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

Name: National Institute of Agrobiological Sciences (NIAS)
Teruo Ishige, President
Address: 2-1-2 Kannondai, Tsukuba-shi, Ibaraki Prefecture

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Rice containing cedar pollen peptide (*7Crp, Oryza sativa* L.) (7Crp#10)
Content of the Type 1 Use of Living Modified Organism	Cultivation [limited in the isolated fields at National Institute of Crop Science (NICS) of National Agriculture and Food Research Organization (NARO) (2-1-18 Kannondai, Tsukuba-shi, Ibaraki Prefecture) and National Institute of Agrobiological Sciences (NIAS) (2-1-2 Kannondai, Tsukuba-shi, Ibaraki Prefecture) with proper separation distances, time difference in flowering or other such proper provisions made to eliminate possible crossing with the rice crops cultivated in the neighborhoods], processing (limited to the processes up to rice milling in the above described isolated fields), storage, transportation, disposal and acts incidental to them
Method of the Type 1 Use of Living Modified Organism	—

Outline of the Biological Diversity Risk Assessment Report

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

1. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

a. Composition of expression cassettes of the donor nucleic acid that was used for the production of rice containing cedar pollen peptide (*7Crp*, *O. sativa* L.) (7Crp#10) and the origins of component elements are shown in Table 2.

Table 2 Sizes and functions of component elements of donor nucleic acid

Component elements	Size (kb)	Origin and function
Cedar pollen peptide (7Crp) expression cassette		
Glutelin GluB-1 promoter	2.3kb	A gene promoter encoding the rice seed storage protein glutelin GluB-1. Regulates the expression specific to endosperm tissue in the ripening period of seeds. Derived from rice.
Glutelin GluB-1 signal peptide	0.072kb	Signal peptide sequence of rice storage protein glutelin GluB-1. Takes part in the transportation of glutelin storage protein into the membrane of endoplasmic reticulum. Derived from rice.
7Crp (target gene)	0.288kb	A gene, derived from cedar pollen Cryj I and Cryj II allergen protein genes. It encodes the artificial peptide linking seven-site sequences recognized by the human cedar allergen-specific T cells.
KDEL localizing signal	0.012kb	Signal sequence referring to the retention of transferred gene product to the endoplasmic reticulum. Derived from rice.
Glutelin GluB-1 terminator	0.65kb	A glutelin GluB-1 gene terminator. Regulates the termination of transcription. Derived from rice.
Hygromycin-resistant expression cassette		
CaMV35S promoter	0.8kb	Constitutive expression promoter. Makes the gene linked to the downstream express in the entire plant body. Derived from cauliflower mosaic virus genome DNA.
hpt	1.1kb	Selective marker gene to confer the resistance to hygromycin. Derived from *Escherichia coli* (*E. coli*).
Ag7 terminator	0.3kb	Ag7 gene terminator on the *Agrobacterium* Ti plasmid. Regulates the termination of transcription of transferred gene. Derived from *Agrobacterium* Ti plasmid.

Glossary

T cell: Derived from thymus gland; To be activated as a helper T cell by the stimulated antigen to facilitate the differentiation/proliferation of B cells and the production of IgE antibody.

2) Function of component elements

A living body possesses the defense reaction that recognizes pollens as foreign matter, when inhaled through nose or eyes, and removes the foreign matter. This reaction is known as immune reaction, the foreign matter known as antigen (allergen), and the substance which binds to the antigen and eliminates the antigen known as antibody. The state of exaggerated immune reaction refers to allergy, which appears in the form of allergic disease such as cedar pollinosis.

For the cedar pollinosis, the proteins in the cedar pollens designated as Cryj I and Cryj II are identified as the major antigens. When these antigens enter the human body and they are taken into immunocytes, the T cells, serving as a control tower for immune reaction, are first activated. The activated T cells issues a command to the B cells (one of the components of leukocyte) to urge the B cells to become activated to produce an antibody called IgE. The IgE produced by the B cells binds to the mastocytes and enters the standby state. In the season when the cedar pollens are scattering, the pollen antigens bind to the antibodies contained in the mastocytes and take out various chemical transmitting substances from the mastocytes, which can cause various inflammations. These chemical transmitting substances act to cause swollen capillary vessels, swollen skin and/or secretion of mucus, thereby provoking sneezing, nasal discharge, nasal congestion, itching and other allergic inflammations [13].

