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

Name: Monsanto Japan Limited
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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Low saturated fatty acid and high oleic acid soybean tolerant to glyphosate herbicide (*FAD2-1A*, *FATB1-A*, modified *cp4 epsps*, *Glycine max* (L.) Merr.) (MON87705, OECD UI: MON-877Ø5-6)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

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

1 Information concerning preparation of living modified organisms

Soybean oil is widely utilized in a variety of foods. However, soybean oil is susceptible to oxidization due to the higher contents of polyunsaturated fatty acids. In addition, it is known that in the process of heating soybean oil or hydrogenation of soybean oil for the increased stability, some polyunsaturated fatty acids are transformed into trans fatty acids. Furthermore, it is reported that saturated fatty acids such as palmitic acid and myristic acid contained in soybean oil take part in the increasing LDL cholesterol (WHO, 2003). Then Monsanto Company has developed the low saturated fatty acid and high oleic acid soybean tolerant to glyphosate herbicide (*FAD2-1A*, *FATB1-A*, modified *cp4 epsps*, *Glycine max* (L.) Merr.) (MON87705, OECD UI: MON-877Ø5-6) (hereinafter referred to as "this recombinant soybean"), which features the enhanced versatility in application to food due to the modified fatty acid composition in soybean seed. Soybean oil derived from this recombinant soybean contains the same five (5) major fatty acids [palmitic acid and stearic acid (saturated fatty acid), oleic acid (monounsaturated fatty acid), and linoleic acid and linolenic acid (polyunsaturated fatty acid)] as found in conventional soybean seeds. However, soybean oil from this recombinant soybean features reduced levels of saturated fatty acids and increased levels of oleic acid compared to conventional soybean oil, providing the fatty acid profile similar to the vegetable oils such as canola oil and olive oil, which are versatile and plentiful (Figure 1, p3). Therefore, soybean oil obtained from this recombinant soybean can be utilized in many food applications such as in cooking, salad dressing etc. as the stability of the oil improved oil even without hydrogenation.

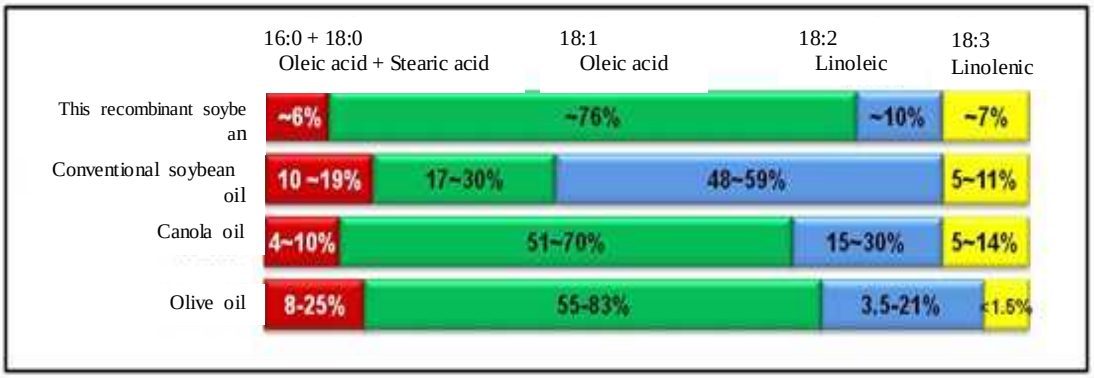

Figure 1 Comparison of fatty acid composition between this recombinant soybean oil and other vegetable oils[1]

(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 soybean are shown in Figure 2 (p5) and Table 1 (p6-9).

This recombinant soybean contains partial sequence of the soybean endogenous genes, *FAD2-1A* and *FATB1-A*, and these genes are referred to as "*FAD2-1A* gene segment" and "*FATB1-A* gene segment", respectively.

This recombinant soybean was produced using *Agrobacterium* transformation method by transferring the plasmid vector PV-GMPQ/HT4404 (Figure 2, p5) that contains two T-DNAs. The transferred T-DNA I and T-DNA II in this recombinant soybean both contain DNA segments designed to suppress expression of the endogenous *FAD2* and *FATB* genes which encode the enzymes in the soybean fatty acid biosynthetic pathway by RNAi **(RNA interference)**. T-DNA I also contains the intron of *FAD2-1A* gene under control of *7Sα'* promoter and the 5'-untranslated region sense chain of *FATB1-A* gene. T-DNA II also contains the intron of *FAD2-1A* gene and the 5'-untranslated region anti-sense chain of *FATB1-A* gene. During the transformation to produce this recombinant soybean, in order to allow the RNA transcribed from *FAD2-1A* and *FATB1-A* gene segments to form a double stranded RNA (dsRNA), Invader assay [2] was used for selection of the plant individuals that contain the *FAD2-1A* and *FATB1-A* gene segments from T-DNA I of the plasmid vector PV-GMPQ/HT4404 (Figure 2, p5) and the *FAD2-1A* and *FATB1-A* gene segments from T-DNA II co-integrated at one locus in the soybean genome in the form of inverted repeat (Figure 6, p23) adjacent to

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

[2] Invader assay is a signal amplification technology for identification of single nucleotide polymorphism (SNP) or genetic variation and/or quantitative analysis of genes. Invader assay allows identifcation of any mutaions based on cleavage process known as Invader® method without the need for gene amplifcastion by PCR. In the cleavage process, target gene sequences are cleaved by the enzyme known as Cleavase® that can specifically identify structures for detection of fluorsescence. Invader® and Cleavase® are registered trademarks of Third Wave Technologies, Inc.

each other.

In addition, amino acid sequence of CP4 EPSPS protein expressed from the *cp4 epsps* gene which was transferred into this recombinant soybean was changed from serine to leucine in the second amino acid position from the N-terminus compared to that of CP4 EPSPS protein from the *Agrobacterium* sp. strain CP4 due to the transferring of restriction enzyme cleavage sites in the process of cloning. Therefore, the *cp4 epsps* gene transferred into this recombinant soybean is referred to as the "modified *cp4 epsps* gene" and the expressed protein is referred to as the "modified CP4 EPSPS protein." This protein is identical to the protein expressed in the soybean tolerant to glyphosate herbicide (*cp4 epsps*, *Glycine max* (L.) Merr.) (40-3-2, OECD UI: MON-Ø4Ø32-6), which has been already approved under Type I Use Regulation for use in other glyphosate tolerant plants developed to date by Monsanto Company. The deduced amino acid sequences of the modified CP4 EPSPS protein expressed in this recombinant soybean are shown in Annex1.

2) Functions of component elements

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

Functions of individual component elements of the donor nucleic acid that were used for the development of this recombinant soybean are shown in Table 1 (p6-9).

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

Figure 2 Map of the plasmid PV-GMPQ/HT4404

In the process of rearing of this recombinant soybean, those plants were selected that contain T-DNA I and T-DNA II regions shown above are co-integrated at one locus in the soybean genome.

Table 1 Composition of the donor nucleic acid and origins and functions of the component elements[3]

Component elements	Origin and function
T-DNA I	
B[Note 1]-Left Border	DNA region from *Agrobacterium tumefaciens*, containing the left border sequence used for transfer of the T-DNA (Barker, *et al.*, 1983).
Intervening Sequence	Sequence used in DNA cloning
P[Note2]-*FMV/EF-1α*[4]	Chimeric promoter consisting of enhancer sequences from the promoter of the Figwort Mosaic Virus (FMV) 35S RNA (Richins, *et al.*, 1987) combined with the *EF-1α* promoter from the *Arabidopsis thaliana* (Axelos, *et al.*, 1989). Involved in the constant expression of the target gene in the entire tissue of plant body.
L[Note 3]- *EF-1α*	Leader (exon 1) sequence of the *EF-1α* gene that encodes the translation elongation factor EF-1 alpha from *A. thaliana* (Axelos *et al.*, 1989). Enhances the expression of the transferred gene.
I[Note 4]- *EF-1α*	Intron sequence of the *EF-1α* gene that encodes the translation elongation factor EF-1 alpha from *A. thaliana* (Axelos *et al.*, 1989). Enhances the expression of the transferred gene.
Intervening Sequence	Sequence used in DNA cloning
TS[Note 5]-*CTP2*	Targeting sequence from the S*hkG* gene encoding the transit peptide region of *A. thaliana* EPSPS protein (Herrmann, 1995; Klee, *et al.*, 1987). Directs transport of the modified CP4 EPSPS protein to the chloroplast from the cytoplasm.
CS[Note 6]-modified *cp4 epsps*	Coding sequence of the *aroA* gene from the *Agrobacterium sp*. strain CP4 encoding the CP4 EPSPS protein (Barry, *et al.*, 1997; Padgette, *et al.*, 1996a).
Intervening Sequence	Sequence used in DNA cloning

[3] All the rights pertinent to the information in the table above and the responsibility for the content remain with Monsanto Japan Limited.

