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

Name: Bayer Crop Science K.K
John Gray, President
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Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Soybean tolerant to glufosinate herbicide (*pat*, *Glycine max* (L.) Merr.) (A2704-12、OECD UI: ACS-GMØØ5-3)
Content of the Type 1 Use of Living Modified Organism	Provision as food, provision as feed, processing, storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

Information collected prior to assessing Adverse Effect on Biological Diversity

I. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition of the donor nucleic acid that was used for the production of the soybean tolerant to glufosinate herbicide (*pat, Glycine max* (L.) Merr., A2704-12, OECD UI: ACS-GM005-3) (hereinafter referred to as "the recombinant soybean A2704-12") and origins of component elements are shown in Table 1.

The *pat* gene transferred in the recombinant soybean A2704-12 is a modified type of the native *pat* gene obtained from *Streptomyces viridochromogenes* whose sequence is modified to fit codons used in the plant. The amino acid sequence of the enzyme which is produced by this modification remains unchanged (References 7, 29, and 44). Structure of nucleotide sequence of the modified *pat* gene is shown in Figure 1.

Table 1 Origin and function of component elements

Component Elements (abbreviation)	Size (kbp)	Position in vector (bp)	Origin and function
pat gene expression cassette			
P35S	0.54	461-1003	35S RNA promoter derived from Cauliflower Mosaic Virus. It expresses modified *pat* genes in plants constitutively (Reference 31).
pat	0.55	1012-1563	It encodes PAT protein and gives tolerance to glufosinate herbicide, derived from *Streptomyces viridochromogenes* (Reference 7).
T35S	0.20	1582-1784	35S RNA terminator derived from Cauliflower Mosaic Virus. It terminates transcription and induces polyadenylation of transcripts (Reference 36).
Others			
bla	0.86	3876-3016	It is an ampicillin resistant gene *(bla)* derived from *E.coli*. It expresses β-lactamase only in bacteria (Reference 42).
ori	0.55	2253-2803	It is the replication origin (ColE1) of pUC19, and initiates replication of plasmid (Reference 47).
RB	0.06	189-243	Right border derived from *Agrobacterium tumefaciens* Ti plasmid pTiAch5 (Reference 11).

Figure 1 Comparison between the native *pat* gene and the *pat* gene transferred in the recombinant soybean A2704-12

2) Function of component elements

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

Functions of individual component elements of donor nucleic acid which were used for the production of the recombinant soybean A2704-12 are shown in Table 1.

(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

In the process of nitrogen metabolism, crops produce ammonia by nitrate reduction, amino acid degradation, photorespiration, and so on. Glutamate synthase plays an important role in detoxification of the ammonia produced, though the glutamate synthase is inhibited if crops are sprayed with glufosinate herbicide, ammonia accumulates, and the crops wither and die.

On the other hand, in the plant body to which the *pat* gene is transferred, phosphinothricin acetyl transferase (PAT protein) is produced, and this enzyme acetylates the glufosinate to transform it to N-acetylglufosinate. This helps prevent the inhibition of glutamine synthase by the glufosinate, ammonia does not accumulate in the plant body, and the crop does not die even if it is sprayed with glufosinate (Figure 2).

It is reported that the PAT protein is not toxic to humans and other animals, and it shows no significant homology except with PAT proteins derived from various other species as a result of search for any homology with amino acid sequence of all proteins registered in the GENBANK database (Reference 29). In addition, as a result of comparison of physico-chemical and biochemical characteristics of PAT protein with known allergen, it was observed that this protein has no possibility to possess allergenicity (Reference 45).

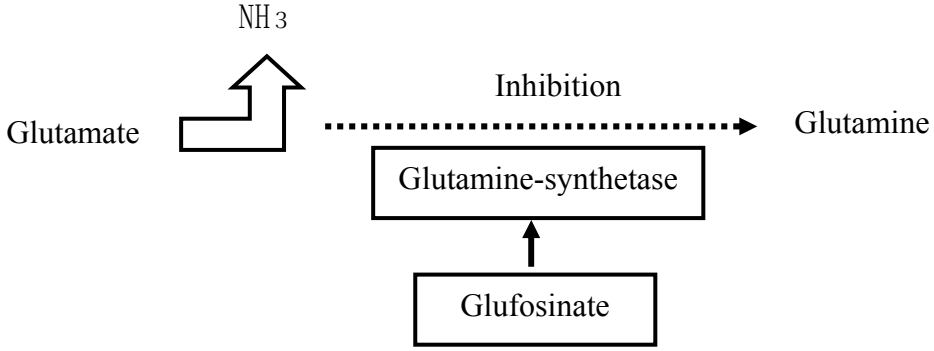
Moreover, based on the nucleotide sequence and amino acid sequence of this protein, overall homology search (EMBL and Swiss Prot) and allergen epitope search were conducted. As a result, this protein did not show any homology with known allergens.

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

The PAT protein encoded by the *pat* gene exhibits a high affinity to glufosinate classified into *L*-amino acid, though it does not cause any acetyl group transfer to the other various amino acids and it has little affinity for the glutamic acid which has specifically high structural similarity and it causes virtually no transfer reaction in any living body (Reference 43). In addition, even in the presence of excessive amount of various amino acids, the acetyl group transfer reaction to glufosinate by PAT protein was never inhibited (References 29 and 46). As a result, it is considered that the PAT protein possesses high substrate specificity and it does not affect the metabolic system of the recipient organism.