A typical approach for treatment of the pollinosis includes the symptomatic therapy using the drugs or immunosuppressive agents to suppress the action, synthesis or release of chemical transmitting substances. The only radical treatment is called desensitization therapy, which is intended to reduce or eliminate the sensitivity to the pollen antigen, the cause of the allergy concerned, by injecting increasing amounts of the antigen. In actuality, however, because of troublesome outpatient treatment of injection required every week, pain or itching caused by the injection of antigen, prolonged period taken for treatment, possible risk of adverse reaction though infrequent, and effectiveness not necessarily established for all the patients, the desensitization therapy has not been widespread adopted [13].

On the other hand, it is reported that injection, nasal or oral administration of antigen or antigen-derived T cell antigen determinant (T cell epitope) helps reduce allergic reaction. The mechanism of action is reportedly based on the unresponsive cedar pollen antigen-specific T cells, deleted T cells themselves, and induced controllable T cells [14), 15)]. Administration of T cell epitopes causes suppressed responsiveness of T cells and decreased command, thereby facilitating the activation of B cells, which leads to the suppressed activation of B cells and decreased amounts of IgE antibody binding to mastocytes. As a result, it is considered that the amount of chemical transmitting substances released from mastocytes is decreased and allergic inflammations are suppressed [17), 18)].

Then, a technique called peptide immunotherapy has been proposed which utilizes the above phenomenon and uses the T cell epitopes. The peptide immunotherapy does not use the allergen concerned and involves administration without containing any B cell epitopes and then, it offers adverse reaction-free and easier to apply features and it is expected as the second-generation antigen-specific immunotherapy.

In addition, the intestinal tract possesses an effective immune mechanism known as the oral immune tolerance. This phenomenon is generally demonstrated by the fact that no immune reaction occurs to foods. This suggests a high probability that oral administration of cedar antigen would more effectively inhibit the allergic reaction to cedar pollen antigen (induction of oral immune tolerance).

For the cedar pollen antigen proteins Cryj I and Cryj II which have been identified as the antigens causing cedar pollen allergy, the T cell epitope (12-19 amino acids), recognized by the cedar allergen specific T cells, has been investigated in detail [16]. Then, based on the idea that if the T-cell epitopes of cedar allergen could be accumulated in the daily ingested rice, "the rice possibly offering the effects of mitigating or curing the Japanese cedar pollinosis by ingestion" could be developed based on the oral immune tolerance phenomenon, the epitope peptide accumulated rice was developed.

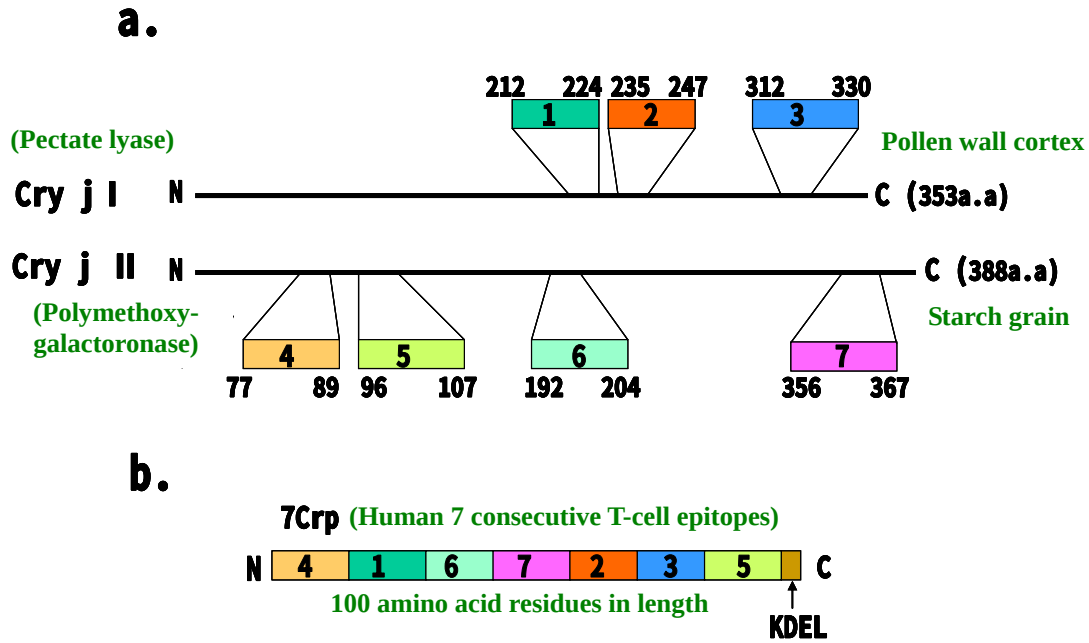

Figure 1 Order of sequences of 7 epitopes among the determinant (epitope) and 7Crp of cedar allergen antigen recognized by the human T cells