[4] *EF-1α* is identifical to *Tsf1* referred to in Table 1 (p33) in Annex 5.

Table 1 (Continued) Composition of the donor nucleic acid and origins and functions of the component elements

Component elements	Origin and function
T-DNA I (Continued)	
T[Note 7]-*E9*	3'-terminal untranslated region of the pea *RbcS2* gene encoding the subunit of ribulose-1, 5-bisphosphate carboxylase from *Pisum sativum*. Directs polyadenylation of the mRNA (Coruzzi, *et al.*, 1984).
Intervening Sequence	Sequence used in DNA cloning
P-*7Sα'*	Promoter and leader sequence from the *Sphas1* gene of *Glycine max* encoding β-conglycinin storage protein (alpha'-bcsp) (Doyle, *et al.*, 1986). Directs mRNA transcription in soybean seed (Chen *et al.*, 1986).
Intervening Sequence	Sequence used in DNA cloning
FAD2-1A[pNote 8]	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the Δ-12 desaturase (Fillatti, *et al.*, 2003).
FATB1-A[p]	Partial sequence from the 5'-untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti *et al.*, 2003).
Intervening Sequence	Sequence used in DNA cloning
B-Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of T-DNA (Depicker, *et al.*, 1982; Zambryski, *et al.*, 1982).

Table 1 (Continued) Composition of the donor nucleic acid and origins and functions of the component elements

Component elements	Origin and function
Backbone region (not present in this recombinant soybean)	
Intervening Sequence	Sequence used in DNA cloning
aadA	Bacterial promoter, coding sequence and 3'-untranslated region for 3'(9)-O-nucleotidyltransferase (aminoglycoside-modifying enzyme) from the transposon *Tn7* (Fling, *et al.*, 1985). Confers resistance to spectinomycin and streptomycin.
Intervening Sequence	Sequence used in DNA cloning
OR[Note 9]*-ori.pBR322*	Replication origin region isolated from pBR322 that permits autonomous replication of vectors in *E. coli* (Sutcliffe, 1979).
Intervening Sequence	Sequence used in DNA cloning
CS-*rop*	Coding sequence for repressor of primary protein from the ColE1 plasmid that maintains plasmid copy number in *Escherichia coli* (Giza and Huang, 1989).
Intervening Sequence	Sequence used in DNA cloning
T-DNA II	
B-Left Border	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of T-DNA (Barker *et al.*, 1983).
Intervening Sequence	Sequence used in DNA cloning
T-*H6*	3'-untranslated region sequence of the *H6* gene from *Gossypium barbadense* (pima cotton) encoding a fiber protein involved in secondary cell wall assembly (John and Keller, 1995).
Intervening Sequence	Sequence used in DNA cloning

Table 1 (Continued) Composition of the donor nucleic acid and origins and functions of the component elements

Component elements	Origin and function
T-DNA II (Continued)	
FAD2-1A[p]	Partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the Δ12 desaturase (Fillatti *et al.*, 2003).
FATB1-A[p]	Partial sequence from the 5'-untranslated region and the plastid targeting sequence from *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti *et al.*, 2003).
Intervening Sequence	Sequence used in DNA cloning
B-Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of T-DNA (Depicker *et al.*, 1982; Zambryski *et al.*, 1982).
Backbone region (not present in this recombinant soybean)	
Intervening Sequence	Sequence used in DNA cloning
OR-*ori* V	Origin of replication from the broad host range plasmid RK2, conferring the autonomous replication to vectors in *Agrobacterium* (Stalker, *et al.*, 1981).
Intervening Sequence	Sequence used in DNA cloning

Note 1 B – Border sequence
Note 2 P – Promoter
Note 3 L – Leader sequence
Note 4 I – Intron
Note 5 TS – Targeting Sequence
Note 6 CS – Coding Sequence
Note 7 T – Transcriptional Termination Sequence
Note 8 [P]– Partial sequence
Note 9 OR – Origin of Replication

(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 which is known to possess any allergenicity

[Expression products from *FAD2-1A* gene segment and *FATB1-A* gene segment]

The RNAs produced from *FAD2-1A* and *FATB1-A* gene segments transferred into this recombinant soybean (Table 1, p6-9), suppresses expression of the endogenous *FAD2* and *FATB* genes. There is no report that RNAs possess any allergic property or toxicity, and nucleic acid has been ingested safely for a long time and approved by the US Food and Drug Administration (FDA) as GRAS (generally recognized as safe) food substance (FDA, 1992).

In this recombinant soybean, expression of the soybean endogenous *FAD2* and *FATB* genes is suppressed due to RNAi by the *FAD2-1A* and *FATB1-A* gene segments. Northern blotting analysis has confirmed that mRNA of the *FAD2-1A* and *FATB1-A* genes in this recombinant soybean is suppressed (Annex 1, Figure 1, p18 and Figure 2, p19).

RNA interference or RNAi is a gene suppression mechanism that typically takes place in eukaryotes for regulation of gene expression. RNAi is triggered by the process in which double stranded RNA (dsRNA) is broken by an enzyme known as Dicer and short interfering RNAs (siRNAs) of 21-26 nucleotides in length is formed. After complexing with RNAi-induced silencing complex (RISC), siRNAs bind to the mRNA containing the target complementary sequence (Figure 3, p11) (Siomi and Siomi, 2009). Degradation of the siRNA bound target mRNA inhibits protein production from the target mRNA. RNAi has high specificity and high suppression effect on gene expression and thus, it has been utilized for conferring specific traits and understanding gene function (Kusaba, 2004).

The *FAD2-1A* gene segment is a partial sequence from intron #1 of the *Glycine max FAD2-1A* gene that encodes the Δ12 desaturase (Fillatti *et al.*, 2003), and the *FATB1-A* gene segment is a partial sequence from the 5'-untranslated region and the plastid targeting sequence of *Glycine max FATB1-A* gene that encodes the palmitoyl acyl carrier protein thioesterase (Fillatti *et al.*, 2003). Since these sequences do not encode any protein translation regions, it is considered unlikely that the transferred genes in this recombinant soybean would produce any new proteins.

