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

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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Glyphosate-induced male-sterile and glyphosate-tolerant maize (Modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (MON87427, OECD UI: MON-87427-7)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

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

1 Information concerning preparation of living modified organisms

The Monsanto Company has developed the glyphosate-induced male-sterile and glyphosate-tolerant maize MON87427 (Modified *cp4 epsps, Zea mays* subsp. *mays* (L.) Iltis) (MON87427, OECD UI: MON-87427-7) (hereinafter referred to as "this recombinant maize") for the purpose of facilitating efficient production of hybrid seeds in maize breeding.

Modified *cp4 epsps* coding sequence was introduced into this recombinant maize under the control of the *e35S* promoter in combination with the *hsp70* intron (*e35S-hsp70*), resulting in a tissue-specific expression of the modified CP4 EPSPS protein in this recombinant maize (Table 3, p21; Table 1, p17, Table 2, p18 and Table 3, p19 of Appendix 2). In this recombinant maize, the modified CP4 EPSPS protein is not or scarcely expressed in the tapetum cells and microspores which are male reproductive tissues (Figure 1, p3 of Appendix 1) but is expressed in the vegetative and female reproductive tissues at levels sufficient for imparting glyphosate herbicide tolerance to the plant (Table 3, p21; Table 1, p17, Table 2, p18 and Table 3, p19 of Appendix 2). Tapetum cells provide nutrition to micropores and pollens throughout the pollen formation process (Goldberg et al., 1993; Huang et al., 2009), while microspores develop into pollens through cell division. It is also known that destruction of tapetum cells generally results in non-viable pollen (Goldberg et al., 1993).

As mentioned above, the modified CP4 EPSPS protein is not or scarcely expressed in the tapetum cells and microspores of this recombinant maize, so when glyphosate herbicide is applied to this recombinant maize twice during the early stage of tassel formation (stages ranging from 8th-leaf (V8) to 13th-leaf (V13) stages), the activities of the tapetum cells and the microspores are inhibited, resulting in inhibition of viable pollen formation (Table 4, p27; Table 5, p29; Figure 5, p27). In general, tassels develop during V8 to V13 stages, but under field conditions, various environmental factors may induce difference in growth speed among maize individuals. In order to ensure that glyphosate herbicide is applied to maize plants when the tapetum cells and microspores are under development in each plant, it is recommended that the application is carried out twice during V8 to V13 stages. Meanwhile, the vegetative and female reproductive

tissues of this recombinant maize express the modified CP4 EPSPS protein (Table 3, p21; Table 1, p17, Table 2, p18 and Table 3, p19 of Appendix 2) and thus are not affected by the glyphosate herbicide application. This unique glyphosate herbicide-tolerance of this recombinant maize would enable efficient production of hybrid seeds from this recombinant maize, as illustrated in Figure 1 (p4).

One of the problems associated with hybrid seed production in maize breeding is that the tassels of both the seed parent and the pollen parent produce pollens during the same period. Under such circumstances, pollens need to be removed from the seed parent to ensure crossing between the pollen parent and the seed parent.

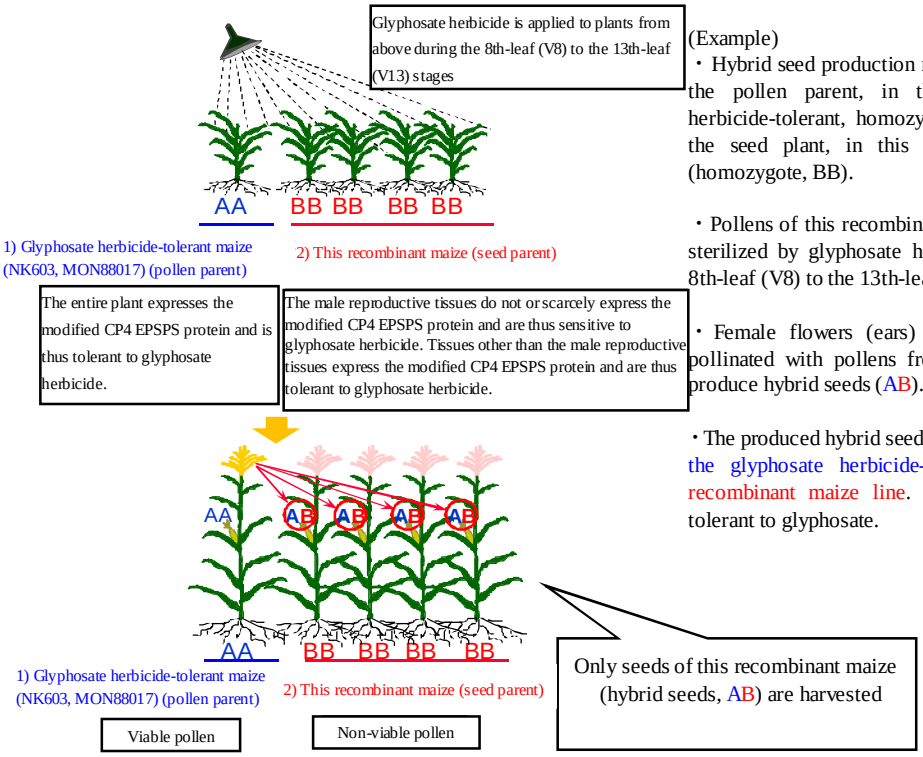
A general approach for removing pollens from the seed parent is the removal of the tassels from the seed parent. However, the conventional emasculation technique requires many hands, as it needs to be carried out within a short time for producing high-purity hybrid seeds. In addition, to ensure the production of intended hybrid seeds using the conventional technique, observation and emasculation procedures need to be repeated, which also requires much labor.

Another approach for producing hybrid seeds is the use of cytoplasmic male sterility, but the technique requires much breeding effort for imparting male sterility to the seed parent. Yet, emasculation is still needed in some cases, as the expression of male sterility may vary depending on the type of male sterility genes or environmental conditions. Moreover, only a certain percentage of existing maize germplasm has been tested to be workable with cytoplasmic male sterility.

Compared to these conventional techniques, the use of this recombinant maize has many benefits in hybrid seed production. The benefits include,reduction of labor, high purity of the hybrid seeds produced and less susceptibility to environmental factors, thereby realizing highly efficient hybrid seed production. Moreover, the effective period for emasculation by glyphosate herbicide application is longer than that by the conventional technique, allowing enough time for emasculation.

Furthermore, glyphosate herbicide can be applied to this recombinant maize at the early vegetative growth stage (corresponding to the optimum application period currently adopted for the use of glyphosate herbicide for weed control, which is indicated on the current Roundup agricultural product label for weed control purposes) for weed control. Application of the herbicide at this period would not affect pollen formation of this recombinant maize, as male reproductive tissues are still undeveloped

at the early vegetative growth stage.



(Example)

・Hybrid seed production requires two parent cultivars: 1) the pollen parent, in this case, another glyphosate herbicide-tolerant, homozygous maize line (AA); and 2) the seed plant, in this case, this recombinant maize (homozygote, BB).

・Pollens of this recombinant maize (seed parent, BB) are sterilized by glyphosate herbicide application during the 8th-leaf (V8) to the 13th-leaf (V13) stages.

・Female flowers (ears) of the seed parent (BB) are pollinated with pollens from the pollen parent (AA) to produce hybrid seeds (AB).

・The produced hybrid seed is a heterozygote (AB) between the glyphosate herbicide-tolerant maize line and this recombinant maize line. All tissues in the hybrid are tolerant to glyphosate.