As shown in Figure 1a, a total of seven (7) T cell epitopes, three (3) from Cryj I and four (4) from Cryj II, have been identified as the major epitopes expressed in rice and recognized by many human cedar allergen specific T cells. Then, the peptide (7Crp; 96 amino acids in length) composed by connecting the 7 epitopes as shown in Figure 1b was constructed, and the peptide of 100 amino acids composed by connecting the KDEL sequence to enhance the accumulation of 7Crp in the

downstream of 7Crp sequence was accumulated in the endosperm of rice [17]. Use of the multiple epitopes from the same allergen is aimed to offer the prospect of higher probability of being recognized as epitopes and wider range of cedar pollinosis patients applicable to treatment with use of multiple epitopes against individual allergens, since recognizable epitopes vary according to the human genotype.

It is reported that the seven-consecutive epitope peptide (7Crp) is recognized by the cedar pollen antigen-specific T cell in 90% or more patients similarly as the original Cryj I or Cryj II [16]. In addition, it has been also clarified that the seven-consecutive epitope peptide does not possess any bonding to the cedar pollen allergy specific IgE antibody. Thus, this peptide is considered far more suitable to safely provoke oral immune tolerance than use of allergen itself.

As one of the tests for effectiveness, the epitope peptide accumulated in rice was extracted and administered to mice to identify the reactivity to proliferation of T cells and as a result, it exhibited the similar reactivity as obtained from the original allergen. Furthermore, to the special mice that recognize one of the seven epitopes as epitope, this epitope-accumulated rice was fed for one month at a rate of 5 to 6 grains per day and then, cedar allergen was sensitized 9 times in total through nose every other day. As a result, the level of IgE antibody was found to have decreased to approx. 30% compared to the mice fed with ordinary rice [17), 18)].

Glossary

Epitope: An antigen peptide composed of 10 to 20 amino acid residues derived from the antigen proteins digested and modified in the antigen-presenting cell (macrophage) to emphasize the antigenicity. This peptide is exposed on the surface of cell membrane as antigen information and transmitted to T cells.

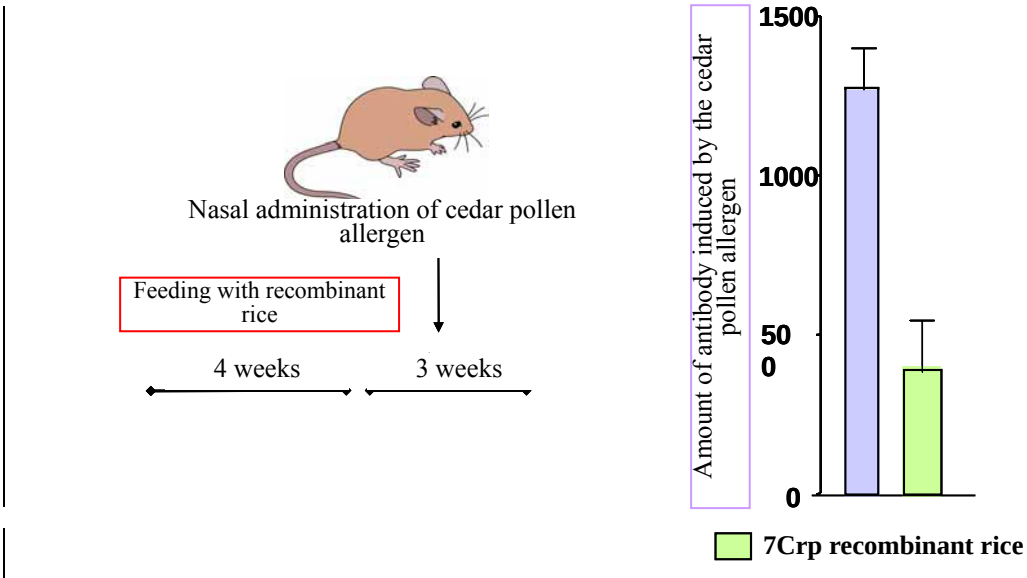

Figure 2 Immune tolerance inducibility due to oral administration of 7Crp epitope peptide accumulated rice

In addition, assuming the typical intake condition of cooked rice, the rice was processed in the boiling water at 100°C for 20 minutes to identify the stability of epitope peptide accumulated in the rice at elevated temperatures and as a result, the epitope peptide was found stable at high temperatures. In addition, it was also confirmed that the expression of epitope peptide does not affect the expression of rice allergen protein gene contained in rice by nature, which causes rice allergy.