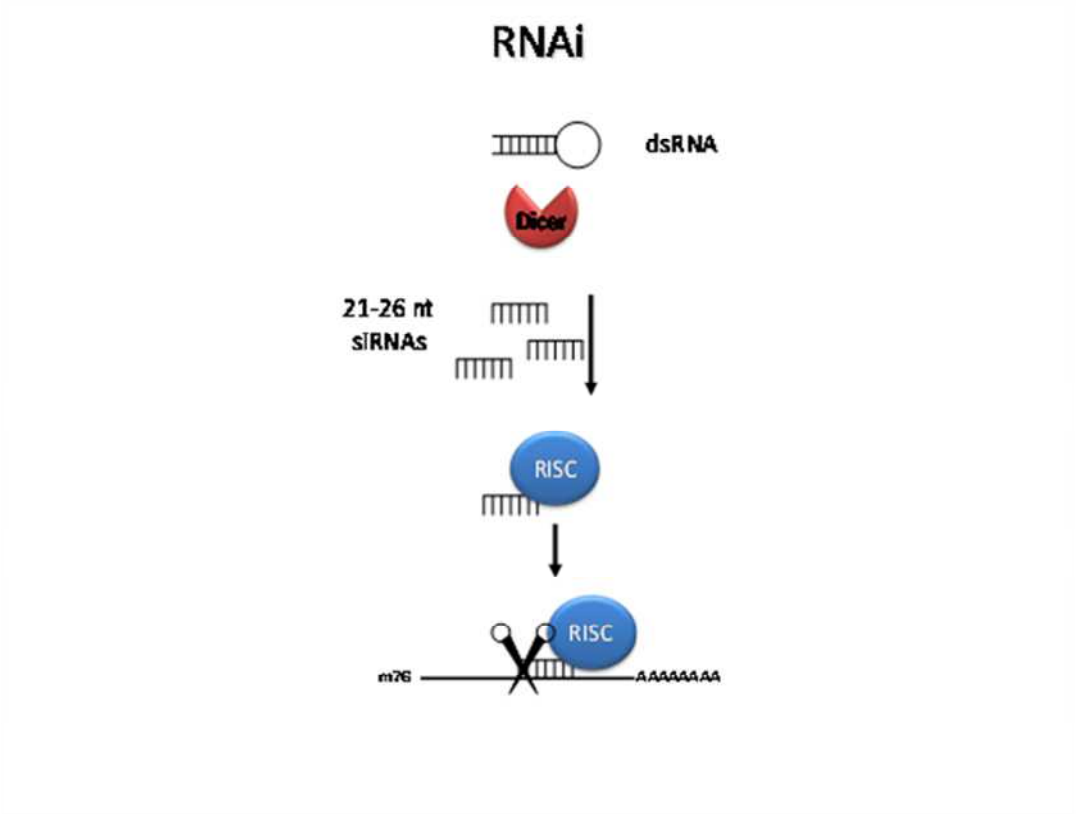

Figure 3 Mechanism of RNAi[5]

[Modified CP4 EPSPS protein]

The modified *cp4 epsps* gene transferred in this recombinant soybean was used as a selection marker in the selection process. Wild-type *cp4 epsps* gene is isolated from the *Agrobacterium* sp. strain CP4, encoding 5-enol-pyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS) protein. The CP4 EPSPS protein offers a high tolerance to glyphosate herbicide. Amino acid sequence of the modified CP4 EPSPS protein expressed in this recombinant soybean is as shown in Annex 1.

Glyphosate herbicide is the active ingredient of Roundup, a nonselective herbicide, and inhibits the activity of 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) (E.C.2.5.1.19), one of the enzymes in the shikimate synthesis pathway for aromatic amino acid biosynthesis, by specifically binding to the enzyme (Haslam, 1993; Steinrücken and Amrhein, 1980). As a result, plants treated with glyphosate cannot synthesize enough aromatic amino acids essential for protein synthesis due to the inhibition of EPSPS, resulting in the death of the plants. On the other hand, the transgenic plants which express the modified CP4 EPSPS protein continue to produce aromatic amino acids in the presence of glyphosate due to the activity of the modified CP4 EPSPS, thus grow normally.

In order to investigate whether the modified CP4 EPSPS protein shares amino acid sequences with known allergens, the modified CP4 EPSPS was compared with the

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allergens in the Allergen Database (AD_2009 [6]) using FASTA type and ALLERGENSEARCH type algorithms. As a result, the modified CP4 EPSPS protein did not have any sequence homology to those of known allergens.

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

[*FAD2-1A* gene segment and *FATB1-A* gene segment]

Biosynthesis pathway of vegetable oil has been well clarified and summarized in typical plant biochemistry textbooks (Buchanan, *et al.*, 2000). Biosynthesis of fatty acids in plants takes place in the plastid via the gradual condensation of two carbon units to the acyl chain (reaction (1) in Figure 4, p14). This reaction is catalyzed by fatty acid synthase in plants, resulting in production of palmitoyl-ACP (16:0-ACP) and stearoyl-ACP (18:0-ACP). In soybeans, most stearoyl-ACP is desaturated by Δ9 desaturase, a soluble enzyme in the plastid, to produce oleoyl-ACP (18:1-ACP) (reaction (2) in Figure 4, p14). These fatty acid chains are separated from ACP by two different acyl-ACP thioesterases, FATA and FATB (reactions (3) and (4) in Figure 4, p14). FATA works mainly to hydrolyze the 18:1-ACP and produce oleic acid (reaction (4) in Figure 4, p14). On the other hand, FATB hydrolyzes the acyl-ACPs which contain saturated fatty acids having 14 to 18 carbon atoms (14:0-ACP to 18:0-ACP) . It mainly hydrolyzes palmitoyl-ACP (16:0-ACP) and stearoyl-ACP (18:0-ACP) and produces palmitic acid and stearic acid (reaction (3) in Figure 4, p14). These free fatty acids are converted to acyl-CoA in the plastid membrane and they are transferred to the endoplasmic reticulum (ER).

Oleic acid separated from ACP by FATA to become a free fatty acid turns to oleoyl-CoA in the plastid membrane and then, leaves the plastid and enters the Kennedy pathway in the lipogenesis system in the endoplasmic reticulum (Figure 4, p14). Polyunsaturation of fatty acids in the endoplasmic reticulum is triggered by two membrane bound enzymes FAD2 and FAD3. FAD2 catalyzes Δ12 desaturation of oleic acid (18:1) to linoleic acid (18:2) (reaction (5) in Figure 4, p14), and FAD3 catalyzes Δ15 desaturation from linoleic acid (18:2) to linolenic acid (18:3) (reaction (6) in Figure 4, p14). Seed oil will be eventually accumulated in the oil body in the seed cells.

In this recombinant soybean, expression of the endogenous *FATB* gene is suppressed due to RNAi by the *FATB1-A* gene segment. As stated earlier, the thioesterase FATB hydrolyzes the acyl-ACP (14:0-ACP to 18:0-ACP) containing saturated fatty acid residues of 14 to 18 carbon number (reaction (1) in Figure 4, p14), and it is known to mainly hydrolyze palmitoyl-ACP (16:0-ACP) and stearoyl-ACP (18:0-ACP); so FATB is an important plastid enzyme for production of saturated fatty acids. It has been reported that as a result of suppression of FATB in soybean, the level of saturated fatty acids, especially palmitic acid (16:0) is decreased in the oil (Kinney, 1996). Similarly, in this recombinant soybean, decrease in FATB primarily induces reduced hydrolysis of palmitoyl-ACP (16:0-ACP) and stearoyl-ACP (18:0-ACP) and as a result, the levels of saturated fatty acids, palmitic acid (16:0) and stearic acid (18:0) has been decreased. , Additionally, due to this, there is a concurrent increase in the levels of unsaturated fatty acids in the oil from this recombinant soybean.

[6] Assembled from sequences found on the Food Allergy Research and Resource Program Database (FARRP) (http://www.allergenonline.com)

In addition, in this recombinant soybean, due to RNAi by the *FAD2-1A* gene segment, expression of the endogenous *FAD2* gene is suppressed. As stated earlier, FAD2 is Δ12 desaturase, which catalyzes the conversion of monounsaturated fatty acids to polyunsaturated fatty acids in the endoplasmic reticulum (reaction (5) in Figure 4, p14). Therefore, in this recombinant soybean, decrease in FAD2 in the endoplasmic reticulum causes a decrease in the desaturation of oleic acid (18:1) to linoleic acid (18:2), resulting in increased level of oleic acid (18:1) accumulation in the oil body in seed cells. This leads to production of a large amount of diacylglycerol which contains oleic acid (18:1). The diacylglycerol then turns to triacyl glycerol by the diacylglycerol acyltransferase (DGAT), resulting in an increased level of oleic acid (18:1) and a decreased level of linoleic acid (18:2) in the oil.

