*Hsp70*	Intron of heat shock protein 70 gene from maize. ZmHsp70 intron is used to enhance the expression of foreign genes in plants (Rochester et al., 1986).
TS-*CTP2*	A sequence encoding the N-terminal chloroplast transit peptide of EPSPS protein in the *Arabidopsis thaliana epsps* gene (Klee et al., 1987). Transfers target proteins from cytoplasm to chloroplast.
CS-modified *cp4 epsps*	5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) gene derived from *Agrobacterium* CP4 strain (Padgette et al., 1996; Barry et al., 1997). To enhance the expression in plants, the second amino acid from the N-terminal in the wild-type CP4 EPSPS protein is modified to leucine, instead of serine.
T-*nos*	3'-untranslated region of nopaline synthase (*nos*) gene derived from *A. tumefaciens* T-DNA. Terminates transcription of mRNA and induces polyadenylation (Bevan et al., 1983).

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

Table 4 Component elements of the plasmid PV-ZMGT32L that were used for the production of NK603, and their origins and functions[7] (continued)

Others (not existing in plant body)	
lac	Consists of partial coding sequence for *lacI* (Farabaugh, 1978), *lac* promoter (Dickson et al., 1975) and partial coding sequence for *lacZ*. Hydrolyzes lactose and expresses β-galactosidase used as a selective marker (Shuman and Silhavy, 2003).
OR [Note 6]-*ori- pBR 322*	The replication origin region isolated from pBR322. Permits autonomous replication of vector in *E. coli* (Sutcliffe, 1979).
nptII	A gene derived from *E. coli* transposon Tn5 (Beck et al., 1982). Encodes neomycin phosphotransferase type II and confers resistance to kanamycin to plants. Also used as a marker to select vectors in *E. coli* or other bacteria (Herrmann et al., 1978).

[Note 1] P – promoter
5 [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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[7]All the rights pertinent to the information in the table above and the responsibility for the contents rest upon Monsanto Japan Limited.

(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

5 **[Modified cold shock protein B (modified CSPB)]**

The modified CSPB expressed in MON87460 is derived from a soil bacterium *Bacillus subtilis*.

Studies have shown that the modified CSPB binds to RNA and disrupts the secondary structure of the RNA. It is also known that the modified CSPB is localized
10 in the nucleus and cytoplasm and is especially abundant in the meristem tissues. These findings are also recognized in proteins containing bacterial cold shock protein (CSP) and plant cold shock domain (CSD) (Fusaro et al., 2007; Sasaki et al., 2007; Chaikam and Karlson, 2008), suggesting that the modified CSPB acts on stress response pathways to confer drought tolerance on MON87460. Photosynthesis rate,
15 stomatal conductance, and quantum efficiency at photosystem II of MON87460 were improved compared with the non-recombinant control maize under water-limited conditions in an environment-controlled greenhouse. The results of the field tests indicated that MON87460 efficiently distributes photosynthates to ears under water-limited conditions, which results in higher ear dry weight, grain count per ear,
20 yield, and harvest index than the non-recombinant control maize. These findings together suggest that the modified CSPB functions as an RNA chaperone under water-limited conditions and hence, maintains the growth and development of MON87460, resulting in a reduction of yield loss.

The modified *cspB* gene has been reported to show tolerance to various
25 environmental stresses (Castiglioni et al., 2008). However, the results of the studies for tolerance of matured plants to drought, low-temperature stress, high-temperature stress, and salt stress conclude that MON87460 obtains drought tolerance through expression of the modified CSPB but does not have tolerance to low temperatures, high temperatures, and salt.

30 The amino acid sequence of the modified CSPB is identical to that in the wild-type CSPB derived from *B. subtilis* (a bacterium widely distributed in soil), with the exception of a single amino acid change from leucine to valine at the second position from the N-terminal. The change was necessary to add restriction enzyme cutting site for cloning purposes.

35

[NPTII protein]】

The *nptII* gene, an antibiotic tolerance marker gene introduced to select transformants, is derived from an *E. coli* transposon Tn5. The encoded NPT II protein inactivates aminoglycoside antibiotics (e.g., kanamycin) through

phosphorylation, conferring tolerance to these antibiotics on the transformant. As a result, application of kanamycin to media will enable selection of transformed cells (Beck et al., 1982; Nap et al., 1992; Shaw et al., 1993).

5 —Insecticidal proteins[8]—

[Cry1A.105 protein]

The Cry1A.105 protein, which is encoded by the *cry1A.105* gene used for the development of MON89034, is a chimeric Bt protein composed of Domains I and II of the Cry1Ab protein, Domain III of the Cry1F protein, and C-terminal Domain of the Cry1Ac protein, and it has been developed to enhance insecticidal activity against target pest insects by combining the different Domains of the Bt protein.

In order to investigate the insecticidal spectrum of the Cry1A.105 protein, the Cry1A.105 protein was added to artificial feeds, which were given to 15 different species of insects including 5 insects of the order Lepidoptera. As a result, the Cry1A.105 protein exhibited insecticidal activity against the larvae of Corn earworm (*Helicoverpa zea*) (MacRae et al., 2005), Black cutworm (*Agrotis ipsilon*) (MacRae, 2005), Fall armyworm (*Spodoptera frugiperda*) (MacRae, 2005), Southwestern corn borer (*Diatraea grandiosella*) (MacRae, 2005), and European corn borer (*Ostrinia nubilalis*) (MacRae et al., 2006a), which are the major pest insects of order Lepidoptera for maize, though it did not exhibit any insecticidal activity against honeybee (Richards, 2006a; Richards, 2006b), ladybug (Paradise, 2006a), and other beneficial insects except insects of the order Lepidoptera.

Based on the above results, it was confirmed that the Cry1A.105 protein exhibits selective insecticidal activity against only insects of the order Lepidoptera similarly to the Cry1Ab protein, the Cry1F protein, and the Cry1Ac protein, which are the component elements of Cry1A.105, and it does not possess any insecticidal activity against other species of insects.

30 **[Modified Cry2Ab2 protein]**

In order to investigate the insecticidal spectrum of the modified Cry2Ab2 protein,

[8]The Bt protein, produced by *Bacillus thuringiensis*, a gram-positive bacterium existing universally in soil, is known to bind to the specific receptors in the midgut epithelium of the target pest insects and form cation-selective pores in the cells and, as a result, inhibit the digestive process, thereby providing insecticidal activity (Hofmann et al., 1988; Slaney et al., 1992; Van Rie et al., 1990). In addition, several studies have shown that the Bt protein is composed of several Domains and what functions individual Domains possess. It has been revealed that the Bt protein is composed of Domains I, II, and III and C-terminal Domain, and Domain I is involved in formation of cation-selective pores to inhibit the digestive process, Domain II is involved in recognition of specific receptors, Domain III is involved in binding to receptors, and C-terminal Domain is involved in the crystal structure of the Bt protein (de Maagd et al., 2001; Masson et al., 2002).

the modified Cry2Ab2 protein was added to artificial feeds, which were given to 15 different species of insects including 4 insects of the order Lepidoptera. As a result, the modified Cry2Ab2 protein exhibited insecticidal activity against the larvae of Corn earworm (MacRae et al., 2006a), Fall armyworm (MacRae et al., 2006b), and European corn borer (MacRae et al., 2006a) among the 4 species of major pest insects of the order Lepidoptera used in the investigation and not against Black cutworm (MacRae et al., 2006b). Also, the modified Cry2Ab2 protein did not exhibit any insecticidal activity against honeybee (Maggi, 2000b; Maggi, 2000a), ladybug (Paradise, 2006b), and other beneficial insects except insects of the order Lepidoptera; therefore, it was confirmed that the modified Cry2Ab2 protein offers specific insecticidal activity against only insects of the order Lepidoptera and not against other species of insects.

