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

Name: DuPont Kabushiki Kaisha
Applicant: Minoru Amou, President (Seal)
Address: 11-1 Nagata-cho, 2-chome, Chiyoda-ku, Tokyo

Approved Type 1 Use Regulation

Name of the Type of Living Modified Organism	Oilseed rape resistant to glyphosate herbicide (*gat4621*, *Brassica napus* L.) (73496, OECD UI:DP-Ø73496-4)
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 Effects on Biological Diversity

1. Information concerning preparation of living modified organisms

(1) Information concerning donor nucleic acid

1) Composition and origins of component elements

The composition and the origins of component elements for the donor nucleic acid in oilseed rape resistant to glyphosate herbicide (*gat4621*, *Brassica napus* L.) (73496, OECD UI:DP-Ø73496-4) (hereinafter referred to as "modified oilseed rape") were shown in Table 1 (page 3). In addition, the nucleotide sequence of the donor nucleic acid was shown in page 10 of Attachment 1 (confidential and non-disclosed).

The *gat4621* gene in the component elements was produced with the following method.

Exploration of *N*-acetyl transferase having an *N*-acetylation activity for glyphosate:

Among the Bacillus microorganisms, ST401 strain, B6 strain and DS3 strain of *Bacillus licheniformis* in which *N*-acetylating activities of glyphosate were shown were selected, and *N*-acetyl transferase gene was cloned from each genome DNA.

Modification for increasing the activity of *N*-acetyl transferase:

It has been reported that the activity of PAT protein, providing a resistant to glyfosinate herbicide, to glyfosinate shows about 5,000 folds of wild type *N*-acetyl transferase (Siehl *et al.*, 2005). Hence, the goal for the modification was determined to be about 5,000 folds of wild type *N*-acetyl transferase of *B. licheniformis* strain.

The modification was performed with DNA shuffling method, using the aforementioned three cloned wild type *N*-acetyl transferase genes of *B. licheniformis* strain (Castle *et al.*, 2004; Keenan *et al.*, 2005; Stemmer, 1994).

1) The cloned gene was fragmented with DNA digesting enzyme, the obtained fragments were bound each other by PCR without adding any primer, then PCR was performed using both end sites of the genes based thereon as a primer to obtain a full length gene.
2) The reconstituted gene was introduced into Escherichia coli to select a colony showing the N-acetylating activity of glyphosate.
3) Several clones having high *N*-acetylating activity were selected therefrom to clone a gene.

As a result of repeating the steps of 1) to 3) for 11 times in which a modification for gene is additionally performed by site-specific mutation, modified *N*-acetyl transferase gene having the target activity (*gat4621* gene) was obtained.

GAT4621 protein expressed from *gat4621* gene exhibits 3,700 to 5,500 folds activity of the original wild type *N*-acetyl transferase (k_{cat}/K_m value[1)]=6,719 min^{-1} mM^{-1}).

2) Functions of component elements

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

The function of individual elements of the donor nucleic acid are shown in Table 1 (p. 3).

Table 1 Composition of the donor nucleic acid used for production of the modified oilseed rape and origins and function of its component element

Component element	Size (bp)	Origin and function
UBQ10 promotor	1,308	Promoter region inducing a transcription of *UBQ10* polyubiquitin gene derived from Arabidopsis (*Arabidopsis thaliana*), which inducing a constitutional expression within the plant body (Norris *et al.,* 1993). Contains a 5'-terminal untranslated region (66 bp) and intron (304 bp).
gat4621	444	*gat4621* was obtained based on genes derived from three strains of *B. licheniformis* (ST401 strain, B6 strain and DS3 strain). The gene encodes *N*-acetyl transferase which *N*-acetylate glyphosate herbicide (GenBank Accession No: CS022547).
*pin*II terminator	310	Terminator region of protease inhibitor II gene derived from potato (*Solanum tuberosum*) (Keil *et al.*, 1986; An *et al.*, 1989), which terminates the transcription.

(2) Functions of proteins produced by the expression of target genes and selective markers, and the fact, if applicable, that the produced protein is homologous with any protein which is known to possess any allergen

a Functions of proteins produced by the expression of the target gene

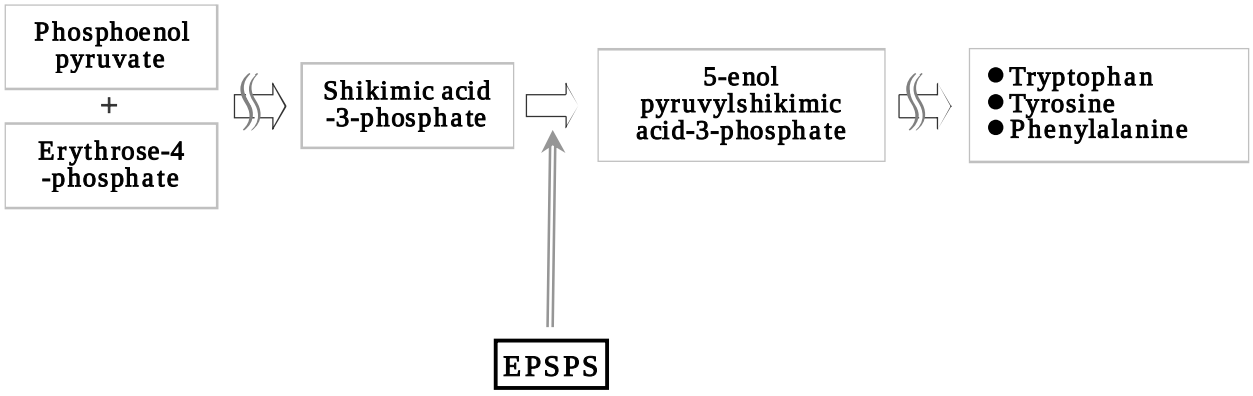
GAT4621 protein produced by the expression of *gat4621* gene is *N*-acetyl transferase which *N*-acetylates the glyphosate herbicide, consisting of 147 amino acids, and having about 17 kDa in molecular weight. The amino acid sequence of this protein is shown in page 5 of Attachment 1 (confidential and non-disclosed).

The GAT4621 protein acetylates NH group of the glyphosate herbicide to convert it into *N*-acetyl glyphosate which does not inhibit an activity of 5-enol pyruvylshikimic acid-3-phosphate synthase (EPSPS), resulting in providing the resistance to the glyphosate herbicide for the plant (

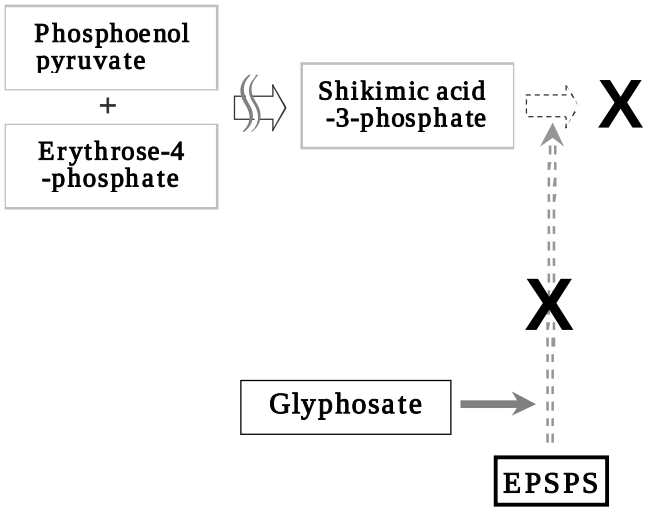
1) k_{cat} indicates a rate constant for catalytic reaction, K_m indicates an affinity for substrate, and k_{cat}/K_m value indicates a catalyst efficiency for substrate.