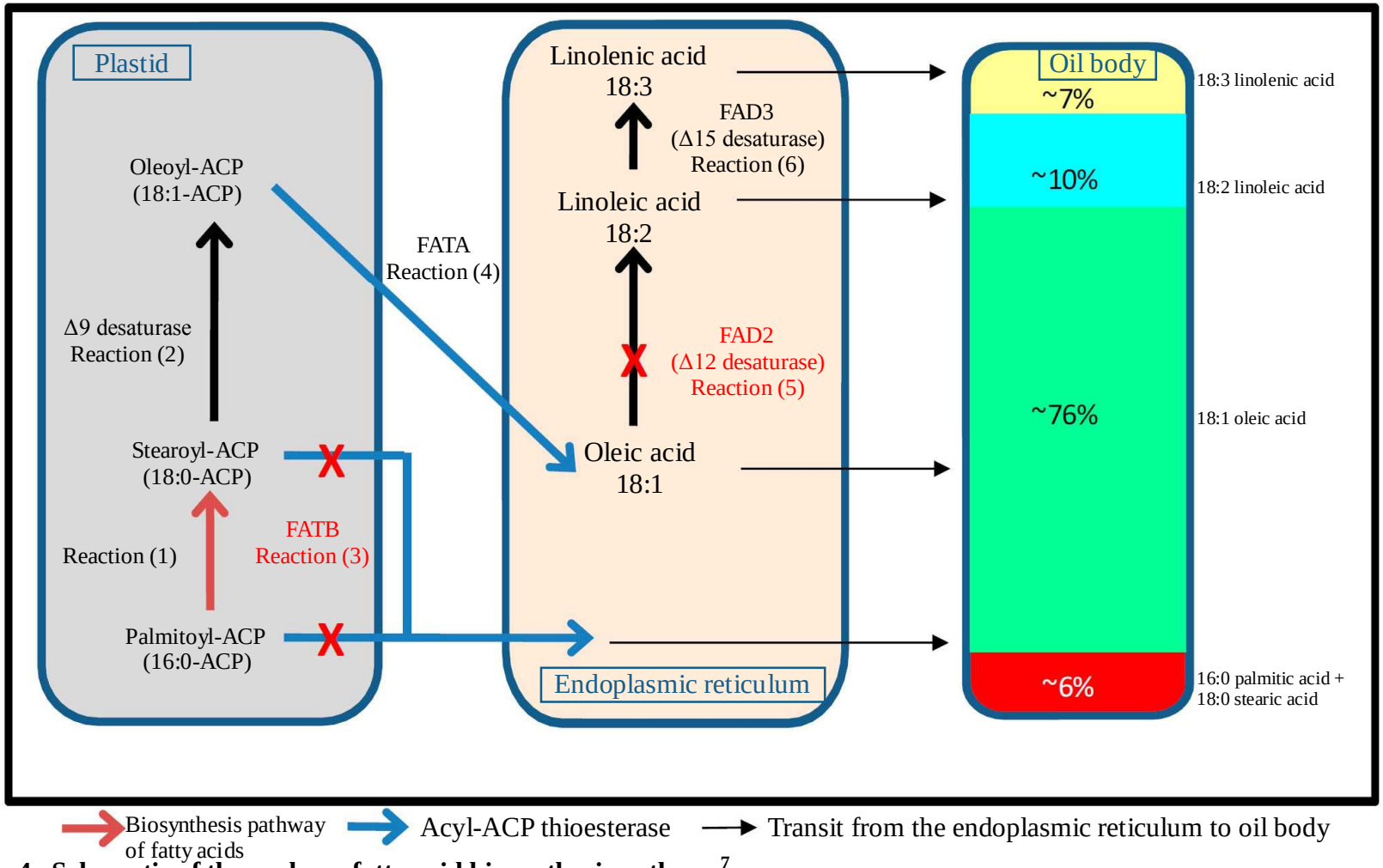

Figure 4 Schematic of the soybean fatty acid biosynthesis pathway[7]

✕ indicates suppression of translation of endogenous enzymes (FATB1-A and FAD2-1A) RNAs in this recombinant soybean seed.

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The seeds of this recombinant soybean, the non-recombinant control soybean and 20 conventional commercial varieties were analyzed for fatty acid composition. As intended reduced levels of saturated fatty acids such as palmitic acid (16:0) and stearic acid (18:0) were observed in this recombinant soybean compared to the non-recombinant soybean due to the suppressed expression of the endogenous *FATB* gene. It was confirmed that percentage of palmitic acid (16:0) in the seed of this recombinant soybean was decreased from 10.83% (non-recombinant control soybean) to 2.36% (this recombinant soybean), and proportion of stearic acid (18:0) decreased from 4.50% (non-recombinant control soybean) to 3.31% (this recombinant soybean) of the total fatty acids. As a result, total saturated fatty acid decreased from approximately 15.3% (non-recombinant control soybean) to 5.7% (this recombinant soybean). In addition, due to the suppressed expression of the endogenous *FAD2* gene in this recombinant soybean, an increased level of monounsaturated fatty acid, oleic acid (18:1), and a decreased levels of linoleic acid (18:2) were observed compared to the non-recombinant soybean (Table 2, p17). Proportion of oleic acid (18:1) to the total fatty acid increased from 22.81% (non-recombinant control soybean) to 76.47% (this recombinant soybean) and proportion of linoleic acid (18:2) to the total fatty acid decreased from 52.86% (non-recombinant control soybean) to 10.10% (this recombinant soybean). As expected a statistically significant difference ($p<0.05$) was observed between this recombinant soybean and the non-recombinant control soybean regarding the level of linolenic acid (18:3) since linolenic acid (18:3) is generated from linoleic acid (18:2) that was decreased due to the suppression of the *FAD2* gene.

[Modified *cp4 epsps* gene]

EPSPS protein is one of the enzymes that catalyze the shikimate pathway for the biosynthesis of aromatic amino acids specific to plants and microorganisms, and it exists in chloroplasts or plastids in plants (Della-Cioppa, *et al.*, 1986). The shikimate pathway is an important metabolic pathway that is considered to be involved in one fifth of carbon fixation in 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 through the production of EPSP, which is catalyzed by EPSPS, to the synthesis of chorismic acid are inhibited or suppressed by metabolic intermediates or end products of this pathway (Herrmann, 1983; Weiss and Edwards, 1980). This suggests that EPSPS is not the rate-determining enzyme in this pathway, and as such it is not considered that enhanced EPSPS activity will increase the concentration of aromatic amino acids, the end products of this pathway (Padgette, *et al.*, 1996a; Ridley, *et al.*, 2002). In addition, the amino acid content in other glyphosate-tolerant crops, conducted as part of the food/feed safety evaluation of glyphosate-tolerant crops (soybean, oilseed rape, cotton, maize, alfalfa and sugar beat) which have been developed by Monsanto Company, have no relevant composition differences from their non-recombinant crops to date.

Besides, EPSPS protein is the enzyme that catalyzes a reversible reaction to produce EPSP and inorganic phosphate (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 that is known to react with EPSPS other than these is shikimate, an analogue of S3P. However, the

reactivity with shikimate is only one two millionth of the reactivity with S3P when calculated based on the article by Gruys *et al.* (1992), therefore it is unlikely that shikimate acts as the substrate of EPSPS in the living body.

5 Consequently, it is considered to be extremely unlikely that the production of the modified CP4 EPSPS protein, which is functionally parallel to plant EPSPS protein, has an effect in any way on the metabolic pathways of plants.