The modified Cry2Ab2 protein, which is encoded by the modified *cry2Ab2* gene, has a single aspartic acid introduced after the methionine at the N-terminal compared to the wild-type Cry2Ab2 protein. The change was necessary to add restriction enzyme cutting site for cloning purposes.

[Modified CP4 EPSPS protein]

Plants treated with glyphosate wither and die, since the 5-enol-pyruvylshikimate-3-phosphate synthase (EPSPS) (E.C.2.5.1.19) is inhibited and the synthesis of aromatic amino acids essential for synthesis of proteins is interrupted. The modified *cp4 epsps* gene, the target gene of NK603, produces the modified CP4 EPSPS protein that has high tolerance to the glyphosate herbicide. The activity of the modified CP4 EPSPS protein produced by the modified *cp4 epsps* gene is not inhibited even under the presence of glyphosate, and thus the recombinant plants that express this protein have normal functions of shikimate synthesis pathway and can grow.

The modified *cp4 epsps* gene has the nucleotide sequence of the wild-type *cp4 epsps* gene modified to enhance the expression level in plants without changing the functional activity of the wild-type CP4 EPSPS protein, with only a single modification introduced to the amino acid sequence: the second amino acid from the N-terminal is modified to leucine, instead of serine. Two modified *cp4 epsps* gene cassettes were transferred to NK603 in order to enhance tolerance to the glyphosate herbicide.

In order to investigate whether the modified CSPB protein, the NPTII protein, the Cry1A.105 protein, the modified Cry2Ab2 protein, and the modified CP4 EPSPS protein expressed in the parent lines share any functionally important amino acid sequences with known contact allergens, they were compared with the allergens in

the database (including AD8, AD 2009, GenBank, EMBL, PIR, and SwissProt). Results showed they did not share structurally related sequences with known allergens.

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

[Modified CSPB]

Bacteria-derived CSPs including CSPB non-specifically bind to RNA and function as RNA chaperones (Herschlag, 1995; Jiang et al., 1997). As a result, CSP plays a role in maintaining translation under conditions where translation is inhibited otherwise (Graumann et al., 1997). CSPB does not have functions to directly induce transcription (Schindler et al., 1999; Weber et al., 2001), and there is no report showing that CSPB has enzymatic activity.

Thus, it is unlikely that the modified CSPB expressed in MON87460 acts as an enzyme to produce new metabolites.

[NPTII protein]

NPTII protein is an enzyme that catalyzes phosphorylation reaction of hydroxyl group in aminoglycosides contained in aminoglycoside antibiotics (Shaw et al., 1993). NPTII protein is reported to have association only in phosphorylation reactions in limited aminoglycoside antibiotics, including neomycin, kanamycin, paromomycin, ribostamycin, and butirosin (Price et al., 1974; Davies and Smith, 1978; Davies, 1986). A study of structural activities of NPTII protein demonstrated that the protein cannot use aminoglycoside antibiotics as a substrate if there are any minute changes (e.g., removal of the hydroxyl group, modification of the amino group) in the aminoglycoside structure in the aminoglycoside antibiotics (Price et al., 1974).

Thus, it is unlikely that expression of NTPII protein in MON87460 results in production of new metabolites.

[Cry1A.105 protein, modified Cry2Ab2 protein]

The Cry1A.105 protein and the modified Cry2Ab2 protein are crystalline insecticidal proteins called Bt proteins derived from *B. thuringiensis*. For the mechanism of insecticidal activity by the Bt proteins, many studies have been made (OECD, 2007), and there is no report available to date that the Bt proteins possess any other functions. Therefore, it is considered unlikely that the Bt proteins possess any enzymatic activity and they would affect the metabolic system of the recipient organism.

[Modified CP4 EPSPS protein]

The EPSPS, identical functionally to the modified CP4 EPSPS protein, is an enzyme protein that catalyzes the shikimate pathway for the biosynthesis of aromatic amino acid. However, it is not a rate-determining enzyme in the pathway, and as such, it is not considered that enhanced EPSPS activity would increase the concentration of aromatic amino acids, the end products of this pathway. In addition, EPSPS is known to react specifically with its substrates, phosphoenolpyruvic acid (PEP) and shikimate-3-phosphate (S3P) (Gruys et al., 1992). It is also known to react with shikimate, an analogue of SP3, but the reactivity with shikimate is only one–two millionth of the reactivity with S3P, when compared in terms of specificity constant k_{cat}/K_m (Gruys et al., 1992). Thus, it is unlikely that shikimate reacts as the substrate of EPSPS in any living organism. Consequently, it is considered unlikely that the modified CP4 EPSPS protein would affect the metabolic system of the recipient organism.

(2) Information concerning vectors

1) Name and origin

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

MON87460: PV-ZMAP595 assembled from plasmids including the vector pBR322 (Sutcliffe, 1979) derived from *E. coli*

MON89034: PV-ZMIR245 assembled from plasmids including the vector pBR322 (Sutcliffe, 1979) derived from *E. coli*

NK603: PV-ZMGT32 assembled from plasmids including the vector pUC119 (Vieira and Messing, 1987) derived from *E. coli*

2) Properties

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

The total number of base pairs in the plasmid vectors used for the production of parent lines is as follows.

MON87460: PV-ZMAP595; 9,379 bp

MON89034: PV-ZMIR245; 17,600 bp

NK603: PV-ZMGT32; 9,308 bp

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

5 The following antibiotic resistance genes were used as selective markers.

MON87460: The *nptII* gene to confer resistance to aminoglycoside antibiotics including kanamycin and neomycin, and the *aadA* gene to confer resistance to spectinomycin and streptomycin

MON89034: The *aadA* gene to confer resistance to spectinomycin and streptomycin

10 NK603: The *nptII* gene to confer resistance to aminoglycoside antibiotics including kanamycin and neomycin

None of these antibiotic resistance genes have been transferred to the recipients, except for *nptII* gene that has been transferred to MON87460.

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

The infectivity of PV-ZMAP595, PV-ZMIR245, and PV-ZMGT32 is not known.