Figure 1, p. 4).

i) Synthetic pathway of aromatic amino acids in non-modified oilseed rape at not spraying herbicides



ii) Synthetic pathway of aromatic amino acids in non-modified oilseed rape at spraying the glyphosate herbicide



iii) Synthetic pathway of aromatic amino acids in the modified oilseed rape at spraying the glyphosate herbicide

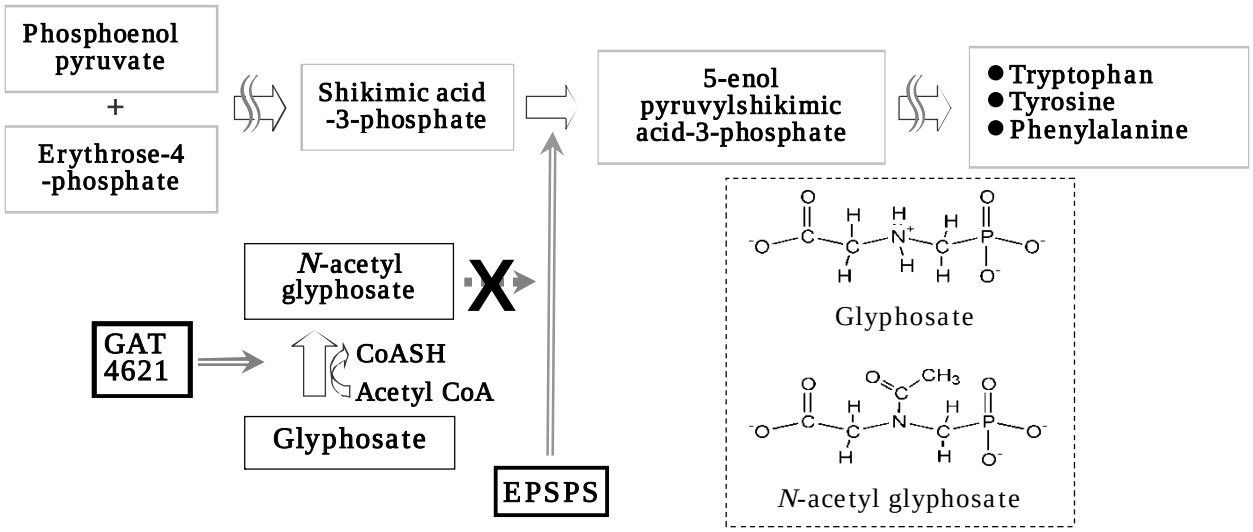


Figure 1 Mechanism of action of the GAT4621 protein on glyphosate in the synthetic pathway of aromatic amino acids

b Fact, if applicable, that the produced protein by the expression of target genes is homologous with any protein which is known to possess any allergen

Searches for peptides having 8 or more amino acids in residual length using Food Allergy Research and Resource Program (FARRP) allergen database (Release 12 - February 2012) and for sequence having 80 or more amino acids in residual length with 35% or more in homology using FASTA35 algorithm (Pearson and Lipman, 1988) were performed. In the database, amino acid sequences of 1,603 known allergens are included. As the result, the known allergen and the like having homology with the GAT4621 protein could not be found in the database.

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

Any possibilities to change the metabolic system in the recipient by the GAT4621 protein was investigated. As the result of confirming compounds which will be a substrate for the GAT4621 protein, it is observed that its catalytic activity is found for 5 types of amino acids. Accordingly, the content of *N*-acetyl amino acid in the modified oilseed rape is analyzed to investigate an effect on compositions of amino acids and free amino acids by increasing *N*-acetyl amino acid.

Compound for GAT4621 protein as substrate

The catalytic activity of compounds which are considered as a possible substrate for the GAT4621 protein is measured. In this experiment, in order to increase an ability to detect, the activity is determined with an amount of Coenzyme A, which is a reaction product in the *N*-acetylation reaction, which is accumulated for 30 minutes. As the substrate, amino acids (21 types), agrochemicals (herbicides, insecticides and germicides, 20 types), and antibiotics (kanamycin, ampicillin and the like, 10 types) are used. As the result, the catalytic ability was found in 5 types of amino acids of aspartic acid, glutamic acid, threonine, serine and glycine in addition to glyphosate using as a control.

Hence, 100 mM KCl was added to a reaction liquid used for measuring the aforementioned k_{cat}/K_m value (I.2.(1) (2), p.2 of this report), and a reaction liquid which is arranged to be a condition of an ion strength similar to the biological body is used to measure the catalytic activity. In this study, 4 types of compounds (D-2-amino-3-phosphonopropionate, L-2-amino-3-phosphonopropionate, DL-2-amino-4-phosphonobutylate, and DL-2-amino-5-phosphonopentanoate), which have similar structure with those in glyphosate, are also used as the substrate. As the result, the k_{cat}/K_m value of the GAT4621 protein for glyphosate is 1,063 $min^{-1}mM^{-1}$, and those for aspartic acid and glutamic acid are 12.1 $min^{-1}mM^{-1}$ and 8.32 $min^{-1}mM^{-1}$, respectively. In addition, those for threonine and serine are 0.605 $min^{-1}mM^{-1}$ or lower, and no catalytic activity is found for glycine and glyphosate-like compounds.

Amino acid compositions

As mentioned above, since the low catalytic activities for the 5 types of amino acids were found, contents of *N*-acetyl amino acid in seed, aboveground plant body and root of the modified oilseed rape were analyzed.

As the result, while 4 types of *N*-acetyl aspartic acid, *N*-acetyl glutamic acid, *N*-acetyl serine and *N*-acetyl threonine were significantly increased in the seed, compared with those in non-modified oilseed rape (P value<0.05), the contents of *N*-acetyl serine and *N*-acetyl threonine are within the variable range of those in our commercially available product (Table 2, p. 9). In addition, 4 types in total of *N*-acetyl aspartic acid, *N*-acetyl glutamic acid, *N*- acetyl glycine and *N*-acetyl threonine in aboveground plant body and root of the modified oilseed rape were statistically significantly increased compared with those in the non-modified oilseed rape (P value<0.05) (Table 3 and Table 4 and p. 10 and 11).

In addition, the composition of the amino acids and free amino acids in seed, aboveground plant body and the root of the modified oilseed rape were analyzed.

As the result, the composition of amino acids and free amino acids in the seed is not observed with a statistically significance (P value<0.05) or is within a variable range of those in our commercially available product, compared with those in the non-modified oilseed rape (Table 5 and Table 6, p. 12 and p. 15). For the aboveground plant body and the root, while a statistically significance between the modified oilseed rape and the non-modified oilseed rape for several items to be analyzed (Table 7 to Table 10, p. 19 to p. 29), it was considered that the increase of *N*-acetyl amino acid does not affect the amino acid pool in the host with biological significance in the aboveground plant body and the root, in view that there is no constant trend for the increase/decrease of the amino acids and the free amino acids in the seed, the aboveground plant body and the root as a whole.

Possibility of the effect on insects

N-acetyl amino acids are also contained in animals and plants such as meat, grain, vegetables, fruits and the like, not a component which is newly produced in the modified oilseed rape (Hession *et al.*, 2008; Attachment 2). Further, as the result of searching documents, there is no report relating to an effect of *N*-acetyl amino acids on insects.

In order to examine a possibility of an effect of the modified oilseed rape on insects, 30 individual cabbageworms (*Pieris rapae*) were bred on leaves of the modified oilseed rape and the non-modified oilseed rape, respectively, for 7 days to measure mortality rates and weights after breeding (Table 11, p. 33; Attachment 10; Confidential and non-disclosed). The content of *N*-acetyl aspartic acid of the leaf of the modified oilseed rape used in this study was 6,591 (μg/g freeze-dried weight)[2)] (Table 12, p. 33), which is

[2)] While the freeze-dried product normally contains water with about 10%, a sufficient amount of the sample is not remained for measuring the water content. Accordingly, the measured value cannot be precisely converted into a dried weight. In a case that a value per freeze-dried weight is converted into a value per

a higher value than 1,640 (μg/g dried weight) which is an upper limit of 95% confidence interval thereof in the seed of the modified oilseed rape, 4,950 (μg/g freeze-dried weight) in those of the aboveground plant body, and 5,390 (μg/g dried weight) of maximum value in the root thereof (Table 2 to Table 4, p. 9 to 11).

As the result of the test, no statistical significance increase in the mortality of the worm fed with the modified oilseed rape was observed compared with those in the non-modified oilseed rape (P value<0.05), and no significant decrease in its weight was also observed.

Therefore, no result affecting any adverse effect of the modified oilseed rape on growth of the cabbageworm was observed.

Further, while an investigation of a degree of a feeding damage was performed in the following 34 field tests in total, no difference between the modified oilseed rape and the non-modified oilseed rape was observed in any of the field tests.

Field test 1: The insect species on the aboveground plant body and the degree of feeding damage were investigated in the field in Canada and US during 2007 to 2010.
The insect species such as fleahopper (*Phyllothreta cruciferae* or *Phyllothreta striolata*), cabbageworm and the like were observed. Also in those of Province of Alberta in which the severe feeding damage was observed, no difference of thereof with the non-modified oilseed rape in the degree of feeding damage for the aboveground plant body was observed (Table 13 and Table 14, p. 34 and 35).