A) Normal Plant

Since glufosinate herbicide inhibits glutamine synthetase, ammominia accumulates in the plant body, causing the plant to die.



B) Recombinant Plant

Glufosinate herbicide is acetylated and becomes N-acetylglufosinate by action of the PAT protein, and the inhibition of the glutamine-synthetase does not occur. Therefore, ammonia does not accumulate in the plant body, and the plant can grow.

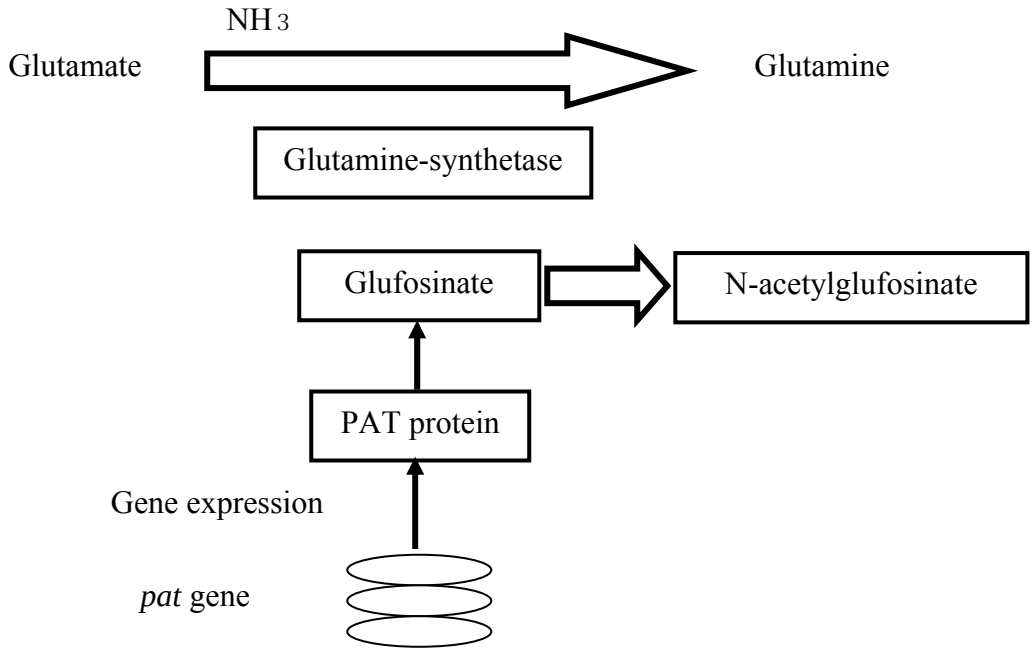


Figure 2 Mechanism of tolerance to glufosinate herbicide by the product of *pat* gene

(Note: All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon the applicant.)

(2) Information concerning vectors

1) Name and origin

The vector used for the production of the recombinant soybean A2704-12 is the plasmid pB2/35SAck (Figure 3), which was constructed based on the plasmid pUC19 derived from *Escherichia coli*.

2) Properties

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

The number of base pairs of the plasmid pB2/35SAck is 4,076 bps. Physical map of the plasmid is shown in Figure 3, and the nucleotide sequence is shown in Annex 3.

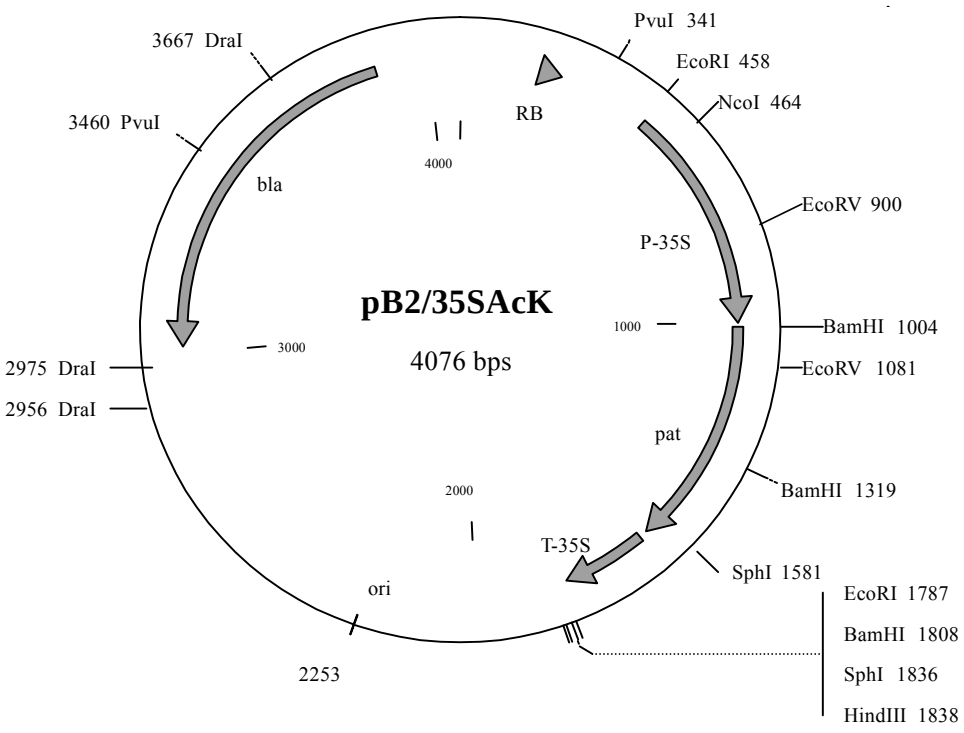


Figure 3 Physical Map of plasmid pB2/35Sack

(Note: All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon the applicant.)