Figure 1　Effective hybrid seed production using this recombinant maize[1]

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

(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 this recombinant maize are shown in Figure 2 (p6) and Table 1 (p7-11).

Compared to the CP4 EPSPS protein derived from the *Agrobacterium* sp. CP4 strain, the modified CP4 EPSPS protein expressed by the modified *cp4 epsps* gene transferred to this recombinant maize has the second residue from the N terminal modified from serine to leucine, as a result of insertion of a restriction enzyme cleavage site during the cloning process. The *cp4 epsps* gene transferred to this recombinant maize is hereinafter referred to as "modified *cp4 epsps* gene" and the protein expressed by the gene as "modified CP4 EPSPS protein". The deduced amino acid sequence of the modified CP4 EPSPS protein expressed in this recombinant maize is shown in Appendix 3.

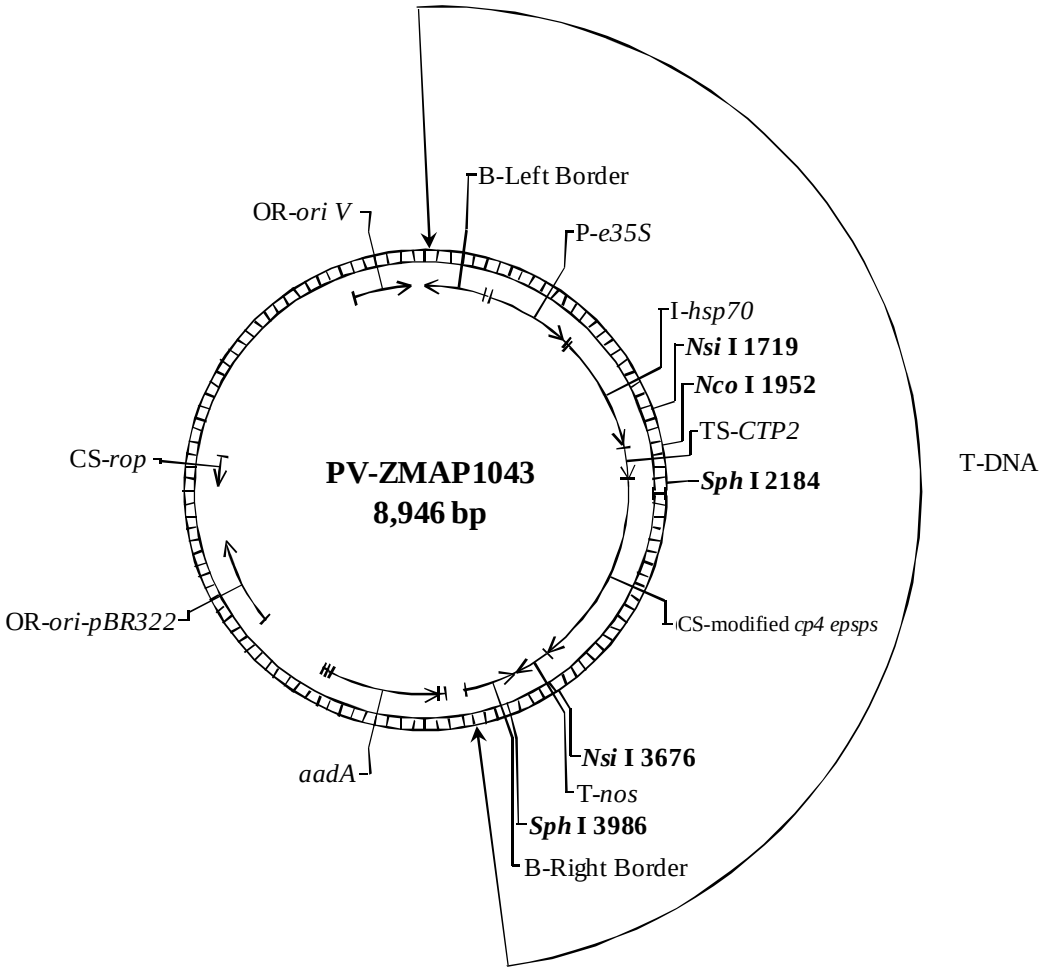


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Figure 2 Physical map of the plasmid PV-ZMAP1043 used for the development of this recombinant maize[2]

[2]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-ZMAP1043 used for the development of this recombinant maize[3]

Component elements	Origin and Function
T-DNA region	
B[Note 1]-Left Border	A DNA fragment derived from *Agrobacterium tumefaciens*, containing the left border sequence. It is used for transferring T-DNA (Barker et al., 1983).
Intervening Sequence	A sequence used for cloning of DNA
P[Note 2]-*e35S*	A promoter developed from a Cauliflower mosaic virus promoter (CaMV 35) (Odell et al., 1985) hardly expressed in maize pollens (Hamilton et al., 1992). It possesses a domain increasing the activity of the CaMV 35S promoter in two tandem repeats (Figure 3, p10; McPherson and Kay, 1994) to achieve an increased transcription activity without losing the tissue-specific expression pattern. As in the case of the CaMV *35S* promoter, the *e35S* promoter has been confirmed to show low activity in pollens and tapetum cells in maize (CaJacob et al., 2004).
Intervening Sequence	A sequence used for cloning of DNA
I[Note 3]-*hsp70*	The intron of a heat shock protein (*hsp70*) of *Z. mays* (maize) (Brown and Santino, 1997).
Intervening Sequence	A sequence used for cloning of DNA
TS[Note 4]-*CTP2*	The sequence encoding chlorophyll transit peptide derived from 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene (*ShkG*) from *Arabidopsis thaliana* (Arabidopsis) (Klee et al., 1987). It directs the modified CP4 EPSP protein to chloroplasts.
CS[Note 5]-modified *cp4 epsps*	The coding sequence of *aroA* (*epsps*) gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) derived from *Agrobacterium* CP4 strain (Barry et al., 2001; Padgette et al., 1996a).
Intervening Sequence	A sequence used for cloning of DNA
T[Note 6]-*nos*	The 3' untranslated region of nopaline synthase gene derived from *A. tumefaciens*. It terminates transcription and causes polyadenylation (Bevan et al., 1983).
Intervening Sequence	A sequence used for cloning of DNA
B-Right Border	A DNA fragment derived from *A. tumefaciens*, containing the right border. It is used for transferring T-DNA (Depicker et al., 1982; Zambryski et al., 1982).

[3]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-ZMAP1043 used for the development of this recombinant maize this recombinant maize (continued)

Outside the T-DNA region	
Intervening Sequence	A sequence used for cloning of DNA
aadA	The bacterial promoter, coding region and 3' untranslated region of 3'(9)-O-nucleotidyltransferase, an aminoglycoside-modifying enzyme, derived from transposon Tn 7 (Fling et al., 1985). It confers spectinomycin/streptomycin resistance.
Intervening Sequence	A sequence used for cloning of DNA
OR[Note 7]-*ori-pBR322*	A region containing replication origin isolated from plasmid pBR322. It confers autonomous replication potential in *Escherichia coli* to the vector (Sutcliffe, 1979).
Intervening Sequence	A sequence used for cloning of DNA
CS-*rop*	A coding sequence of the repressor of the primer protein derived from plasmid ColE1. It regulates the plasmid copy number in *E. coli* (Giza and Huang, 1989).
Intervening Sequence	A sequence used for cloning of DNA
OR-*ori V*	Replication origin segment derived from the broad host range plasmid RK2. It confers autonomous replication potential in *Escherichia coli* to the vector (Stalker et al., 1981).
Intervening Sequence	A sequence used for cloning of DNA

[Note 1] B-Border sequence

[Note 2] P-Promoter

[Note 3] I-Intron

[Note 4] TS-Targeting Sequence

[Note 5] CS-Coding Sequence

[Note 6] T-Transcription Termination Sequence

[Note 7] OR-Origin of Replication

2) Functions of component elements

(a) Functions of target genes, expression-regulating regions, localization signals, selectable markers and other component elements of donor nucleic acid

Functions of component elements of donor nucleic acid which were used for the production of this recombinant maize are shown in Table 1 (p7-11).

