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	Maize tolerant to glufosinate herbicide（*pat*, *Zea mays* subsp.*mays* (L.) Iltis） (T25,OECD UI:ACS-ZMØØ3-2)
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 a recipient organism or the species to which the recipient organism belongs

(1) Taxonomical position and state of distribution in natural environment

i) The common name and the scientific name

The common name: Maize, Corn
The scientific name: *Zea mays* subsp.*mays* (L.) Iltis

ii) Type of the recipient organism

The recipient organism is He/89, the strain derived from tissue culture line, which belongs to dent type (var. *indentata*).

iii) Wild habitat in natural environment in Japan and abroad

The origin of maize is still not clear, but many theories about the origin have been raised so far. However, the teosinte-origin theory that "humans selected teosinte and made it a cultured plant" is gaining wide acceptance at present.

Since modern maize has been cultivated as a crop for a long time , it cannot propagate without the intervention of humans, and it cannot survive as a wild plant. Species related to maize are the teosinte (*Zea mays* subsp. *mexicana*) and the genus *Tripsacum* (*Tripsacum*, 2n=36). The teosinte is distributed naturally in the valleys of Mexico and Guatemala, and in the Mexican Highlands, but it is not grown naturally in the corn-belt zone of the US, European countries, Africa, Australia and Asia. The genus *Tripsacum* is a perennial plant, and there are 16 species of the genus *Tripsacum*. *Tripsacum floridanum* is distributed in the south end of Florida, *Tripsacum australe* and other 2 species are distributed in South America, and the other 12 species are distributed in Mexico and Guatemala, the same place as the wild habitat of teosinte.

There are more than 40 local varieties of maize in Mexico and approximately 250 local varieties of maize in the north-south Americas.

There is no report of natural distribution in Japan.

(2) History and present state of Use

i) History of Type 1 Use in Japan and abroad

Tiwacan Valley in Mexico is representative as a site from where remains relevant to maize were excavated in large quantities. From the excavation of the ears of primitive maize, it is thought that maize first appeared at around 6800 BC to 5000 BC. Also it is thought that full-scale farming of maize started at the time of 5000

BC to 3000 BC, and the ears were still primitive but getting larger. At the time of 1500 BC to 200 BC, the ears of maize became very large and the maize became the fine cultivation type with many rows of grain which can be seen at the present day. Maize was introduced from Mexico and meso-America to the various regions of the north-south Americas. It is understood that in the process of the introduction, various types such as dent, pop, sweet and flint were differentiated. In addition, maize was introduced to European countries through Spain after the discovery of the new continent by Colombus, and then maize was spread all over the world.

The first introduction of maize to Japan is said to have been made by the Portuguese, who are thought to have introduced the flint type in 1579 to Nagasaki or Shikoku. Then in the Meiji era (1868-1912), dent type and flint type were introduced to Hokkaido from the US and they were spread all over Japan. Since then, maize has been cultivated mainly at the foot of Mount Aso in Kyushu region, at the foot of Mount Fuji in Tokai region, in Nagano and in the north part of Kanto region.

From a long time ago, the grain of maize has been used for feed, fresh food, quasi-staple food in the mountain areas and so on. However, in Japan, grains such as rice, wheat, millet, buckwheat and others have grown well, and also the high humidity climate is not suitable for maize cultivation. Consequently, maize cultivation for food has not been established.

Also, the area for the cultivation of maize for seeds and for feed has decreased rapidly since the end of World War II, and now Japan depends mostly on imports. Meanwhile, the need for soiling maize for silage has been increasing with the rapid increase in stock breeding, and the domestic production is now barely sufficient.

In addition, since around 1948, the starch industry, which uses maize as a raw material, has been increasing rapidly, and maize has been used widely for processing in the textile industry, manufacturing industry, food industry and others.

ii) Main cultivation area, cultivation method, current status of distribution and use

Maize is one of the three major grain crops, together with wheat and rice, and it has been cultivated in a wide area including the US, Mexico, Argentina, Brazil, European countries, India, China, South Africa and others. In 2002, the total amount of production in the world was 602.59 million tons. The top five countries were the US (228.81 million tons), China (123.18 million tons), Brazil (35.48 million tons), Mexico (17.5 million tons) and France (16.01 million tons).

The US is currently the main maize producing country in the world, and maize is cultivated in the area called the “corn belt,” which includes Indiana, Ohio, Illinois, Iowa and Missouri.

In advanced countries, maize is cultivated in areas with high rainfall and fertile soil. Massive technology input has been intensively adopted, and large-scale machine cultivation of maize has been performed by commercial producers.

On the other hand, the statuses in developing countries are varied. For example, in almost all countries in Latin America, maize has been cultivated in small units, and more than half of the producers in Mexico have neither adopted technology input nor used improved varieties. However, in Brazil, Argentina and Chile, commercial producers have adopted technology input and have performed large-scale cultivation in the same way as in advanced countries. In Asia, China is the main maize cultivating country, and the amount of production is the second largest in the world, next to the US, but the farmland per household is small, and the use of improved varieties and technology input has been limited.

In Japan, mechanization was adopted early on in Tohoku region and Nagano. Before the war, the Western Style Plough Agriculture Technique, which utilizes the power of livestock, was adopted in Hokkaido, but mechanization took hold after the war.