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

The plasmid pB2/35SAck contains the *bla* gene to confer the resistance to ampicillin. The *bla* gene was used as a selective marker for construction of this plasmid, though this gene does not possess any promoter which functions in plants and therefore it does not function in the cells of soybean. Moreover, the plasmid pB2/35SAck has been cut by the restriction enzyme PvuI before the transformation, and the *bla* gene is split to such extent that it cannot function (Figure 4). Northern blotting analysis using the *bla* gene as a probe was conducted regarding RNA extracted from the leaves, stems, roots and seeds of the recombinant soybean A2704-12 (R4 generation). As a result, it was confirmed that the transcript of *bla* gene was not detected in any tissue

(limit of detection of 1 pg) and this gene was not expressed in any plant body (Annex 4, Figure 8).

(c) Presence or absence of infectious characteristics of vector and the information concerning the region of recipient organism if the infectivity of vector is found present

The plasmid pB2/35SAck does not possess a transferring ability, and therefore noninfectious. In addition, it is known that the range of recipient organisms for the autonomous replication of plasmid pUC19, the basis for construction of this plasmid, is limited to *Escherichia coli* and a few gram-negative bacteria.

(3) Method of preparing living modified organisms

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

The plasmid pB2/35SAck was treated with the restriction enzyme Pvu I and the *bla* gene was cut into two (2), large and small, fragments. Structure of the transferred T-DNA is shown in Figure 4.

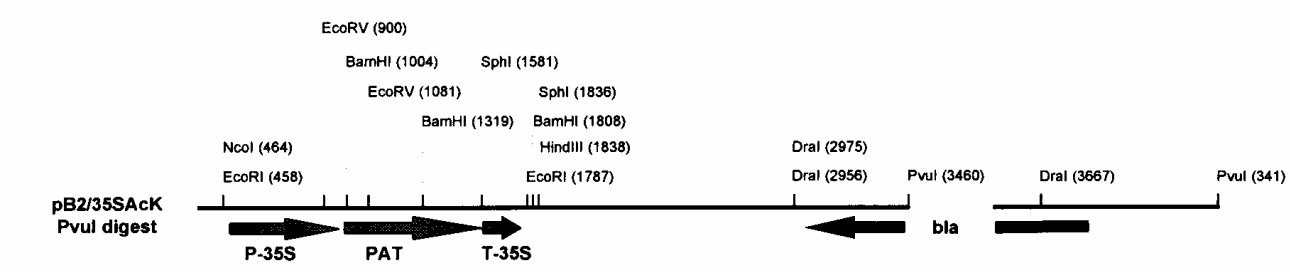


Figure 4 Structure of the nucleic acid transferred into the recipient organism
The "PAT" in the figure refers to the *pat* gene.

(Note: All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon the applicant.)

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

The two, large and small, fragments of plasmid pB2/35SAck, which was cut at two sites by the restriction enzyme PvuI (Figure 4), were transferred into the tissue cells of 4 to 8 mm long shoot apex of the soybean by the particle gun bombardment.

3) Processes of rearing of living modified organisms

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

After transformation, the nucleic acid transferred cells were formed into protoplasts, which were incubated in the Kao's medium of 10 mL (Reference 17) for eight (8) days in the darkness at room temperature. Then, Kao's medium of 5 mL was added by dropping method and the cells were additionally incubated at room temperature under a low level of lighting of 2,000 lux. Addition of Kao's medium was repeated a total of eight (8) days by dropping the volume equivalent to a half of the culture solution every day. Then the cells were moved to a solid medium containing glufosinate. Two (2) to three (3) weeks later, the glufosinate-tolerant soybean callus that survived among the transformed cells was selected. The selected callus was moved to the MS medium containing plant hormone and regenerated to the young plant bodies. In addition, the regenerated young plant bodies were screened by glufosinate herbicide and the glufosinate-tolerant individuals were acclimatized, planted in pots, and grown in a greenhouse.

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

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(c) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line to which confirmed the state of existence of replication products of transferred nucleic acid, 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 recipient species A2704 was transformed by the plasmid pB2/35SAck to obtain the current generation of the recombinant soybean A2704-12. Then, self-pollination was repeated to obtain the plant bodies in individual generations used in the tests. The pedigree tree of the recombinant soybean A2704-12 is shown in Figure 5.

In addition, the approvals received from organizations in Japan regarding the recombinant soybean A2704-12 are listed below.