Modified *cp4 epsps* coding sequence was introduced into this recombinant maize under the control of the *e35S* promoter in combination with the *hsp70* intron (*e35S-hsp70*), resulting in a tissue-specific expression of the modified CP4 EPSPS protein in this recombinant maize. The details of the promoter and the intron are described below.

The *e35S* promoter in this recombinant maize was developed from a Cauliflower mosaic virus (CaMV) *35S* promoter.

Although it is generally known that the CaMV *35S* promoter drives constitutive expression of the target gene in all tissues, some reports suggest that it is not always expressed in all cell or tissue types (Benfey and Chua, 1989a; Terada and Shimamoto, 1990; Williamson et al., 1989; Yang and Christou, 1990). It has also been reported that the use of the CaMV *35S* promoter resulted in very low expression of reporter genes[4] in pollens of various plant species (Sunilkumar et al., 2002; Wilkinson et al., 1997) and that the activity of the CaMV *35S* promoter is particularly low in maize pollens (Hamilton et al., 1992). The extremely low expression of the CaMV *35S* promoter in pollens could be attributed to the absence of the cis-acting elements regulating pollen-specific expression in the sequence of the CaMV *35S* promoter (Eyal et al., 1995).

The CaMV *35S* promoter consists of domain A and domain B (Figure 3, p10; Benfey et al., 1989b); the former contains at least one sequence that plays the role as a promoter and at least one sequence that enhances the promoter activity, while the latter contains several sequences enhancing the promoter activity (Benfey et al., 1989b; Fang et al., 1989; Odell et al., 1985). Meanwhile, the *e35S* promoter used in this recombinant maize has two tandem repeats of domain B upstream of domain A.

[4]In general, reporter genes express readily visible and quantifiable gene products (King and Stansfield, 1997) and are used for demonstrating the expression patterns of various promoters. A promoter region is coupled to a reporter gene sequence to identify its expression pattern in various cell types.

Except for the presence of this additional domain B, there is no difference between the *e35S* promoter and the CaMV35S promoter (Figure 3, p10; McPherson and Kay, 1994). Thus, the *e35S* promoter is considered to have an increased transcription activity but retain the tissue-specific expression pattern of the CaMV35S promoter. This was actually demonstrated by the low expression of the modified CP4 EPSPS protein in pollens and tapetum cells of another recombinant maize, which had been transformed using the same *e35S* promoter as this recombinant maize (CaJacob et al., 2004).

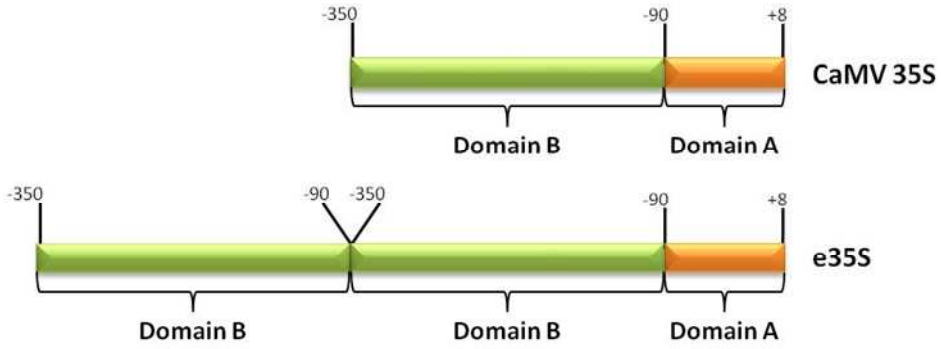

Figure 3 Comparison of the CaMV*35S* promoter and the *e35S* promoter[5]
The numbers in the figure indicate the nucleotide position from the transcription initiation site of the CaMV*35S* promoter.

The *hsp70* intron was introduced into this recombinant maize to increase the expression of the modified CP4 EPSPS protein in vegetative and female reproductive tissues (Callis et al., 1987) without changing the expression pattern of the protein in this recombinant maize (Brown and Santino, 1997).

The combination of the *e35S* promoter and the *hsp70* intron (*e35S-hsp70*) used for developing this recombinant maize is not new, as it has already been used for developing other recombinant crops (NK603 and MON810) that have been approved for Type-1 use (Appendix 4).

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

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

Glyphosate herbicide inhibits the activity of endogenous 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimate pathway for biosynthesis of endogenous aromatic amino acid in plants, to induce cell death (Franz et al., 1997). This recombinant maize has been conferred tolerance to glyphosate herbicide by the modified CP4 EPSPS protein expressed by the modified *cp4 epsps* gene introduced into the maize.

In order to investigate whether the modified CP4 EPSPS protein shares functionally important amino acid sequences with known allergens, the proteins were compared with allergens in the allergen database (AD_2010[6]), using the FASTA algorithm and the 8- contiguous amino acid search. The results showed that the modified protein did not share structurally related sequences with any known allergens examined.

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

EPSPS protein, present in plants, bacteria, and fungi, is one of the enzymes involved in the shikimate pathway for the biosynthesis of aromatic amino acids, , and is located in chloroplasts or plastids in plants (della-Cioppa et al., 1986). The shikimate pathway is an important metabolic pathway considered to be involved in one fifth of carbon fixation by plants (Haslam, 1974; 1993). This pathway is regulated by 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase, which is involved in the first step of the pathway, but it has been clarified to be extremely unlikely that the stages from DAHP to the synthesis of chorismic acid are inhibited or suppressed by metabolic intermediates or end products of this pathway (Weiss and Edwards, 1980; Hermann, 1983). This suggests that EPSPS is not the rate-determining enzyme in this pathway, and thus it is considered that enhanced EPSPS activity will not increase the concentration of aromatic amino acids, the end products of this pathway (Padgette et al., 1996b; Ridley et al., 2002). Actually, it has

[6] FARRP (Food Allergy Research and Resource Program): Database holding 1,471 amino acid sequences registered in the Allergen Online database (FARRP, 2009) as of December, 2009.

been reported that plant cells producing 40 times as much EPSPS as compared to normal do not synthesize excessive aromatic amino acid (Smart et al., 1985). Moreover, amino acid composition in seeds was determined as part of a food/feed safety evaluation conducted for glyphosate herbicide-tolerant crops (soybean, rapeseed, cotton, maize, alfalfa, sugar beet) commercialized as products of Monsanto Company, but no difference was observed in the contents of aromatic amino acids between seeds of genetically modified crops and those of non-recombinant crops, which also supported that EPSPS is not a rate-determining enzyme of this pathway.