(3) Method of preparing living modified organisms

20

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

25 The component elements of the plasmid vectors PV-ZMAP595, PV-ZMIR245, and PV-ZMGT32 transferred to the recipient organisms for development of MON87460, MON89034, and NK603, respectively, are listed in Table 1 Component elements of the plasmid PV-ZMAP595 that were used for the production of MON87460, and their origins and functions to Table 3 (p. 7–13), and the positions of the component elements of the donor nucleic acid and sites cleaved by restriction enzymes in the vector are shown in Figure 1 to Figure 3 (p. 4–6).

30

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

35 Transferring nucleic acid into the recipient organism was based on the following method.

MON87460: The T-DNA region of the plasmid vector PV-ZMAP595 was transferred by the *Agrobacterium* method.

MON89034: The T-DNA I and T-DNA II regions of the plasmid vector

NK603: PV-ZMGT32L, which is a DNA fragment of the plasmid vector PV-ZMGT32, was transferred by the particle gun method.

3) Process of rearing of living modified organisms

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

5 Selection of transformed cells was made on the medium containing the followings.

MON87460: Paromomycin
MON89034: Paromomycin
NK603: Glyphosate

10

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

In the development of MON87460 and MON89034, *Agrobacterium* was removed 15 by adding carbenicillin to the medium. Absence of remaining *Agrobacterium* in MON87460 and MON89034 was confirmed by transferring MON87460 or MON89034 to the carbenicillin-free medium and then observing that no colony of *Agrobacterium* is formed on the medium. In the development of NK603, nucleic acid was transferred into the recipient organism by the particle gun method and not by the 20 *Agrobacterium* method.

(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 25 tests; and the line used for collection of other necessary information for assessment of Adverse Effect on Biological Diversity

In the development of MON87460, transformed R_0 (regenerated plants) was bred with a conventional maize line LH59 and then self-crossed. The subsequent R_1 plants 30 were screened for the expression of the modified CSPB, tolerance to kanamycin, and homozygosity of the inserted gene. Only the progeny of the screened plants were subjected to the analysis of the inserted genes and morphological assessments, and the results were used to select MON87460 line as the final commercial line.

35 In the development of MON89034, R_0 (regenerated plants) plants were bred with a conventional maize line LH172 to obtain LH172BC0F$_1$ plants. Based on the PCR method, the LH172BC0F$_1$ plants were subsequently selected for the presence of the T-DNA I region and the absence of T-DNA II region, and those containing the T-DNA II region were discarded. Regarding the selected individuals, further

selection was carried out based on the analysis of transferred genes and the expression levels of the Cry1A.105 protein and the modified Cry2Ab2 protein. Tests in a climate chamber and greenhouse were then carried out, and actual pest insect resistance and agronomic characters (e.g., morphological and growth characteristics, yield-related characteristics, and sensitivity to disease or pest insects) were examined in outdoor field tests. MON89034 was selected upon comprehensive evaluation of these results.

For NK603, commercial cultivars of yellow dent corn were crossed with a wide variety of cultivars, evaluation for line selection started in 1997, and field experiments were carried out regarding morphological and growth characteristics at a total of 103 field sites from 1997 to 1999. In addition, analyses for expression of the modified CP4 EPSPS protein and the transferred gene were conducted, and the final excellent line was selected.

[Process of rearing of MON87460 × MON89034 × NK603]

This stack line maize is an F1 hybrid developed using inbred lines of MON87460, MON89034, and NK603 as parents (Figure 4, p.22).

5

10

15 [Confidential: Not made available or disclosed to unauthorized person]

20

25 Figure 4 Process of rearing of this stack line maize

(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

5

It has been confirmed that the transferred genes in MON87460, MON89034, and NK603 exist on the maize genome.

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

10

[MON87460]

A Southern blot analysis of the transferred genes confirmed that one copy of the transferred gene region exists at one site in the genomic DNA of MON87460. It was also confirmed by Southern blot analysis on multiple generations that the transferred genes are inherited stably by offspring.

15

Analysis of the base sequence of MON87460 revealed that the right border region of the transferred gene region (2,816-3,172 bp of PV-ZMAP595) and the upstream 733 bp of the subsequent P-Ract1 region (3,205-3,937 bp of PV-ZMAP595) were missing.

20

[MON89034]

A Southern blot analysis of the transferred genes confirmed that one copy of the transferred gene region exists at one site in the genomic DNA of MON89034. It was also confirmed by Southern blot analysis on multiple generations that the transferred genes are inherited stably by offspring.

25

As a result of analysis on the nucleotide sequence of the transferred genes in MON89034, it was found that the 5'-terminal region of P-*e35S* controlling the expression of the *cry1A.105* gene and the neighboring right border region have been replaced by the left border region in 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, this homologous recombination did not take place in the protein encoding regions, even in the Cry1A.105 protein encoding region, the nearest open reading frame, which has been confirmed by the normal expression of the Cry1A.105 protein in individual tissues. Consequently, it was concluded that this homologous recombination could not cause formation of any new open reading frame.

30

35

[NK603]

A Southern blot analysis of the transferred genes confirmed that one copy of the transferred gene region composed of the modified *cp4 epsps* gene cassettes (a) and (b) exists at one site in the genomic DNA of NK603. It was also confirmed by Southern blot analysis on multiple generations that the transferred genes are inherited stably by offspring.

For NK603, it was confirmed that a 217 bp fragment of *Ract1* promoter exists in the reverse direction near the 3'-terminal of the transferred gene by Southern blot analysis and nucleotide sequence analysis of the 3'-terminal.

Regarding the 217 bp fragment near the 3'-terminal, strand-specific RT-PCR was conducted. In the result, a transcription product was found that was considered to start from either the *Ract1* promoter of the transferred gene or the *e35S* promoter and to read through the *nos* 3' terminator. However, only the modified CP4 EPSPS protein was detected in NK603, and no fusion protein containing the modified CP4 EPSPS protein was detected. This is likely to be explained by preservation of a static codon in the upstream of the terminator in the transcription product generated from the reading through of the terminator. It was concluded that this reading through does not affect the safety evaluation. Therefore, it was approved by the Ministry of Agriculture, Forestry and Fisheries and the Ministry of the Environment of Japan in November 2004, concerning the use in accordance with the Type I Use Regulation for Living Modified Organisms (provision as food, provision as feed, cultivation, processing, storage, transportation, disposal, and acts incidental to them) based on the Law concerning 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, each of the 456th base and the 641th base from the 5'-terminal of the coding region in the modified *cp4 epsps* gene induced by the *e35S* promoter was changed from thymine (T) to cytosine (C) compared to the base in the plasmid for expression in plant. It was proved that the change of the 456th base does not cause any change of amino acid, but in the modified CP4 EPSPS expressed by the *e35S* promoter, due to the change of the 641st base, leucine changes to proline in the 214th amino acid from the N-terminal in the original CP4 EPSPS protein (hereinafter this protein is referred to as "L214P").