[Environmental safety]

In 1998, based on the “Guideline for the use of recombinant in agriculture, forestry and fisheries”, conducting isolated field test was approved by the Ministry of Agriculture, Forestry and Fisheries. In addition, in May 1999,

based on the "Guideline for the use of recombinant in agriculture, forestry and fisheries", the compatibility to the guideline regarding recombinant being imported to Japan (used for processing and feed) was confirmed by the Ministry of Agriculture, Forestry and Fisheries.

[Feed safety]

Based on the "Procedures for feed safety Assessment of feed and feed additives derived from Recombinant-DNA technology", safety of use for feed was approved by the Ministry of Agriculture, Forestry and Fisheries in March 27, 2003.

[Food safety]

Based on the "Procedures for food safety Assessment of food and food additives derived from Recombinant-DNA technology", safety of use for food was approved by the Ministry of Health, Labour and Welfare in July 8, 2002.

Confidential: Not made available or disclosed to unauthorized person

Figure 5 Pedigree tree of the recombinant soybean A2704-12

(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

As a result of glufosinate herbicide-spraying tests using the individuals (R2 generation) grown by self-crossing of the R1 generation of the recombinant soybean A2704-12, a heterozygote with regard to the *pat* gene locus, a segregation ratio of 3:1 was obtained between glufosinate-tolerant and glufosinate-sensitive strains (Table 2-1). Then, the seeds of R3 generation obtained by self-crossing of the glufosinate-tolerant individuals were sown to the rows by pedigree and the germinated seedlings were sprayed with glufosinate herbicide to examine the segregation ratio between glufosinate-tolerant and glufosinate-sensitive plants. As a result, a segregation ratio of 1:2 was obtained between the strains exhibiting tolerance to glufosinate in all seedlings (homozygote) and the strains containing glufosinate-tolerant seedlings and glufosinate-sensitive seedlings in proportions of 3:1 (heterozygote) (Table 2-2). This result corresponds to the segregation ratio expected in single-gene dominant inheritance, therefore it is considered that the transferred gene exists in one chromosome.

Table 2-1 Segregation data on the glufosinate herbicide-tolerant individuals in the R2 generation obtained by self-crossing of heterozygote (1995, US)

Generation	Glufosinate-tolerant strain	Glufosinate-sensitive strain	Estimated segregation ratio	χ^{2} [a]
R2	67	24	3:1	0.10

R2: Generation obtained by self-crossing of R1 (heterozygote).

[a]: No significant difference was observed by the χ^{2} tests (P=0.05). (Defined significant at P=0.05 when $\chi^{2}>3.84$ and

df=1)

(Note: All the rights pertinent to the information in the table above and the responsibility for the contents rest upon the applicant.)

Table 2-2 Segregation ratio between glufosinate-tolerant and glufosinate-sensitive individuals in the progeny (R3) obtained by self-crossing of glufosinate herbicide-tolerant individuals of R2 generation (1995, US)

Generation	Number of rows in which all seedlings exhibited tolerance to glufosinate	Number of rows containing both glufosinate-tolerant and glufosinate-sensitive seedlings	Estimated segregation ratio	χ^{2} [a]
R3	24	45	1:2	0.06

R3: Generation obtained by self-crossing of glufosinate herbicide-tolerant individuals among those in the R2 generation.

[a]: No significant difference was observed by the χ^{2} tests (P=0.05). (Defined significant at P=0.05 when $\chi^{2}>3.84$ and df=1)

(Note: All the rights pertinent to the information in the table above and the responsibility for the contents rest upon the applicant.)

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

In order to identify the number of copies of T-DNA transferred into the recombinant soybean A2704-12, Southern blotting analysis using the genome DNA obtained from the recombinant soybean A2704-12 (R4 generation) was conducted (Annex 4, Figures 2 to 7). As a result, it was suggested that two (2) copies of *pat* gene cassettes are transferred in the recombinant soybean A2704-12 in the same direction, and that one copy of sequences each in the regions of 3'-terminal and 5'-terminal of *bla* gene separated at the section cut by the restriction enzyme PvuI is transferred between the two copies. In addition, the 5' *bla* fragment is transferred in the opposite direction to that in the plasmid pB2/35SAck, and it was considered that the complete *bla* gene is not intact. Furthermore, as a result of sequence analysis, the nucleotide sequence in the region transferred in the genome of soybean was determined (Annex 5).

In addition, in order to confirm the stability of transferred gene, Southern blotting analysis was conducted for the individual genome DNAs obtained from R3, R4 and R5 generations of the recombinant soybean A2704-12. As a result, two (2) fragments of same size were detected in the individual generations. Therefore, it was confirmed that transferred nucleic acid is stably inherited in the posterity (Annex 6).

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

It was confirmed that the recombinant soybean A2704-12 contains two (2) copies of *pat* gene expression cassettes repeated in the same direction, between which there exist 3'-terminal region of *bla* gene separated at the section cut by the restriction enzyme PvuI in the same direction as in the plasmid pB2/35SAck and the 5'-terminal region in the opposite direction. The transferred region is shown in Figure 6.