Table 2 Fatty acid profile in soybean oil from this recombinant soybean, the non-recombinant control soybean (A3525) and conventional varieties [18]

	This recombinant soybean Mean (%) [Range (%)]	Non-recombinant control soybean Mean (%) [Range (%)]	p-value	Conventional commercial varieties (Range (%)) [99% Tol. Int.[2]]
Fatty acid composition (percent to total fatty acid				
16:0 Palmitic acid	2.36 [2.25 - 2.44]	10.83 [10.51 - 11.08]	<0.001	(8.78 - 11.51) [7.62, 12.55]
18:0 Stearic acid	3.31 [3.07 - 3.82]	4.50 [4.24 - 4.85]	<0.001	(3.82 - 7.21) [2.87, 7.15]
18:1 Oleic acid	76.47 [73.13 - 79.17]	22.81 [21.41 - 25.08]	<0.001	(20.77 - 27.19) [18.40, 30.22]
18:2 Linoleic acid	10.10 [7.85 - 12.42]	52.86 [51.68 - 53.89]	<0.001	(48.62 - 54.74) [47.75, 56.46]
18:3 Linolenic acid	6.69 [5.55 - 7.81]	8.02 [6.86 - 8.60]	<0.001	(5.89 - 9.11) [4.97, 9.93]

[1] Based on gas chromatography analysis for seed samples obtained from fields at 5 sites in Chile and statistical analysis using ANOVA (n=5).
[2] The tolerance interval includes 99% of the values expressed in population of conventional commercial varieties at the confidence level of 95%. The lower limit was set at 0.

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(2) Information concerning vector

1) Name and origin

The vector PV-GMPQ/HT4404 used for the production of this recombinant soybean is assembled from plasmids including pBR322 derived from *E. coli*.

2) Properties

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

The total number of base pairs of PV-GMPQ/HT4404 used for the production of this recombinant soybean is 13,088bp.

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

This vector contains the *aadA* gene derived from *E. coli* transposon *Tn7* as a selection marker gene for construction vectors outside of T-DNA region, which confers resistance to spectinomycin and streptomycin.

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

The infectivity of this vector is not known.

(3) Method of preparing living modified organisms

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

Component elements of the plasmid vector transferred into the recipient organism are shown in Table 1 (p6-9). In addition, position of each component element of donor nucleic acid and restriction enzyme cleavage sites in the vector is shown in Figure 2 (p5).

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

The plasmid vector PV-GMPQ/HT4404 was transferred into the embryo cell derived from the non-recombinant soybean variety A3525 by *Agrobacterium* method.

3) Processes of rearing living modified organisms

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

Meristem tissues were excised from the embryos of conventional soybean variety A3525. After co-culturing with the *Agrobacterium tumefaciens* strain ABI carrying the plasmid vector PV-GMPQ/HT4404, the meristems were placed on the tissue culture medium (TCM) containing glyphosate for selection of transformed cells.

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

Agrobacterium used for transformation was removed by placing the embryos on the TCM containing carbenicillin and cefotaxime. Additionally, as a result of PCR analysis targeting outside backbone region of the plasmid vector PV-GMPQ/HT4404 at R3 generation of this recombinant soybean, it was found that the outside backbone region of the plasmid vector PV-GMPQ/HT4404 is not present in this recombinant soybean (Annex 3). Consequently, the absence of *Agrobacterium* used for transformation was confirmed in this recombinant soybean.

(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 R_0 plants regenerated through the above-mentioned transformation were self-pollinated, and the subsequent R_1 plants were screened for the zygosity of the transferred genes. Only the R_1 plants that are homozygous for the transferred genes, and produced seeds with the desired fatty acid composition were advanced for development. Their progeny was subjected to further transferred gene analysis and morphological characteristics examination. As a result, the MON 87705 line was finally selected as a commercialization line.

The process of rearing of this recombinant soybean is shown in Figure 5 (p20). The application for approval of Type I Use Regulation refers to R_3 generation of this recombinant soybean and all the progeny hybrids derived from the R_3 generation.

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

Figure 5 Process of rearing of this recombinant soybean

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

1) Place where the replication product of transferred nucleic acid exists

In order to investigate whether the transferred genes in this recombinant soybean are present on the chromosome, statistical analysis was conducted based on the Chi-square (χ^2) test for segregation ratio through multiple generations.

As a gene that is present in the transferred gene and not present in the soybean genome, *H6* terminator was selected as an indicator for investigation of segregation pattern. R_4 generation of this recombinant soybean was crossed with soybean variety which does not contain *H6* terminator (A3525) to obtain F_1 generation. The F_1 generation was self-pollinated to yield F_2 generation. Then, the plants that are heterozygous for *H6* terminator were selected based on Invader assay and self-pollinated to produce F_2, F_3, F_4 and F_5 generations, which were then used to investigate the segregation pattern of the transferred genes in this recombinant soybean. As a result, at F_3 generation, a statistically significant difference was observed between the observed and expected values of segregation ratio (1:2:1). However, no statistically significant difference was observed based on the Chi-square (χ^2) test between the observed and expected values of segregation ratio (1:2:1) at F_2, F_4 and F_5 generations. Based on the data for 3 generations (F_2, F_4 and F_5), it is considered that the transferred genes in this recombinant soybean exist on the chromosome (Annex 4).

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

As a result of the Southern blotting analysis of the transferred genes, it was confirmed that this recombinant soybean contains a single copy of T-DNA I region and T-DNA II region in one site in the genome adjacently to each other (Annex 5, Figure 5 to Figure 7, p44-46). In addition, no backbone region was detected (Annex 5, Figure 8, p47 and Figure 9, p48), and the transferred genes were confirmed to have been stably inherited to the progeny as a result of Southern blotting analysis for multiple generations (R_3, R_4, R_5 and R_6 generations) (Annex 5, Figure 15, p59).

In addition, as a result of DNA sequence analysis of the transferred genes, the transferred genes in this recombinant soybean were found 7,251 bp. Except a deletion of 30 bp observed at 3'-terminus of *FATB1-A* segment derived from T-DNA II, DNA sequence of the transferred genes was found identical to that of individual component elements of the plasmid vector PV-GMPQ/HT4404. However, it was confirmed that the deletion would not affect the function of the gene suppression cassette (RNAi).

Schematic representation of the transferred genes in this recombinant soybean is shown in Figure 6 on p.23.

Table 3 Segregation pattern of *H6* terminator gene in this recombinant soybean[9]

					1:2:1 segregation ratio				
Gene-ration [1]	Total plants tested [2]	Observed value Transferred genes Homozygous Positive	Observed value Transferred genes Heterozygous Positive	Observed value Transferred genes Negative	Expected value Transferred genes Homozygous Positive	Expected value Transferred genes Heterozygous Positive	Expected value Transferred genes Negative	χ^2	p-value
F2	4197	1009	2091	1097	1049.25	2098.5	1049.25	3.7	0.1538
F3	81	30	35	16	20.25	40.5	20.25	6.3	0.0421
F4	266	68	126	72	66.50	133.0	66.50	0.9	0.6514
F5	175	44	88	43	43.75	87.5	43.75	0.0	0.9915

[1] F_2 generation was obtained from self-pollination of F_1 generation that was yielded from crossing between the soybean variety (A3525) that does not contain *H6* terminator and R_5 generation of this recombinant soybean. F_3, F_4 and F_5 generations were obtained from self-pollination of parent generations that are heterozygous for *H6* terminator gene.

[2] Existence of *H6* terminator was determined based on Invader assay. "Total plants tested" refer to the total number of plants for which the zygosity was identified.

F_3, F_4 and F_5 generations were subjected to tests using the seeds obtained from any single plant of parent generation.

[9] All the rights pertinent to the information in the table above and the responsibility for the content remain with Monsanto Japan Limited.

