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

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Approved Type 1 Use Regulation

| | |
|---|---|
| Name of the type of Living Modified Organism | Cotton tolerant to glufosinate herbicide (*bar, Gossypium hirsutum* L) (LLCotton25, OECD UI:ACS-GH001-3) |
| 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

## I. Information concerning the preparation of the living modified organism

### 1. Information concerning the donor nucleic acid

#### (1) Composition and origins of component elements

The composition of the donor nucleic acid that was used for the development of the cotton tolerant to glufosinate herbicide (*bar, Gossypium hirsutum* L.) (hereinafter referred to as "LLCotton25") and the origins of component elements are shown in Table 1.

Table 1 DNA used for introducing LLCotton25

| Component element (abbr.) | Position in vector | Size (bp) | Origin and function |
|---|---|---|---|
| *bar* cassette | | | |
| P35S3 | 250-1634 | 1385 | Promoter region derived from cauliflower mosaic virus 35S transcript gene. Initiates transcription. |
| Modified *bar* | 1635-2186 | 552 | Bialaphos resistance (*bar*) gene derived from *Streptomyces hygroscopicus*. Confers tolerance to glufosinate herbicide. The two codons at the N-terminus of wild *bar* gene are replaced by ATG and GAC respectively. |
| 3'nos | 2206-2465 | 260 | 3' untranslated region of nopaline synthase gene derived from T-DNA of pTiT37. Terminates transcription and induces 3'-polyadenylation. |
| Others | | | |
| RB | 198-222 | 25 | Right border repetitive sequence of T-DNA derived from pTiB6S3. |
| LB | 2520-2544 | 25 | Left border repetitive sequence of T-DNA derived from pTiB6S3. |
| *aad*A | 2544-4618 | 2075 | Sequence including a gene tolerant to streptomycin/spectinomycin, derived from Tn7 transposon. |
| p VS1ori | 4619-8389 | 3780 | Replication origin of plasmid pVS1 derived from *Pseudomonas*. |
| ColE1 | 8390-9555 | 1165 | Sequence including replication origin ColE1 ori derived from plasmid pBR322 |

Since the wild *bar* gene obtained from *Streptomyces hygroscopicus* contains a large amount of G:C (guanine:cytosine) rarely found in plants, it was modified from GTC to ATG for making the codon suitable for use in plants and from AGC to GAC for enhancing the efficiency of translation. In the modification from GTG to ATG, the amino acid to be actually translated remains methionine, but in the modification from AGC to GAC, the amino acid changes from serine to aspartic acid.

#### (2) Functions of component elements

i) Functions of respective component elements of donor nucleic acid

The functions of the respective components elements of the donor nucleic acid that was used for the development of LLCotton25 are shown in Table 1.

ii) Function and allergenicity of the protein produced by the expression of target gene, etc.

In the process of nitrogen metabolism, plants produce ammonia by nitrate reduction, amino acid degradation, photorespiration, and so on. Glutamine synthetase plays an important role in detoxication of the ammonia produced, but if glufosinate herbicide is applied, glutamine synthetase is inhibited and ammonia accumulates, causing the plant to die.

The phosphinothricin acetyl transferase (PAT protein) encoded by the modified *bar* gene introduced acetylates glufosinate to make N-acetylglufosinate, and inactivates the inhibitory action of glufosinate to the glutamine synthetase. By virtue of this mechanism, ammonia is not accumulated, and the plant does not die, even if it is sprayed with glufosinate herbicide (Figure 1).

Glufosinate is classified as an amino acid, but the acetyl group transfer reaction to glufosinate by the PAT protein was not inhibited even in the presence of excessive amounts of various amino acids. Furthermore, it is confirmed that the PAT protein does not catalyze the transfer reaction even to especially structurally similar glutamic acid, methionine sulfoximine and the like either. These facts suggest that the PAT protein has high substrate specificity.