Regarding L214P, the following are considered: 1) proline which is the 214th amino acid from the N-terminal is not included in the seven amino acids essential for activating the EPSPS protein family; 2) this change of the amino acid does not affect the active site and the three-dimensional structure of the EPSPS protein; 3) as the traits of enzyme activity and immune response of the L214P and the modified CP4 EPSPS protein are substantially the same, the structure and function of the L214P protein and the modified CP4 EPSPS protein are substantially the same.

In order to investigate whether the L214P protein shares functionally important amino acid

sequences with known contact allergens, it was compared with contact allergens in the database. Results showed the L214P protein did not share structurally related homologous sequences with any of the known allergens examined.

The change of the base was confirmed in multiple generations and stably descended to the progeny.

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

This item is not applicable because there was only one copy each in MON87460, MON89034, and NK603.

(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 was identified as follows:

MON87460: Confirming the expression of proteins by ELISA method

MON89034: Confirming the expression of proteins by Western blot analysis

NK603: Confirming the expression of the modified CP4 EPSPS protein in multiple generations by glyphosate herbicide spraying test during the growth

(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 nucleic acids transferred to MON87460, MON89034, and NK603 do not contain any sequence that allows gene transmission, and thus, there is no possibility that the transferred nucleic acids could be transmitted to any other wild animals or plants through virus infection and/or other routes.

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

MON87460, MON89034, and NK603 can be specifically detected by using the DNA sequences of the transferred genes and the nearby regions of the plant genome as primers. For detection and identification of this stack line maize, the above-mentioned method 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 stack line maize is given the traits as described below:

MON87460: Drought resistance due to the modified CSPB which is derived from the transferred gene

MON89034: Resistance to insects of the order Lepidoptera due to the Cry1A.105 protein and the modified Cry2Ab2 protein which are derived from the transferred genes

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

The modified CSPB expressed in MON87460 is derived from *B. subtilis*. Bacteria-derived CSPs including CSPB nonspecifically bind to RNA and function as RNA chaperons (Herschlag, 1995; Jiang et al., 1997). CSPB does not have functions to directly induce transcription (Schindler et al., 1999; Weber et al., 2001), and there is no report showing that CSPB has enzymatic activity. The modified CSPB expressed in MON87460 also functions as a RNA chaperon, and there is no report that the modified CSPB directly induces transcription or has any enzymatic activity.

The NPTII protein introduced into MON87460 as a selective marker has high substance specificity (Price et al., 1974; Davies and Smith, 1978; Davies, 1986).

The Cry1A.105 protein and the modified Cry2Ab2 protein expressed in MON89034 are crystalline insecticidal proteins derived from *B. thuringiensis* (Bt proteins). For the mechanism in which Bt proteins develop insecticidal activity, a number of studies have been made (OECD, 2007), and there is no report published to date that Bt proteins possess any other functions. Based on the above understanding, Bt proteins are considered not to offer any enzymatic activity. The Cry1A.105 protein and the modified Cry2Ab2 protein, which are Bt proteins, exhibit their insecticidal activities by binding to the specific receptors on the cell membranes of midgut epithelium of the insects of the order Lepidoptera such as European corn borer and Fall armyworm, and thus exhibit selective insecticidal activities against insects of the order Lepidoptera. To date, there is no report that combined use of different Bt proteins or Bt formulations showing insecticidal activities against insects of the same order resulted in broadening of their insecticidal spectrum to other orders. Therefore, it is considered unlikely that the insecticidal activity would be broadened in this stack line maize.

The EPSPS protein expressed in NK603, which is functionally identical to the modified

CP4 EPSPS protein, is one of the enzymes in the shikimate pathway for aromatic amino acid biosynthesis. Since EPSPS has high substrate specificity and is not the rate-determining enzyme in the pathway of shikimate synthesis, the enhanced EPSPS activity due to the expression of the modified CP4 EPSPS protein would not increase the
5 concentrations of aromatic amino acids, the end products of this pathway.

Based on the above results, it was considered that the modified CSPB protein, the NPTII protein, the Bt protein, and the modified CP4 EPSPS protein expressed in this stack line maize function independently from each other through different mechanisms.

10 As mentioned above, the modified CSPB expressed in this stack line maize does not directly induce transcription, has no enzymatic activity, and binds to RNA in a non-specific manner; these facts suggest that the modified CSPB protein would not enhance or reduce particular traits. Thus, it is unlikely that the modified CSPB protein expressed in this stack line maize would specifically affect the resistance to insects of the
15 order Lepidoptera or the tolerance to glyphosate herbicide. Moreover, since the NPTII protein, the Cry1A.105 protein, the modified Cry2Ab2 protein, and the modified CP4 EPSPS protein expressed in this stack line maize have no enzymatic activity or have high substrate specificity, they would not affect the metabolic pathway of plants. Thus, it is unlikely that the expressed proteins derived from individual parent lines have a new effect
20 on the metabolic pathway of plants.

Based on the above understanding, it is unlikely that the expressed proteins derived from individual parent lines exhibit interaction with each other in this stack line maize.

25 To actually confirm that the expressed proteins derived from individual parent lines do not interact with each other, bioassays were carried out in 2006–2007, 2007, and 2007 to confirm the lack of differences between this stack line maize and MON87460 regarding yield, this stack line maize and MON89034 regarding resistance to insects of the order Lepidoptera, and this stack line maize and NK603 regarding tolerance to glyphosate
30 herbicide, respectively.

[Yield]

In order to check whether the yield remained unchanged by crossing MON87460,
35 MON89034, and NK603 using the traditional crossbreeding method, this stack line maize, MON87460, and non-recombinant control maize (HCL301 × LH59) were grown under two soil moisture conditions (well-watered and water-limited) to compare their yield. This stack line maize and MON87460 used in the assay shared the same genetic background

(HCL301 × LH59).

In 2006–2007, four maize varieties, that is, this stack line maize, MON87460, non-recombinant control maize, and a commercial cultivar, were cultivated at four field locations (Calera de Tango [CT], Colina [CL], Lumbreras [LUM], and Quillota [QUI]) in Chile under two soil moisture conditions (well-watered and water-limited). Three replicates were made for each condition. The test plots designated for the well-watered conditions received proper levels of irrigation to achieve optimum yield, while irrigation in the test plots designated for the water-limited conditions was limited in the period from the later vegetative stage (V10) to the early reproductive stage (R2) to impose drought stress to the test plants (Table 5 Cumulative irrigation volume during the cultivation period under well-watered and water-limited conditions (2006–2007, Chile), p. 30). Based on the report that moderate drought stress reduces maize yield by 15% or more every year (Barker et al., 2005), a minimum of 15% reduction in yield of the commercial cultivar grown under the water-limited conditions compared to that under the well-watered conditions was used as the index of the presence of drought stress. In addition, days to silking, ear height, and culm length were also examined, since decrease in these items are also reported to serve as drought stress indicators in maize (Campos et al., 2006). Results showed the commercial cultivar grown under the water-limited conditions in fields in CT, CL, and LUM exhibited 15% or more reduction in yield compared to that grown under the well-watered conditions and also suffered growth inhibition in terms of other morphological characteristics. Meanwhile, the commercial cultivar grown under the water-limited conditions in field in QUI did not show 15% or more reduction in yield compared to that grown under the well-watered conditions and did not suffer any growth inhibition in terms of other morphological characteristics (Table 6 Morphological and growth characteristics of a commercial variety under well-watered and water-limited conditions (2006–2007, Chile), p. 31). Therefore, results from the field in QUI were excluded from the statistical processing of the results of all fields. ANOVA by the linear mixed model was carried out using the results obtained from all lines cultivated in the three fields other than QUI, and the least mean square calculated by ANOVA was used to compare the results between the three populations, that is, this stack line maize, MON87460, and the non-recombinant control maize, by Fisher's LSD.