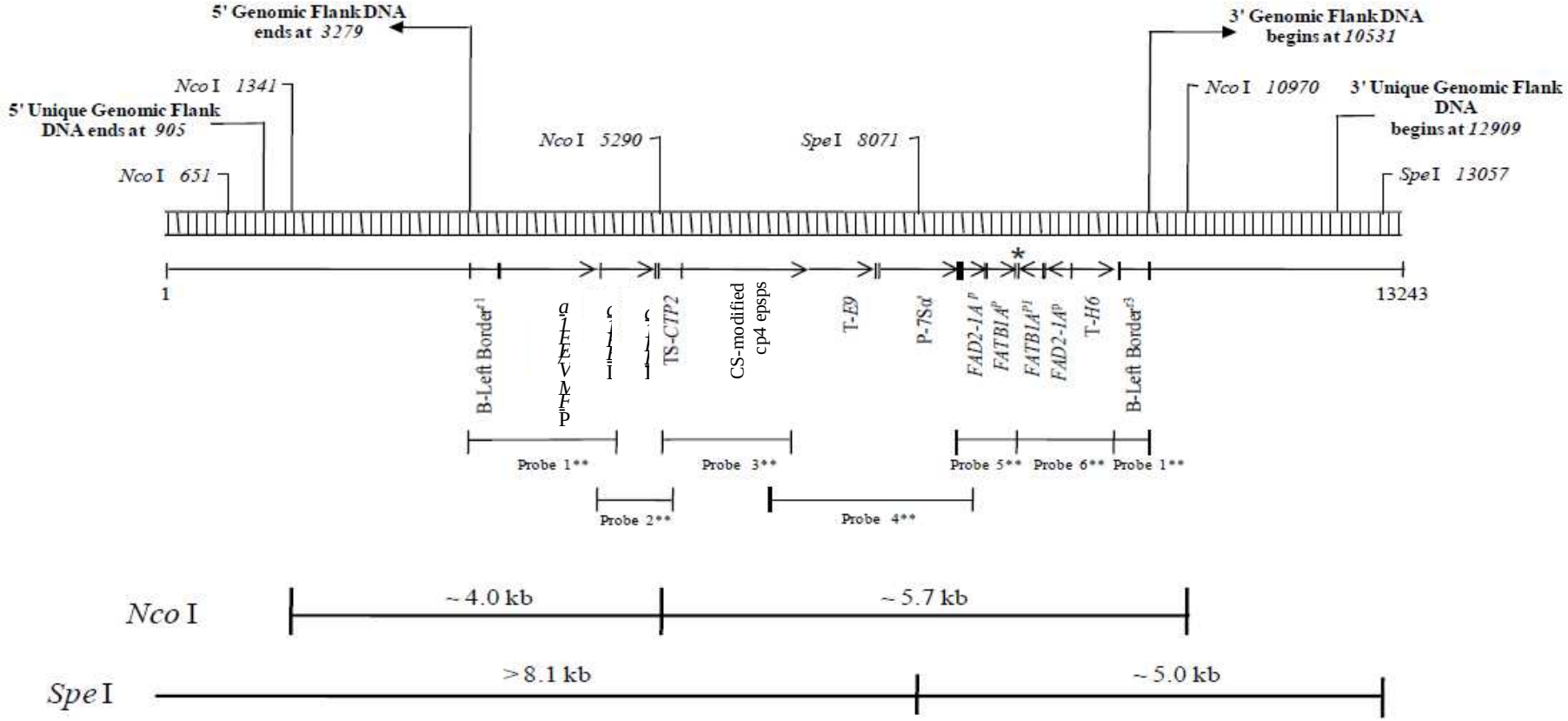

Figure 6 Schematic representation of the transferred genes in this recombinant soybean [10]

[10] *EF-1α* shown in the diagram is identifical to *Tsf1* referred to in Table 3 (p41) in Annex 5.

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

This item is not applicable because of one copy.

4) Inter-individual or inter-generational expression stability under a natural environment with respect to the characteristics referred to specifically in (6)-1)

As stated in I-2-(1)-2)-(b) (p10), it was confirmed that the levels of mRNA of *FAD2-1A* gene and *FATB1-A* gene in this recombinant soybean are significantly lower compared to the non-recombinant control soybean (Annex 2, Figure 1, p18 and Figure 2, p19).

Expression level of the modified CP4 EPSPS protein in this recombinant soybean was determined based on the ELISA method. In the test, leaves (over-season leaf; OSL 1 to 4), above-ground parts, roots and harvested seeds of this recombinant soybean and the non-recombinant control soybean collected in the fields at five (5) sites in Chile in 2007/2008 (3 sites in Santiago Metropolitan Region and 2 sites in O'Higgins Region) were used. As a result, expression level of the modified CP4 EPSPS protein in this recombinant soybean was found between 40 and 1,000 μg/g dwt (dry weight). The average expression level of the modified CP4 EPSPS protein was found highest in leaves (range from 200 to 530 μg/g dwt) followed by above-ground parts (120 μg/g dwt), seeds (110 μg/g dwt) and roots (77 μg/g dwt) (Table 4, p25; Annex 6, Table 1, p17).

Stable expression of the modified CP4 EPSPS protein across multiple generations (R_3, R_4, R_5, and R_6) of this recombinant soybean was confirmed by Western blot analysis (Annex 7, Figure 1, p15).

In addition, in the process of rearing, selection was conducted by confirming saturated fatty acid contents, oleic acid content and expression of the modified CP4 EPSPS protein in soybean oil from this recombinant soybean at individual generations.

5) 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

Regarding the plasmid vector PV-GMPQ/HT4404, the region of the recipient organism, which allows autonomous replication, is limited to gram-negative bacterium such as *E. coli* and *A. tumefaciens*. Therefore, there is no possibility that the transferred nucleic acid might be transmitted to any wild animals and plants under natural environment.

Table 4 Expression of the modified CP4 EPSPS protein in this recombinant soybean tissue (Grown in 2007/2008 in Chile)[11]

Tissue type [1]	Modified CP4 EPSPS µg/g fwt (SD)[2,4]	Range [3] (µg/g fwt)[4]	Modified CP4 EPSPS µg/g dwt (SD)[2,5]	Range [3] (µg/g dwt)[5]	LOQ/LOD[6] (µg/g fwt)[4]
OSL-1	36 (14)	16-65	200 (72)	84-340	0.57/0.26
OSL-2	110 (51)	60-230	530 (230)	290-1000	0.57/0.26
OSL-3	51 (21)	11-84	220 (94)	47-350	0.57/0.26
OSL-4	51 (21)	27-94	210 (92)	110-410	0.57/0.26
Above-ground	32 (5.3)	22-40	120 (24)	77-160	0.57/0.10
Root	24 (6.4)	14-34	77 (24)	41-120	0.57/0.11
Harvested seed	100 (39)	35-190	110 (44)	40-210	0.34/0.26

[1] OSL-1 to 4 means as follows: OSL1: 3- to 4-leaf stage, OSL2: 6- to 8-leaf stage, OSL3: 10- to 12-leaf stage and OSL4: 14- to 16-leaf stage. Leaf samples were collected at individual leaf stages. Above-ground parts and roots were collected at R5 stage (grain thickening period), and harvested seeds were collected at R8 stage (maturation period).
[2] Mean value and standard deviation (SD) were calculated for each tissue type across all sites (n=15 for all other tissues, except OSL-2 where n=12 and OSL-3 where n=19).
[3] Minimum and maximum values were calculated for each tissue type across sites.
[4] Protein expression levels on a fresh weight (fwt) basis are expressed as protein weight (µg) per tissue weight (g).
[5] Protein expression levels on a dry weight (dwt) basis were calculated by dividing the protein expression levels on an fwt basis by the dry weight conversion factors obtained from moisture analysis data.
[6] LOQ: Below the limit of quantification, LOD: Below the limit of detection

[11] All the rights pertinent to the information in the table above and the responsibility for the content remain with Monsanto Japan Limited.

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

It is possible to detect and identify MON 87705 by PCR analysis (Annex 8). This method offers sufficient sensitivity for detection on a single grain basis. Recommended concentration of DNA for analysis is 5 to 10 ng for one PCR reaction. Reproducibility of this method was examined by using 44 plants of this recombinant soybean and 46 plants of the non-recombinant soybean (Annex 8).