EPSPS is an enzyme that catalyzes a reversible reaction to produce EPSP and inorganic phosphates (Pi) from phosphoenolpyruvate (PEP) and shikimate-3-phosphate (S3P) (Levin and Sprinson, 1964) and is known to specifically react with these substrates (Gruys et al., 1992). The only substance known to react with EPSPS other than these substrates is shikimate, an analogue of S3P, but the reaction specificity of EPSPS with shikimate is only one-two millionth of that with S3P, as compared using the specificity constant (k_{cat}/K_m) values representing the readiness of the reaction (Gruys et al., 1992). Thus, it is unlikely that shikimate reacts as the substrate of EPSPS.

(2) Information concerning vector

1) Name and origin

The plasmid vector PV-ZMAP1043 used for developing this recombinant maize has been constructed based on the *E. coli* vector pBR322 (Sutcliffe, 1979), etc.

2) Properties

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

The total number of base pairs of the plasmid vector PV-ZMAP1043 used for developing this recombinant maize is 8,946 bp.

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

As a marker gene for selection in *E. coli*, the constructed vector contains the *aadA* gene, which is derived from *E. coli* transposon Tn7 and confers spectinomycin/streptomycin resistance, outside the T-DNA region.

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

This vector does not possess any infectious characteristic.

(3) Method of preparing living modified organisms

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

The component elements of this plasmid vector transferred into the recipient are shown in p12-11. The position of the component elements of the donor nucleic acid and the restriction enzyme cleavage sites in the vector are shown in Figure 2 (p6).

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

The T-DNA region of PV-ZMAP1043 was introduced by the *Agrobacterium* method into immature embryo cells of a conventional maize cultivar classified into dent type【Confidential: not made available or disclosed to unauthorized persons】×HiII.

3) Processes of rearing of living modified organisms

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

Immature embryos of the conventional maize cultivar【Confidential: not made available or disclosed to unauthorized persons】×HiII were co-cultured with *A. tumefaciens* ABI strain carrying the plasmid vector PV-ZMAP1043 and subsequently transferred to a tissue-culture medium containing glyphosate and carbenicillin. Glyphosate herbicide was used for selecting the transformants.

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

Agrobacterium used for transformation had been removed by the addition of carbenicillin to the tissue-culture medium. Complete removal of *Agrobacterium* from this recombinant maize was confirmed by transferring this recombinant maize to a carbenicillin-free medium and checking the absence of *Agrobacterium* colony formation.

(c) Processes 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 Effect on Biological Diversity

The R0 plants generated through the transformation process were transferred to soil. The R0 plants were crossed with the conventional maize cultivar【Confidential: not made available or disclosed to unauthorized persons】to produce F1 hybrids which were subsequently backcrossed three times with the conventional cultivar. Tolerance to glyphosate herbicide was confirmed during these processes. Homozygous state of the transferred gene was achieved through self-pollination, and progenies of the selected plants were subjected to analysis and morphological characterization. Ultimately, this recombinant maize was selected as the line to be commercialized.

The existence and stability of the expression of the transferred gene in this recombinant maize, as well as the generations subjected to field tests in Japan are shown in Figure 4 (p15). The subjects of this application are the【Confidential: not made available or disclosed to unauthorized persons】BC3F4 generation and all cross progeny lines derived from the【Confidential: not made available or disclosed to unauthorized persons】BC3F4 generation.

【Confidential: Not made available or disclosed to unauthorized persons】

Figure 4 Process of rearing of this recombinant maize

【Confidential: Not made available or disclosed to unauthorized persons】

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

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

The existence of the transferred gene on the chromosome of this recombinant maize was confirmed by analyzing the segregation ratio of the transferred gene using a chi-square test in multiple generations of this recombinant maize.

For producing the three generations used for the test (Figure 4, p15), first, the 【Confidential: not made available or disclosed to unauthorized persons】BC3F4 generation of this recombinant maize homozygous for the modified *cp4 epsps* gene was crossed with a conventional commercial cultivar 【Confidential: not made available or disclosed to unauthorized persons】not carrying the modified *cp4 epsps* gene to produce the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC0F1 generation. The obtained TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC0F1 generation was backcrossed using the【Confidential: not made available or disclosed to unauthorized persons】as a recurrent parent to produce the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC1F1 generation. Then, TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC1F1 generation plants heterozygous for the modified *cp4 epsps* gene were selected by glyphosate herbicide application and backcrossed again to produce the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC2F1 generation. Finally, the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC2F1 generation plants heterozygous for the modified *cp4 epsps* gene were selected by glyphosate herbicide application and self-pollinated to produced the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC2F2 generation.

Glyphosate herbicide was applied to these three generations (the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC1F1 generation, the TI:[【Confidential: not made available or disclosed to unauthorized persons】] BC2F1 generation and the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC2F2 generation) to check the presence or absence of the modified *cp4 epsps* gene for calculating the segregation ratio for the gene. The obtained segregation ratios were analyzed using a

chi-square test. Since the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC1F1 and the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC2F1 generations had been produced by crossing their respective previous generations heterozygous for the modified *cp4 epsps* gene with the【Confidential: not made available or disclosed to unauthorized persons】not carrying the modified *cp4 epsps* gene, the segregation ratio for the modified *cp4 epsps* gene was expected to be 1:1. Meanwhile, the TI:[【Confidential: not made available or disclosed to unauthorized persons】BC3F4] BC2F2 generation had been produced by selecting the previous generation heterozygous for the modified *cp4 epsps* gene and self-pollinating these selected lines, so the segregation ratio for the modified *cp4 epsps* gene was expected to be 3:1.

The result of the chi-square test revealed no statistically significant difference between the observed and expected values of the segregation ratio in the three generations analyzed (Table 2, p18; Appendix 5). Therefore, it was considered that the transferred gene exists on the chromosome of this recombinant maize.

Table 2　Segregation ratio for the transferred gene in this recombinant maize[7]

Generation	Number of plants tested [1]	Observed value[2]		Expected value		χ^2	p value[3]
		Positive	Negative	Positive	Negative		
TI:[【Confidential: not available or disclosed to unauthorized persons】 BC3F4]BC1F1	238	109	129	119	119	1.68	0.194
TI:[【Confidential: not available or disclosed to unauthorized persons】 BC3F4]BC2F1	290	145	145	145	145	0.00	1.000
TI:[【Confidential: not available or disclosed to unauthorized persons】 BC3F4]BC2F2	1107	820	287	830	277	0.50	0.476

[1]The 238, 290 and 1107 plants of the TI: [【Confidential: not available or disclosed to unauthorized persons】 BC3F4] BC1F1 generation, the TI: [【Confidential: not available or disclosed to unauthorized persons】BC3F4] BC2F1 generation and the TI: [【Confidential: not available or disclosed to unauthorized persons】BC3F4] BC2F2 generation, respectively, were obtained from 3, 2 and 6 plants of their respective parent generations, respectively.

[2]The presence or absence of the modified *cp4 epsps* gene was checked by applying glyphosate herbicide (1.89 kg a. e. /ha) to the plants 14 days after sowing. Plants that were surviving or found dead 5 days after the herbicide application were considered positive and negative for the presence of the transferred gene, respectively.

[3]The segregation ratios obtained in these three generations were analyzed using a chi-square test ($p \leq 0.05$).

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

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

Results of Southern blotting analysis of the transferred gene demonstrated that one copy of T-DNA region was integrated at one genomic site in this recombinant maize (Figures 4-6 of Appendix 6, p38-40). It was also confirmed that no region outside the T-DNA region had been inserted. (Figure 7 of Appendix 6, p41).