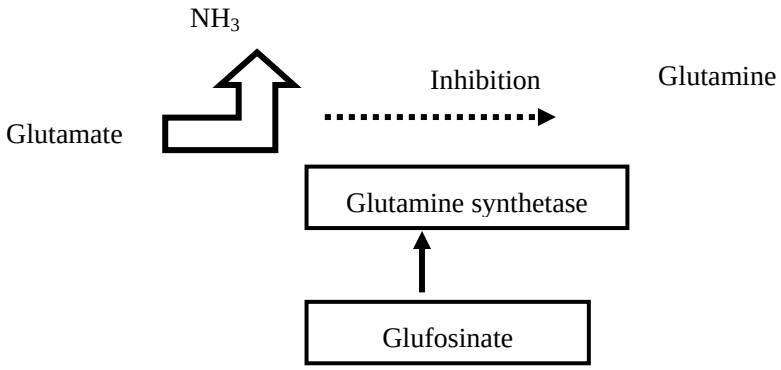
Moreover, based on the amino acid sequence of the modified *bar* gene product, overall homology search (Swiss Prot, trEMBL, GeneSeq-Prot, PIR, PDB, DAD, and GenPept) and allergen epitope search were conducted. As a result, this protein did not show any homology with known toxins or allergens. So, it was suggested that the protein does not have allergenicity.

iii) Effect on the metabolic pathway of gene product

The PAT protein has very high substrate specificity, and there exists no other protein serving as a substrate than glufosinate among plants. Therefore, it cannot be considered that the PAT protein affects the metabolic pathway of the recipient organism

a) Normal Plant

Since glufosinate herbicide inhibits glutamine synthetase, ammonia 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,

to prevent the glutamine synthetase from being inhibited. Therefore, ammonia does not accumulate in the plant body, and the plant can grow.

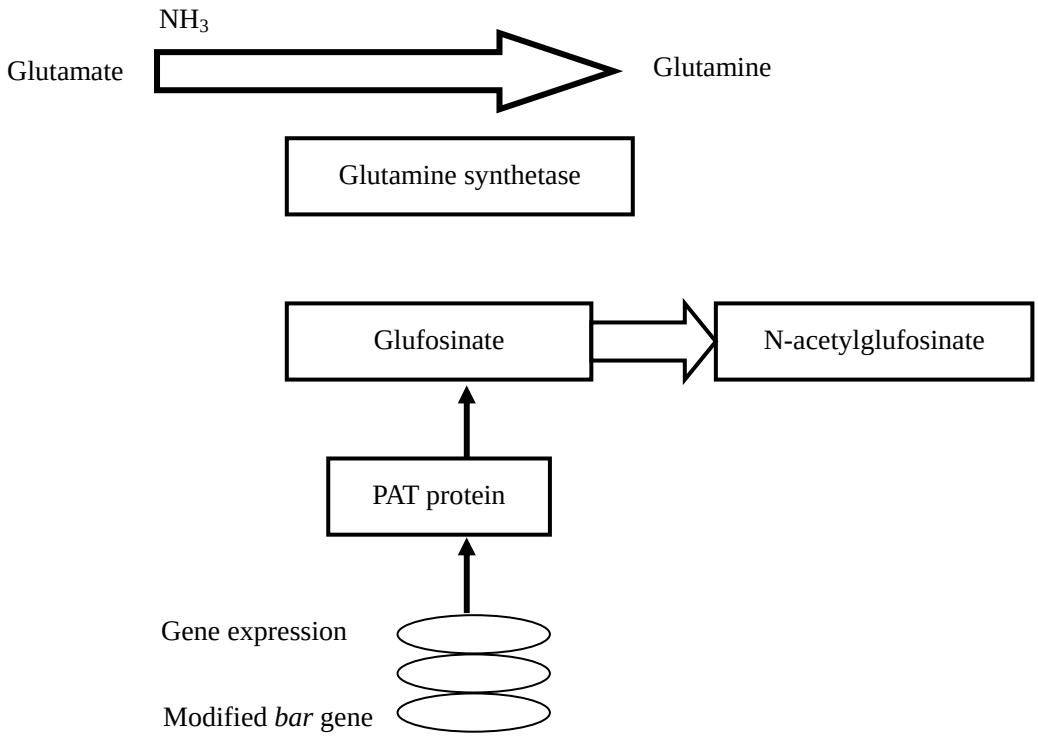



Figure 1 Mechanism of tolerance to glufosinate herbicide by the product of modified *bar* gene

2. Information concerning the vector

(1) Name and origin

The plasmid vector pGSV71 used for the production of LLCotton25 was constructed based on the plasmid pBR322 derived from *Escherichia coli* and the plasmid vector pVS1 derived from *Pseudomonas aeruginosa*.