In Japan, approximately 280 thousand tons of sweet type for food, and approximately 4.87 million tons of soiling maize for feed were harvested in 2002. Also, 16.4 million tons of maize were imported to Japan in 2002, 12.32 million tons of which were for feed. The main importing countries were the US, Brazil, China and Argentina in that order.

In advanced countries, maize is used mainly for feed and as a raw material for industrial products. Persons in charge of breeding in the US and EU have set targets for agronomic character for the feed industry, and for industrial genetic character, including for the production of high-fructose corn syrup, fuel alcohol, starch, glucose, dextrose and others.

In almost all countries in Latin America and sub-Sahara Africa, maize has been used as staple food, while in Asia maize has been mainly used for feed.

(3) Physiological and ecological properties

i) Environmental conditions allowing inhabiting or growth

The optimum germination temperature of maize is 32-36°C and the minimum germinating and minimum growing temperature is 6-10°C. In practice, the optimum sowing season is considered to be the period when the temperature is 13-14°C. Under conditions of low temperature and high humidity, germination of seeds would delay, and in most cases the seeds would decay and die before germinating. Moreover, even if seeds germinate, they cannot subsist under conditions of exposure to temperatures below 0°C for 6-8 hours or over once the growing point reaches the above-ground level (5th-7th stage).

For germination of the seeds, temperatures of 10°C and above and moderate water supply are required in general, but maize would germinate even under conditions of fairly severe dryness. The results of a test to use sweet type at a dry place in California showed that 80% or more of the maize will germinate if the humidity in soil is slightly above the withering point of the soil (8.6% in sand, 14.9% in clay).

ii) Mode of propagation or reproduction

(a) Shedding habit, mode of dispersion, dormancy and longevity of the seeds

Shedding habit of maize is considered to be extremely low, because seeds of maize are closely attached and held firmly on the cob of a big ear. Also, even if a seed which is covered with a bract falls down to the ground under good conditions for germination, it would decay before germinating. Moreover even if seeds germinate, it would be impossible for them to grow to the bearing stage, because hundreds of seeds on an ear would germinate and the competition between seeds would become too fierce.

The dormancy of the seed is extremely low, and it will germinate if the soil temperature reaches 10°C.

The longevity of seeds is influenced by the activity of enzymes through respiration, and the respiration rate is mainly related to temperature and humidity. If the seeds are under conditions with 55% or less relative humidity, the respiration rate is low, but if the relative humidity is over 65%, the respiration rate increases rapidly and the activity of the seeds rapidly decreases. Seeds can be stored for 6-8 years under conditions where they contain 12% water, at a temperature of 10°C, and with the relative humidity maintained as 55% or less.

(b) Mode of vegetative propagation and budding property in natural condition

The only tissue that can regenerate the plant body in natural conditions is the seed.

(c) Degree of selfing and out-crossing, presence or absence of self-incompatibility, and hybridization with related plants

Maize is originally a plant which propagates 100% through cross-pollination. Maize does not have self-incompatibility.

Related plants which can be hybridized are the teosinte (genus *Zea*) and the genus *Tripsacum.* Maize and annual teosinte have high compatibility of reproduction with each other and they produce a fertile hybrid. However, the natural distribution area of teosinte is limited, and from factors including geographical separation, flowering time, growing property, structure of reproductive organs, and others, it is considered that the possibility of natural hybridization is low. In addition, there is incompatibility between some maize and teosinte, and it is difficult to form hybrids.

Also, species of the genus *Tripsacum* (*T.dactyloides, T.floridanum, T.lanceolatum, T.pilosum*) can rarely be hybridized with maize, but the hybrids are genetically unstable, becoming infertile with high probability . In addition, it is reported that since the number of chromosomes of the genus *Tripsacum* is different to that of maize, the hybridization rate becomes extremely low.

(d) Production, fertility, shape, mode of pollination, dispersion distance and longevity of pollen

It is supposed that a tassel of maize produces 18 million pollen grains. The diameter of matured pollen is 90-120 μ, and the average is 100 μ. Pollen is carried by wind, and the rate of hybridization becomes 0.003% when 200m apart from the origin of the pollen, and the possibility of hybridization becomes extremely low. The longevity of pollen differs with environmental conditions, but in a field condition in high summer pollen loses its reproduction ability within 24 hours.

iii) Productivity of harmful substances

Regarding maize, the productivity of harmful substances that can affect the growth or inhabiting of other wild animals and wild plants has not been reported.

iv) Other information

It has not been reported so far that maize seeds which were spilled during transportation, etc., on locations other than cultivation fields have grown.

2. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

i) Composition and origins of component elements

The composition of donor nucleic acid and the origins of component elements used for the development of maize tolerant to glufosinate herbicide (*pat*, *Zea mays* subsp. *mays* (L.) Iltis) (hereinafter referred to as “the recombinant maize T25”) are shown in Table 1.