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

a. 7Crp expression cassette

i) GluB-1 promoter

A rice-derived seed storage protein glutelin promoter, the specific nucleotide sequence on the DNA to initiate the mRNA synthesis based on the DNA as template. It expresses specifically in the endosperm in the seed ripening period.

ii) GluB-1 signal peptide

Nucleotide sequence of signal peptide of rice-derived seed storage protein glutelin. The signal peptide acts to facilitate the adhesion of synthesized protein to endoplasmic reticulum and the passage through the membrane of endoplasmic reticulum, and it is cleaved by signal peptidase after passing through the membrane.

iii) *7Crp*

Nucleotide sequence to express the part of amino acid sequence contained in the antigen proteins Cryj I and Cryj II in the cedar pollens causing pollen allergy and recognized by the human cedar pollen antigen-specific T cell (hereinafter referred to as "human T cell epitope") (see Figure 1).

At a total of seven (7) sites, three (3) in Cryj I and four (4) in Cryj II, the human T-cell epitopes, composed of 12 to 19 amino acid residues in length respectively, have been identified. In order to express the artificial peptide (7Crp) composed of 96 amino acid residues by linking the seven-site epitopes (amino acid sequence), the artificial gene was synthesized in accordance with the amino acid sequence of T cell epitope (see Figure 1). For the synthesis, the frequently used codon was selected among the gene cluster which encodes the major rice seed storage proteins [17), 18)].

iv) KDEL

Nucleotide sequence composed of four (4) amino acids serving to localize the proteins to endoplasmic reticulum. The proteins containing KDEL sequence (amino acids) at C-terminal are localized

in the endoplasmic reticulum.

v) GluB-1 terminator

A rice-derived protein glutelin terminator; the nucleotide sequence required to terminate the synthesis of mRNA.

b. Hygromycin-resistant expression cassette

i) CaMV35S promoter

A promoter derived from cauliflower mosaic virus, the specific nucleotide sequence on the DNA to initiate the synthesis of mRNA based on the DNA as template. It expresses in the entire plant tissue.

ii) *hpt*

A gene derived from *E. coli* K-12 strain to exhibit the resistance to the antibiotic hygromycin.

iii) Ag7 terminator

A terminator derived from *Agrobacterium tumefaciens* C58 strain, the nucleotide sequence required to terminate the synthesis of mRNA.

(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 (excluding the allergenicity as food)

a. 7Crp

7Crp refers to the human T-cell epitopes of the cedar pollen antigen proteins, Cryj I and Cryj II. However, it does not possess ability of binding to any specific IgE antibody required for a sequence of allergic reactions nor contains any B-cell epitope and thus, it is considered extremely unlikely to cause any allergy.

b. hpt

It produces the enzyme which phosphorylates the hygromycin. Due to this enzyme, hygromycin is phosphorylated and inactivated. The hygromycin-resistant enzyme is found not to have any homology with allergen protein and thus it is considered very unlikely to cause any allergic reaction.

(2) Information concerning vectors

1) Name and origin

Binary vector pGTV-35S-HPT [21]. Derived from *E. coli* K12 strain and *Agrobacterium tumefaciens* C58 strain.

2) Properties

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

The total number of base pairs is 13,900bps. For the nucleotide sequence and other information, refer to Reference 19.

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

There exist the hygromycin-resistant gene (*hpt*) as a drug-resistant gene and the cauliflower mosaic virus-derived CaMV 35S promoter and *Agrobacterium tumefaciens* C58 strain-derived Ag7 terminator as regulatory factors for expression of *hpt* gene.

(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

It has not been known that the vector possesses any infectious characteristics.