The yields of this stack line maize and MON87460 were 13.7 MT/ha and 13.9 MT/ha, respectively, under the well-watered conditions and were 7.9 MT/ha and 7.2 MT/ha, respectively, under the water-limited conditions (エラー！参照元が見つかりません。, p. 31). In either soil moisture condition, no statistically significant difference was observed in yield between this stack line maize and MON87460 ($p > 0.05$) (Table 8 Paired comparison

of yields among this stack line maize, MON87460, and non-recombinant control maize, p. 31).

Table 5 Cumulative irrigation volume during the cultivation period under well-watered and water-limited conditions (2006–2007, Chile)[9]

	Irrigation volume (inch)							
	CL[1]		CT[1]		LUM[1]		QUI[1]	
Month	Well-watered	Water-limited	Well-watered	Water-limited	Well-watered	Water-limited	Well-watered	Water-limited
December	0.9	0.9	2.8	3.8	2.8	2.8	1.9	1.9
January	10.3	10.3	9.4	9.4	9.4	9.4	10.3	10.3
February	8.5	4.7	8.5	2.8	8.5	2.8	8.5	1.9
March	9.4	5.6	10.3	6.6	10.3	6.6	9.4	5.6
April	2.8	2.8	2.8	2.8	1.9	2.8	1.9	1.9
May	0	0	0	0	0	0	0	0
Total irrigation volume	31.9	24.3	33.8	25.4	32.9	24.4	32.0	21.6

No rainfall was observed during the cultivation.

[1] Test fields in Chile: CL = Colina; CT = Calera de Tango; LUM = Lumbreras; QUI = Quillota

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

Table 6 Morphological and growth characteristics of a commercial variety under well-watered and water-limited conditions (2006–2007, Chile)[10]

	CL[1]		CT[1]		LUM[1]		QUI[1]	
Morphological and growth characteristics	Well-watered	Water-limited	Well-watered	Water-limited	Well-watered	Water-limited	Well-watered	Water-limited
Days to 50% silking	63.1	63.8	66.2	67.3	70.3	73.7*	67.7	67.1
Ear height (inch)	63.4	50.9*	55.0	46.0	50.4	41.8*	63.5	63.4
Culm length (inch)	110.7	79.7*	105.9	92.1	97.9	75.0*	112.0	112.8
Yield (bushel/acre)	185.5	82.3*	236.5	152.3*	213.9	94.4*	203.1	196.3
Loss of yield (%)		56%		36%		56%		3%

Statistical analysis was carried out by Fisher's LSD using the least mean square calculated by ANOVA by the linear mixed model (n = 3, 3 replicates/field) (significant at p ≦ 0.05).
* Indicates statistically significant differences between well-watered and water-limited conditions in each field (p ≦ 0.05).
[1] Test fields in Chile: CL = Colina; CT = Calera de Tango; LUM = Lumbreras; QUI = Quillota

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

Table 7 Mean values and standard errors of yields of this stack line maize, MON87460, and non-recombinant control maize (2006–2007, Chile) [1] [11]

Soil moisture condition	Test plant	Mean ± Standard error (bu/acre)	Mean ± Standard error (MT/ha)
Well-watered	This stack line maize	218.6±9.02	13.7±0.57
	MON87460	220.7± 7.87	13.9±0.49
	Non-recombinant control maize	220.0±10.19	13.8±0.64
Water-limited	This stack line maize	125.4±15.84	7.9±0.99
	MON87460	114.5±16.04	7.2±1.01
	Non-recombinant control maize	86.7±14.17	5.4±0.89

[1]Results from fields in CT, CL, and LUM

Table 8 Paired comparison of yields among this stack line maize, MON87460, and non-recombinant control maize[12]

Comparison	Soil moisture condition	P-value
This stack line maize vs. MON87460	Well-watered	0.875
	Water-limited	0.411
This stack line maize vs. non-recombinant control maize	Well-watered	0.916
	Water-limited	0.009
MON87460 vs. non-recombinant control maize	Well-watered	0.958
	Water-limited	0.048

Statistical analysis was carried out by Fisher's LSD using the least mean square calculated by ANOVA by the linear mixed model (n = 9, 3 replicates/field, 3 fields) (significant at $p \leqq 0.05$).

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

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

[Resistance to insects of the order Lepidoptera]

In order to check whether the resistance to insects of the order Lepidoptera, a trait derived from MON89034, remained unchanged by crossing MON87460, MON89034, and NK603 using the traditional crossbreeding method, feeding damage by Fall armyworm (*Spodoptera frugiperda*), a major pest insect of order Lepidoptera for maize, was evaluated to compare the resistance to insects of order Lepidoptera in this stack line maize, MON89034, and non-recombinant control maize. In this assay, experiments were conducted twice under the same condition, and the data obtained from the two experiments were analyzed together. The assay was conducted using 12 plants each (3 plants/plot × 2 replicates × 2 experiments) of this stack line maize, MON89034, and non-recombinant control maize (HCL301 × LH59). This stack line maize and MON89034 used in the assay shared the same genetic background (HCL301 × LH59).

At the 5th leaf stage, the first instar larvae of Fall armyworm were inoculated (25 larvae/plant). On the 7th day after inoculation of the Fall armyworm, the severity of feeding damage was determined based on the leaf damage rate (LDR) on a 10-step scale from 0 (no damage) to 9 (serious damage) proposed by Davis et al. (1992). On this scale, 0 indicates that no feeding damage was observed, while 9 indicates that the leaf was seriously damaged, having thin scratches or holes of various sizes. Due to the unevenness of variance, the data were subjected to rank transformation prior to statistical processing. The transformed data were used to perform ANOVA by the linear mixed model, and multiple comparison was performed by Fisher's protected LSD.

Results showed the severity of feeding damage by pest insects of order Lepidoptera in this stack line maize and MON89034 was 0.33 (Table 9, p. 33), and no statistically significant difference was observed ($p > 0.05$) (エラー! 参照元が見つかりません。, p. 33).