(2) Properties

(i) Number of base pairs and nucleotide sequence of vector

The plasmid map is shown in Figure 2. The number of base pairs of the plasmid vector pGSV71 used for the production of LLCotton25 is 9555 bp. Among them, the vector is a portion excluding the T-DNA region (from 198 bp at the end of RB to 2544 bp at the beginning of LB in Fig. 2) (for the positions of the respective component elements in the vector, see Table 1)

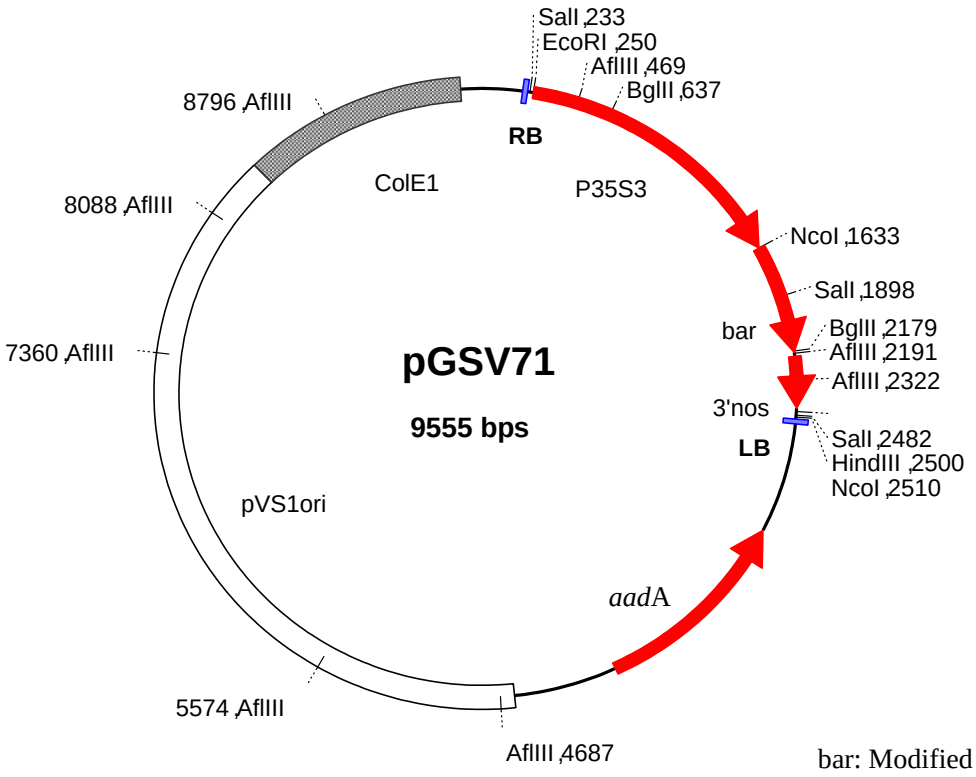



Figure 2　pGSV71 plasmid map and restriction enzyme cleavage sites

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

The plasmid pGSV71 has a selectable marker gene (*aadA* gene derived from *E. coli*) that confers tolerance to streptomycin and spectinomycin. The *aadA* gene is used as a selectable marker when the plasmid pGSV71 for transformation of Cotton is constructed using *E. coli*, and since this gene does not have a promoter functioning in a plant, it cannot be considered that the gene is expressed in a plant. Furthermore, the *aadA* gene is positioned outside the T-DNA region (Figure 2), and it is not considered that the gene is introduced into the plant genome DNA.