(6) Difference between the modified organism and the recipient organism or the species to which the recipient organism belongs

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

In this recombinant soybean, expression of soybean endogenous *FAD2* and *FATB* genes is suppressed due to RNAi by expression of the *FATB1-A* and *FATB1-A* gene segments. Fatty acid composition analysis showed that the saturated fatty acids (palmitic acid and stearic acid) in this recombinant soybean was reduced to 5.7% whereas in the non-recombinant control soybean it was found to be approximately 15.3%. In addition, the oleic acid content of this recombinant soybean was found to be 76.47%, where as that in non-recombinant control soybean was 22.81%. The increase in oleic acid content was accompanied by decrease in linoleic acid content in this recombinant soybean. This linoleic acid level in this recombinant soybean was 10.10% compared to 52.86% in the non-recombinant control soybean (Table 2, p17).

The modified *cp4 epsps* gene in this recombinant soybean confers tolerance to glyphosate herbicide by expressing the modified CP4 EPSPS protein.

2) 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[12]

Isolated field tests were carried out in Kawachi Research Farm (KRF), Monsanto Japan Limited, in 2009 to 2010 using this recombinant soybean. The soybeans tested included the R_6 generation of this recombinant soybean (Figure 5, p20). The mother plant of this recombinant soybean to which the genes were transferred, A3525, was used as the non-recombinant control soybean. Cold-tolerance tests were carried out at Monsanto Company (U.S.A.).

(a) Morphological and growth characteristics

The differences in morphological and growth characteristics between this recombinant soybean and the non-recombinant control soybean were investigated. A total of 20 conventional commercial varieties were also included in this study. The following characteristics were evaluated based on the designatation for registration of seeds and

[12] All the rights pertinent to the information in (a) to (g) of this item and the responsibility for the content remain with Monsanto Japan Limited.

seedlings: [initiation of germination, date of germination, uniformity of germination, number of germinated plants, germination rate, shape of leaflet, trichome quantity, time of flower initiation, time of flower completion, elongation type, maturation period, main stem length, number of main stem nodes, number of branches, the lowest main stem node height of podding, plant shape, weight of plant at harvest time, and shape of harvested seed (seed hull color, uniformity of seeds and seed shape)]. Among the items subjected to statistical analysis in the number of germinated plants and number of branches (number of germinated plants, main stem length, number of main stem nodes, number of branches, the lowest main stem node height of podding, and weight of plant at harvest time), a statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean. In addition, regarding date of germination and uniformity of germination among the items not subjected to statistical analysis (initiation of germination, date of germination, uniformity of germination, germination rate, shape of leaflet, trichome quantity, time of flower initiation, time of flower completion, elongation type, maturation period, plant shape, and shape of harvested seed (seed hull color, uniformity of seeds and seed shape)), a difference was observed between this recombinant soybean and the non-recombinant control soybean (Annex 9, Table 2, p9).

The number of germinated plants was 944 for this recombinant soybean and 879 for the non-recombinant control soybean. The date of germination was July 12nd, for this recombinant soybean and July 13rd for the non-recombinant control soybean, and the uniformity of germination was July 13rd for this recombinant soybean and July 14th for the non-recombinant control soybean, showing a difference of only one day between the both plants in the respective items. In addition, the number of branches was 7.0 for this recombinant soybean and 6.1 for the non-recombinant control soybean (Annex 9, Table 2, p9).

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

Cold-tolerance tests at the early stage of growth were carried out in a climate chamber at Monsanto Company (U.S.A.). This recombinant soybean, the non-recombinant control soybean A3525 and six (6) conventional commercial varieties were grown in a greenhouse, and the seedlings on the 20th day after seed sawing were transferred to a climate chamber set at alternating temperatures of 15°C day/8°C night for 20 days to compare plant vigor, main stem length, growth stage, fresh weight and dry weight. In all the items tested, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean (Annex 10, Table 3, p6).

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

This recombinant soybean and the non-recombinant control soybean raised in an isolated field were left to grow even after the maturation period to observe the growth conditions in winter season in Japan. Both this recombinant soybean and the control soybean both found dead (Annex 9, Figure 6, p12) when observed on January 5, 2010.

(d) Fertility and size of the pollen

Pollen was sampled from this recombinant soybean and the non-recombinant control

soybean, and the samples were stained with iodine potassium iodide solution to observe their fertility and sizes for investigation. No difference was observed in the fertility of the pollen between this recombinant soybean and the non-recombinant control soybean. Also regarding the shape and size of the pollen, no difference was observed between the both plants (Annex 9, Figure 7, p13).

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

The differences were investigated between this recombinant soybean and the non-recombinant control soybean cultivated under the same condition regarding the characteristics for seed production (the number of ripe pods, approximate grain weight per plant, precise grain weight per plant and 100-kernel weight). A statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean in precise grain weight per plant and 100-kernel weight (Annex 9, Table 3, p16). The precise grain weight per plant was 41.3 g for this recombinant soybean and 44.6 g for the non-recombinant control soybean, and the 100-kernel weight was 18.2 g for this recombinant soybean and 19.4 g for the non-recombinant control soybean (Annex 9, Table 3, p16).

Regarding the shedding habit, this recombinant soybean and the non-recombinant control soybean were harvested during the maturation period, and the plant body was left to air-dry in a vinyl house to examine whether or not the pod became broken. This recombinant soybean and the non-recombinant control soybean showed no difference in pod breaking habit. (Annex 9, Table 3, p16).

To investigate the dormancy and germination rate, seeds immediately after harvesting were placed in a Petri dish and incubated at 25°C to examine the number of germinated plants over time. As a result, this recombinant soybean and the non-recombinant control soybean both exhibited a high germination rate of 98.9%, and no statistically significant difference was observed in the final number of germinated plants (Annex 9, Table 4, p16).

(f) Crossability

In order to examine the crossability between this recombinant soybean and the non-recombinant control soybean, the frequency of occurrence of hybrids in the harvested seeds of the non-recombinant control soybean was identified, while this recombinant soybean was defined as pollen parent. Identification of the hybrid was based on tolerance to glyphosate herbicide that the pollen parent, possesses.

From the rows in the plot for the non-recombinant control soybean cultivated in the plot for investigation of morphological and growth characteristics, which are adjacent to this recombinant soybean, seeds were harvested (except three plants each at the both ends of each row). The non-recombinant soybeans were 1.65 m away from this recombinant soybean in the neighboring plot that extended southeast or northwest (Annex 9, Figure 2, p5). The plot was not protected with any insect screen during the flowering period. Four hundred and eighty (480) seeds were selected at random from the harvested seeds and sawn in pots in a greenhouse. When the plants were at 2- to 3-leaf stage, glyphosate herbicide (Product name: Roundup Max Load, a 1 to 100 solution) was sprayed. On the 21st day after herbicide spraying, the number of individual plants which were found

surviving due to the tolerance to glyphosate was counted.

Among 480 seeds tested, there were no plants that survived glyphosate herbicide Therefore it was concluded that the crossability was 0% in the investigation (Annex 9, p17).

(g) Productivity of harmful substances

To confirm whether or not this recombinant soybean produces any substances affecting soil microbes and other plants, soil microflora tests, plow-in tests and succeeding crop tests were conducted. As a result, in the number of microorganisms in soil, and the number of germinated plants and dry weight of radish, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean. Also regarding germination rate of radish which was not subjected to statistical analysis, no difference was observed (Annex 9, Table 5 to Table 7, p20).

Results of the review by the Committee for Discussing Biological Diversity Risk Assessment

Name: Low saturated fatty acid and high oleic acid soybean tolerant to glyphosate herbicide (*FAD2-1A*, *FATB1-A*, modified *cp4 epsps*, *Glycine max* (L.) Merr.) (MON87705, OECD UI: MON-877Ø5-6)

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

Applicant: Monsanto Japan Limited

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

This recombinant soybean was developed by transferring the PV-GMPQ/HT4404 assembled based on the plasmid pBR322 from *E. coli* and other vectors through the *Agrobacterium* method.