Table 9 Mean values and standard errors of the leaf damage rate (LDR) in this stack line maize, MON89034 and non-recombinant control maize (SEM)[13]

Samples tested	Mean value ± Standard error of leaf damage rate (LDR)[1]
This stack line maize	0.33 ± 0.19
MON89034	0.33 ± 0.14
Non-recombinant control maize	6.17 ± 0.29

[1] On the 7th day after inoculation, the leaf damage rate was determined on a scale proposed by Davis et al. (1992). On this scale, 0 indicates that no feeding damage was observed, while 9 indicates that the leaf was seriously damaged, having thin scratches or holes of various sizes.

Table 10 Paired comparison of leaf damage rate among this stack line maize, MON89034, and non-recombinant control maize[14]

Comparison	P-value
This stack line maize vs. MON89034	0.969
This stack line maize vs. non-recombinant control maize	< 0.001
MON89034 vs. non-recombinant control maize	< 0.001

After carrying out ANOVA by the linear mixed mode, multiple comparison test was performed by Fisher's protected LSD (n = 4, 3 individuals/plot, 4 replicates) (significant at $p \leqq 0.05$).

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

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

[Tolerance to glyphosate herbicide]

In order to check whether the tolerance to glyphosate herbicide, a trait derived from NK603, remained unchanged by crossing MON87460, MON89034, and NK603 using the traditional crossbreeding method, a glyphosate herbicide spraying test was performed. The test was conducted using 5 plants each (1 plant/plot × 5 replicates) of this stack line maize, NK603, and the non-recombinant control plant (HCL301 × LH59) which were cultivated in pots in a greenhouse. This stack line maize, NK603, and the non-recombinant control maize used in the test shared the same genetic background (HCL301 × LH59).

At the 4th leaf stage, glyphosate herbicide (Product name: Roundup WeatherMAX) was either not sprayed or sprayed at two different concentrations (0.84 kg acid equivalent[15] (a.e.)/ha (normal dosage) and 27 kg a.e./ha (32-time higher dosage than the normal dosage)). On the 7th and 14th days after spraying glyphosate, the severity of injury by spraying of the herbicide to plant bodies was evaluated based on a 11-step scale from 0 (no injury) to 10 (nearly the entire plant withered and died due to the injury). The obtained data were used to perform ANOVA by the linear mixed mode, and multiple comparison was performed by Fisher's protected LSD. The data obtained on the 14th day after spraying showed no variation and were thus excluded from the statistical processing.

From the statistical processing of the data obtained on the 7th day after spraying, no statistically significant difference was observed between this stack line maize and NK603 regarding the severity of injury by spraying of the herbicide at either dosage at any time of observation ($p > 0.05$) (Table 11 Levels of herbicide injury to this stack line maize, NK603, and non-recombinant control maize by spraying of glyphosate herbicide (normal dosage1 and 32-times higher dosage2)[3] to Table 11 Paired comparison of levels of herbicide injury to this stack line maize, NK603, and non-recombinant control maize on 7th day after spraying, p. 36).

[15] Acid equivalent. Herbicide active ingredients are formulated either as the active ingredients themselves or in the form of their salts. In active ingredients formulated as their salts, the acid moieties serve as the active components, and the base moieties vary between formulations. If the dosage of a herbicide is expressed as the amount of the salt of the active ingredient formulated, the amount of the active component cannot be precisely compared between formulations with different base moieties. Thus, acid equivalent was used as the unit for the active component.

Table 11 Levels of herbicide injury to this stack line maize, NK603, and non-recombinant control maize by spraying of glyphosate herbicide (normal dosage[1] and 32-times higher dosage[2])[3] [16]

Observation day	Normal dosage			32-times higher dosage than the normal dosage		
	This stack line maize	NK603	Non-recombinant maize	This stack line maize	NK603	Non-recombinant maize
7th day after spraying	0.0 ± 0.00	0.0 ± 0.00	8.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	8.2 ± 0.20
14th day after spraying	0.0 ± 0.00	0.0 ± 0.00	10.0 ± 0.00	1.0 ± 0.00	1.0 ± 0.00	10.0 ± 0.00

The values represent mean value ± standard error.

[1] Normal dosage is 0.84 kg a.e./ha.

[2] 32-times higher dosage is 27 kg a.e./ha.

[3] Evaluation was made on a scale from 0 (no injury) to 10 (nearly the entire plant withered and died due to the injury).

Table 11 Paired comparison of levels of herbicide injury to this stack line maize, NK603, and non-recombinant control maize on 7th day after spraying[17]

Dosage	Comparison	P-value
Normal dosage	This stack line maize vs. NK603	1.000
	This stack line maize vs. non-recombinant control maize	<0.001
	NK603 vs. non-recombinant control maize	<0.001
32-times higher dosage	This stack line maize vs. NK603	1.000
	This stack line maize vs. non-recombinant control maize	<0.001
	NK603 vs. non-recombinant control maize	<0.001

After carrying out ANOVA by the linear mixed mode, multiple comparison test was performed by Fisher's protected LSD (n = 5, 1 individual/plot, 5 replicates) (significant at $p \leqq 0.05$).

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

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

Based on the above findings, it was concluded that the individual proteins expressed in the relevant parent lines do not interact with each other and that the traits acquired through the transferred genes remain unchanged in this stack line maize.

Consequently, with regard to the differences in physiological or ecological characteristics between this stack line maize and maize, the taxonomic species to which the recipient organism belongs, the evaluation was conducted based on the results of individual examinations of the parent lines (MON87460, MON89034, and NK603) at the times of their approval.

(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[18]

The evaluation results of adverse effect on biological diversity in Japan of the parent lines (MON87460, MON89034, and NK603) of this stack line maize are provided below.

i. Test conducted under typical cultivation conditions with irrigation

a Morphological and growth characteristics

For the morphological and growth characteristics, comparison was made between the parent lines (MON87460, MON89034, and NK603) and their respective non-recombinant controls regarding the items listed in Table 12 Investigational results of morphological and growth characteristics in MON87460, MON89034, and NK603 (p. 38).

In the results, a difference was observed between MON87460 and its non-recombinant control maize in the flowering period (Table 3 of Annex 1, p. 12).No statistically significant difference was observed between MON89034 or NK603 and their non-recombinant control maize (Table 2 of Annex 3, p. 8; Table 3-2 of Annex 4, p. 17).

[18]All the rights pertinent to the information in a-g under i, a-b under ii, and a-b under iii below and the responsibility for the contents rest upon Monsanto Japan Limited.

Table 12 Investigational results of morphological and growth characteristics in MON87460, MON89034, and NK603[19]

	MON87460	MON89034	NK603
Uniformity of germination	○	○	○
Number of plants germinated	—	○	—
Germination rate	○	○	○
Time to tasseling	○	○	○
Time to silking	○	○	○
Start of flowering	○	—	—
Flowering period	○*	○	—
Culm length	○	○	○
Culm diameter	—	○	—
Plant shape	○	○	○
Tiller number	○	○	○
Ear height	○	○	○
Maturation time	○	○	○
Weight of above-ground parts at harvest	○	○	○
Grain shape	○	○	○
Grain color	○	○	○

○ : Examined
— : Not examined
* A statistically significant difference or a difference was observed.