Table 1 Component elements of donor nucleic acid in vector pUC/Ac, and their position, size and functions

Component elements (abbreviation)	Position in vector	Size (Kbp)	Origin and function
pat cassette			
P-35S	1746-1217	0.52	35S RNA promoter derived from cauliflower mosaic virus. Makes *pat* genes expressed in plants constitutively.
Pat	1188-637	0.53	It encodes PAT protein and gives tolerance to glufosinate herbicide, derived from *Streptomyces viridochromogenes.*
T-35S	618-412	0.20	35S RNA terminator derived from cauliflower mosaic virus. It terminates transcription and induces polyadenylation of transcripts.
Others			
Bla	3783-2923	0.86	It is an ampicillin resistant gene derived from *E.coli*. It expresses β-lactamase only in bacteria.
ori-pUC	2164-2714	0.55	It is the replication origin (ColE1) of pUC18, and initiates replication of plasmid

Since the natural *pat* gene obtained from *Streptomyces viridochromogenes* includes many G:C (Guanine : Cytosine), the introduced synthetic *pat* gene is a modified type of the natural *pat* gene whose sequence is modified to adjust the codon used in the plant. Also, the amino acid sequence of the enzyme which is produced by this modification has not changed.

ii) Function of component elements

(a) Functions of each component element of donor nucleic acid

Functions of component elements of donor nucleic acid which were used for the development of the recombinant maize T25 are shown in Table 1.

(b) Function and the presence or absence of allergenicity of the protein produced by the expression of target gene and selectable marker

In the process of nitrogen metabolism, plants produce ammonia by nitrate reduction, amino acid degradation, photosynthesis, and so on. Glutamate synthetase plays an important role in detoxication of the ammonia produced, but if glufosinate herbicide is applied, glutamate synthetase is inhibitted, ammonia accumulates, and the plant dies.

The synthetic *pat* gene introduced produces phosphinothricin acetyl transferase (PAT), and this enzyme acetylates glufosinate to make N-acetylglufosinate, and inactivates the inhibitory action of glufosinate to the glutamate synthetase. Thanks to this mechanism, ammonia is not accumulated, and the recombinant maize does not die, even if it is sprayed with glufosinate. (Figure 1)

It is confirmed that the PAT protein produced by the synthetic *pat* gene shows a high affinity to glufosinate. Also, the PAT protein shows specific reaction only to glufosinate, without showing acetyl group transfer reaction to the other various amino acids under the condition of the existence of excessive various amino acids. As a result, it was suggested that the PAT protein possesses high substrate specificity.

Moreover, the synthetic *pat* gene sequence was compared with all nucleotide which were published in the EMBL database (European Molecular Biology Laboratory, Germany, Release 40.0, September 1994). In addition, regarding PAT protein sequence, homology search was performed by SWISSPROT database (Geneva, Switzerland, Release 30.0, September 1994).
The result in both cases shows no significant homology except with PAT proteins derived from various other species. Consequently, no homology to known toxin and allergen was confirmed.

In addition, physico-chemical and biochemical characteristics of PAT protein were compared with known allergen. As a result, it was confirmed that the possibility of this protein to possess allergenicity is extremely low.

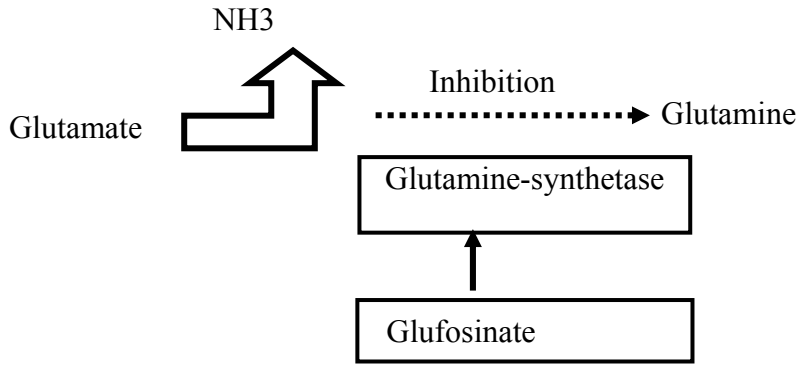
bla gene gives resistance to ampicillin, and it was used as a selectable marker at the time of constructing plasmid by using *E.coli*. This gene does not express in plants, because it does not possess a promoter to work in plants. Since the *bla* gene was divided at the time of insertion into the genome of maize, it was confirmed that it does not possess any function in the recombinant maize.

(c) Effect on the metabolic system of recipient organism

Since the PAT protein possesses high substrate specificity, it is not considered that the acetyl group is transferred to chemical compounds other than glufosinate. Consequently, it is considered that the PAT protein does not affect the metabolic system of the recipient organism.

a) Normal Plant

The plant dies if ammonia accumulates in the plant body due to the inhibition of glutamine-synthetase caused by the effect of glufosinate herbicide.



b) Recombinant plant

Glufosinate herbicide is acetylated and becomes N-acetylglufosinate by work 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.