Moreover, as a result of Southern blotting analysis using the DNA extracted from multiple generations (【Confidential: not made available or disclosed to unauthorized persons】BC3F3,【Confidential: not made available or disclosed to unauthorized persons】BC3F4,【Confidential: not made available or disclosed to unauthorized persons】BC3F6,【Confidential: not made available or disclosed to unauthorized persons】BC3F7 and [【Confidential: not made available or disclosed to unauthorized persons】BC3F7×【Confidential: not made available or disclosed to unauthorized persons】] F1), it was confirmed that the transferred gene was stably inherited across multiple generations (Figure 14 of Appendix 6, p49).

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

This item is not applicable because only one copy was inserted (Figures 4-6 of Appendix 6, p38-40).

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

The results of the Western blotting analysis conducted on multiple generations of this recombinant maize (【Confidential: not made available or disclosed to unauthorized persons】BC3F3,【Confidential: not made available or disclosed to unauthorized persons】BC3F4,【Confidential: not made available or disclosed to unauthorized persons】BC3F6,【Confidential: not made available or disclosed to unauthorized persons】BC3F7 and [【Confidential: not made available or disclosed to unauthorized persons】 BC3F7×【Confidential: not made available or disclosed to unauthorized persons】]F1) demonstrated that the modified CP4 EPSPS protein was stably expressed in all generations tested (Figure 1 of Appendix 7, p15).

This recombinant maize was cultivated in 3 replicates each in 5 fields in the United States (Arkansas, Iowa, Illinois, Indiana and Nebraska), and the amount of expression of the modified CP4 EPSPS protein in various tissues of this recombinant

maize was analyzed by the ELISA method. Samples collected from one particular plot in the Indiana field, potentially contaminated by other events, were excluded from the analysis, so 14 samples were analyzed in total. The expression levels of the modified CP4 EPSPS protein in various tissues of this recombinant maize are shown in Table 3 (p21) (Table 1 p17, Table 2, p18 and Table 3, p19 of Appendix 2).

Regarding the expression level of the modified CP4 EPSPS protein in pollens, the average values (or ranges) were below the detection limit in 6 samples and were 0.49 µg/g fwt (0.18-1.1 µg/g fwt) in 6 samples, among the 14 samples tested (Table 1 of Appendix 2, p17). In the remaining 2 samples, average values of duplicate analysis could not be determined, as the protein was detected in the first analysis but was below the detection limit in the second. The trace amount of the modified CP4 EPSPS protein detected in the pollens of the 6 samples suggests that the modified CP4 EPSPS protein was slightly expressed in the pollens, although the collected samples might have been contaminated by anthers expressing the modified CP4 EPSPS protein (CaJacob et al., 2004; Hamilton et al., 1992).

Table 3 Expression levels of the modified CP4 EPSPS protein in various tissues of this recombinant maize cultivated in fields in the United States (2008, the United States)[8]

Tissue[1]	Growth stage[2]	Days after sowing	Modified CP4 EPSPS protein (Standard deviation) Range		Detection limit/ quantification limit
			(μg/g fresh weight)[3]	(μg/g dry weight)[4]	(μg/g fresh weight)
leaf (OSL-1)	2nd-5th leaf	20-28	100 (21) 75 – 140	680 (170) 400 – 940	0.069/0.137
Leaf (OSL-2)	6th-8th leaf	32-46	83 (25) 30 –110	410 (130) 130 – 560	0.069/0.137
Leaf (OSL-3)	10th-12th leaf	41-67	61 (19) 35 –95	290 (74) 210 – 410	0.069/0.137
Leaf (OSL-4)	Tasseling	54-73	95 (30) 17 – 140	370 (120) 70 – 520	0.069/0.137
Kernel	Harvest	118-182	3.6 (0.73) 2.6 – 5.3	4.2 (0.89) 2.8 – 6.2	0.16/0.228
Pollen[5]	Pollinating	58-81	< LOD (NA) NA 0.49 (0.36) 0.18 – 1.1	<LOD (NA) NA 0.87 (0.70) 0.25 – 2.2	0.099/0.137
Silk	Pollinating	58-76	9.4 (0.97) 8.1 – 11	100 (12) 90 – 120	0.121/0.137
Aerial parts	Yellow ripe	83-116	38 (14) 8.3 – 57	120 (48) 21 – 200	0.069/0.137
Foliage	Maturation	124-180	14 (6.3) 5.9 – 26	43 (27) 13 – 98	0.069/0.137
Root (OSR-1)	2nd-5th leaf	22-28	18 (5.3) 8.1 – 27	140 (46) 58 – 210	0.033/0.068
Root (OSR-2)	6th-8th leaf	32-46	16 (6.8) 8.3 –29	110 (62) 48 – 240	0.033/0.068
Root (OSR-3)	10th-12th leaf	41-67	12 (4.3) 4.9 –19	73 (28) 22 – 110	0.033/0.068
Root (OSR-4)	Tasseling	54-73	15 (5.7) 5.6 – 23	83 (36) 23 – 140	0.033/0.068
Root (Initial yellow ripe)	Initial yellow ripe	83-116	15 (5.2) 8.6 – 24	72 (23) 39 – 100	0.033/0.068
Root (Immediately after harvest)	Maturation	124-180	16 (8.3) 5.9 – 29	72 (37) 26 – 130	0.033/0.068
Aerial parts (OSWP-1)	2nd-5th leaf	22-28	50 (8.3) 37 – 66	500 (190) 310 – 840	0.069/0.137
Aerial parts (OSWP-2)	6th-8th leaf	32-46	46 (7.6) 33 – 58	360 (42) 300 – 420	0.069/0.137
Aerial parts (OSWP-3)	10th-12th leaf	41-67	43 (7.1) 28 – 56	380 (78) 230 – 500	0.069/0.137
Aerial parts (OSWP-4)	Tasseling	54-73	37 (6.3) 23 – 47	240 (42) 160 – 340	0.069/0.137

[1] OSL= over-season leaf (leaf); OSR= over-season root (root); OSWP= over-season whole plant (aerial parts)

[2] Growth stages of tissue samples collected

[3] Protein expression level expressed as average value and standard deviation (shown in parentheses). Protein weight expressed as μg per 1g of tissue on a fresh weight basis. The average value, standard deviation and range (minimum – maximum) were calculated for each tissue across all sites (n=14 for all tissues, except for root (initial yellow ripe) (n=11) and pollen (n=6)). NA: Not Applicable; LOD=Limit of detection

[4] Protein expression level expressed as mean value and standard deviation (shown in parentheses). Protein weight expressed as μg per 1g tissue on a dry weight basis. Dry weight was calculated by dividing the fresh weight by

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the dry weight conversion factor obtained from moisture analysis data. NA: Not Applicable; LOD=Limit of detection

[5]The expression levels of the modified CP4 EPSPS protein in pollens of this recombinant maize were either below the detection limit (upper row; n=6) or extremely low (lower row; n=6) in all fields tested. Pollen samples collected from 2 fields producing unclear results were excluded from the calculation.

(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 transferred nucleic acid does not contain any sequence allowing transmission and thus, it is unlikely that the nucleic acid transferred to this recombinant maize could be transmitted to any other wild animals and plants through virus infection and/or other routes.

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

Detection and identification of this recombinant maize is available by the PCR method (Appendix 8).

The recommended DNA concentration for the test is 5-10ng per PCR. The test can be conducted using leaf discs.