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

Cold tolerance of MON87460 was evaluated under four different temperature conditions (optimum temperature: 30ºC/22ºC (day/night), mildly low temperature: 20ºC/15ºC, moderately low temperature: 15ºC/10ºC, severely low temperature: 4ºC/4ºC). Results showed MON87460 withered due to the low temperature treatment at the early stage of growth similarly to its non-recombinant control (Table 2 of Annex 2, p. 17). Under the optimum temperature condition, statistically significant differences were observed in growth stages and dry weights at the 4th and 8th day after transfer to a climate chamber (Table 2 of Annex 2, p. 17).

MON89034 and NK603 both withered and died due to the low temperature treatment at the early stage of growth similarly to their non-recombinant control

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

plants (Figure 6-2 of Annex 3, p. 14; Table 3-4 of Annex 4, p. 24).

c Wintering ability and summer survival of the mature plant

5 Maize is a summer type annual plant, and after ripening, it usually dies out in winter, and it does not re-grow and propagate vegetatively, or produce seeds. Actually, at the end of isolated field tests, start of withering and death after ripening was observed.

10 d Fertility and size of the pollen

MON87460, MON89034, and NK603 all exhibited high fertility of pollen, similarly to their non-recombinant control plants, and no difference was observed regarding the shape and size of pollens (Figure 6 of Annex 1, p. 15; Figure 8-1 to Figure 8-2 of Annex
15 3, p. 19; Table 3-3 and Photo 4-1 to Photo 3-4a-b of Annex 4, p. 21–22).

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

Regarding seed production, comparison was conducted between MON87460,
20 MON89034, and NK603 and their non-recombinant control plants for the characteristics listed in Table 13 Investigational results of seed production in MON87460, MON89034, and NK603 (p. 39).

Results showed statistically significant differences between MON89034 and its non-recombinant control plant regarding ear diameter and grain number per ear, and
25 between NK603 and its non-recombinant control plant regarding 100-kernel weight. (Table 2, p. 8 and Table 6, p. 20 of Annex 3; Table 3-2 of Annex 4, p.17). Meanwhile, no statistically significant difference was observed between MON87460 and its non-recombinant control plant (Table 4 of Annex 1, p. 17).

30 Table 13 Investigational results of seed production in MON87460, MON89034, and NK603[20]

	MON87460	MON89034	NK603
Number of ears	○	○	○
Number of effective ears/Total number of effective ears	○	○	○
Ear length	○	○	○
Ear diameter	○	○*	○

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

Number of grains per ear	○	○*	—
Row number per ear	○	○	○
Grain number per row	—	○	○
100-kernel weight	○	○	○*

○ : Examined
— : Not examined
* A statistically significant difference was observed

Regarding shedding habit of the seed, it was not observed in natural environment, since the ears of MON87460, MON89034, and NK603 and their non-recombinant control plants were covered with bracts at the time of harvesting.

Regarding germination rate, in order to identify the dormancy of harvested seeds, germination test was carried out for the seeds harvested from MON87460, MON89034, and NK603 and their non-transgenic control plants. Results showed no difference, and no dormancy of the seed was identified (Table 4 and Table 5 of Annex 1, p. 17; Table 3 to Table 4 of Annex 3, p. 16; Table 3-4 of Annex 4, p. 24).

f Crossability

Crossability test was not performed for the parent lines MON87460, MON89034, and NK603, since no wild relatives that can be crossed grow in Japan.

g Productivity of harmful substances

Soil microflora tests, plow-in tests, and succeeding crop tests were performed for MON87460, MON89034, and NK603. Results showed no statistically significant difference (Table 6 to Table 8 of Annex 1, p. 19; Table 7 to Table 9 of Annex 3, p. 23; Table 3-5 to Table 3-7 of Annex 4, p. 26–28).

ii. Test conducted under typical cultivation conditions without irrigation

In order to evaluate the drought tolerance of MON87460 under environmental conditions in Japan, MON87460 and its non-recombinant control maize were cultivated under normal conditions without irrigation, and comparison was made regarding (a) morphological and growth characteristics and (b) production and

shedding habit of the seed.

a Morphological and growth characteristics

For the morphological and growth characteristics, comparison was made between MON87460 and the non-recombinant control maize regarding 14 items (uniformity of germination [date], germination rate [%], time to tasseling [date], time to silking [date], start of flowering [date], flowering period [date], culm length [cm], ear height [cm], tiller number, plant shape, maturation time [rate], weight of above-ground parts at harvest [kg], grain shape, and grain color). Among the statistically analyzed items (germination rate, culm length, ear height, tiller number, and weight of above-ground parts at harvest), a statistically significant difference was observed between MON87460 and its non-recombinant control plant in ear height (Table 9 of Annex 1, p. 25). Among the items not statistically analyzed (uniformity of germination, time to tasseling, time to silking, start of flowering, flowering period, plant shape, maturation time, grain shape, and grain color), a difference was observed in start of flowering between MON87460 and the non-recombinant control maize (Table 9 of Annex 1, p. 25).

b Production and shedding habit of the seed

Regarding seed production, comparison was made between MON87460 and its non-recombinant control plant for the following items: total number of effective ears, ear length (cm), ear diameter (cm), row number per ear, number of grains per ear, and 100-kernel weight (g). Results showed statistically significant differences between MON87460 and its non-recombinant control plant regarding total number of effective ear, ear length and number of grains per ear (Table 10 of Annex 1, p. 28).

Regarding shedding habit of the seed, it was not observed in natural environment, since the ears of MON87460 and its non-recombinant control plant were covered with bracts at the time of harvest.

iii. Test conducted with no agronomical treatment

In order to evaluate the self-seeding ability of MON87460, MON87460, and its non-recombinant control plant were cultivated without any agronomical treatment such as irrigation, fertilization, disease, and pest insect control or weeding, and comparison was made regarding (a) morphological and growth characteristics and (b) production and shedding habit of the seed. In response to abundance of weeds,

nutrient deficiency due to the lack of fertilization, drought stress, feeding damage by pest insects, and other types of stresses, the survival rate was extremely low; 18 out of 33 MON87460 plants tested and 26 out of 33 non-recombinant control plants tested withered and died (Table 13 of Annex 1, p. 35). Moreover, the total numbers of effective ears recovered from 33 MON87460 plants and 33 non-recombinant control plants were 9 and 2, respectively (Table 13 of Annex 1, p. 35). Because of the low values, the test results were not statistically processed.

a Morphological and growth characteristics

For the morphological and growth characteristics, comparison was made between MON87460 and the non-recombinant control maize regarding five items (uniformity of germination [date], germination rate [%], culm length [cm], maturation time [date], and weight of above-ground parts at harvest [kg]). The results suggested that there is no difference between MON87460 and the non-recombinant control maize (Table 14 of Annex 1, p. 36).

b Production and shedding habit of the seed

Regarding seed production, comparison was made between MON87460 and its non-recombinant control plant for the following items: total number of effective ears, ear length (cm), ear diameter (cm), row number per ear, and number of grains per ear. Results showed differences between MON87460 and its non-recombinant control plant regarding total number of effective ears and number of grains per ear (Table 15 of Annex 1, p. 38).