Field test 2: The degree of feeding damage for the aboveground plant body was investigated in the fields in Canada and US at 2009 and 2010.
No statistical significantly difference (P value<0.05) of thereof with the non-modified oilseed rape in the degree of feeding damage for the aboveground plant body was observed (Table 15 and Table 16, p. 36 and 37). Also in the field of Washington on 2009 in which the severe feeding damage was especially observed, no difference of thereof with the non-modified oilseed rape was observed (Table 15, p. 36)

Since the following facts were recognized that:

- *N*-acetyl amino acids are contained in various plants, and there is no report relating to any effects of *N*-acetyl amino acids on insects;
- It is considered that the increasing the content of *N*-acetyl amino acids does not affect the pool of amino acids with biologically significance;
- In the feeding test using the leaf in which the content of *N*-acetyl aspartic acid is higher than the upper limit of 95% confidence interval or the maximum value, the modified oilseed rape did not adversely affect on the growth of cabbageworm; and
- In the 34 field test in total, no difference between the modified oilseed rape and the

dried weight, the corresponding value will be increased by about 10%.

non-modified oilseed rape in the degree of feeding damage was observed, it was considered that there is little possibility that the increasing the content of *N*-acetyl amino acids in the modified oilseed rape affects on insects, upon comprehensively discussing them.

In order to accumulate knowledgement relating to toxicity of *N*-acetyl amino acids, we voluntarily performed a reverse mutation test using bacteria, a micronucleus test using mice, an acute oral toxicity test using rats, and repeated oral administration toxicity test for 28 days by administering mixed feeds using rats. In addition, we also performed, for *N*-acetyl aspartic acid, a repeated oral administration toxicity test for 90 days by administering mixed feeds using rats and two-generations reproductive toxicity test by administering mixed feeds using rats. It was considered, from the results of each aforementioned toxicity test, that *N*-acetyl amino acids, which was observed to increase in the modified oilseed rape, do not affect an adverse effect on the animal health (Delaney *et. al.*, 2008; Delaney, 2010; Harper *et. al.*, 2009; Harper *et. al*, 2010; Karaman *et. al.*, 2009; Karaman *et. al.*, 2011a; Karaman *et. al.*, 2011b; van de Mortel *et. al.*, 2010a; van de Mortel *et. al.*, 2010b).

Metabolites of the herbicide glyphosate

The main metabolite of the herbicide glyphosate in the modified oilseed rape is *N*-acetyl glyphosate, and other metabolites include amino methyl phosphonic acid (AMPA) and *N*-acetyl amino methyl phosphonic acid (*N*-acetyl AMPA).

For the safety of these metabolites, in the case of *N*-acetyl glyphosate, its toxicity is equivalent to those of glyphosate, and there is no concern on the toxicology for AMPA, and it was considered that the toxicity of *N*-acetyl AMPA is low (OECD, 1999; US EPA, 2008).

After the herbicide glyphosate was sprayed to the modified oilseed rape which was cultivated in US and Canada with maximum amount and maximum times (2 or 3 times) according to the standards determined in both countries[3)], the residual amount thereof was measured in the mature seed. As the result, the residual amount of glyphosate itself was 2.5 mg/kg (ppm) when it was sprayed for 3 times, which is less than the Japan standard value of residual agrichemical in rapeseed (10 ppm; The Japan Food Chemical Research Foundation, 2012). In addition, the residual amounts in total of glyphosate and three metabolites were 3.9 mg/kg (converted into glyphosate).

3) Sprayed for 2 times: 1,754 g acid equivalent, pre-emergent (a.e.; equivalent amount of free acid of glyphosate)/ha, 6-leaf stage 620 g a.e./ha.
Sprayed for 3 times: Pre-emergent 675 g a.e./ha, 6-leaf stage 675 g a.e./ha, 7 days before harvesting 900 g a.e./ha.

Table 2 *N*-acetyl amino acids in seed of the modified oilseed rape

(μg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
N-acetyl aspartic acid	Mean value	1.24	1480	0.00861 - 4.43
	Minimum value - Maximum value	0.377 - 5.39	1200 - 1770	
	Confidence interval	0 - 9.38	1340 - 1640	
	P value		<0.0001 1)	
N-acetyl glutamic acid	Mean value	0.628	32.8	0.0968 - 5.37
	Minimum value - Maximum value	0.428 - 1.46	20.3 - 61.1	
	Confidence interval	0.00000752 - 2.50	24.4 - 42.5	
	P value		<0.0001 1)	
N-acetyl glycine	Mean value	0.0751	0.0825	0.0240 - 0.338
	Minimum value - Maximum value	0.0481 - 0.125	0.0424 - 0.182	
	Confidence interval	0.0540 - 0.105	0.0592 - 0.115	
	P value		0.454	
N-acetyl serine	Mean value	0.843	1.04	0.0524 - 27.2
	Minimum value - Maximum value	0.389 - 3.05	0.491 - 3.55	
	Confidence interval	0.437 - 1.63	0.542 - 2.01	
	P value		0.0035 1)	
N-acetyl threonine	Mean value	0.110	0.546	0.0140 - 1.74
	Minimum value - Maximum value	0.0531 - 0.212	0.260 - 1.64	
	Confidence interval	0.0665 - 0.181	0.331 - 0.902	
	P value		<0.0001 1)	

Non-modified oilseed rape: 5536×1822 lines.
Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).
Condition for cultivation: 2009, 3 places including Manitoba, Canada, 5 places in total, including North Dakota, Washington in US. 4 plots/field, n=20.
Statistical analysis: Variance analysis using linear mixed model.
1) Statistical significance (P value<0.05).

Table 3 *N*-acetyl amino acids in aboveground plant body of the modified oilseed rape

(μg/g freeze-dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
N-acetyl aspartic acid	Mean value	0.705	4560
	Minimum value - Maximum value	0.404 - 1.23	3730 - 5340
	Confidence interval	0.649 - 0.766	4190 - 4950
	P value		<0.0001 1)
N-acetyl glutamic acid	Mean value	2.04	26.0
	Minimum value - Maximum value	1.45 - 3.27	21.0 - 35.9
	Confidence interval	1.87 - 2.22	23.8 - 28.3
	P value		<0.0001 1)
N-acetyl glycine	Mean value	0.152	0.344
	Minimum value - Maximum value	0.122 - 0.193	0.247 - 0.445
	Confidence interval	0.139 - 0.166	0.316 - 0.376
	P value		<0.0001 1)
N-acetyl serine	Mean value	14.0	13.0
	Minimum value - Maximum value	10.2 - 21.9	9.17 - 22.0
	Confidence interval	12.3 - 15.9	11.5 - 14.7
	P value		0.406
N-acetyl threonine	Mean value	1.92	7.67
	Minimum value - Maximum value	1.60 - 2.50	5.43 - 11.8
	Confidence interval	1.76 - 2.11	7.00 - 8.39
	P value		<0.0001 1)

Non-modified oilseed rape: 5536×5676 lines.
Modified oilseed rape: F1*[5] generation (Figure 3, p. 40; Confidential and non-disclosed).
Condition for cultivation: Cultivate in greenhouse, and harvest at flower bud differentiation. n=15.
Statistical analysis: Variance analysis using linear mixed model.
1) Statistical significance (P value<0.05).

Table 4 *N*-acetyl amino acids in the root of the modified oilseed rape

(μg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
N-acetyl aspartic acid	Mean value	0.472	4980
	Minimum value - Maximum value	0.338 - 0.867	4770 - 5390
	Standard deviation	0.222	254
	P value		0.00794 1)
N-acetyl glutamic acid	Mean value	0.914	37.8
	Minimum value - Maximum value	0.544 - 1.39	28.7 - 45.9
	Standard deviation	0.312	7.65
	P value		0.00794 1)
N-acetyl glycine	Mean value	0.491	0.796
	Minimum value - Maximum value	0.392 - 0.612	0.663 - 0.949
	Standard deviation	0.0828	0.132
	P value		0.00794 1)
N-acetyl serine	Mean value	12.5	13.0
	Minimum value - Maximum value	7.96 - 21.8	9.23 - 15.8
	Standard deviation	5.67	3.25
	P value		0.548
N-acetyl threonine	Mean value	2.93	8.39
	Minimum value - Maximum value	2.40 - 3.62	6.92 - 11.1
	Standard deviation	0.494	1.60
	P value		0.00794 1)

Non-modified oilseed rape: 5536×5676 lines.

Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).

Condition for cultivation: Cultivate in greenhouse at Iowa, US, 2012, and collect at the time of flowering period. 40 individuals were analyzed collectively with each 8 individuals (each 5 samples of those in the non-modified oilseed rape and the modified oilseed rape).

Statistical analysis: Mann-Whitney U test.

1) Statistical significance (P value<0.05).

Table 5 Amino acid composition in the seed of the modified oilseed rape (1/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
Alanine	Mean value	1.14	1.12	0.588 - 1.90
	Minimum value - Maximum value	1.01 - 1.37	0.970 - 1.32	
	Confidence interval	1.02 - 1.28	0.996 - 1.25	
	P value		0.0446 1)	
Arginine	Mean value	1.62	1.57	0.741 - 3.07
	Minimum value - Maximum value	1.30 - 1.98	1.32 - 1.92	
	Confidence interval	1.42 - 1.85	1.37 - 1.79	
	P value		0.0502	
Aspartic acid	Mean value	2.00	2.06	0.980 - 3.52
	Minimum value - Maximum value	1.26 - 2.54	1.77 - 2.50	
	Confidence interval	1.71 - 2.33	1.77 - 2.40	
	P value		0.152	
Cystine	Mean value	0.606	0.618	0.311 - 1.24
	Minimum value - Maximum value	0.505 - 0.751	0.523 - 0.722	
	Confidence interval	0.516 - 0.712	0.526 - 0.726	
	P value		0.592	
Glycine	Mean value	1.38	1.35	0.688 - 2.52
	Minimum value - Maximum value	1.09 - 1.61	1.17 - 1.54	
	Confidence interval	1.25 - 1.52	1.22 - 1.49	
	P value		0.162	
Glutamic acid	Mean value	5.05	5.01	1.99 - 11.9
	Minimum value - Maximum value	2.48 - 6.68	4.30 - 6.40	
	Confidence interval	4.23 - 6.02	4.20 - 5.97	
	P value		0.783	
Histidine	Mean value	0.800	0.801	0.342 - 1.72
	Minimum value - Maximum value	0.644 - 0.966	0.667 - 0.939	
	Confidence interval	0.712 - 0.899	0.713 - 0.900	
	P value		0.945	
Isoleucine	Mean value	1.08	1.06	0.533 - 1.95
	Minimum value - Maximum value	0.869 - 1.30	0.922 - 1.25	
	Confidence interval	0.958 - 1.22	0.935 - 1.19	
	P value		0.113	

See p. 14 for footnote.

Table 5 Amino acid composition in the seed of the modified oilseed rape (2/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
Leucine	Mean value	1.88	1.83	0.910 - 3.42
	Minimum value - Maximum value	1.52 - 2.26	1.59 - 2.19	
	Confidence interval	1.66 - 2.12	1.62 - 2.07	
	P value		0.0498 1)	
Lysine	Mean value	1.65	1.64	0.729 - 3.16
	Minimum value - Maximum value	1.25 - 2.02	1.48 - 1.97	
	Confidence interval	1.45 - 1.87	1.45 - 1.86	
	P value		0.669	
Methionine	Mean value	0.464	0.472	0.258 - 0.857
	Minimum value - Maximum value	0.383 - 0.546	0.402 - 0.546	
	Confidence interval	0.408 - 0.529	0.415 - 0.538	
	P value		0.596	
Phenylalanine	Mean value	1.12	1.10	0.545 - 2.12
	Minimum value - Maximum value	0.901 - 1.35	0.927 - 1.28	
	Confidence interval	1.01 - 1.26	0.985 - 1.23	
	P value		0.167	
Proline	Mean value	1.63	1.59	0.717 - 3.36
	Minimum value - Maximum value	1.46 - 1.98	1.38 - 1.95	
	Confidence interval	1.43 - 1.87	1.39 - 1.83	
	P value		0.123	
Serine	Mean value	1.12	1.12	0.576 - 2.08
	Minimum value - Maximum value	0.719 - 1.34	0.985 - 1.31	
	Confidence interval	1.01 - 1.25	1.00 - 1.25	
	P value		0.971	
Threonine	Mean value	1.11	1.11	0.619 - 1.95
	Minimum value - Maximum value	0.847 - 1.29	0.997 - 1.26	
	Confidence interval	1.02 - 1.22	1.01 - 1.21	
	P value		0.687	
Tryptophan	Mean value	0.325	0.312	0.153 - 0.537
	Minimum value - Maximum value	0.242 - 0.429	0.236 - 0.427	
	Confidence interval	0.256 - 0.382	0.239 - 0.371	
	P value		0.212	

See p. 14 for footnote.

Table 5 Amino acid composition in the seed of the modified oilseed rape (3/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
Tyrosine	Mean value	0.635	0.620	0.337 - 1.20
	Minimum value - Maximum value	0.552 - 0.757	0.508 - 0.737	
	Confidence interval	0.573 - 0.704	0.560 - 0.688	
	P value		0.205	
Valine	Mean value	1.39	1.36	0.682 - 2.49
	Minimum value - Maximum value	1.04 - 1.66	1.20 - 1.59	
	Confidence interval	1.23 - 1.57	1.20 - 1.54	
	P value		0.159	

Non-modified oilseed rape: 5536×1822 lines.

Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).

Condition for cultivation: 2009, 3 places including Manitoba, Canada, 5 places in total, including North Dakota, Washington. 4 plots/field, n=20.

Statistical analysis: Variance analysis using linear mixed model.

1) Statistical significance (P value<0.05).

Table 6 Free amino acid composition in the seed of the modified oilseed rape (1/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
α-Amino butyric acid	Mean value	<0.00606 2)	<0.00606 2)	0 - 3.00
	Minimum value - Maximum value	<0.00606 2)	<0.00606 2)	
	Confidence interval	NA	NA	
	P value		NA	
γ-Amino butyric acid	Mean value	1.19	1.04	0 - 3.00
	Minimum value - Maximum value	0.840 - 1.53	0.740 - 1.57	
	Confidence interval	1.02 - 1.39	0.893 - 1.22	
	P value		0.0668	
Alanine	Mean value	0.200	0.204	0.00816 - 3.26
	Minimum value - Maximum value	0.104 - 0.499	0.0920 - 0.512	
	Confidence interval	0.105 - 0.381	0.107 - 0.389	
	P value		0.860	
Arginine	Mean value	0.166	0.167	0.0164 - 1.62
	Minimum value - Maximum value	0.108 - 0.240	0.113 - 0.292	
	Confidence interval	0.125 - 0.220	0.126 - 0.222	
	P value		0.754	
Asparagine	Mean value	0.559	0.436	0.148 - 1.92
	Minimum value - Maximum value	0.442 - 0.720	0.318 - 0.580	
	Confidence interval	0.462 - 0.677	0.361 - 0.528	
	P value		<0.0001 1)	
Aspartic acid	Mean value	0.250	0.195	0.0396 - 2.16
	Minimum value - Maximum value	0.115 - 0.441	0.0513 - 0.463	
	Confidence interval	0.124 - 0.506	0.0963 - 0.394	
	P value		0.0954	
Cystine	Mean value	<0.00606 2)	0.00879	0 - 0.0158
	Minimum value - Maximum value	<0.00606 2)	0.00858 - 0.00899	
	Confidence interval	NA	NA	
	P value		NA	
Ethanolamine	Mean value	0.0997	0.0970	0.00369 - 0.833
	Minimum value - Maximum value	0.0766 - 0.121	0.0768 - 0.121	
	Confidence interval	0.0863 - 0.115	0.0840 - 0.112	
	P value		0.479	

See p. 18 for footnote.