It has been confirmed based on the gene segregation and Southern blotting analysis that this recombinant soybean has a single copy of T-DNA I and T-DNA II regions containing the *FAD2-1A* gene segment which encodes Δ-12 desaturase from *Glycine max*, the *FATB1-A* gene segment which encodes palmitoyl acyl carrier protein thioesterase, and the modified *cp4 epsps* gene which encodes 5-enol-pyruvyl-shikimate-3-phosphate synthase protein from *Agrobacterium* sp. strain CP4 integrated on the chromosome adjacently to each other and it is stably inherited through multiple generations. It has been also confirmed based on the Northern blotting analysis that expression of the *FAD2-1A* and *FATB1-A* genes in this recombinant soybean is suppressed due to RNAi by the transferred *FAD2-1A* and *FATB1-A* gene segments. In addition, for expression of the modified *cp4 epsps* gene, it has been identified based on the Western blot analysis that the modified CP4 EPSPS protein is stably expressed through multiple generations. Based on the above understanding, it was concluded that the transferred nucleic acid exists on the chromosome and it is stably inherited and expressed.

(1) Competitiveness

The soybean plant (*Glycine max* (L.) Merr.), the biological species to which the recipient organism belongs, has been cultivated for a long time in Japan, but there is no report that it grows voluntarily in Japan.

As a result of examination in the isolated fields in Japan regarding the characteristics relating to competitiveness of this recombinant soybean, a statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean in the number of germinated plants, number of branches, precise grain weight per plant and 100-kernel weight. In addition, in the items not subjected to statistical analysis, a difference was observed between this recombinant soybean and the non-recombinant control soybean regarding date of germination and uniformity of germination.

The number of germinated plants was found 944 for this recombinant soybean and 879

for the non-recombinant control soybean. However, in the number of germinated plants from mature seeds, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean. Furthermore, also in the germination tests conducted in the US, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean.

The date of germination was July 12nd for this recombinant soybean and July 13rd for the non-recombinant control soybean, and the uniformity of germination was July 13rd for this recombinant soybean and July 14th for the non-recombinant control soybean, showing a difference of only one day in the both items.

The number of branches was 7.0 for this recombinant soybean and 6.1 for the non-recombinant control soybean. However, in the items relating to seed production (the number of ripe pods, approximate grain weight per plant, precise grain weight per plant and 100-kernel weight), any difference that would increase seed production of this recombinant soybean was not observed.

The precise grain weight per plant was 41.3 g for this recombinant soybean and 44.6 g for the non-recombinant control soybean. However, it was considered unlikely that the smaller precise grain weight per plant would increase seed production of this recombinant soybean.

The 100-kernel weight was 18.2 g for this recombinant soybean and 19.4 g for the non-recombinant control soybean. However, the average 100-kernel weight of this recombinant soybean was found within the previously published range of 100-kernel weight of conventional soybeans.

Based on the above findings, it was considered unlikely that observed differences could increase the competitiveness of this recombinant soybean.

In this recombinant soybean, due to RNAi by the transferred *FAD2-1A* and *FATB1-A* gene segments, saturated fatty acid levels in seed are reduced and the degree of desaturation from oleic acid to linoleic acid is decreased, resulting in increased oleic acid content and reduced linoleic acid. Typically it is known that oil contents in soybean seed are stored as energy source in soybean seed and utilized mainly in germination. However, there is no report that reduced saturated fatty acid contents and increased oleic acid content in the seeds of this recombinant soybean would influence germination. In addition, this recombinant soybean is given the trait to be tolerant to glyphosate herbicide due to the constitutive expression of the modified *cp4 epsps* gene. However, it is considered unlikely that this trait causes this recombinant soybean to enhance the competitiveness under the natural environment..

Based on the above understanding, it was determined that the conclusion by the applicant that the wild animals and wild plants are not likely to be affected and that the use of this recombinant soybean poses no significant risk of Adverse Effect on Biological Diversity attributable to competitiveness is reasonable.

(2) Productivity of harmful substances

Regarding the plant species of soybean to which the recipient organism belongs, there is no report that it produces a harmful substance to wild animals and wild plants.

This recombinant soybean is given the ability to produce the modified CP4 EPSPS protein, though there is no report that this protein would be a harmful substance, and it has been confirmed not to have any amino acid sequence similarity with known allergens. The modified CP4 EPSPS protein works as a similar enzyme as the EPSPS protein in the shikimate pathway, the biosynthesis pathway for aromatic amino acids, though EPSPS protein is not the rate-determining enzyme in this pathway and the modified CP4 EPSPS protein has high substrate specificity. Therefore, it was considered extremely low that the proteins would affect the metabolic system of the recipient organism and produce any new harmful substances. For the transferred *FAD2-1A* and *FATB1-A* gene segments, RNAi triggered by these gene segments only acts to suppress expression of *FAD2-1A* and *FATB1-A* genes in this recombinant soybean, and it is considered unlikely to cause production of any new proteins.

In the isolated fields in Japan, this recombinant soybean was investigated for productivity of any harmful substances (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) based on the soil microflora test, plow-in test and succeeding crop test and as a result, no statistically significant difference was observed between this recombinant soybean and the non-recombinant control soybean.

Based on the above understanding, it was determined that the conclusion by the applicant that the wild animals and wild plants are not likely to be affected and that the use of this recombinant soybean poses no significant risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is reasonable.

(3) Crossability

It is known that *Glycine soja* is closely related to soybean and the both plants have the same chromosome number 2n=40 and thus they can cross with each other. Therefore, *Glycine soja* was specified as a wild plant likely to be affected, and the following examination was performed.

Since there is no specific obstacle identified to the growth of any hybrid produced by artificial crossing between soybean and *Glycine soja*, there is a possibility that the hybrid, if produced by crossing between this recombinant soybean and *Glycine soja* in the natural environment in Japan, would grow and that the gene transferred in this recombinant soybean through backcross of the hybrid with *Glycine soja* would spread without remaining in a low content in the population of *Glycine soja*. In addition, since *Glycine soja* ranges widely throughout the country and grows voluntarily in river beaches, banks, in the vicinity of farmlands, orchards and other places, it can cross with this recombinant soybean when it is raised adjacent to the recombinant soybean.
However,

1) There is a report that no genetic marker has been detected suggesting emergence

of hybridized progenies as a result of follow-up survey of *Glycine soja* populations for several years in the surroundings of soybean fields at many places in Japan to investigate hybridization between soybean and *Glycine soja* and penetration of genes into progeny.

2) It is generally known that the flowering time of soybean and *Glycine soja* is unlikely to match with each other, and even in the case when the both plants are cultivated alternately at a planting distance of 50 cm by artificially matching the flowering time, the rate of crossability is reportedly 0.73%.

3) As a result of crossability test in which the flowering time of herbicide glyphosate tolerant soybean line 40-3-2 and *Glycine soja* was matched with each other and the both plants were cultivated adjacent to each other and raised with *Glycine soja* wound around soybean, one grain of 32,502 harvested seeds of *Glycine soja* was reportedly crossed with soybean.

In addition, as a result of investigation for natural crossing between this recombinant soybean and the non-recombinant control soybean which were cultivated in the test plots adjacent to each other in the isolated field in Japan, no hybridization of this recombinant soybean with the non-recombinant soybean was observed. As a result of examination for the traits relating to reproduction (pollen fertility, pollen shape, and seed production), the characteristics of this recombinant soybean were found not to exceed the range of the species, and it was estimated that crossability between this recombinant soybean and *Glycine soja* would be extremely low similarly to that between conventional soybean varieties and *Glycine soja*.

Furthermore, if this recombinant soybean crosses with *Glycine soja*, the obtained hybrid is considered to possess the trait to be tolerant to herbicide glyphosate due to the modified *cp4 epsps* gene. However, this trait is considered unlikely to increase the competitiveness, and even if hybrid that possesses glyphosate tolerance emerges, it is considered unlikely that the hybrid could dominate the population of *Glycine soja*.

Based on the above understanding, it was determined that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to crossability is reasonable.

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 soybean in accordance with Type 1 Use Regulation causes Adverse Effect on Biological Diversity. It was determined that the conclusion above made by the applicant is reasonable.

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