Moreover, the plasmid pGSV71 has the replication origin ColE1ori derived from the plasmid pBR322 of *E. coli* and the replication origin pVS1ori of the plasmid vector pVS1 of *Pseudomonas aeruginosa*, and though they function to cause autonomous replication in *E. coli* and *P. aeruginosa* respectively, they do not function in plants. These replication origins are positioned outside the T-DNA region (Figure 2), and it is not considered that they are introduced into the plant cells.

(iii)Whether or not the vector is infections, and information concerning recipient organisms

The recipient organisms allowing the autonomous replication of the vector pGSV71 are limited to *E. coli* and some gram-negative bacteria such as *A. tumefaciens*.

3. Method of preparing the living modified organism

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

For the production of LLCotton25, used is the plasmid vector pGSV71 in which modified *bar* gene expression cassette ([P35S]-[modified *bar*]-[3'nos]) for conferring the trait of tolerance to glufosinate herbicide to the recipient organism is placed between RB and LB of the vector. The positions and directions of the component elements of the donor nucleic acid and the restriction enzyme cleavage sites in the vector are shown in Figure 2.

(2) Method of transferring the nucleic acid transferred in the recipient organism

For introducing the gene of plasmid pGSV71 into the recipient organism, a binary vector method using *A. tumefaciens* was used. A tissue fragment (region from hypocotyl to radicle) excised from an immature of cultivar Coker312 was exposed to and infected with a culture solution of *A. tumefaciens* harboring Ti plasmid pGV3000 and binary vector pGSV71, for integrating the T-DNA region placed between RB and LB on the plasmid pGSV71 into the cotton genome.

(3) Process of rearing the living modified organism

(i) Method of selecting the cells containing the transferred nucleic acid

Plant indivisuals were reproduced from the tissue fragment transplanted into a reproduction medium, and furthermore, a reproduction medium containing glufosinate was used to select strains tolerant to glufosinate.

(ii) Whether or not *Agrobacterium* remains

*Agrobacterium* is removed by a reproduction medium containing 500 mg/L claforan. Therefore, *Agrobacterium* does not remain.

(iii) Process of rearing and pedigree tree

The LLCotton25 was self-crossed or crossed with the cultivars owned by Bayer Crop Science for selective breeding.

4. State of existence of the nucleic acid transferred in cells and stability in the expression of trails caused by the nucleic acid

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

A transferred DNA, if once integrated in a plant chromosome, follows the Mendel's law of inheritance like other plant genes.

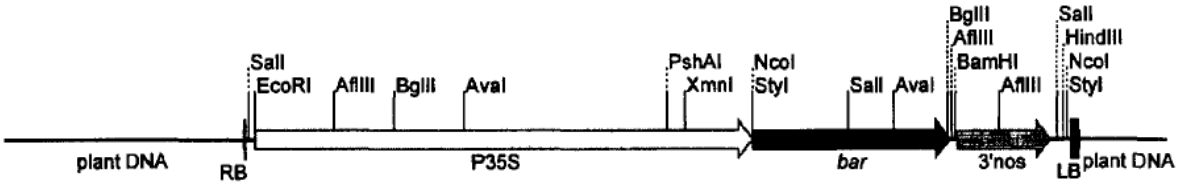
Individuals having the modified *bar* gene of LLCotton25 pedigree were self-crossed, or back-crossed with the non-recombinant, to obtain progeny individuals, and their segregation characteristics were examined. As a result, segregation ratios of 3:1 and 1:1 respectively corresponding to segregation patterns of single dominance according to the Mendel's law of inheritance were shown. So, it is considered that the transferred nucleic acid is positioned on one chromosome.

(2) Number of copies of the transferred nucleic acid and stability of transferring in multiple generations

To investigate the number of copies of the DNA inserted into LLCotton25, the genome DNA of LLCotton25 was cleaved with restriction enzymes Sal I, EcoR I, Afl III, Bgl II and Nco I, and

Southern blotting analysis was performed using the entire T-DNA region as a probe, to analyze the hybridized fragments obtained by treatment with various restriction enzymes. As a result, it was demonstrated that a complete one copy of the gene cassette was integrated in the plant genome. Furthermore, PCR analysis was used to examine the boundary region of the inserted DNA in detail, and as a result, it can be considered that the T-DNA region is integrated in LLCotton25 in such a mode as illustrated in Figure 3.