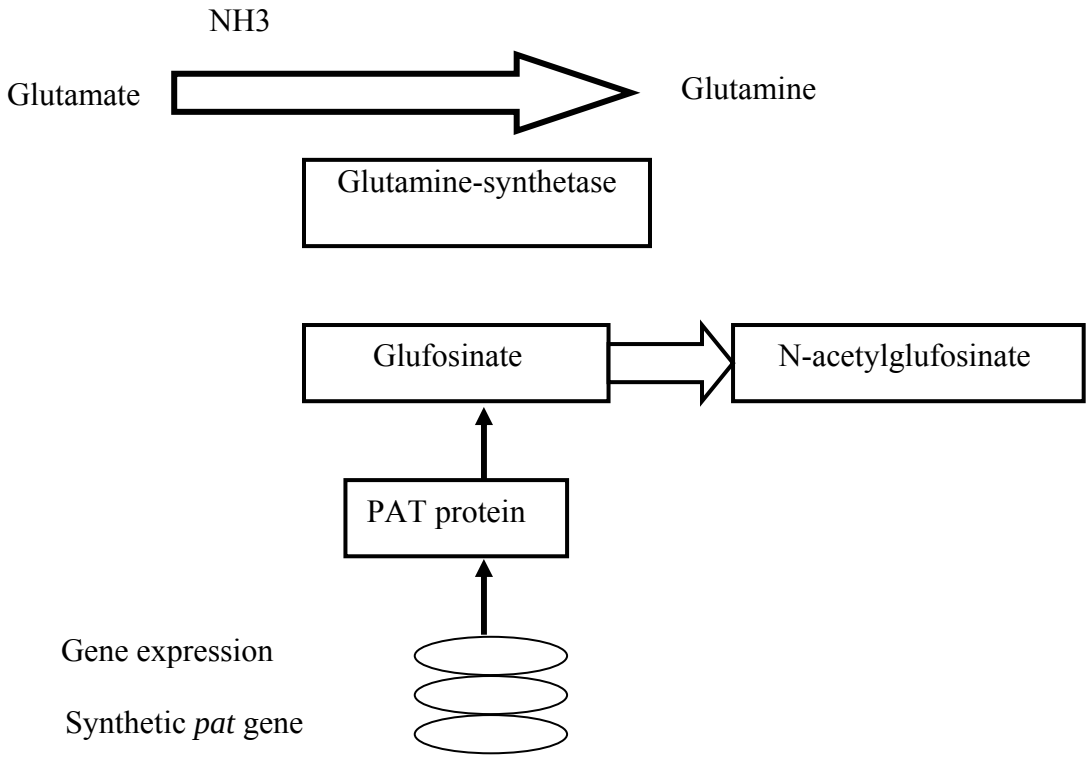


Figure 1 Mechanism of tolerance to glufosinate herbicide by the product of synthetic *pat* gene

(2) Information concerning vectors

i) Name and origin

The vector used for the production of the recombinant maize T25 is plasmid pUC/Ac, which is inserted into the synthetic *pat* gene at the section of Sal I between the 35S promoter of pDH51 produced from pUC18 derived from K12 strain of *Escherichia coli* and the terminator.

ii) Properties

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

Plasmid map of the plasmid pUC/Ac is shown in Figure 2.

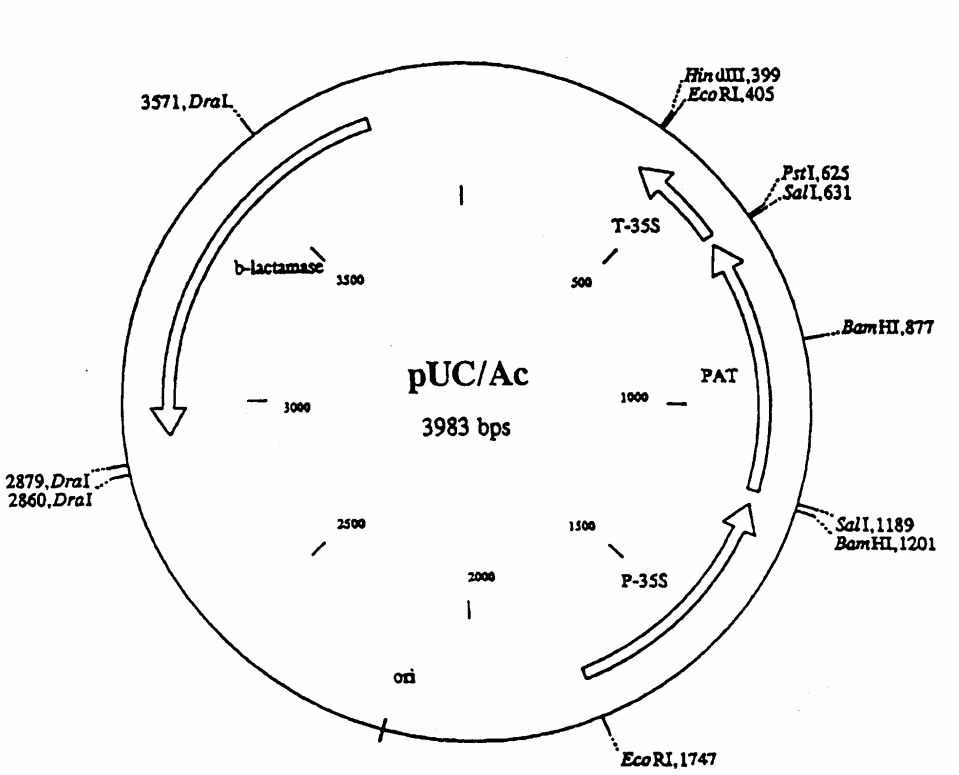


Figure 2 Vector pUC/Ac used for the transformation of the recombinant maize T25

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

The characteristics of all genes existing in plasmid pUC/Ac have become clear, and they do not contain known harmful base sequences.