Table 6 Free amino acid composition in the seed of the modified oilseed rape (2/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
Glutamic acid	Mean value	0.439	0.326	0 - 6.79
	Minimum value - Maximum value	0.0504 - 1.03	0.0329 - 0.811	
	Confidence interval	0.128 - 1.51	0.0949 - 1.12	
	P value		0.0376 1)	
Glutamine	Mean value	0.0648	0.0482	0.00512 - 0.770
	Minimum value - Maximum value	0.0245 - 0.189	0.0191 - 0.125	
	Confidence interval	0.0381 - 0.110	0.0283 - 0.0819	
	P value		0.0241 1)	
Glycine	Mean value	0.0407	0.0426	0.00765 - 0.188
	Minimum value - Maximum value	0.0303 - 0.0640	0.0288 - 0.0762	
	Confidence interval	0.0311 - 0.0534	0.0325 - 0.0559	
	P value		0.513	
Histidine	Mean value	0.0411	0.0361	0.0110 - 0.145
	Minimum value - Maximum value	0.0284 - 0.0564	0.0245 - 0.0599	
	Confidence interval	0.0333 - 0.0507	0.0292 - 0.0445	
	P value		0.00461 1)	
Hydroxy proline	Mean value	<0.00606 2)	<0.00606 2)	0 - 0.0194
	Minimum value - Maximum value	<0.00606 2)	<0.00606 2)	
	Confidence interval	NA	NA	
	P value		NA	
Isoleucine	Mean value	0.0291	0.0235	0.00573 - 0.120
	Minimum value - Maximum value	0.0181 - 0.0484	0.0136 - 0.0407	
	Confidence interval	0.0189 - 0.0446	0.0153 - 0.0360	
	P value		<0.0001 1)	
Leucine	Mean value	0.0283	0.0251	0.00575 - 0.119
	Minimum value - Maximum value	0.0188 - 0.0428	0.0150 - 0.0431	
	Confidence interval	0.0204 - 0.0394	0.0181 - 0.0349	
	P value		0.0153 1)	
Lysine	Mean value	0.0558	0.0506	0.0103 - 0.254
	Minimum value - Maximum value	0.0346 - 0.432	0.0341 - 0.0719	
	Confidence interval	0.0466 - 0.0669	0.0422 - 0.0606	
	P value		0.417	

See p. 18 for footnote.

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Table 6 Free amino acid composition in the seed of the modified oilseed rape (3/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
Methionine	Mean value	0.0112	0.0115	0 - 0.0264
	Minimum value - Maximum value	<0.00606 [2)]- 0.0216	<0.00606 [2)]- 0.0224	
	Confidence interval	0.00811 - 0.0156	0.00830 - 0.0159	
	P value		0.764	
Ornithine	Mean value	<0.00606 [2)]	<0.00606 [2)]	0.00891 - 0.937
	Minimum value - Maximum value	<0.00606 [2)]	<0.00606 [2)]	
	Confidence interval	NA	NA	
	P value		NA	
Phenylalanine	Mean value	0.101	0.0887	0.0202 - 0.439
	Minimum value - Maximum value	0.0592 - 0.166	0.0450 - 0.150	
	Confidence interval	0.0690 - 0.146	0.0609 - 0.129	
	P value		<0.0001 [1)]	
Proline	Mean value	0.0873	0.0618	0.0108 - 0.831
	Minimum value - Maximum value	0.0443 - 0.253	0.0401 - 0.0935	
	Confidence interval	0.0634 - 0.120	0.0449 - 0.0850	
	P value		0.00227 [1)]	
Serine	Mean value	0.0934	0.0787	0.0186-0.520
	Minimum value - Maximum value	0.0398 - 0.142	0.0386 - 0.109	
	Confidence interval	0.0811 - 0.107	0.0684 - 0.0906	
	P value		0.0149 [1)]	
Taurine	Mean value	<0.00606 [2)]	<0.00606 [2)]	NC
	Minimum value - Maximum value	<0.00606 [2)]	<0.00606 [2)]	
	Confidence interval	NA	NA	
	P value		NA	
Threonine	Mean value	0.0658	0.0662	0.0187 - 0.181
	Minimum value - Maximum value	0.0550 - 0.0812	0.0513 - 0.0827	
	Confidence interval	0.0579 - 0.0747	0.0583 - 0.0753	
	P value		0.576	
Tryptophan	Mean value	0.0536	0.0530	0.0106 - 0.209
	Minimum value - Maximum value	0.0354 - 0.0968	0.0293 - 0.110	
	Confidence interval	0.0350 - 0.0823	0.0345 - 0.0813	
	P value		0.607	

See p. 18 for footnote.

Table 6 Free amino acid composition in the seed of the modified oilseed rape (4/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape	Variable range in our commercially available product
Tyrosine	Mean value	0.0371	0.0344	0.00994 - 0.176
	Minimum value - Maximum value	0.0245 - 0.0778	0.0242 - 0.0527	
	Confidence interval	0.0301 - 0.0457	0.0279 - 0.0425	
	P value		0.0488 1)	
Valine	Mean value	0.0597	0.0545	0.0185 - 0.396
	Minimum value - Maximum value	0.0408 - 0.0899	0.0340 - 0.0926	
	Confidence interval	0.0417 - 0.0854	0.0381 - 0.0780	
	P value		0.00738 1)	

Non-modified oilseed rape: 5536×1822 lines.
Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).
Condition for cultivation: 2009, 3 places including Manitoba, Canada, 5 places in total, including North Dakota, Washington in US. 4 plots/field, n=20.
Statistical analysis: Variance analysis using linear mixed model.
NA: Not statistical analysis to be allowed.
NC: Not calculated.
1) Statistical significance (P value<0.05).
2) Less than lower limit of quantification.

Table 7 Amino acid composition in the aboveground plant body of the modified oilseed rape (1/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Alanine	Mean value	1.63	1.61
	Minimum value - Maximum value	1.55 - 1.73	1.52 - 1.74
	Standard deviation	0.0756	0.0912
	P value		0.690
Arginine	Mean value	2.12	1.66
	Minimum value - Maximum value	1.93 - 2.39	1.51 - 1.90
	Standard deviation	0.176	0.151
	P value		0.00794 1)
Aspartic acid	Mean value	2.71	3.01
	Minimum value - Maximum value	2.57 - 2.85	2.85 - 3.35
	Standard deviation	0.119	0.197
	P value		0.0159 1)
Cystine	Mean value	0.428	0.382
	Minimum value - Maximum value	0.384 - 0.478	0.367 - 0.410
	Standard deviation	0.0420	0.0195
	P value		0.0635
Glycine	Mean value	1.38	1.39
	Minimum value - Maximum value	1.31 - 1.48	1.32 - 1.50
	Standard deviation	0.0650	0.0699
	P value		0.889
Glutamic acid	Mean value	5.65	5.90
	Minimum value - Maximum value	5.14 - 6.21	5.23 - 6.72
	Standard deviation	0.474	0.579
	P value		0.548
Histidine	Mean value	0.759	0.670
	Minimum value - Maximum value	0.697 - 0.838	0.634 - 0.744
	Standard deviation	0.0519	0.0447
	P value		0.0317 1)
Isoleucine	Mean value	1.19	1.17
	Minimum value - Maximum value	1.16 - 1.24	1.11 - 1.27
	Standard deviation	0.0313	0.0618
	P value		0.421

See p. 21 for footnote.

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Table 7 Amino acid composition in the aboveground plant body of the modified oilseed rape (2/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Leucine	Mean value	2.20	2.15
	Minimum value - Maximum value	2.11 - 2.30	2.03 - 2.35
	Standard deviation	0.0744	0.124
	P value		0.548
Lysine	Mean value	1.98	1.92
	Minimum value - Maximum value	1.91 - 2.04	1.82 - 2.13
	Standard deviation	0.0534	0.124
	P value		0.222
Methionine	Mean value	0.584	0.500
	Minimum value - Maximum value	0.527 - 0.657	0.466 - 0.533
	Standard deviation	0.0514	0.0303
	P value		0.0317 1)
Phenylalanine	Mean value	1.50	1.45
	Minimum value - Maximum value	1.40 - 1.63	1.37 - 1.58
	Standard deviation	0.0915	0.0838
	P value		0.516
Proline	Mean value	1.54	1.18
	Minimum value - Maximum value	1.44 - 1.65	1.07 - 1.29
	Standard deviation	0.101	0.0966
	P value		0.00794 1)
Serine	Mean value	1.44	1.29
	Minimum value - Maximum value	1.36 - 1.55	1.21 - 1.41
	Standard deviation	0.0727	0.0764
	P value		0.0317 1)
Threonine	Mean value	1.28	1.24
	Minimum value - Maximum value	1.20 - 1.36	1.18 - 1.34
	Standard deviation	0.0596	0.0623
	P value		0.286
Tryptophan	Mean value	0.480	0.385
	Minimum value - Maximum value	0.424 - 0.565	0.339 - 0.464
	Standard deviation	0.0663	0.0479
	P value		0.0476 1)

See p. 21 for footnote.