Moreover, to examine the stability of the inserted DNA in multiple generations of LLCotton25 and in multiple cultivars as different genetic backgrounds, restriction enzyme *Ncol* having two cleavage sites in the inserted DNA was used for cleaving, and Southern blotting analysis was performed using the aforesaid T-DNA region as a probe. The individuals used in this test were of LLCotton25 (T4 generation), LLCotton25 (T5 generation) and LLCotton25/A (cultivar obtained by back-crossing cultivar A three times to the T1 generation, and further self-crossing three times). Twenty or more individuals were used for each generation. As a result, in all the test samples, the predicted two bands were confirmed, and the stability of LLCotton25 in multiple generations at the genome level could be confirmed.




bar: Modified *bar* gene

Figure 3 Structure of T-DNA region

(3) Stability of expression under natural conditions

To confirm the stability in the expression of the introduced gene under natural conditions, glufosinate herbicide was sprayed over five individuals each of LLCotton25 and the non-recombinant, and the development was observed. As a result, all the individuals of LLCotton25 showed tolerance, but all the individuals of the non-recombinant died. Meanwhile, in USA on 2000 and 2001, the tolerance of LLCotton25 of T5 generation and T6 generation to glufosinate herbicide was examined, and it was confirmed that the introduced gene was stably expressed even after generations.

Furthermore, the PAT protein in the seeds, lint coat and lint of LLCotton25 was determined by means of ELISA. As a result, the PAT protein was detected in all of seeds, lint coat and lint. The results suggest that the modified *bar* gene is constitutively expressed in the respective tissues due to the function of 35S promoter.

(4) Whether or not the transferred nucleic acid is likely to be transferred to wild animals and wild plants, and how likely to occur the transfer is

Since the recipient organisms allowing the autonomous replication of the plasmid pGSV71 are

limited to *E. coli* and some gram-negative bacteria such as *A. tumefaciens*, it cannot be considered that the transferred nucleic acid is transferred to wild animals and wild plants under natural conditions.

5. Methods of detection and discrimination of the living modified organism and their sensitivity and reliability

A PCR method using respective pairs, each consisting of a 20-mer primer and a 21-mer primer, obtained by using the DNA inserted in LLCotton25 and its surrounding genome sequences can be used to specifically identify this event. Furthermore, if a template DNA of usually 50ng is used, efficient detection can be made. If a very slight amount of a seed or plant body of LLCotton25 is available, detection and identification can be made. In replicated tests, highly reproducible results could be obtained. This PCR method is effectively used also for actually testing the purity of seeds of LLCotton25.

6. Difference from the recipient organism or the species to which the recipient organism belongs

(1) Physiological and ecological properties conferred by the expression of the replication product of transferred nucleic acid

With the PAT protein produced due to the expression of the inserted modified *bar* gene, LLCotton25 shows tolerance to glufosinate herbicide. When the susceptibility to the herbicide was tested in isolated fields, 100% of the non-recombinant showed susceptibility, while 100% of LLCotton25 showed tolerance to the herbicide as the property of the protein.

(2) Difference between the living modified organism and the species of recipient organism

The National Agricultural Research Center for Kyushu Okinawa Region, National Agricultural Research Organization carried out isolated field tests in FY 2003, to examine the difference between LLCotton25 (T5 generation) and the non-recombinant control.

For reference, the results of the following tests are attached hereto and quoted as required: the tests conducted in 2000 in USA for comparing LLCotton25 (T5 generation) and the non-recombinant control, the comparison tests conducted in 2001 in USA for comparing LLCotton25 (T6 generation), cultivars (BC3/F3 or BC3/F4) obtained by conferring the trait of LLCotton25 to five commercial cultivars different in genetic background, and respective non-recombinant control cultivars, and the reproductive test (T5 generation) conducted in 2001 in France.