The reliability of the method has been verified by collecting 3 leaf discs per plant and analyzing them in triplicates by the method to identify the plant as this recombinant maize or a non-recombinant maize. Then, 45 leaf discs from 1-3 plants identified as this recombinant maize and 45 leaf discs from 1-3 plants identified as non-recombinant maize were collected and analyzed by this method. The results showed that 44 and 1 among the 45 samples of this recombinant maize were positive and false-negative, respectively, whereas 44 and 1 out of 45 samples of non-recombinant maize plants were negative and false-positive, respectively. The false-negative rate and the false-positive rate were both within the acceptable range (within 5%), so further test was not conducted for the 2 samples yielding the false-negative/false-positive results.

(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

Due to the tissue-specific expression of the modified CP4 EPSPS protein, this recombinant maize exhibits male sterile phenotype upon glyphosate herbicide application, while its vegetative and female reproductive tissues exhibit glyphosate herbicide tolerance. This characteristic of this recombinant maize was confirmed by the

following tests.

【Assessment of pollen viability and total number of pollens】

The expression of male sterility in this recombinant maize after glyphosate herbicide application was confirmed in a greenhouse test conducted in the United States in 2010, in which the pollen viability and the total number of pollens was examined in the HC50 BC6F4 generation after glyphosate herbicide application (Figure 4, p15). The pollen viability and the number of pollens of this recombinant maize were evaluated in the following three groups: the first group did not have glyphosate herbicide applied; the second group had a normal dosage (0.84 kg a.e.[9]/ha) of glyphosate herbicide applied only at the 3rd-leaf stage (V3) corresponding to the optimum application period conventionally adopted for weed control; and the third group had a normal dosage (0.84 kg a.e. /ha) of glyphosate herbicide applied at the 3rd-leaf (V3), the 8th-leaf (V8), and the 10th-leaf (V10) stages corresponding to the early stage of tassel formation (Appendix 9). Five florets, each collected from tassels of individual plants, were stained with Alexander's stain (Alexander, 1969) to examine pollen viability, while the rest of the florets were used for counting the total number of pollens (sum of viable and non-viable pollens) (Appendix 9). The pollen viability was evaluated as the ratio (%) of viable pollens to all pollens observed under a microscope after Alexander staining. The total number of pollens were obtained by extracting a portion of pollens from the florets (excluding those used for the pollen viability test) collected from the individual plants, counting the extracted pollens and calculating the total number of pollens for the entire tassel based on the number of the extracted pollens.

The results of the examination showed that there was no statistically significant difference ($p>0.05$) either in the pollen viability or the total number of pollens between the plot in which glyphosate herbicide was not applied and the plot in which glyphosate herbicide was applied at the 3rd-leaf stage (V3). Meanwhile, statistically significant differences ($p<0.05$) were observed both in the pollen viability and in the total number of pollens between the plot in which glyphosate herbicide was not applied and the plot in which the herbicide was applied at the 3rd-leaf (V3), the 8th-leaf (V8) and the 10th-leaf (V10) stages (Table 4, p27). The viability of pollens of this recombinant

[9] a.e.; Acid equivalent. In herbicide formulations, active ingredients may exist either in free states or in the form of salts. For those existing in the form of salts, acids serve as their active components, while the base portions differ among formulations. If the herbicide dosage is expressed in terms of the salt of the active ingredient in the formulation, the amount of the active components cannot be accurately compared between formulations having different base portions. Thus, acid equivalent was used as the unit for representing the active components.

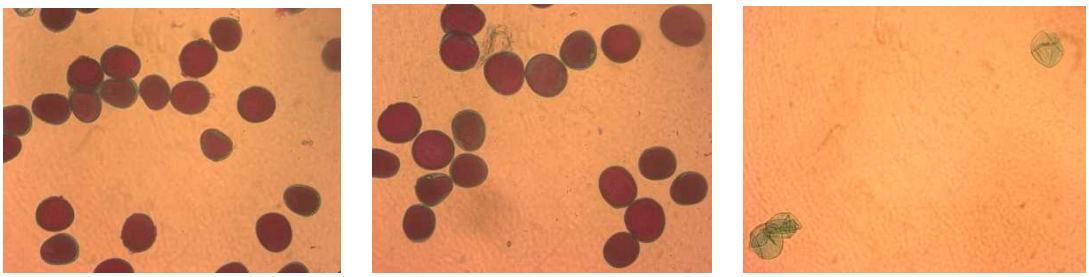
maize after glyphosate herbicide application is shown in Figure 5 (p27). When pollens stained with the Alexander's stain were observed under a microscope, viable pollens were viewed as purple-stained, round grains (Figure 5, p27: A and B), whereas non-viable pollens were viewed as unstained or blue/green, shrunken grains (Figure 5, p27: C).

Table 4 Examination of pollen viability (%) and total number of pollens after glyphosate herbicide application to this recombinant maize[10]

	This recombinant maize No application		**This recombinant maize** Application at 3rd-leaf (V3) stage		**This recombinant maize** Application at 3rd-leaf (V3), 8th-leaf (V8) and 10th-leaf (V10) stages	
Pollen characteristics	**Average**	**Standard error**	**Average**	**Standard error**	**Average**	**Standard error**
Pollen fertility (%)	96.9	1.1	90.7	5.3	0.0*	0.0
Total number of pollens	2,515,000	96,640	2,781,111	209,459	1,188,571*	151,743

1 plant/plot, 10 replicates, n=10

* indicates that a statistically significant difference was observed between the plot in which glyphosate herbicide was not applied and the plot in which the herbicide was applied at the 3rd-leaf (V3), the 8th-leaf (V8) and the 10th-leaf (V10) stages ($p \leq 0.05$).



A. No application B. Application at V3 C. Application at V3, V8 and V10

Figure 5 Pollens of this recombinant maize after glyphosate herbicide application (Alexander staining)[11]

A. Glyphosate herbicide was not applied.

B. A normal dosage of glyphosate herbicide (0.84 kg a.e./ha) was applied at the 3rd-leaf (V3) stage.

C. A normal dosage of glyphosate herbicide (0.84 kg a.e./ha) was applied at the 3rd-leaf (V3), the

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

8th-leaf (V8) and the10th-leaf (V10) stages.

【Assessment of pollen viability based on anther extrusion rate】

The fertility of pollens of this recombinant maize after glyphosate herbicide application was confirmed by examining the anther extrusion rate.

In general, anther extrusion is used as an index of pollen fertility among those engaged in breeding and hybrid seed production of maize. The absence of anther extrusion is generally considered to reflect the sterility of pollens of cytoplasmic male-sterile plants (Beckett, 1971). As in cytoplasmic male-sterile plants, the formation of fertile pollens is also arrested in this recombinant maize if glyphosate herbicide is applied at the initial stage of tassel formation, so the absence of anther extrusion was examined to confirm male sterility in this recombinant maize.

Anther extrusion is a part of the process through which pollens are released from maize plants; thus, no pollen would be released if anthers are not extruded.

Anther extrusion study of this recombinant maize was performed in a greenhouse test conducted in the United States in 2010, in which the anther extrusion ratio was examined in the HC50 BC6F4 generation after glyphosate herbicide application (Figure 4, p15). The pollen viability was evaluated based on the anther extrusion rate calculated from the anther extrusion data obtained for the following three groups: the first group did not have glyphosate herbicide applied; the second group had a normal dosage (0.84 kg a.e. /ha) of glyphosate herbicide applied only at the 3rd-leaf stage (V3); and the third group had a normal dosage of the herbicide applied at the 3rd-leaf (V3), the 8th-leaf (V8), and the 10th-leaf (V10) stages (Appendix 9).