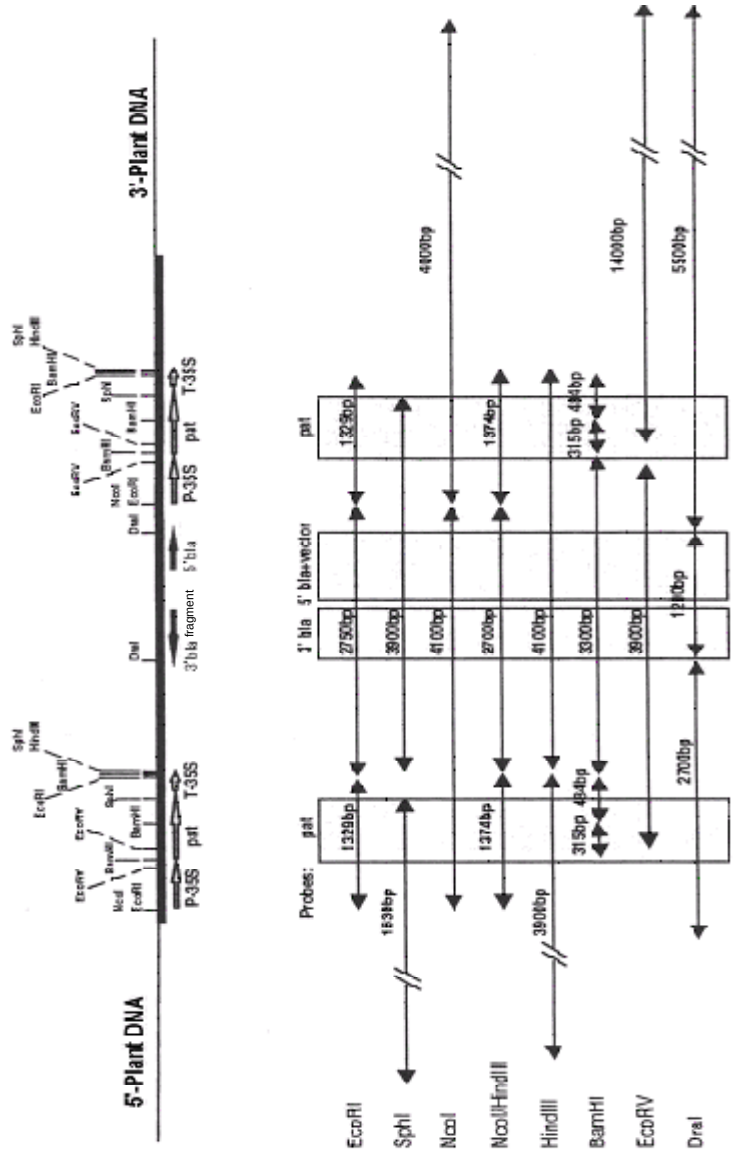

Figure 6 Genetic map of the transferred gene in the recombinant soybean A2704-12

A schematic view of restriction enzyme fragments detected by Southern blotting analysis using the *pat*, 3' *bla* and 5' *bla*+vector as probes

(Note: All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon the applicant.)

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

In 2002 in the US, ELISA analysis was conducted on the PAT protein in the roots, stems and leaves of five (5) individuals of each of the recombinant soybean A2704-12 and the recipient species A2704 (hereinafter referred to as "the non-recombinant soybean"). As a result, PAT protein was detected at all sites in the entire plant body in the recombinant soybean A2704-12, though PAT protein was not detected from the non-recombinant soybean (Table 3-1). In addition, as a result of ELISA analysis of PAT protein in the seeds of the recombinant soybean A2704-12 cultivated in US and Canada, PAT protein was detected in all the tests conducted (Table 3-2).

Table 3-1 Measurement of PAT protein in the roots, stems and leaves of the recombinant soybean A2704-12 based on the of ELISA method

Plant body	Site	Mean PAT protein content (μg/g fresh weight)±SD	Crude protein/Fresh weight (%)	PAT protein/Crude protein (%)
Recombinant soybean A2704-12	Root	2.23±1.29	1.95	0.011
	Stem	7.63±2.20	3.58	0.021
	Leaf	14.5±2.4	5.96	0.024
Non-recombinant soybean	Root	<LOD	1.61	-
	Stem	<LOD	4.15	-
	Leaf	<LOD	5.22	-

2002, US
(n=5)
LOD : Limit of Detection, Root 5.12 ng/g, Stem 2.48 ng/g, and Leaf 4.16 ng/g

(Note: All the rights pertinent to the information in the table above and the responsibility for the contents rest upon the applicant.)

Table 3-2 Measurement of PAT protein in the harvested seeds of the recombinant soybean A2704-12 based on the ELISA method

Sample No.	PAT (ng/g sample) Mean±SD	Crude protein content (%)	PAT/Crude protein (%)
1	1057	-	-
2	573	-	-
3	862±268	38.03	0.000227
4	2138±33	43.5	0.00049

－No data available
Sample No. 1: A mean of two (2) measurements of the samples obtained from individual plots in the field tests at three (3) locations and extracted once per each for three (3) repeats
Sample No. 2: A mean of measurements of the samples obtained in the field tests and extracted twice (a mean of two (2) measured values, LOD 2.0 ng/g sample)
Sample No. 3: A mean of two (2) measurements of the samples obtained from individual plots in the field tests at four (4) locations and extracted twice per each for three (3) repeats (a mean of a total of 48 measured values, LOD 6.6 ng/g sample)
Sample No. 4: A mean of two (2) measurements of the samples extracted twice (a mean of a total of four (4) measured values, LOD 4.0 ng/g sample)

(Note: All the rights pertinent to the information in the diagram above and the responsibility for the contents rest upon the applicant.)