(i) Morphological and growth characteristics

LLCotton25 and the non-recombinant were compared with each other in the following morphological and growth characteristics: 1. Germination characteristics (uniformity of germination, and germination rate); 2. Plant body characteristics (plant type, plant height, total number of branches, and number of nodes); 3. Leaf morphology (leaf shape, and leaf size); 4. Floral organ morphology (flower color, flower shape, petal color); 5. Flowering characteristics (flowering date, and number of appearing flower buds); 6. Boll morphology (boll (fruit) shape, boll size, number of segments per

boll, and number of seeds per boll); 7. Boll opening characteristics (boll opening time, fiber color (lint color), seed color and shape, and harvesting time); and 8. Final morphology at harvesting time (number of harvested bolls per plant, number of non-harvested bolls, total number of bolls, fresh weight per boll, and weights of aerial part and subterranean part at harvesting time).

As a result, in none of the traits other than plant height and number of nodes, any significant difference was confirmed between LLCotton25 and the non-recombinant control.

With regard to the plant height, a significant difference was observed only at the 60$^{th}$ day after sowing, and LLCotton25 showed a value of about 6 cm lower on the average than the non-recombinant control. However, at the other points of time (30$^{th}$ day, 90$^{th}$ day and 120$^{th}$ day after sowing), no significant difference was observed.

With regard to the number of nodes, at the 60$^{th}$ day after sowing, LLCotton25 was larger by 0.6 on the average than the non-recombinant control, and at the 120$^{th}$ day, the non-recombinant control was larger by 1.7 on the average than LLCotton25, not showing any constant trend. Furthermore, at the 30$^{th}$ day and 90$^{th}$ day after sowing, no significant difference was observed.

As for the number of non-harvested bolls per plant, the total number of bolls per plant, and the weights of aerial part and subterranean part at harvesting time, in terms of mean values, LLCotton25 tended to show higher values than the non-recombinant, but the variation were large between individuals and between replications. So, no significant difference could be detected between LLCotton25 and the non-recombinant. As the cause of the irregularity, it can be considered that the cultivation conditions may have been different from test individual to test individual owing to the site conditions of isolated test fields, etc.

Meanwhile, as a reference, fieldtestscarried out in 2000 and 2001 in USA is also quoted. Significant differences were confirmed in the first flowering day in the tests of 2000 and in percent stand count of seedlingin the tests of 2001, but no statistically significant difference was confirmed in the other traits.

(ii) Chilling tolerance at the early stage of growth

Twenty seedlings (two-leaf stage) of LLCotton25 were placed in an incubator of 4°C having 12-hour lightening interval and the reaction to the low temperature was observed with the lapse of time. As a result, on the 6$^{th}$ day after they were placed, all the individuals died, showing no chilling tolerance.

(iii)Overwintering ability of the matured plant

The matured plant of LLCotton25 grown in the isolated field completely died due to the low temperatures and frost occurring till the last 10 days of December, showing no overwintering ability.

(iv)Fertility and size of the pollen

In Japan, this item is not tested. In the cultivation tests conducted in 2001 in France, the survival rates and germination rates of the pollen obtained from the LLCotton25 and the non-recombinant grown in greenhouses were investigated, and the shapes of the pollen and germinated pollen were

microscopically observed.

No significant difference was observed in pollen survival rate or germination rate. Furthermore, both of them had a pollen diameter of 200 μm on the average, and they were not different in the shape of germinated pollen either.

(v) Production, shedding habit, dormancy, and germination rate of the seeds

In the isolated field tests conducted in FY 2003 in Japan, the following traits concerning the production of seeds were investigated: boll size, number of sections per boll, number of seeds per boll, boll shape, fresh weight per boll, number of non-harvested bolls per plant, number of harvested bolls per plant and total number of bolls per plant. In none of the traits, any significant difference was confirmed between LLCotton25 and the non-recombinant control. Meanwhile, with regard to the number of non-harvested bolls per plant and the total number of bolls, as also described in "(i) Morphological and growth characteristics," variations between individuals and between replications were large in the test results, and no significant difference could be detected between LLCotton25 and the non-recombinant control.