As a result, in the group in which glyphosate herbicide was not applied and the group in which the glyphosate herbicide was applied only at the 3rd-leaf (V3) stage, the anther extrusion rates were 100% in all periods examined, demonstrating the fertility of the plants. Meanwhile, the anther extrusion rate of this recombinant maize to which the herbicide was applied at the 3th-leaf (V3), the 8th-leaf (V8) and the 10th-leaf (V10) stages was 0%, demonstrating the male sterility of the plants (Table 5, p29).

Table 5 Examination of the anther extrusion rate after glyphosate herbicide application to this recombinant maize[12]

Period of glyphosate herbicide application	Anther extrusion rate Average[1, 2, 3] (%)		
	Period of observation		
	S90[4]	S90 + 3[5]	S90 + 6[6]
No application	100	100	100
Application at 3rd-leaf (V3) stage	100	100	100
Application at 3rd-leaf (V3), 8th-leaf (V8) and 10th-leaf (V10) stages	0	0	0

[1]1 plant/plot, 10 replicates, n=10

[2]Average of anther extrusion rate was calculated using the formula: [(LP×0.25) + (MP×0.5) + (HP×1.0)] / number of plants×100%, in which LP (Light Partial) represents the number of plants in which 10 or less anthers were extruded per plant, MP (Medium partial) represents the number of plants in which 11 or more anthers were extruded per plant but the overall anther extrusion rate was below 25%, and HP (Heavy partial) represents the number of plants in which 25% or more anthers were extruded in a plot (Appendix 9).

[3]Average of anther extrusion rate was calculated for all plants examined for each period of glyphosate herbicide application.

[4] S90: Day by which 90% or more plants showed extrusion of silks

[5] S90 + 3: Three days after S90

[6] S90 + 6: Six days after S90

Based on these results, regarding the pollen viability, total number of pollens and anther extrusion rate, no difference was observed between this recombinant maize to which glyphosate herbicide was not applied and this recombinant maize to which the herbicide was applied at the 3rd-leaf (V3) stage, demonstrating that glyphosate herbicide application to this recombinant maize at the 3rd-leaf (V3) stage would not induce male sterility. Furthermore, regarding the pollen viability, total number of pollens and anther extrusion rate, differences were observed between this recombinant maize to which glyphosate herbicide was not applied and this recombinant maize to which the herbicide was applied three times in total at the 3rd-leaf (V3), the 8th-leaf (V8) and the 10th-leaf (V10) stages, demonstrating that glyphosate herbicide application to this recombinant maize at the 3rd-leaf (V3), the 8th-leaf (V8) and the 10th-leaf (V10) stages would induce male sterility.

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(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[13]

In 2010, an isolated field test of this recombinant maize was conducted in an isolated field at the Kawachi Research Farm of Monsanto Japan Limited, with the use of the [【Confidential: not made available or disclosed to unauthorized persons】 BC3F7×【Confidential: not made available or disclosed to unauthorized persons】] F1 generation of this recombinant maize (Figure 4, p15).【Confidential: not made available or disclosed to unauthorized persons】 sharing the genetic background of this recombinant maize was used as the non-recombinant control maize. For assessment of cold-tolerance, a test was conducted in a climate chamber of Monsanto Company (U.S.) in 2009.

a. Morphological and growth characteristics

The following 12 items for morphological and growth characteristics were examined between this recombinant maize and the non-recombinant control maize: date of germination uniformity (date), germination rate (%), tassel emergence date (date), silk emergence date (date), main stem height (cm), height of ear setting (cm), number of tiller, flag leaf angle, maturation date (date), plant weight at harvest (kg), grain shape and grain color. The items were evaluated based on the test guidelines of individual agricultural, forestry and aquatic plant species established for variety registration.

As a result, a statistically significant difference was found between this recombinant maize and the non-recombinant control maize only for the weight of aerial parts at harvest (Table 3 of Appendix 10, p10). Regarding the weight of aerial parts at harvest, the average value for this recombinant maize was 0.72 kg, which was lower than that for the non-recombinant control maize (0.78 kg) (Table 3 of Appendix 10, p10).

b. Cold-tolerance or heat-tolerance at the early stage of growth

In order to assess the cold tolerance at the early stage of growth in this recombinant

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maize and the non-recombinant control maize, plants at the 3rd-leaf stage (V3) were cultivated for 20 days at alternating temperatures of 12ºC day/5ºC night and subsequently examined for plant height, growth stage, growth vigor and weight of aerial parts (fresh or dry weight). For reference, 4 commercial cultivars were also examined for these items.

The results revealed no statistically significant difference between this recombinant maize and the non-recombinant control maize in any of the items examined (Table 4 of Appendix 11, p19).

c. Wintering ability or summer survival of the matured plant

Maize is a summer annual plant, and after seed set, it usually dies out in winter, never showing regrowth, vegetative reproduction nor seed production thereafter. Actually, when this recombinant maize and the non-recombinant control maize were left in the field after they reached maturation in a wintering ability test zone of an isolated field, all were found withered and dead by November 8, 2010 (Figure 5 of Appendix 10, p12).

d. Fertility and size of the pollen

Both this recombinant maize and the non-recombinant control maize showed high pollen fertility. There was no substantial difference in fertility, shape and size of the pollens between this recombinant maize and the non-recombinant control maize (Figure 6 of Appendix 10, p13).

In 2008, fertility and size were examined in pollens collected from this recombinant maize and the non-recombinant control maize cultured in a field in Missouri, U.S. As a result, a statistically significant difference was observed for pollen fertility between this recombinant maize and the non-recombinant control maize ($p<0.05$) (Table 2 of Appendix 12, p11). The average value of pollen fertility for this recombinant maize was 99.7%, which was higher than that for the non-recombinant control (98.9%) and fell outside the range of the average values for the 4 commercial cultivars (99.2-99.6%) examined for reference (Table 2 of Appendix 12, p11). Meanwhile, no statistically significant difference was observed for pollen size (Table 2 of Appendix 12, p11; Figures 1~3 of Appendix 12, p12-13).

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

Seed production was evaluated by examining the following 6 items in this recombinant maize and the non-recombinant control maize: total number of effective ears, ear length (cm), ear diameter (cm), row number per ear, grain number per row and 100-kernel weight (g).

As a result, no statistically significant difference was observed between this recombinant maize and the non-recombinant control maize in any of the items examined (Table 4 of Appendix 10, p14).

For comparing shedding habit, this recombinant maize and the non-recombinant control maize were visually examined for the presence of husks covering the ears and the occurrence and degree of shedding after husk removal at harvest.

In both this recombinant maize and the non-recombinant control maize, the ears were covered with husk at harvest, and shedding was not observed under natural conditions. In addition, the seeds hardly shed even after husks were removed from the ears, and no difference was observed between the recombinant maize and the non-recombinant maize in terms of the shedding habit of seeds (Table 4 of Appendix 10, p14).

To evaluate dormancy and germination rate, seeds harvested 15 days beforehand were sown on a petri dish incubated in an incubator at 25ºC, and the number of germinated seeds was counted at several time points.