In addition, twenty (20) seeds per each of the recombinant soybean A2704-12 (R5 generation) and the non-recombinant soybean were sown in an isolated field and the germinated seedlings were sprayed with the Basta ® herbicide (containing glufosinate as an active ingredient) diluted to a factor of 500. As a result of visual observation ten (10) days after spraying test, it was confirmed that the seedlings of the non-recombinant soybean all withered and died, though the individuals of the recombinant soybean A2704-12 all exhibited tolerance to the herbicide (Annex 1).

Moreover, as a result of spraying of glufosinate herbicide to the seedlings germinated from the seeds of the recombinant soybean A2704-12 (R6 generation) and the non-recombinant soybean subjected to the special screened greenhouse test, it was confirmed that the seedlings of the non-recombinant soybean all withered and died, though the seedlings of the recombinant soybean A2704-12 all exhibited tolerance to glufosinate and survived. In addition, as a result of the same examination using the seeds of the next generation recombinant soybean A2704-12 and the non-recombinant soybean cultivated and harvested in the same special screened greenhouse, it was also confirmed that the seedlings of the non-recombinant soybean all withered and died and the seedlings of the recombinant soybean A2704-12 all survived (Annex 2).

Based on the results discussed above, it was confirmed that the *pat* gene in the recombinant is stably expressed among individuals and 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

The recombinant soybean A2704-12 contains no DNA sequence which possesses transferring factor and therefore, there is no possibility of transmission of nucleic acid transferred to wild animals and wild plants under a natural environment.

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

Specific detection method for the recombinant soybean A2704-12 is available by PCR method using the transferred DNA in the recombinant soybean A2704-12 and its surroundings as 20-mer and 21-mer primers. The PCR method has been typically used as an effective means for control of cultivation of the recombinant soybean A2704-12 (Annex 8).

(6) Difference from 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

With the production of the PAT protein due to the expression of the transferred *pat* gene, tolerance to glufosinate herbicide is conferred to the recombinant soybean A2704-12.

2) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between recombinant crop and the taxonomic species to which the recipient organism belongs, and the degree of difference, if any

In the isolated field tests conducted in FY 1998 at National Agriculture Research Center for Hokkaido Region, the Ministry of Agriculture, Forestry and Fisheries, comparison was made for differences between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 1). In addition, with regard to wintering ability of the matured plant, shedding habits of the seeds, germination rate of the seeds and production of harmful substances, examination was conducted in FY 2005 in a special screened greenhouse in Japan (Annex 2). Regarding the fertility and size of the pollen, comparison was made in 2002 at Aventis CropScience (the present Bayer CropScience) in France between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 7). In all of the tests mentioned above, the A2704 line was used as the control non-recombinant soybean.

(a) Morphological and growth characteristics

Comparison between the recombinant soybean A2704-12 and the non-recombinant soybean was made for the initiation of germination and germination period in the investigation in germination (Annex 1, Table 2), the time of flower initiation; flowering period; color of flowers; main stem length; the number of main stem nodes; thickness of stem; the number of branches; leaf length; leaf width; leaf color; trichome color and trichome quantity in the investigation at flowering time (Annex 1, Tables 3-1 and 3-2), and the plant type; growth habit; difficulty in pod bursting; aerial weight of a plant; weight of pod seeds per plant; the number of ripe pods per plant; pod color; grain weight per plant; the number of perfect grains per plant; the weight of perfect grains per plant; the number of grains per pod; 100-grain weight; seed hull color and hilum color in the investigation during maturation period and at harvesting time (Annex 1, Tables 4-1 and 4-2). As a result, none of the items showed difference between the recombinant soybean A2704-12 and the non-recombinant soybean.

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

Cold-tolerance test at the early stage of growth of the plant body was not conducted. Instead, in order to examine the wintering ability of the seeds, the seeds were taken from the recombinant soybean A2704-12 and the non-recombinant soybean grown in an isolated field, dried up and then placed in the earth to a depth of 10 cm in the isolated field in December 7, 1998, then recovered sixty (60) days later and observed. As a result, it was confirmed that the seeds were all decayed when they absorbed water and they lost the germinating ability. Therefore, it is considered that the recombinant soybean A2704-12 and the non-recombinant soybean fail to germinate and grow and survive the winter season under natural condition in the winter season.

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

For the recombinant soybean A2704-12 and the non-recombinant soybean sown in August 17, 2005 and grown in a special screened greenhouse, the matured plants were left to stand in the special screened greenhouse in the winter season even after maturation and they were observed February 2 in the next year. As a result, it was confirmed that all the individuals withered and died (Annex 2), and no difference was observed between the recombinant soybean A2704-12 and the non-recombinant soybean in the wintering ability.

(d) Fertility and size of the pollen

Based on the fact that the recombinant soybean A2704-12 and the non-recombinant soybean were found equivalent to each other in terms of fertility and germination of the pollen in the tests conducted in 2002 in France, it is considered that there is no difference in fertility of the pollen between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 7). In addition, as a result of comparison for the shape of the pollen between the recombinant soybean A2704-12 and the non-recombinant soybean, no difference was observed between them (Annex 7).