(c) Presence or absence of infectious characteristics of vector

Since plasmid pUC/Ac does not possess a transferring trait, the infectious characteristics of this vector is not known. Also, it is known that recipient organisms for the self-replication of the vector pUC18 is limited to such as *E. coli* and some gram-negative bacteria.

(3) Method of preparing living modified organisms

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

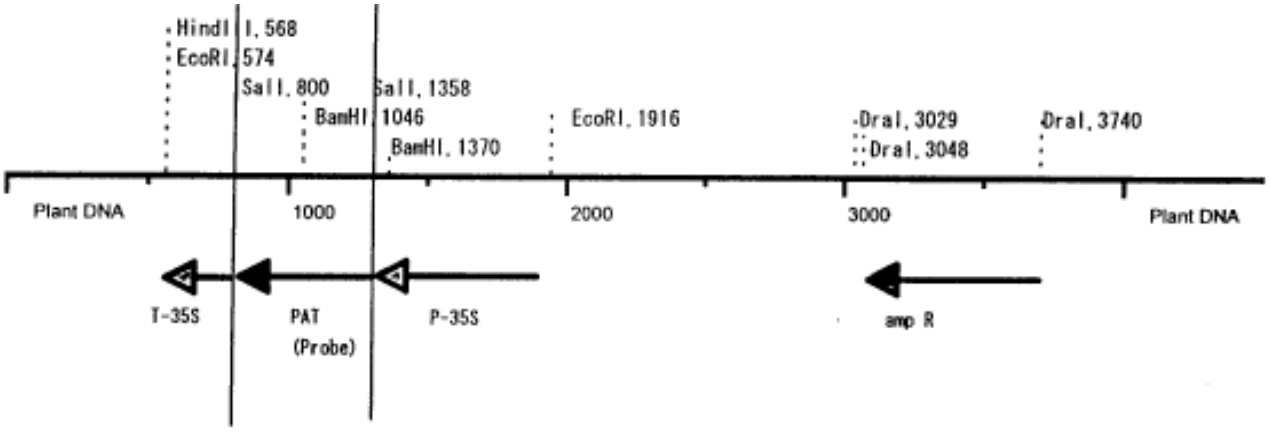


Figure 3 Structure of the inserted gene

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

Protoplast of He/89 strain derived from tissue culture was used for transferring the gene by Polyethylene-glycol Method.

iii) Processes of rearing of living modified organisms

(a) Mode of selection of the cell in which nucleic acid is transferred

After transformation, and after forming microcolonies from 20-50 protoplasts, they were moved to a solid medium. Then, after the selection by glufosinate herbicide, they were regenerated to the plant body.

(b) Presence or absence of remaining Agrobacterium

This item is not applicable.

(c) Processes of rearing and pedigree tree

The recombinant maize T25 and yellow dent type commercial cultivars or the cultuvars which Bayer CropScience possesses were hybridized, and selection was performed.

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

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

In back cross of the recombinant maize T25, 1:1 segregation ratio was shown for tolerance to glufosinate. In addition, as a result of Southern blotting analysis, it was suggested that the replication product of transferred nucleic acid was inserted into the chromosome [Refer to ii) The number of copies of replication product of transferred nucleic acid and the stability of transferring in multiple generations].

ii) The number of copies of replication product of transferred nucleic acid and the stability of transferring in multiple generations

Southern blotting analysis was conducted to examine the number of copies of transferred nucleic acid. DNA (15μg) was digested with BamH I (lane 1), EcoR I (lane 2), Dra I (lane 3), Hind III (lane 4) and Sal I (lane 5). The bacteriophage λ DNA digested withPst I was used for the size marker, and the synthetic *pat* gene (Sal I section of 552 bp), which was labelled with ^{32}P, was used as prove (Figure 4).

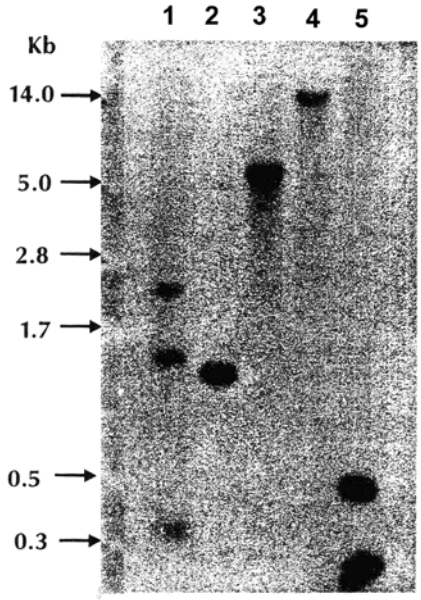


Figure 4 Southern blotting analysis to show the number of copies of the recombinant maize T25

In BamH I (lane 1), two bands (0.3 kbp and 1.5 kbp) were detected. This 0.3 kbp band is the fragment which was cut out from plasmid pUC/Ac. The 1.5 kbp band is derived from the cleavages of one BamH I site in integrated vector and one BamH I site in adjacent plant DNA.. This one 1.5 kbp band supports the evidence that one copy of synthetic *pat* gene cassette was integrated in plant genome. A band was seen in 2.5 kbp, but this band is derived from the incomplete digestion with BamH I.