Furthermore, in the cultivation tests in USA, as traits relating to the number of non-harvested bolls per plant and the total number of bolls, investigated were the boll holding rate (total number of bolls/number of bearing shoots), total number of seeds per plant and yield of seed cotton, but in none of the traits, any significant difference was observed between LLCotton25 and the non-recombinant control.

With regard to the shedding habit, the seeds of cotton are unlikely to be separated from each other since the lints are entangled with each other, and the shedding habit of seeds is considered to be low. In isolated fields, LLCotton25 and the non-recombinant control were compared in boll opening characteristics. As a result, there was no difference between LLCotton25 and the non-recombinant control in the boll opening time or the degree in the progress of boll opening. Furthermore, from photographs showing the cotton lint of the opened bolls of LLCotton25, it was observed that the seeds were unlikely to be separated from each other since the lints were entangled with each other as in the non-recombinant control. Therefore, it is considered that the shedding habit of LLCotton25 is equivalent to that of the non-recombinant.

With regard to the dormancy, it is reported that the presently cultivated cotton cultivars do not have deep dormancy. Furthermore, in general, dormancy breaking in cotton sowing is not necessarily required, but sometimes for enhancing the germination rate, low temperature treatment is employed as the case may be. For the dormancy and germination rate of LLCotton25, when natural crossing rates was investigated, in the second 10-day period of September, seeds were taken from LLCotton25 and the non-recombinant control cultivated in isolated fields, and on October 10, the seeds were sown in small vinyl greenhouses installed in isolated fields. As a result, all the seeds of both germinated. Moreover, whenthe chilling tolerance was examined in the early stage of growth, in the second 10-day period of September, seeds were taken from the LLCotton25 cultivated

similarly in an isolated field and on November 4, they were sown in pots in a small vinyl greenhouse installed in an isolated field. All the seeds easily germinated. From these facts, it is considered that LLCotton25 is shallow in the dormancy of seeds like the non-recombinant control and that even in the germination rate, LLCotton25 is equivalent to the non-recombinant control.

(vi)Crossability

In the second 10-day period of September corresponding to the latter half of the flowering stage, 180 seeds were selected at random from 30 individuals of the non-recombinant control cultivated at a place of 1 m apart from LLCotton25, and furthermore, 20 seeds were taken at random from 12 individuals of LLCotton25. They were stored at room temperature as they were. The seeds were sown in a vinyl greenhouse, and in the two-leaf stage, glufosinate herbicide was sprayed to examine their survival rates. As a result, the individuals sampled from LLCotton25 did not show any chemical injury, but on the contrary, all the individuals sampled from the non-recombinant died. Within the scope of this test, no possibility that crossing occurred was observed. Meanwhile, in the flowering stage, it was confirmed that *Parnara guttata* and *Hymenia recurvalis* as insects likely to transmit pollen visited LLCotton25 and the non-recombinant control.

(vii)Productivity of harmful substances

To examine the Adverse Effect on Biological Diversity attributable to the possibility that LLCotton25 might produce harmful substances, the following tests were carried out to compare LLCotton25 and the non-recombinant contorl: succeeding crop tests for examining the substances secreted from the roots with a threat to affect other plants, plowing-in tests 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 soil samples taken from the cultivation fields of LLCotton25 and the non-recombinant control after harvesting crops were sieved to remove the residues of plant bodies. The remaining soil samples were packed into nursery pots. Seeds of *Raphanus sativus* were sown and grown in the pots and examined the number of sprouts, germination rate, plant height, root length and aerial part weight. As a result, no significant difference was observed in those data between the soils from LLCotton25 and non-recombinant control.

Plant body plowing-in tests: Seeds of *Raphanus sativus* were sown and grown in soils for nursery cells of vegetable, respectively containing 0.5% of a dried plant body powder of LLCotton25 or a dried plant body powder of the non-recombinant control, to comparethe soils in the number of sprouts, germination rate, plant height, root length and aerial part weight. As a result, no significant difference was observed between both the lines.