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Table 7 Amino acid composition in the aboveground plant body of the modified oilseed rape (3/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Tyrosine	Mean value	0.664	0.561
	Minimum value - Maximum value	0.576 - 0.706	0.527 - 0.631
	Standard deviation	0.0507	0.0403
	P value		0.0159 1)
Valine	Mean value	1.54	1.52
	Minimum value - Maximum value	1.49 - 1.61	1.45 - 1.65
	Standard deviation	0.0444	0.0800
	P value		0.421

Non-modified oilseed rape: 5536×5676 lines.
Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).
Condition for cultivation: 2012, cultivate in greenhouse at Iowa, US and collect at the time of flowering. 40 individuals were analyzed collectively with each 8 individuals (each 5 samples of the non-modified oilseed rape and the modified oilseed rape).
Statistical analysis: Mann-Whitney U test.
No variable range in our commercial available product.
1) Statistical significance (P value<0.05).

Table 8 Free amino acid composition in the aboveground plant body of the modified oilseed rape (1/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
α-Amino butyric acid	Mean value	0.0730	0.0945
	Minimum value - Maximum value	0.0606 - 0.0896	0.0660 - 0.107
	Standard deviation	0.0106	0.0168
	P value		0.0952
γ-Amino butyric acid	Mean value	3.02	3.03
	Minimum value - Maximum value	0.871 - 5.08	2.00 - 4.15
	Standard deviation	2.02	0.943
	P value		1.00
Alanine	Mean value	1.68	1.77
	Minimum value - Maximum value	1.43 - 1.93	1.50 - 2.06
	Standard deviation	0.178	0.259
	P value		0.730
Arginine	Mean value	12.5	4.83
	Minimum value - Maximum value	10.6 - 14.3	3.81 - 5.81
	Standard deviation	1.80	0.774
	P value		0.00794 [1)]
Asparagine	Mean value	3.91	4.56
	Minimum value - Maximum value	3.32 - 4.39	3.50 - 5.62
	Standard deviation	0.445	0.768
	P value		0.151
Aspartic acid	Mean value	0.287	0.315
	Minimum value - Maximum value	0.227 - 0.331	0.275 - 0.360
	Standard deviation	0.0446	0.0380
	P value		0.310
Cystine	Mean value	0.0202	0.0269
	Minimum value - Maximum value	<0.00596 [2)]-0.0359	0.0181 - 0.0350
	Standard deviation	0.0117	0.00640
	P value		0.421
Ethanol amine	Mean value	0.433	0.528
	Minimum value - Maximum value	0.395 - 0.467	0.502 - 0.569
	Standard deviation	0.0294	0.0261
	P value		0.00794 [1)]

See p. 25 for footnote.

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Table 8 Free amino acid composition in the aboveground plant body of the modified oilseed rape (2/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Glutamic acid	Mean value	1.16	1.06
	Minimum value - Maximum value	0.964 - 1.35	0.635 - 1.99
	Standard deviation	0.150	0.539
	P value		0.222
Glutamine	Mean value	43.4	37.9
	Minimum value - Maximum value	39.2 - 47.1	30.5 - 43.7
	Standard deviation	2.96	5.74
	P value		0.0952
Glycine	Mean value	0.251	0.284
	Minimum value - Maximum value	0.199 - 0.308	0.256 - 0.311
	Standard deviation	0.0388	0.0202
	P value		0.0794
Histidine	Mean value	2.96	1.28
	Minimum value - Maximum value	2.64 - 3.20	0.919 - 1.58
	Standard deviation	0.270	0.255
	P value		0.00794 [1)]
Hydroxyproline	Mean value	<0.00586 [2)]	<0.00586 [2)]
	Minimum value - Maximum value	<0.00586 [2)]	<0.00586 [2)]
	Standard deviation	NA	NA
	P value		NA
Isoleucine	Mean value	0.906	0.634
	Minimum value - Maximum value	0.732 - 1.29	0.529 - 0.809
	Standard deviation	0.221	0.109
	P value		0.0317 [1)]
Leucine	Mean value	0.817	0.505
	Minimum value - Maximum value	0.653 - 1.19	0.360 - 0.686
	Standard deviation	0.218	0.118
	P value		0.0317 [1)]
Lysine	Mean value	0.368	0.267
	Minimum value - Maximum value	0.299 - 0.427	0.225 - 0.349
	Standard deviation	0.0583	0.0505
	P value		0.0317 [1)]

See p. 25 for footnote.

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Table 8 Free amino acid composition in the aboveground plant body of the modified oilseed rape (3/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Methionine	Mean value	0.307	0.305
	Minimum value - Maximum value	0.282 - 0.327	0.263 - 0.339
	Standard deviation	0.0224	0.0361
	P value		0.952
Ornithine	Mean value	0.219	0.0831
	Minimum value - Maximum value	0.164 - 0.254	0.0743 - 0.0976
	Standard deviation	0.0344	0.00943
	P value		0.00794 1)
Phenylalanine	Mean value	0.760	0.424
	Minimum value - Maximum value	0.653 - 0.954	0.356 - 0.487
	Standard deviation	0.122	0.0508
	P value		0.00794 1)
Proline	Mean value	3.68	0.769
	Minimum value - Maximum value	2.03 - 4.99	0.366 - 1.95
	Standard deviation	1.29	0.665
	P value		0.00794 1)
Serine	Mean value	7.79	5.07
	Minimum value - Maximum value	7.20 - 8.38	4.09 - 6.06
	Standard deviation	0.513	0.798
	P value		0.00794 1)
Taurine	Mean value	<0.00596 2)	<0.00596 2)
	Minimum value - Maximum value	<0.00596 2)	<0.00596 2)
	Standard deviation	NA	NA
	P value		NA
Threonine	Mean value	1.60	1.06
	Minimum value - Maximum value	1.37 - 1.86	0.934 - 1.28
	Standard deviation	0.175	0.139
	P value		0.00794 1)
Tryptophan	Mean value	0.433	0.226
	Minimum value - Maximum value	0.379 - 0.584	0.183 - 0.290
	Standard deviation	0.0856	0.0444
	P value		0.00794 1)

See p. 25 for footnote.

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Table 8 Free amino acid composition in the aboveground plant body of the modified oilseed rape (4/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Tyrosine	Mean value	0.661	0.424
	Minimum value - Maximum value	0.568 - 0.815	0.302 - 0.523
	Standard deviation	0.0930	0.0903
	P value		0.00794 1)
Valine	Mean value	1.05	0.881
	Minimum value - Maximum value	0.905 - 1.24	0.762 - 1.08
	Standard deviation	0.127	0.120
	P value		0.0556

Non-modified oilseed rape: 5536×5676 lines.
Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).
Condition for cultivation: 2012, cultivate in greenhouse at Iowa, US, and collect at the time of flowering. 40 individuals were analyzed collectively with each 8 individuals (each 5 samples of those in the non-modified oilseed rape and the modified oilseed rape).
Statistical analysis: Mann-Whitney U test.
NA:Not statistical analysis to be allowed.
No range in variation for our commercially available product.
1) Statistical significance (P value<0.05).
2) Less than lower limit of quantification.

Table 9 Amino acid composition in the root of the modified oilseed rape (1/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Alanine	Mean value	0.545	0.626
	Minimum value - Maximum value	0.517 - 0.603	0.552 - 0.676
	Standard deviation	0.0385	0.0454
	P value		0.0317 1)
Arginine	Mean value	0.416	0.466
	Minimum value - Maximum value	0.395 - 0.440	0.394 - 0.505
	Standard deviation	0.0220	0.0425
	P value		0.151
Aspartic acid	Mean value	1.02	1.50
	Minimum value - Maximum value	0.915 - 1.20	1.36 - 1.59
	Standard deviation	0.111	0.0858
	P value		0.00794 1)
Cystine	Mean value	0.256	0.236
	Minimum value - Maximum value	0.243 - 0.272	0.214 - 0.255
	Standard deviation	0.0124	0.0171
	P value		0.111
Glycine	Mean value	0.511	0.604
	Minimum value - Maximum value	0.482 - 0.548	0.528 - 0.647
	Standard deviation	0.0323	0.0463
	P value		0.0317 1)
Glutamic acid	Mean value	2.25	2.00
	Minimum value - Maximum value	1.90 - 2.67	1.87 - 2.15
	Standard deviation	0.303	0.118
	P value		0.198
Histidine	Mean value	0.298	0.316
	Minimum value - Maximum value	0.283 - 0.311	0.282 - 0.334
	Standard deviation	0.0121	0.0204
	P value		0.135
Isoleucine	Mean value	0.503	0.551
	Minimum value - Maximum value	0.477 - 0.547	0.499 - 0.573
	Standard deviation	0.0282	0.0311
	P value		0.0952

See p. 28 for footnote.