(3) Method of preparing living modified organisms

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

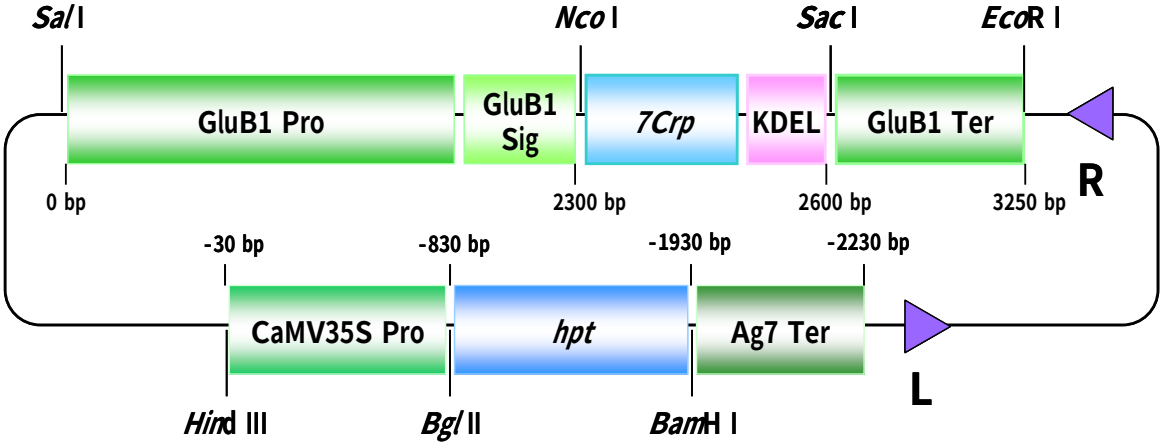

Figure 3 Map of the entire nucleic acid (expressed plasmid) transferred to the recipient organism

GluB1 Pro: GluB-1 promoter, GluB1 Sig: GluB-1 signal peptide,

CaMV35S Pro: CaMV35S promoter, Ag7 Ter: Ag7 terminator,
R: Right border, L: Left border,
For detail description about individual component elements, refer to Table 2.

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

Agrobacterium method

3) Processes of rearing of living modified organisms

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

Agrobacterium, to which the target gene had been transferred, was infected with the rice seed callus, and the cells to which the nucleic acid had been transferred, were selected on the selective medium containing hygromycin (50 mg/L).

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

The seeds of recombinant rice (T_1 generation) were crushed into fine powder with Multi-Beads Shocker, mixed with sterile water and the supernatant after centrifugation was applied to the YEB medium containing rifampicin (20 mg/L) and incubated at 28°C. Three (3) days later, observation was made for *Agrobacterium* and as a result, growth of *Agrobacterium* was not observed. Based on this result, it was judged that there is no residue of *Agrobacterium* used for transferring of gene in the posterity of recombinant rice.

(c) Processes of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line with which the state of existence of replication products of transferred nucleic acid was confirmed, the line subjected to tests in closed and non-containment greenhouse; and the line used for collection of other necessary information for assessment of Adverse Effect on Biological Diversity

In 2001, gene introduction experiment was started, and in the process of safety tests in the closed system (based on the classification in accordance with the former “Guideline for Recombinant DNA Experiment” by the Ministry of Education, Culture, Sports, Science and Technology), four (4) lines were selected. For the four lines, self-fertilization has been repeated to advance the generations, and additional analyses have been conducted to collect the required information for assessment of Adverse Effect on Biological Diversity.

In 2003, safety test was started in the non-containment plots (based on the classification in accordance with the former “Guideline for Recombinant DNA Experiment” by the Ministry of Education, Culture, Sports, Science and Technology), and the "rice containing cedar pollen peptide (*7Crp*, *Oryza sativa* L.) (7Crp#10) (hereinafter referred to as "7Crp#10") was selected based on the fact that it contains the 7Crp peptide most accumulated in the seeds. In May

2005, in accordance with Article 4, Paragraph 2 of the Law concerning the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms, use in the isolated field [(National Institute of Agrobiological Sciences (NIAS)]was approved by the Ministry of Agriculture, Forestry and Fisheries. In FY 2005, T_7 plants were cultivated in the isolated field and Assessment of Adverse Effect on Biological Diversity was conducted. Additionally in FY 2006, T_8 plants were cultivated in the isolated field and the assessment has been repeated. The pedigree tree and the generations and tests conducted are presented in the figures below.

In all the tests, Kita-ake, the recipient organism of the recombinant rice, was used as the control cultivar.