In EcoR I (lane 2), the expected 1.3 kbp fragment was detected, and it shows that the synthetic *pat* gene cassette (35S promoter, *pat*, 35S terminator) of vector pUC/Ac was intergrated.

One band (approximately 5 kb) in Dra I (lane 3) is derived from the cleavages of one Dra I site in vector and one Dra I site in the adjacent plant DNA.

One band (approximately 14 kb) in Hind III (lane 4) is derived from the cleavages of one Hind III site in vector and one Hind III site in the adjacent plant DNA.

In Sal I (lane 5), only a 0.5 kbp fragment from inside of the vector was detected.

The following two facts: 1) only one band was detected in the cleavage by Sal I, EcoR I, Dra I or Hind III, which do not have cleavage site in the synthetic *pat* gene, and 2) the expected band is detected from digest by BamH I, support the evidence that one copy of pUC/Ac was integrated in the plant genome. Based on the above understanding, the presumed form of the integrated gene is shown in Figure 3 (p. 11).

To confirm the stability of inserted nucleic acid in multiple generations, Southern blotting analysis was conducted for the third generation of maize which was obtained from the back cross of the recombinant maize T25. In this analysis, the genome was digested with EcoR I or BamH I, separated by agarose gel electrophoresis, and transferred to nylon membrane. Then genome DNA was hybridized with ^{32}P -labeled synthetic *pat* gene (552bp Sal I fragment). The autoradiography of blotting is shown in Figure 5 (p. 14). Figure 5 shows that the pattern of hybridization has not changed in multiple generations, and the stability of the inserted nucleic acid in multiple generations was proved.

In addition, the *bla* gene was divided into two pieces, part of it disappeared, and a 35S promoter like sequence was located in 5' terminal of the *bla* gene fragment. However, the divided *bla* gene and the 35S promoter like sequence are both incomplete, and it is not considered that the *bla* gene is expressed in the plant transferred by these sequences. To confirm this fact, Northern blotting analysis using the *bla* gene as a probe was conducted regarding RNA extracted from the recombinant maize T25 and it was confirmed that the transcript of the *bla* gene was not included. In addition, to confirm the fact that it was not transcribed nor translated to form active protein, activity assay of β-lactamase was conducted. As a result, the activity of β-lactamase was under the limit of detection. Based on the understanding above, it was confirmed that the incomplete *bla* gene and the 35S promoter like sequence did not function.

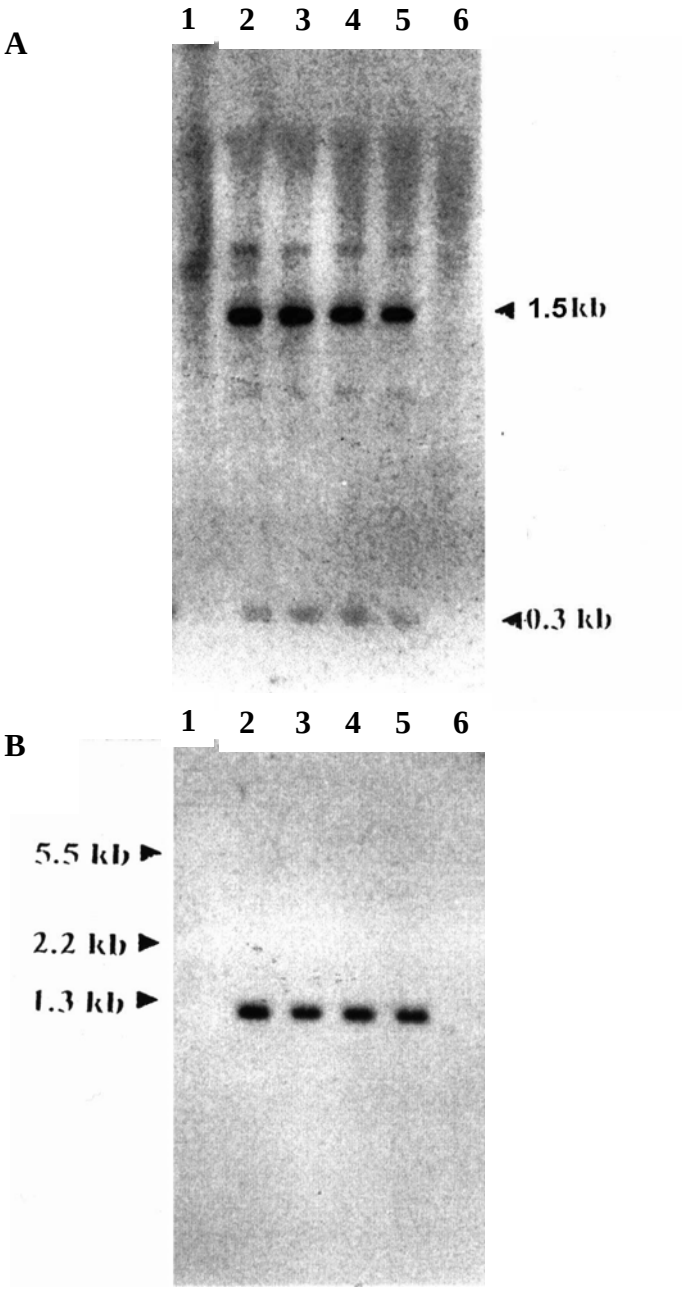


Figure 5 Southern blotting analysis to show the stability of the recombinant maize T25