Table 9 Amino acid composition in the root of the modified oilseed rape (2/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Leucine	Mean value	0.773	0.897
	Minimum value - Maximum value	0.732 - 0.846	0.793 - 0.940
	Standard deviation	0.0484	0.0608
	P value		0.0317 1)
Lysine	Mean value	0.767	0.935
	Minimum value - Maximum value	0.710 - 0.894	0.796 - 0.988
	Standard deviation	0.0810	0.0789
	P value		0.0317 1)
Methionine	Mean value	0.290	0.269
	Minimum value - Maximum value	0.274 - 0.313	0.240 - 0.289
	Standard deviation	0.0183	0.0188
	P value		0.286
Phenylalanine	Mean value	0.463	0.536
	Minimum value - Maximum value	0.441 - 0.491	0.462 - 0.571
	Standard deviation	0.0231	0.0440
	P value		0.0317 1)
Proline	Mean value	0.524	0.475
	Minimum value - Maximum value	0.466 - 0.603	0.434 - 0.503
	Standard deviation	0.0499	0.0264
	P value		0.0952
Serine	Mean value	0.610	0.644
	Minimum value - Maximum value	0.554 - 0.674	0.569 - 0.681
	Standard deviation	0.0460	0.0435
	P value		0.310
Threonine	Mean value	0.492	0.565
	Minimum value - Maximum value	0.466 - 0.539	0.507 - 0.589
	Standard deviation	0.0302	0.0340
	P value		0.0159 1)
Tryptophan	Mean value	0.160	0.154
	Minimum value - Maximum value	0.154 - 0.166	0.148 - 0.157
	Standard deviation	0.00483	0.00344
	P value		0.0714

See p. 28 for footnote.

Table 9 Amino acid composition in the root of the modified oilseed rape (3/3)

(% dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Tyrosine	Mean value	0.344	0.345
	Minimum value - Maximum value	0.339 - 0.349	0.310 - 0.367
	Standard deviation	0.00397	0.0212
	P value		0.246
Valine	Mean value	0.614	0.691
	Minimum value - Maximum value	0.578 - 0.678	0.619 - 0.722
	Standard deviation	0.0409	0.0420
	P value		0.0317 1)

Non-modified oilseed rape: 5536×5676 lines.

Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).

Condition for cultivation: 2012, cultivate in greenhouse at Iowa, US, and collect at the time of flowering. 40 individuals were analyzed collectively with each 8 individuals (each 5 samples of those in the non-modified oilseed rape and the modified oilseed rape).

Statistical analysis: Mann-Whitney U test.

No range in variation for our commercially available product.

1) Statistical significance (P value<0.05).

Table 10 Free amino acid composition in the root of the modified oilseed rape (1/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
α-Amino butylic acid	Mean value	0.0962	0.0731
	Minimum value - Maximum value	0.0359 - 0.214	0.0262 - 0.119
	Standard deviation	0.0723	0.0434
	P value		0.690
γ-Amino butylic acid	Mean value	3.66	3.13
	Minimum value - Maximum value	3.04 - 4.15	2.43 - 3.82
	Standard deviation	0.568	0.611
	P value		0.206
Alanine	Mean value	1.20	0.897
	Minimum value - Maximum value	1.01 - 1.35	0.741 - 1.16
	Standard deviation	0.134	0.163
	P value		0.0317 [1)]
Arginine	Mean value	0.668	0.464
	Minimum value - Maximum value	0.403 - 0.994	0.388 - 0.536
	Standard deviation	0.226	0.0699
	P value		0.0556
Asparagine	Mean value	2.28	1.05
	Minimum value - Maximum value	1.44 - 3.25	0.709 - 1.53
	Standard deviation	0.646	0.303
	P value		0.0159 [1)]
Aspartic acid	Mean value	0.384	0.317
	Minimum value - Maximum value	0.270 - 0.585	0.224 - 0.443
	Standard deviation	0.126	0.0897
	P value		0.548
Cystine	Mean value	<0.00596 [2)]	0.00497
	Minimum value - Maximum value	<0.00596 [2)]	<0.00596 [2)]-0.00808
	Standard deviation	NA	NA
	P value		NA
Ethanol amine	Mean value	0.589	0.572
	Minimum value - Maximum value	0.530 - 0.625	0.527 - 0.617
	Standard deviation	0.0417	0.0440
	P value		0.548

See. p. 32 for footnote.

Table 10 Free amino acid composition in the root of the modified oilseed rape (2/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Glutamic acid	Mean value	0.825	0.564
	Minimum value - Maximum value	0.465 - 1.39	0.289 - 1.01
	Standard deviation	0.360	0.323
	P value		0.222
Glutamine	Mean value	18.0	8.96
	Minimum value - Maximum value	13.6 - 24.0	6.55 - 11.3
	Standard deviation	4.07	1.85
	P value		0.00794 1)
Glycine	Mean value	0.363	0.283
	Minimum value - Maximum value	0.222 - 0.464	0.211 - 0.393
	Standard deviation	0.108	0.0796
	P value		0.151
Histidine	Mean value	1.22	0.540
	Minimum value - Maximum value	1.00 - 1.48	0.486 - 0.651
	Standard deviation	0.193	0.0660
	P value		0.00794 1)
Hydroxyproline	Mean value	<0.00586 2)	<0.00586 2)
	Minimum value - Maximum value	<0.00586 2)	<0.00586 2)
	Standard deviation	NA	NA
	P value		NA
Isoleucine	Mean value	0.904	0.463
	Minimum value - Maximum value	0.532 - 1.34	0.329 - 0.815
	Standard deviation	0.291	0.200
	P value		0.0159 1)
Leucine	Mean value	0.483	0.308
	Minimum value - Maximum value	0.243 - 0.733	0.177 - 0.531
	Standard deviation	0.176	0.136
	P value		0.222
Lysine	Mean value	0.241	0.271
	Minimum value - Maximum value	0.142 - 0.329	0.188 - 0.324
	Standard deviation	0.0700	0.0516
	P value		0.548

See p. 32 for footnote.

Table 10 Free amino acid composition in the root of the modified oilseed rape (3/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Methionine	Mean value	0.158	0.169
	Minimum value - Maximum value	0.136 - 0.173	0.141 - 0.211
	Standard deviation	0.0154	0.0260
	P value		0.690
Ornithine	Mean value	0.257	0.317
	Minimum value - Maximum value	0.189 - 0.372	0.246 - 0.404
	Standard deviation	0.0864	0.0607
	P value		0.310
Phenylalanine	Mean value	0.376	0.289
	Minimum value - Maximum value	0.281 - 0.493	0.245 - 0.398
	Standard deviation	0.0826	0.0651
	P value		0.0952
Proline	Mean value	2.18	0.267
	Minimum value - Maximum value	0.976 - 3.81	0.179 - 0.549
	Standard deviation	1.19	0.159
	P value		0.00794 1)
Serine	Mean value	4.20	2.15
	Minimum value - Maximum value	3.42 - 5.17	1.69 - 2.56
	Standard deviation	0.706	0.416
	P value		0.00794 1)
Taurine	Mean value	<0.00596 2)	<0.00596 2)
	Minimum value - Maximum value	<0.00596 2)	<0.00596 2)
	Standard deviation	NA	NA
	P value		NA
Threonine	Mean value	1.01	0.775
	Minimum value - Maximum value	0.816 - 1.27	0.645 - 0.912
	Standard deviation	0.165	0.103
	P value		0.0317 1)
Tryptophan	Mean value	0.233	0.223
	Minimum value - Maximum value	0.126 - 0.407	0.171 - 0.319
	Standard deviation	0.109	0.0621
	P value		1.00

See p. 32 for footnote.

Table 10 Free amino acid composition in the root of the modified oilseed rape (4/4)

(mg/g dried weight)

Items to be analyzed		Non-modified oilseed rape	Modified oilseed rape
Tyrosine	Mean value	0.378	0.344
	Minimum value - Maximum value	0.279 - 0.506	0.258 - 0.425
	Standard deviation	0.0970	0.0743
	P value		0.548
Valine	Mean value	1.18	0.621
	Minimum value - Maximum value	0.684 - 1.81	0.477 - 0.996
	Standard deviation	0.405	0.216
	P value		0.0159 1)

Non-modified oilseed rape: 5536×5676 lines.
Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).
Condition for cultivation: 2012, cultivate in greenhouse at Iowa, US and collect at the time of flowering. 40 individuals were analyzed collectively with each 8 individuals (each 5 samples of those in the non-modified oilseed rape and the modified oilseed rape).
Statistical analysis: Mann-Whitney U test.
NA:Not statistical analysis to be allowed.
No range in variation for out commercially available product.
1) Statistical significance (P value<0.05).
2) Less than lower limit for quantification.