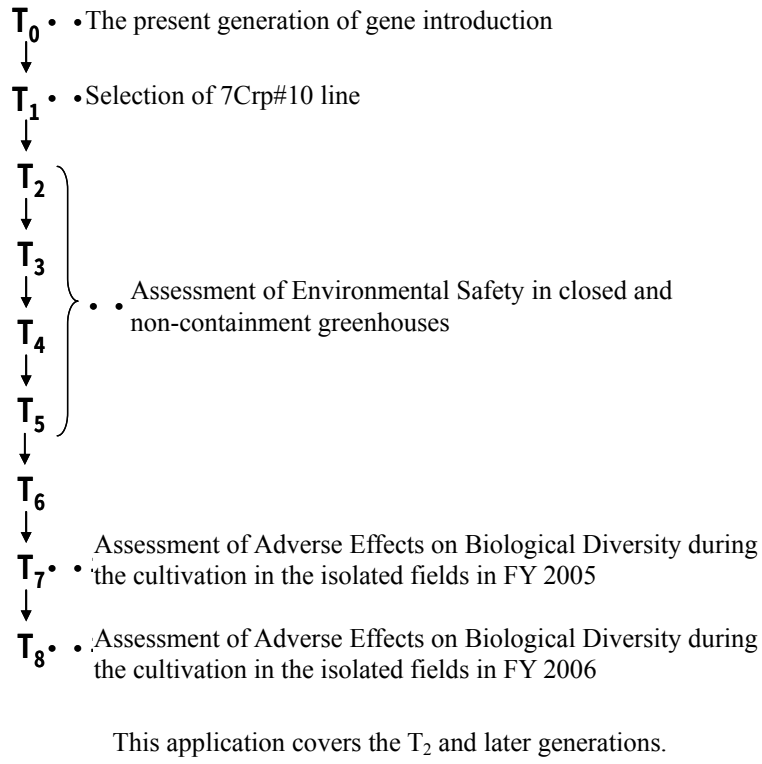

Figure 4 Pedigree tree of rice plants used in the tests

Table 3 Generations and tests conducted

Test items	Generation						
	T_0	T_1	T_2	T_3	T_4	T_5	T_7
Gene existence condition (Southern blotting analysis)			○			○	○
(PCR)		○	○	○	○		
Gene expression condition		○	○	○	○	○	○
Site-specific expression			○				
Residual *Agrobacterium*		○					
Morphological and ecological characteristics					○		○
Cold-tolerance at the early stage of growth						○	
Fertility and size (diameter) of the pollen					○		○
Production, germination rate, dormancy and shedding habit of the seed					○	○	○
Production of harmful substances					○		○

(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 Southern blotting analysis using the genome DNA, it was confirmed that the nucleic acid transferred is introduced on the chromosome. In addition, it was judged that the nucleic acid transferred exists on the chromosome based on the Southern blotting analysis which demonstrated the agreement of band patterns between the generations.

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

(a) The number of copies of nucleic acid

As a result of Southern blotting analysis of genome DNA of T_2 and T_5 generations, it was estimated that *7Crp* and *hpt* genes are retained stably on the genome and the number of copies is four (4).

(b) Stability of inheritance through multiple generations

As a result of PCR analysis of T_1 to T_4 generations and as a result of Southern blotting analysis of genome DNA of T_2, T_5 and T_7 generations, the genes are retained stably in the individual generations.

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

As a result of Southern blotting analysis, it was found that four copies of cassettes were introduced and the genes were arranged adjacent to each other in units of two of the four copies. It was estimated that the copies are introduced at two positions or two loci.

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

As a result of Western blotting analysis of the T_2 to T_4 seeds of 7Crp#10 collected in the closed system, T_5 seeds collected in the non-containment greenhouse and T_8 seeds collected in the isolated field, stable expression was confirmed.

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

There does not exist any applicable virus.

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

The PCR method using the oligoprimers (7Crp-F, 7Crp-2R) for proliferation of the entire length of *7Crp* gene can be used for specific detection and identification of *7Crp* gene. The PCR method using this set of primers specifically detects the transferred genes from the recombinant rice rather than proliferates band when the DNA of the non-transformed plant Kita-ake is used as a template.

(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

As a result of Western blotting analysis on the glumes and leaves of T_1 plant of 7Crp#10 and the embryo and endosperm of T_2 seeds, it was confirmed that the 7 consecutive peptide connecting the 7 human T-cell epitopes composed of 12 to 19 amino acid residues derived from the cedar pollen antigen proteins, Cryj I and Cryj II expressed specifically in the endosperm after the ripening period.

The glutelin promoter used for the expression of *7Crp* gene is specific to endosperm tissue and thus, it is considered that the *7Crp* gene does not express in the pollens of 7Crp#10 [17, 20), 23)].