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

Regarding the characteristics referring to the production of seeds, weight of pod seeds per plant, the number of ripe pods per plant, grain weight per plant, the number of perfect grains per plant, the weight of perfect grains per plant, the number of grains per plant and 100-grain weight, no significant difference was observed between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 1, Tables 4-1 and 4-2).

With regard to the shedding habit, as a result of examination on the difficulty in pod bursting, the recombinant soybean A2704-12 and the non-recombinant soybean were both difficult to burst the pods, so no difference was observed between them in the shedding habit (Annex 1). In addition, in a special screened greenhouse test, the recombinant soybean A2704-12 and the non-recombinant soybean were planted in six (6) pots for each and kept grown in the period up to one month after the maturing time to examine the rate of

pods burst among the total number of pods per plant. As a result, it was observed that the rate of the number of burst pods was relatively smaller in the recombinant soybean A2704-12 compared to the non-recombinant soybean, though it involved larger variations between plants and no significant difference was observed. Then, regarding the difficulty in pod bursting, it was considered that the recombinant soybean A2704-12 and the non-recombinant soybean both fall under the classification of "Difficult" similarly as the result of judgment based on the isolated field tests mentioned above (Annex 2, Tables 3 and 4).

With regard to the dormancy and germination rate, as a result of examination by sowing twenty (20) seeds per each harvested from the recombinant soybean A2704-12 and the non-recombinant soybean cultivated in an isolated field, the germination rates were 100% for the both plants (Annex 1). In addition, the seeds of the recombinant soybean A2704-12 and the non-recombinant soybean harvested in a special screened greenhouse test were sown by thirty (30) seeds per each of three (3) replications (a total of ninety (90) seeds per each plant) to determine the germination rates. As a result, the germination rates were 98.9% for the recombinant soybean A2704-12 and 100% for the non-recombinant soybean, and no significant difference was observed in germination rate (Annex 2, Tables 1 and 2). Based on the above understanding that the recombinant soybean A2704-12 and the non-recombinant soybean both show higher germination rates similarly to each other immediately after harvesting, it is considered that the recombinant soybean A2704-12 and the non-recombinant soybean both have lower levels of seed dormancy.

(f) Crossability

One thousand and sixty six (1,066) seeds derived from the non-recombinant soybean cultivated adjacent to the recombinant soybean A2704-12 at an inter-row spacing of 60 cm were sown and grown in an isolated greenhouse and then sprayed with glufosinate herbicide to identify the number of individuals that exhibit tolerance to glufosinate. As a result, six (6) individuals were found free from any effects of herbicide and thus, the crossability was considered 0.56% (Annex 1). It is reported that the outcrossing rate of soybean ranges between 0.5 and 1% (Reference 4), and the obtained result falls within the range. Consequently, it is considered that the crossability of the recombinant soybean A2704-12 due to dispersion of the pollens does not exceed the existing findings.

(g) Productivity of harmful substances

With regard to the characteristics referring to the productivity of harmful substances of the recombinant soybean A2704-12, the following tests were carried out to compare the recombinant soybean A2704-12 and the non-recombinant soybean: succeeding crop tests using radish, a plant relatively high sensitive to growth-inhibiting substances, for examining the substances secreted from the roots with a threat to affect the growth of other plants; plow-in tests using radish for examining the substances contained in the plant

tissues with a threat to affect other plants after they die; and soil microflora tests for examining the substances secreted from the roots with a threat to affect soil microbes.

[Succeeding crop tests]

The recombinant soybean A2704-12 and the non-recombinant soybean grown in a special screened greenhouse for about three (3) months were sieved after harvesting to remove the plant tissues and other residues of plant bodies and obtain the soil. In the soil, the seeds of radish were sown and grown to examine the germination rate, plant height, root length, fresh weight and dry weight. As a result, no significant difference was observed in all items examined between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 2, Tables 5 to 10).

[Plow-in tests]

The plant bodies including the roots of the recombinant soybean A2704-12 and the non-recombinant soybean harvested after cultivation in a special screened greenhouse for about three (3) months were dried, crushed and mixed with new soil at a rate of about 1% by weight, then packed into nursery pots. Seeds of radish were sown in and grown in the pots to examine the germination rate, plant height, root length, fresh weight and dry weight. As a result, no significant difference was observed in all items examined between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 2, Tables 11 to18).

[Soil microflora tests]

The soils shaken off from the roots of plant bodies of the recombinant soybean A27104-12 and the non-recombinant soybean harvested after cultivation in a special screened greenhouse for about three (3) months were compared to examine the number of bacteria, the number of actinomyces and the number of filamentous fungi. As a result, no significant difference was observed in all items examined between the recombinant soybean A2704-12 and the non-recombinant soybean (Annex 2, Tables 19 to 21).

Based on the above results, it is considered that there is no difference between the recombinant soybean A2704-12 and the non-recombinant soybean in the productivity of harmful substances.

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

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

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

(1) Competitiveness

The plant of soybean (*G. max*) 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.