Regarding shedding habit of the seed, it was not observed in natural environment, since the ears of MON87460 and its non-recombinant control plant were covered with bracts at the time of harvest.

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

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

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

Maize tolerant to drought, resistant to Lepidoptera, and tolerant to glyphosate herbicide (including the progeny lines isolated from the maize lines, MON87460, MON89034, and NK603, that contain a combination of any of the transferred genes in the individual maize lines [except those already granted approval regarding Type 1 Use Regulation]) (hereinafter referred to as this stack line maize) was developed by crossing the following three recombinant maize lines, using the traditional crossbreeding method:

(a) drought-tolerant maize (MON87460) due to the modified *cspB* gene that encodes the modified CSPB protein (modified cold shock protein B) and the *nptII* gene that encodes the NPTII protein (neomycin phosphotransferase type II);

(b) maize resistant to Lepidoptera (MON89034) due to the *cry1A.105* gene that encodes the Cry1A.105 protein and the modified *cry2Ab2* gene that encodes the modified Cry2Ab2 protein;

(c) maize tolerant to glyphosate herbicide (NK603) due to the modified *cp4 epsps* gene that encodes the modified CP4 EPSPS protein (5-enol-pyruvylshikimate-3-phosphate synthase).

These three parent lines were individually judged at the Committee for Review on the Biological Diversity Risk Assessment as causing no Adverse Effect on Biological Diversity when used in line with Type 1 Use described in the application for this stack line maize.

The modified CSPB protein is known to disrupt the secondary structure of the RNA, but it does not induce transcription, has no enzymatic activity, and binds to RNA in a non-specific manner. These facts suggest that the modified CSPB protein expressed in this stack line maize would not specifically affect the resistance to insects of the order Lepidoptera or the tolerance to glyphosate herbicide. Moreover, since the NPTII protein and the modified CP4 EPSPS protein expressed in this stack line maize have high substrate specificity, and since the Cry1A.105 protein and the modified Cry2Ab2 protein have no enzymatic activity, they would not affect the metabolic pathway of plants. Thus, it is unlikely that these proteins expressed in this

stack line maize would interact with each other to change the metabolic pathway of the recipient and result in production of new metabolites.

In addition, based on the bioassays, the tolerance to drought, the resistance to Lepidoptera, and the tolerance to glyphosate herbicide in this stack line maize were found at similar levels as exhibited by the individual parent lines.

Consequently, it is considered unlikely that the proteins expressed in this stack line derived from individual parent lines would cause functional interaction in the plant body of this stack maize line, and it is considered unlikely that notable changes in traits have occurred in this stack maize line except for the traits that it received from the parent lines.

1) Competitiveness

Maize, the taxonomical species to which the recipient organism belongs, has been long used in Japan, though there is no report that it has become self-seeding in the natural environment in Japan.

From investigation for various characteristics referring to competitiveness of MON87460, MON89034, and NK603, the parent lines of this stack line maize, statistically significant differences were observed between the parent lines of this stack line maize and the non-recombinant maize in some of the items examined. However, the differences were judged not to be so large as enhancing the competitiveness of this stack maize line.

This stack line maize is given the tolerance to drought due to the modified CSPB protein and, under conditions with limited soil moisture levels, shows less decrease of yield compared to conventional maize cultivars. However, it is considered that this trait would not enhance the competitiveness of this stack line maize compared to conventional maize cultivars. In addition, this stack line maize is given the trait to be resistant to insects of the order Lepidoptera due to the Cry1A.105 protein and the modified Cry2Ab2 protein. However, it is not generally considered that the insect damage by Lepidopteran insects is the major factor to inhibit the growth of maize under the natural environment in Japan. Moreover, this stack line maize is also given the tolerance to glyphosate herbicide due to the modified CP4 EPSPS protein. However, it is considered unlikely that, in the natural environment less expected to suffer spraying of herbicides, the tolerance to glyphosate herbicide would increase the competitiveness of this stack line maize.

Based on the above understanding, it was judged that the following conclusion made by the applicant is valid: Regarding this stack line maize, there are no specific wild animals and wild plants that are possibly affected by this stack line maize, and it would pose no risk of Adverse Effect on Biological Diversity that is attributable to competitiveness.

2) Productivity of harmful substances

Maize, the taxonomical species to which the recipient organism belongs, has been long used in Japan, though it is not generally known to produce any harmful substances that could affect wild animals and wild plants.

It has been confirmed that the proteins expressed in this stack maize line (the
5 modified CSPB protein, the Cry1A.105 protein, the modified Cry2Ab2 protein, and the modified CP4 EPSPS protein) do not have any homology with any of the known allergens. In addition, for examining the ability of the parent lines of this stack line maize to produce any harmful substances (the substances secreted from the roots that can affect other plants and microorganisms in soil, the substances existing in the plant
10 body that can affect other plants after dying), plow-in tests, succeeding crop tests, and the soil microflora tests were conducted. In all tests, there was no difference suggesting increase in productivity of harmful substances. Consequently, it is considered unlikely that this stack line maize possesses productivity of unintended harmful substances.

15 The Cry1A.105 protein and the modified Cry2Ab2 protein expressed in this stack line maize exhibit insecticidal activity against insects of the order Lepidoptera. Therefore, the Lepidopteran insects were specified as wild animals and wild plants that are possibly affected by this recombinant maize. Then, there is a concern about possible effects on the specified species of Lepidopteran insects that could directly
20 eat this stack line maize or eat pollens dispersed from this stack maize line and attached to their food plants. However, it is considered unlikely that the Lepidopteran insects inhabit locally near the fields for cultivation of this stack line maize and thus, it is considered very unlikely that they could be affected in the level of population.

Based on the above understanding, it was judged that the following conclusion
25 made by the applicant is valid: This stack line maize would pose no risk of Adverse Effect on Biological Diversity that is attributable to productivity of harmful substances.

3) Crossability

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

35 2. Conclusion based on the Biological Diversity Risk Assessment Report

Based on the above understanding, the Biological Diversity Risk Assessment Report concluded that there is no risk that the use of this stack line maize, 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 valid.

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List of Annexes

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Annex 2 An Assessment of the Effect of Cold Stress on Drought Tolerant Corn MON87460 Under Growth Chamber Conditions in 2008 (MSL0021509) (Confidential)

Annex 3 Biological Diversity Risk Assessment Report on maize resistant to Lepidoptera (*cry1A.105*, modified *cry2Ab2*, *Zea mays* subsp. *mays* (L.) Iltis) (MON89034, OECD UI: MON89034-3) in isolated field (Confidential)

Annex 4 Environmental Safety Assessment of maize line NK603 tolerant to glyphosate herbicide in simulated environment (Confidential)