2) With respect to the physiological or ecological characteristics, 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 order to examine the morphological and growth characteristics, the T_4 generation of 7Crp#10 and the recipient organism Kita-ake were transplanted to a closed-end paddy field in the non-containment greenhouse (inside dimensions 7.2 × 1.1 m) at a planting density of 25 × 15 cm in May 2, 2003. For fertilization, NPK was applied 4 kg/10a for each. The transplanting used the seedlings sown in March 24, 2003 and raised in the closed system greenhouse.

In order to examine the production of harmful substances, the T_4 generation of 7Crp#10 and the Kita-ake were transplanted into 1/5000a pot packed with the paddy field soil in the non-containment greenhouse in April 24, 2003.

In order to examine the morphological and growth characteristics, wintering ability and productivity of harmful substances in the isolated field at National Institute of Agrobiological Sciences (NIAS), the T_7 generation of 7Crp#10 was sown in May 19, 2005, and the seedlings were raised in the non-containment greenhouse and then transplanted to the growth investigation plot in the isolated field in July 8. The seedlings were planted based on the single planting at a planting density of 30 cm × 18 cm and fertilized with NPK of 5.26 kg/10a for each and fused phosphate of 56 kg/10a.

(a) Morphological and growth characteristics

As a result of examination in the non-containment greenhouse, no difference was observed in heading time, and no statistically significant difference was observed in culm length, ear length and number of ears between 7Crp#10 and Kita-ake.

In the isolated field, morphological and growth characteristics were examined. The first heading time was delayed two days in 7Crp#10. In addition, in the items, ear length, number of ears, husked rice length, husked rice thickness, a statistically significant difference was observed between 7Crp#10 and Kita-ake.

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

The seedlings at 2-leaf stage were treated in the dark at 5°C for 10 days and then raised in the non-containment greenhouse for 2 weeks for examination. As a result, for both 7Crp#10 and Kita-ake, approx. 80% individuals survived. In all the surviving individuals, the main stems withered, and tillers occurred 1.1 on average in addition to the main stem. No difference was observed between 7Crp#10 and Kita-ake.

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

Rice is a perennial plant in the tropical regions, though in the cultivation districts in Japan, it is known to die naturally in the winter season after seed

setting. Actually, in the isolated fields after harvesting in September 14, observation was made on the offshoots from the stump. As a result, the offshoots of 7Crp#10 grew similarly as those of Kita-ake, the growth stopped around November 30, and the withering process progressed in union up to around December 12. Consequently, it was judged that the wintering ability is very low similarly as the control cultivar.

(d) Fertility and size of the pollen

The 7Crp#10 and Kita-ake had the spherical pollens of similar sizes to each other and no difference was observed in the shape of pollen, and no statistically significant difference was observed in the size (diameter) of pollen. Also regarding the fertility of the pollen, no difference was observed between the both rice plants.

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

Regarding the measured results of the number of grains per ear, shedding habit, dormancy (ear germinating ability) and germination rate of the rice cultivated in the non-containment greenhouse, no difference was observed between 7Crp#10 and Kita-ake.

For the rice cultivated in the isolated fields, examination was made on the number of grains per ear, fertility, winnowed paddy weight per plant, husked rice weight and 1000-kernel weight. The number of grains per ear was found statistically significantly larger in the 7Crp#10, though fertility, paddy weight per plant, husked rice weight and 1000-kernel weight exhibited no statistically significant difference.

As a result of shedding habit examination conducted by holding the ears in the maturation period, 7Crp#10 and Kita-ake were both found difficult to shed and no difference was observed between the both rice plants.

As a result of examination on the germination of ears to identify dormancy, 7Crp#10 and Kita-ake both exhibited easy germination of ears (in the non-containment system) or medium germination of ears (in the isolated fields) and no difference was observed between the both rice plants.

The seeds immediately after harvesting in the isolated fields and the seeds stored at 5°C for approx. 6 months were examined for germination rate and as a result, the germination rate was found 95% or more and no statistically significant difference was observed between 7Crp#10 and Kita-ake.

(f) Crossability

Crossability test was not conducted because no wild relatives are growing voluntarily in Japan that show crossability with this recombinant rice.

(g) Productivity of harmful substances

Regarding the T_3 generation of 7Crp#10 and Kita-ake cultivated in the closed system, gas chromatography analysis for volatile constituents and high-performance liquid chromatography analysis for constituents in plant body were carried out and as a result, no difference was observed between 7Crp#10 and Kita-ake.