As a result, seeds of this recombinant maize and the non-recombinant control maize exhibited high germination rates, with no statistically significant difference between the two lines (Table 4 and Table 5 of Appendix 10, p14-15). The seeds of this recombinant maize and the non-recombinant control maize showed no dormancy.

f. Crossability

Crossability test was not performed since no wild relatives that can be naturally crossed with maize of the recipient organism are growing in Japan.

g. Productivity of harmful substances

In order to check that this recombinant maize does not produce any substances which can affect other plants and/or microorganisms in soil, soil microflora test, plow-in test and succeeding crop test were carried out. As a result, no statistically significant difference

was observed between this recombinant maize and the non-recombinant control for the number of microorganisms in soil and the germination rate and dry weight of radish (Tables 6-8 of Appendix 10, p17).

5

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

Name of the Type of Living Modified Organism: Glyphosate-induced male-sterile and glyphosate-tolerant maize (Modified *cp4 epsps*, *Zea mays* subsp. *mays* (L.) Iltis) (MON87427, OECD UI: MON-87427-7)

Content of the Type 1 Use: Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them

Applicant: Monsanto Japan Limited

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

This recombinant maize was produced by introducing the T-DNA region of the plasmid PV-ZMAP1043, constructed based on the *E. coli* plasmid pBR322 etc., by the *Agrobacterium* method.

The results of gene segregation analysis and Southern blotting analysis demonstrated that 1 copy of the T-DNA region containing the modified *cp4 epsps* gene etc. encoding the modified CP4 EPSPS protein (5-enolpyruvylshikimate-3-phosphate synthase), derived from *Agrobacterium* CP4 strain, was integrated into the chromosome of this recombinant maize and was stably inherited across multiple generations. In addition, the results of the Western blotting analysis demonstrated that these genes were stably expressed across multiple generations.

In this recombinant maize, the expression of the modified *cp4 epsps* gene is controlled by the *e35S* promoter for inducing male sterility by glyphosate herbicide application. Therefore, the modified CP4 EPSPS protein in this recombinant maize is not or scarcely expressed in the tapetum cells and microspores but is expressed in the vegetative and female reproductive tissues at levels sufficient for imparting glyphosate herbicide tolerance to the plant.

(A) 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.

In an isolated field test conducted in Japan in 2010, various traits related to competitiveness were examined in this recombinant maize. A statistically significant difference was observed for the average weight of aerial parts at harvest, which was

0.72 kg for this recombinant maize and 0.78 kg for the non-recombinant control maize. However, this was merely a slight difference, and since no statistically significant difference was observed in any other items related to morphological and growth characteristics as well as seed production, which were also examined during the same period, the observed difference was not considered to increase the competitiveness of this recombinant maize.

In a field trial conducted in the United States in 2008, among the pollen-related items examined, a statistically significant difference was observed for pollen viability between this recombinant maize (99.7%) and the non-recombinant control maize (98.9%). Nevertheless, both this recombinant maize and the non-recombinant control maize showed high pollen viability. In addition, the pollen viability of this recombinant maize merely slightly exceeded the range of the average values for the 4 commercial cultivars. Therefore, the difference in pollen viability is unlikely to provide any competitive advantage to the this recombinant maize.

This recombinant maize produces the modified CP4 EPSPS protein, which confers glyphosate herbicide tolerance, in the vegetative and female reproductive tissues, but since glyphosate herbicide is not likely to be applied under natural conditions, it is unlikely that glyphosate herbicide tolerance would increase the competitiveness of this recombinant maize.

Based on the above understanding, it was judged that the conclusion made by the applicant that the wild animals and plants likely to be affected cannot be specified and that the use of this recombinant maize poses no risk of Adverse Effect on Biological Diversity attributable to competitiveness is valid.

(B) Productivity of harmful substances

Maize, the taxonomical species to which the recipient organism belongs, has been long used in Japan, though there is no report that it produces any harmful substances.

This recombinant maize produces the modified CP4 EPSPS protein, which confers glyphosate herbicide tolerance, in the vegetative and female reproductive tissues, but it has been confirmed that the protein does not contain any sequence which is structurally analogous to known allergens. Moreover, the modified CP4 EPSPS protein acts as an enzyme catalyzing the shikimate pathway for the biosynthesis of aromatic amino acids but is not the rate-determining enzyme in this pathway. It has been confirmed that enhanced EPSPS activity does not increase the concentration of aromatic amino acids, the end products of this pathway.

In isolated fields in Japan, productivity of harmful substances (including the substances secreted from the roots which can affect other plants and microorganisms in soil, and the substances existing in the plant body which can affect other plants after dying) of this recombinant maize was examined by conducting soil microflora test, plow-in test and succeeding crop test. As a result, no statistically significant difference was observed between this recombinant maize and the non-recombinant control.

Based on the above understanding, it was judged that the conclusion made by the applicant that the wild animals and plants likely to be affected cannot be specified and that the use of this recombinant maize poses no risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is valid.

(C) Crossability

In Japan, neither maize nor its cross-compatible wild relative, teosinte, has been found self-seeding in a natural environment. Therefore, no wild animal or plant species likely to be affected by crossability of this recombinant maize could be specified.

Based on the above understanding, it was judged that the conclusion made by the applicant that the use of this recombinant maize poses no risk of Adverse Effect on Biological Diversity attributable to crossability is valid.

(2) Conclusion based on the Biological Diversity Risk Assessment Report

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

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Appendix list for
Glyphosate-induced male-sterile and glyphosate-tolerant maize
(Modified *cp4 epsps, Zea mays* subsp. *mays* (L.) Iltis)
(MON87427, OECD UI: MON-87427-7)

Appendix 1	CP4 EPSPS Immunolocalization Study in MON 87427 (Confidential)
Appendix 2	Assessment of CP4 EPSPS Protein Level in Corn Tissues Collected from MON 87427 Produced in U.S. Field Trials During 2008 (MSL0022370) (Confidential)
Appendix 3	Deduced Amino Acid Sequence of the Modified CP4 EPSPS Protein Based on the Nucleotide Sequence of the Modified *cp4 epsps* Gene Used for Developing This Recombinant Maize (Confidential)
Appendix 4	Molecular Map of the Gene Transferred into the Glyphosate Herbicide-Tolerant Maize NK603 (Confidential)
Appendix 5	Heritability and Stability of Coding Sequences Present in MON 87427 Across Multiple Generations (RPN-09-275) (Confidential)
Appendix 6	Molecular Characterization of MON 87427 (MSL0021822) (Confidential)
Appendix 7	Demonstration of the Presence of CP4 EPSPS Protein in Corn Leaf and Seed Samples of MON 87427 Across Multiple Generations by Western Blot Analysis (MSL0022026) (Confidential)
Appendix 8	Corn RHS HAM027 Event Specific EndPoint TaqMan PCR (BQ-QC-10456-01) (Confidential)
Appendix 9	MON 87427 Pollen Viability, Total Pollen Number and Anther Extrusion 2010 North America (RAR-10-404) (Confidential)
Appendix 10	Report on the Results from Biological Diversity Risk Assessment on Glyphosate-induced Male-sterile and Glyphosate-tolerant Maize (Modified *cp4 epsps, Zea mays* subsp. *mays* (L.) Iltis) (MON87427, OECD UI: MON-87427-7) in Isolated Field (Confidential)
Appendix 11	Assessment of the Effect of Cold Stress on the Growth of MON 87427

Under Growth Chamber Conditions (MSL0022023) (Confidential)

Appendix 12 Pollen Viability and Morphology Evaluation of MON 87427 in a U.S. Field Trial During 2008 (MSL0021913) (Confidential)

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