A is digested with BamH I.
B is digested withEcoR I.
Lane 1: Non-recombinant control maize He/89 (recipient organism)
Lane 2: Recombinant maize T25 (T_0 generation)
Lane 3-5: Each individual obtained from the three times of back cross to T_0 generation of recombinant maize
Lane 6: Non-recombinant commercial cultivar

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

This item is not applicable.

iv) Stability of expression for the characteristics which were given by the expression of replication product of transferred nucleic acid

In an isolated field test, glufosinate was sprayed to the seedlings of maize which were germinated from seeds sown on a plate. As a result, all recombinant plants survived and the non-recombinant plants died. In addition, glufosinate was sprayed to 18 plants of T25 recombinant maize, and the results showed 100% tolerance to glufosinate. Consequently, the stability of expression was confirmed in natural conditions.

Also, in safety studies in the US in 1992 and after, enzyme activity of the PAT protein in each part of the plant was measured using roots, leaves, stems, matured pollen and matured seeds of the recombinant maize T25. As a result, PAT protein activity was highest in leaves, low in seeds, and not detected in pollen.

v) Presence or absence, and degree of transmission of transferred nucleic acid to wild animals and wild plants

Transmission of this item is not applicable, because there is no transferring factor in the vector used for transformation.

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

Specific detection method for the recombinant maize T25 is available by PCR method using the DNA sequence of the plant genome of the inserted gene and its surroundings as 22bp primers. By using 20-50ng DNA, nearly 100% was able to be detected, so if the seeds and plant body of the recombinant maize T25 are very small in quantity, detection and identification is possible. In addition, the result of high reproducibility was obtained in replicated tests.

In practice, this PCR method has been used effectively in breeding of the recombinant maize T25.

(6) Difference from the recipient organism or the species to which the recipient organism belongs

i) Characteristics to be conferred by the expression of transferred nucleic acid

With the production of the PAT protein due to the expression of the transferred synthetic *pat* gene, tolerance to glufosinate herbicide is conferred to the recombinant maize T25. The mechanism of tolerance to herbicide by the PAT protein is shown in Figure 1 (p.9).

ii) Difference from the recipient organism or the species to which the recipient organism belongs

Glassland Research Center of the National Institute of Livestock and Glassland Science, in the National Agriculture and Bio-oriented Research Organization conducted isolated field tests in FY2003, and confirmation of the compatibility to the guideline regarding recombinant in the field of Agriculture, Forestry and Fisheries followed.

(a) Morphological and growth characteristics

For the recombinant maize T25 and the non-recombinant control maize, evaluation was conducted regarding uniformity of germination, germination rate, time of tassel exertion, time of silking, culm length, plant type, tiller number, height of ear, maturation time, number of ears, number of effective ears, ear length, ear diameter, row number per ear, grain number per row, grain color, 100-kernel weight, grain shape and fresh weight at harvesting time. As a result, no statistically significant difference was observed between recombinant and non-recombinant control maize line in any of the characteristics.

(b) Chilling-tolerance at the early stage of growth

Chilling-tolerance of the recombinant maize T25 and non-recombinant control maize was evaluated. Seedlings of both plants were exposed to low temperature in an incubator at 4℃, and their growth was observed. As a result, the elongation growth of all of the seedlings of both plants was retarded. In addition, all of the seedlings which were left in the field in winter died in a few days. No difference was observed between the recombinant maize T25 and the non-recombinant control maize.

(c) Wintering ability of the matured plant

Maize is an annual plant, and the recombinant maize T25 and the non-recombinant control maize which were left in the field after ripening, both died out in winter naturally.

(d) Fertility and size of the pollen

Pollen was observed under a microscope to examine its size. Also, pollen was stained with acetocarmine and its maturity was observed under a microscope. The fertility of pollen was then studied. Regarding the diameter and fertility of pollen, the recombinant maize T25 and the non-recombinant control maize indicated almost equal values, and no significant difference was observed between the recombinant maize T25 and non-recombinant control maize.

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

The recombinant maize T25 was compared with non-recombinant control maize regarding row number per ear, grain number per row and 100-kernel weight, and both plants indicated equal values. Consequently, regarding the

production of the seeds, no significant difference was confirmed between the recombinant maize T25 and non-recombinant control maize.

Since seeds of maize are covered with bracts and the cob holds the seeds firmly, the shedding habit is considered to be extremely low. So, the test for shedding habit has not been carried out.

In the germination test of harvested seed, the recombinant maize T25 and the non-recombinant control maize germinated immediately, and almost 100% of the seeds of both plants indicated germination. It was therefore assumed that there is no dormancy of the seed.