This recombinant soybean is given the tolerance to glufosinate herbicide because of the transferred *pat* gene, but it is hard to consider that glufosinate exerts a selective pressure under a natural environment. In addition, various characteristics relating to the competitiveness of this recombinant soybean have been investigated in Japanese isolated fields, and no significant difference from the non-recombinant soybean has been observed.

Based on the above understanding, it was judged that the conclusion by the applicant that the wild animals and wild plants likely to be affected cannot be specified 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 of soybean (*G. max*) 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 produces the PAT protein having the tolerance to glufosinate, but it is not reported that the protein is a harmful substance. In addition, the PAT protein has high substrate specificity and exhibits little affinity except to glufosinate; therefore it is considered that the PAT protein does not affect the metabolic system of the recipient organism.

In addition, the ability of this recombinant soybean to produce harmful substances (the substances secreted from the roots to affect other plants, the substances secreted from the roots to affect microorganisms in soil, and the substances contained in the plant bodies to affect other plants after dying out) was investigated, and no significant difference between this recombinant soybean and the non-recombinant soybean was observed.

Based on the above understanding, it was judged that the conclusion by the applicant that the wild animals and wild plants likely to be affected cannot be specified 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

1) Identification of wild animals and wild plants likely to be affected

Since it is known that if the *Glycine soja* Sieb. & Zucc. that grows voluntarily in Japan is crossed with soybean(*G. max*), it produces fertile seeds, the *G. soja* was specified as a wild plant likely to be affected, to perform the following examination.

2) Evaluation of concrete details of adverse effect

Existing documents do not show any obstacle to the growth and reproduction of the hybrid obtained from soybean and *G. soja*. So, in the case where this recombinant soybean and *G. soja* are crossed with each other in the Japanese natural environment, there is possibility that the hybrid grows and that the gene transferred into this recombinant soybean through the back crossing from the hybrid to *G. soja* diffuses among the population of *G. soja* without remaining at a low level.

3) Evaluation of likelihood of adverse effect

G. soja grows voluntarily and widely throughout Japan in sunny fields, on the roadsides and the like. So, in the case where this recombinant soybean grows near *G. soja*, it cannot be denied that there are chances where both plants cross with each other. However:

A. There is no plan of cultivation of this recombinant soybean in the future in Japan. It is considered that any seeds may be spilled during transportation and such spilled seeds germinate and grow, though there is no report so far that soybean becomes self-seeding.

B. Even in the case where this recombinant soybean grows voluntarily near *G. soja* as a rare case:

(a) Both G. max and G. soja are typical autogamous plants engaged in cleistogamy＊.

(b) According to existing documents, even when G. soja was grown adjacent to G. max under such a condition that the G. soja pedigree and G. max pedigree flowered at the same time, the crossing rate was less than 1%.

(c) Among the individuals derived from the seeds harvested from the non-recombinant soybean cultivated closely to this recombinant soybean in Japanese isolated fields, there were some individuals that are free from any effects of glufosinate herbicide, though they accounted for such a low proportion of 0.56%. Consequently, it is considered that the crossability

of this recombinant soybean with G. soja is similarly low as that of conventional soybean.

(d) It is considered to be low that the tolerance to glufosinate conferred with the expression of the *pat* gene works dominantly for the selection pressure under the natural environment.

In view of the above, it is considered to be very low that this recombinant soybean and *G. soja* could cross with each other and that the transferred gene could diffuse among the population of *G. soja* without remaining at a low level.

4) Judgment of existence of Adverse Effect on Biological Diversity

As described above, the probability that this recombinant soybean and *G. soja* cross with each other is very low. Even if crossing occurs, it is hard to consider that the hybrid produced from this recombinant soybean and *G. soja* ousts wild plants. In addition, the possibility that the transferred gene diffuses among the population of *G. soja* without remaining at a low level is also considered to be very low stochastically. Consequently, it was judged that the conclusion made by the applicant that there is no risk of Adverse Effect on Biological Diversity attributable to crossability is 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 I Use Regulation causes Adverse Effect on Biological Diversity. It was judged that the conclusion above made by the applicant is reasonable.

* Cleistogamy means self-pollination within a flower that does not open that occurs in angiosperms. It involves a very low probability of cross-pollination with the pollens of other plants due to the physical obstacles of flower bud (petal/calyx). However, it does not mean any physiological incompatibility, so it may allow cross-pollination with the pollens of other plants transmitted by insects, etc.

Reference

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Annex List

Annex 1： FY 1998 Isolated Field Test Report on Genetically Modified Soybean A2704-12
Confidential: Not made available or disclosed to unauthorized person

Annex 2： Environmental Safety Evaluation Test on Glufosinate Herbicide-Tolerant Soybean A2704-12 in Special screened greenhouse
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Annex 3： The Entire Nucleotide Sequence of Plasmid pB2/35SAck
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Annex 4： Transformation System and Genetic Characterization of Glufosinate Resistant Soybean Event A2704-12
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Annex 5： Determination of the Sequence Transferred into the Recombinant Soybean A2704-12
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Annex 6： Molecular Demonstration of the Stability of the Integration of *Glycine max* Transformation Event A2704-12

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Annex 7： Shape and Fertility of Pollen of the Recombinant Soybean A2704-12 (Reproductive Biology Data Glufosinate-tolerant Soybean Event A2704-12)
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Annex 8： Event Identifying Method
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