As a result of succeeding crop test using the soil after pot cultivation in the non-containment greenhouse and plow-in test using the plant body in the maturation period, no statistically significant difference was detected between 7Crp#10 and Kita-ake regarding possible effects on the germination of radish.

In addition, as a result of examination on the number of filamentous fungi, actinomyces and bacteria in the soil after cultivation, no statistically significant difference was observed between 7Crp#10 and Kita-ake.

The succeeding crop test using the soil after harvesting in the isolated fields and the plow-in test using the dried leaves and stems after harvesting and the husked rice in which the target product of peptide has been accumulated were carried out using the seeds of lettuce. As a result, regarding the germination rate and fresh weight of lettuce, no statistically significant difference was observed between 7Crp#10 and Kita-ake. In addition, as a result of examination on the number of filamentous fungi, actinomyces and bacteria before rice transplanting, at the heading time and after harvesting in the soil collected from cultivation plots of 7Crp#10 and Kita-ake in the growth investigation area, no statistically significant difference was observed between 7Crp#10 and Kita-ake.

Furthermore, in the isolated field cultivation experiments, investigation on visiting insect fauna, rearing of insect using *Leptocorisa chinensis* Dallas, and survival analysis were conducted in order to identify possible effects on insects of the substances produced by 7Crp#10. As a result, regarding the visiting insect fauna to the growth investigation area, no difference was observed, and regarding the survival rate of *Leptocorisa chinensis* Dallas sucking the 7Crp#10 and Kita-ake, no statistically significant difference was observed.

(h) Additional information (Reference 1)

In the isolated field cultivation experiments in FY 2005, a total of 48 Hakucho-mochi in pot were arranged adjacent to Kita-ake and 7Crp#10, and the seeds collected from Hakucho-mochi were investigated for possible crossing with Uruchi (non-glutinous, ordinary cooking rice) in the cases when xenia is present and absent. Kita-ake, 7Crp#10 and Hakucho-mochi slightly differed from each other in heading date, but reached the high flowering period at almost same timing. Among the total of 23,952 grains borne in Hakucho-mochi, there was no grain observed that had been crossed with 7Crp#10 or Kita-ake due to the dispersed pollens and then, it was considered the degree of pollen dispersion was low similarly in the both rice cultivars.

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

This recombinant rice is given traits to produce the cedar pollen peptide due to the transferred *7Crp* gene and to be resistant to hygromycin due to the *hpt* gene. However, it is not generally considered that these characteristics cause this recombinant rice to become competitive in a natural environment.

As a result of investigation on the various traits relating to the competitiveness of this recombinant rice in the Japanese isolated fields, a difference from the control was observed in heading date, and a significant difference from the control was observed in ear length, number of ears, number of grains per ear and seed size. However, the differences are found to fall within the variable ranges for the results of cultivation experiments of Japanese type rice plants conducted so far in the areas. Consequently, it is unlikely that these characteristics cause this recombinant rice to become competitive in a natural environment.

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 rice poses no significant risk of Adverse Effect on Biological Diversity attributable to competitiveness is reasonable.

(2) Productivity of harmful substances

This recombinant rice produces the 7Crp peptide, though it is clarified that it consists of only the epitopes recognized by the human T cells and does not exhibit any binding to the human IgE antibody involved in the cedar allergy. In addition, there is no report that even in the case of oral administration of this recombinant rice to mice, the growth of mice is inhibited or allergy condition is observed. Based on the above understanding, it is considered unlikely that this recombinant rice produces any substances harmful to human and mice.

There is no report to indicate the possibility that human T cell epitopes react with other mammals and birds.

The tests have been conducted to check the harmful substances productivity (the substances excreted from the roots of the recombinant rice which can affect other plants, the substances excreted from the roots which can affect microorganisms in soil, and the substances exist in the plant body which can affect other plants after dying), at the isolated field in Japan. As a result, there is no significant difference from the control.

In addition, it was feared that the 7Crp peptide might affect the grain-sucking insects since it expresses specifically to endosperm tissue. However, as a result of feeding test using *Leptocorisa chinensis* Dallas, no significant difference was observed from the control.

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 rice poses no significant risk of Adverse Effect on Biological Diversity attributable to productivity of harmful substances is reasonable.

(3) Crossability

In the Japanese natural environment, there are no wild relatives growing voluntarily including *O. nivara* and *O. rufipogon* that can cross with rice. In the fields and levees, weed rice can grow in some cases, though weed rice is considered to come from cultivated species. Therefore, 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 rice poses no significant 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 rice 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.

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