Soil microflora tests: Respectively before planting, in growth period and at harvesting time, surface layer soil samples were taken from an LLCotton25 growing plot and a non-recombinant growing plot, and the samples were stored at 5°C and tested adequately. Furthermore, for

investgating the microfloras around lateral roots, the plant bodies pulled out at harvesting time were used. The investigation items were the ATP biomass by the luciferin-luciferase reaction method, the plate count by the dilution plate method (from the counts obtained by using a bouillon medium and a diluted bouillon medium), the plate count of *Actinomyces* (from the count obtained by using a diluted bouillon medium), the number of spores of mold fungi (from the count obtained by using a rose bengal medium), and fluorescent *Pseudomonas* count (from the count obtained by using a Kato's P-1 medium). The average values of three replications were obtained for the respective investigation items and for the respective plots as the results of the investigation, to examine the significant difference. As a result, no significant difference was observed between LLCotton25 and the non-recombinant control in any of the investigation items or in either of the plots.

From the above results, it can be considered that LLCotton25 is equivalent to the non-recombinant control 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 applied 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 cotton (*Gossypium hirsutum* L.), to which the recipient organism belongs, is very sensitive to low temperatures in winter in Japan and its seeds are very shallow in dormancy. So, it is considered that cotton can not grow voluntarily in Japan. Cotton is commercially distributed as cotton seeds in Japan for a long time, but there is no report that cotton has grown voluntarily in Japan.

In isolated fields in Japan, various traits (morphological and growth characteristics, the overwintering ability of seedlings and matured plants, productivity of seeds, etc.) relating to the competitiveness were investigated, and from the results as well as from the results of field tests in USA, it is not considered that the recombinant cotton improves in reproductivity and survival ability in the natural environment and becomes more competitive than the non-recombinant cotton. Furthermore, this recombinant cotton has the tolerance to glufosinate herbicide because of the transferred modified *bar*, but it is hard to consider that glufosinate becomes a selection pressure in the natural environment. Therefore, it is likely that LLCotton25 does not have high competition nature by the obtained character as compared with the recipient cotton.

In view of the above, 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 cotton poses no significant risk of Adverse Effect on Biological Diversity attributable to competitiveness is reasonable.

(2) Productivity of harmful substances

Regarding the cotton (*Gossypium hirsutum* L.), to which the recipient organism belongs, there is no report that it produces harmful substances to affect wild animals and wild plants. This recombinant cotton produces phosphinothricin acetyl transferase (PAT protein) that inactivates glufosinate, but it is not reported that this protein is harmful to wild animals and wild plants. Furthermore, regarding the PAT protein, it was confirmed that it does not transfer acetyl groups to various amino acids which are similar in structure to glufosinate, and that even if various amino acids exist in excessive quantities, the transfer reaction of acetyl groups to glufosinate is not inhibited. It was shown that the PAT protein has high substrate specificity. Consequently, it is not considered that the PAT protein affects the metabolic system of the recipient organism.

In the isolated field tests in Japan, the ability of this recombinant cotton to produce harmful substances (the substances secreted from the roots with a threat to affect other plants, the substances secreted from the roots with a threat to affect soil microbes, and the substances contained in the plant bodies with a threat to affect other plants after they die) was investigated, and no significant difference between this recombinant cotton and the non-recombinant cotton was observed.

In view of the above, 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 cotton poses no significant risk of Adverse Effect on Biological Diversity attributable to the productivity of harmful substances is reasonable.

(3) Crossability

The applicant concluded that in the Japanese natural environment, there are no related wild species which can hybridize with cotton, therefore that there are no specific wild plants or wild animals that are possibly affected by the crossability of this recombinant cotton, and that the use of this recombinant cotton poses no significant risk of Adverse Effect on Biological Diversity attributable to crossability. This conclusion was judged to be reasonable.

2. Conclusion

In view of the above, it was judged that the conclusion of the Biological Diversity Risk Assessment Report that in the case where this recombinant cotton is used according to Type 1 Use Regulation, the use of this recombinant cotton poses no significant risk of Adverse Effect on Biological Diversity is reasonable.