(f) Crossability

Crossability test was not performed since no wild relatives that can be hybridized grow in Japan.

(g) Productivity of harmful substances

Regarding productivity of harmful substances, the following tests were carried out for the recombinant maize T25 and non-recombinant control maize. The succeeding crop test was performed to evaluate the effect of the secretion from roots on other plants; the plow-in test using plant residue was performed to evaluate the effect of the substances in the plant body on other plants after dying; and the biota test of microorganisms in rhizosphere soil was performed to evaluate the effect of secretion from roots on the microorganisms in soil. In the succeeding crop test and the plow-in test, the length of leaves and the fresh weight and dry weight of the radish used as the test plant were measured in each test, and no significant difference was confirmed. In addition, in the biota test of microorganisms in soil, no significant difference between either plant regarding the number of bacteria, number of actinomyces and the number of filamentous fungi was confirmed.

3. Information concerning the Use of living modified organisms

(1) Content of the Use

Provision as food, provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them

(2) The result of the use in the similar environment with which the Type I Use is going to perform

Isolated field tests were performed in the National Agriculture Research Center for Hokkaido Region in 1997. Isolated field tests were also performed in Grassland Research Center of the National Institute of Livestock and Grassland Science, in the National Agriculture and Bio-oriented Research Organization in 2003.

(3) Information obtained from Use abroad

Information obtained from Use abroad is shown in Table 2.

Table 2 Information obtained from Use abroad

Country	Approval organization	Time of approval	Content of approval
US	US Department of Agriculture (USDA)	June, 1995	Confirming environment safety
	Food and Drug Administration (FDA)	December, 1995	Safety of food/feed
Canada	Canada Food Inspection Agency	May, 1996	Confirming environment safety
		March, 1997	Safety of feed
	Health Canada	April, 1997	Safety of food
EU	European Commission Directorate General Environmental Protection	August, 1998	Approval of import
		July, 1998	Approval of cultivation
	England	February, 1997	Safety of feed (only import of corn gluten)
		March, 2004	Approval of cultivation
	European Commission Directorate General Health and Consumer Protection	January, 1998	Safety of food (only oil, starch, cooked food, and fermented food with the use of dry-milling fraction)
Swiss	Swiss Federal Bureau of Health	October, 1998	Safety of imported feed (only corn gluten)
Bulgaria	Ministry of Agriculture	July, 1999	Safety of food, feed, and cultivation
Argentina	Agriculture and Stockraising Biotechnology National Advisory Committee	February, 1998	Environment safety (confirming out of regulation)
	National Food and Agriculture Quality and Safety Service	June, 1998	Safety of food/feed

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

1. Competitiveness

Regarding the maize (*Zea mays* subsp. *mays* (L.) Iltis.), to which the recipient organism belongs, cultivation has been conducted in Japan, but there is no report that it has grown voluntarily in Japanese natural environment so far.

This recombinant maize T25 is given a trait to be tolerant to glufosinate herbicide by transferred modified *pat*, but it is not generally considered that the glufosinate exerts pressure for selection under a natural environment. In addition, it was judged that the use of this recombinant maize T25 in the isolated field posed no statistically significant differences in any traits concerning to competitiveness..

Based on the above understanding, no wild animals or wild plants are specified to be possibly affected and,it was judged that the use of the recombinant maize T25 posed no risk of Adverse Effect on Biological Diversity that is attributable to competitiveness.

2. Productivity of harmful substances

Regarding the maize (*Zea mays* subsp. *mays* (L.) Iltis.), to which the recipient organism belongs, there is no report that it produces harmful substances to affect wild animals and wild plants.

This recombinant maize T25 possesses a trait to produce phosphinothricin acetyl transferase (PAT protein), but this protein is not reported as a harmful substance to affect wild animals and wild plants. Regarding PAT protein, the following was confirmed: a) it does not transfer acetyl group to various amino acids which are similar in structure to glufosinate, and b) even if various amino acids exist in excessive quantities, the transfer reaction of acetyl group to glufosinate is not inhibited. It was shown that the PAT protein possesses high substrate specificity. Consequently, it is considered that there is no risk of the PAT protein affecting the metabolic system of the recipient organism.

In Japan, the productivity of harmful substances of this recombinant maize to affect other plants and microorganism has been investigated in isolated fields, and no significant difference between this recombinant maize T25 and the non-recombinant control maize has been confirmed.

Based on the above understanding, no wild animals or wild plants are specified to be possibly affected, and it was concluded that there is no risk of Adverse Effect on Biological Diversity attributable to the productivity of harmful substances.

3. Crossability

In Japan, the voluntary growth of wild species that can be hybridized with maize in the natural environment has not been reported. Therefore, it was concluded that no wild species can be specified as having any effect, and that there is no risk of an Adverse Effect on Biological Diversity from crossability.

III. Comprehensive assessment to Adverse Effect on Biological Diversity

Consequently, it was judged that there is no risk of Adverse Effect on Biological Diversity in Japan attributable to the use of this recombinant maize for provision as food, for provision as feed, cultivation, processing, storage, transportation, disposal and acts incidental to them.