Table 11 Mortality rate and weight of cabbageworm in the feeding test

Items to be examined		Non-modified oilseed rape	Modified oilseed rape
mortality rate (%)[1)]		10	6.7
	P value[2)]		0.8234
Weight (mg)[3)]	Mean value	75.1	90.7
	95%Confidence interval	62.5 - 87.8	83.3 - 98.1
	Minimum value - Maximum value	3.7 - 123.1	39.4 - 114.7
	P value[4)]		0.9823

Non-modified oilseed rape: 5536×5676 lines.
Modified oilseed rape: F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).
Number of worms to be provided: Each 30 individuals.
1) Number of mortal of worm: Non-modified oilseed rape 3, Modified oilseed rape 2.
2) Statistical analysis: Fisher's exact test.
3) Weight of living worm.
4) Statistical analysis: t test.

Table 12 *N*-acetyl amino acids in the leaf used for the feeding test

(μg/g freeze-dried weight)

Items to be analyzed	Non-modified oilseed rape	Modified oilseed rape
N-acetyl aspartic acid	0.6125	6591
N-acetyl glutamic acid	4.662	47.38
N-acetyl glycine	0.1086	0.2593
N-acetyl serine	5.721	10.44
N-acetyl threonine	3.092	10.12

The leaves of 15 individuals cultivated in greenhouse are collected to analyze them for 3 times and to calculate its mean value.

Table 13 Living insect and degree of feeding damage in the field test at Canada of the modified oilseed rape

Year at cultivation	Region (State)	Living insects	Degree of feeding damage	Difference with the non-modified oilseed rape
2008	Morden (Manitoba)	Flea beetle and Lygus bug	Mild	None
	Rosebank (Manitoba)	Flea beetle and Lygus bug	Mild	None
	Crystal City (Manitoba)	Flea beetle	Mild	None
	Carman (Manitoba)	Flea beetle	Mild	None
	Georgetown (Ontario)	Flea beetle, large white and Lygus bug	Mild	None
2009	Fort Saskatchewan (Alberta)	Locust and Cutworm	Mild to severe	None
	Gibbons (Alberta)	Locust andCutworm	Mild to severe	None
	Riviere Qui Barre (Alberta)	Flea beetle, Thrips and Cutworm	Mild	None
	Minto (Manitoba)	Large white, cabbage aphid, Flea beetle and Thrips	Mild	None
	Rosebank (Manitoba)	Flea beetle	Mild	None
	Portage la Prairie (Manitoba)	Flea beetle	Mild	None
	Wellwood (Manitoba)	Flea beetle	Mild	None
	Franklin (Manitoba)	Flea beetle	Mild	None
	Dundurn (Saskatchewan)	Alfalfa looper	Mild	None

Modified oilseed rape: 2008, T3 generation, 2009, F1*4 generation (Figure 3, p. 40; Confidential and non-disclosed).

Non-modified oilseed rape: 1822 lines.

14 fields in total.

Insect observation was conducted for one or more time per 4 weeks and evaluate the degree of feeding damage using following scales.

Mild: Less than 10% in the degree of feeding damage (few damage).

Moderate: 10% or 30% in the degree of feeding damage (remarkable damage).

Severe: 30% or more in the degree of feeding damage (severe damage).

Thrips: *Thrips tabaci*
Cabbage aphid: *Brevicoryne brassicae*
Alfalfa looper: *Autographa californica*
Large white: *Pieris brassicae*
Cutworm:
Euxoa ochrogaster
Cabbage moth: *Plutella xylostella*

Fleahopper: *Phyllothreta cruciferae*
Or *Phyllothreta striolata*
Locust: *Melanoplus sanguinipes*
Lygus bug: *Lygus sp.*
Green peach aphid: *Myzus persicae*
Cabbage white: *Pieris rapae*
Cabbage armyworm: *Mamestra brassicae*

Table 14 Living insect and degree of feeding damage in the field test at US of the modified oilseed rape

Year at cultivation	County (State)	Living insects	Degree of feeding damage	Difference with the non-modified oilseed rape
2007	Imperial (California)	Flea beetle and green peach aphid	Mild to Moderate	None
2008	Imperial (California)	Flea beetle, green peach aphid and cabbage aphid	Mild	None
2009	McHenry (North Dakota)	Flea beetle	Mild	None
	Grant (Washington)	Large white	Mild	None
	Imperial (California)	Flea beetle and green peach aphid	Mild	None
2010	Cass (North Dakota)	Locust, cabbage aphid, cabbage moth, cabbage white and cabbage armyworm	Mild to Moderate	None
	Ward (North Dakota)	Flea beetle	Mild	None
	Grand Forks (North Dakota)	Flea beetle, cabbage moth and cabbage aphid	Mild	None
	McHenry (North Dakota)	Flea beetle	Mild	None
	Grant (Washington)	Green peach aphid	Mild	None

Modified oilseed rape: T3 generation in 2007 and 2008, F1*[4] generation in 2009, F1*[5] generation, 2010 (Figure 3, p. 40; Confidential and non-disclosed).

Non-modified oilseed rape: 1822 lines in 2007, 2008 and 2009, 5536×1822 lines in 2010.

10 fields in total.

See the footnote in Table 13 (p. 34) for standards for evaluation.

Table 15 Feeding damage in the field test at North America in 2009

Mean value (minimum value – maximum value)

States to be tested	Non-modified oilseed rape	Modified oilseed rape	P value
(i) Saskatchewan (Canada) (4 repeated)	8 (6 – 9)	9 (8 – 9)	—
(ii) Saskatchewan (Canada) (3 repeated)	8 (7 – 8)	8 (8 – 8)	—
(iii) Washington (US) (4 repeated)	3 (3 – 3)	3 (3 – 3)	—
All fields (11 repeated in total)	6 (3 – 9)	7 (3 – 9)	0.165

Non-modified oilseed rape: 1822 lines.
Modified oilseed rape: T5 generation (Figure 3, p. 40; Confidential and non-disclosed).
For (iii), the insecticides were sprayed at 2 months after seeding.
The damage was evaluated with the following 9 scales, during the end of flowering to the matured stage.

1: Serious damage, the plant body is not functioned or fired.
2: Serious damage, the plant body is not functioned as a normal state.
3: Large damage, the plant body is suffered with high stress.
4: Large damage, the health of the plant is lowered.
5: Observable damage, the plant is suffered from the sign of stress.
6: Observable damage, the plant body is healthy.
7: Few observable damage, the plant body is healthy.
8: Few damage, the plant body is healthy.
9: No damage, the plant body is healthy.

Statistical analysis: Variance analysis using linear mixed model.

Table 16 Feeding damage in the field test at North America in 2010

Mean value (minimum value – maximum value)

States to be tested	Non-modified oilseed rape	Modified oilseed rape	P value
(i) Manitoba (Canada)	9 (9 - 9)	9 (9 - 9)	—
(ii) North Dakota (US)	8 (7 - 8)	8 (8 - 8)	—
(iii) North Dakota (US)	8 (8 - 8)	8 (8 - 8)	—
(iv) North Dakota (US)	7 (6 - 8)	8 (7 - 9)	—
(v) Saskatchewan (Canada)	9 (9 - 9)	9 (9 - 9)	—
(vi) Saskatchewan (Canada)	9 (9 - 9)	9 (8 - 9)	—
(vii) Washington (US)	9 (9 - 9)	9 (9 - 9)	—
All fields	8 (6 - 9)	9 (7 - 9)	0.502

4 repeated in each filed, 28 repeated in total.
Non-modified oilseed rape: 5536×1822 lines.
Modified oilseed rape: F1*[5] generation (Figure 3, p. 40; Confidential and non-disclosed).
The insecticides were sprayed at 19 days after seeding for (i), at 2 months after seeding for (iv), at 10 days after seeding for (vii), respectively.
See the footnote in Table 15 (p. 36) for standards for evaluation.
Statistical analysis: Generalized Cochran-Mantel-Haenszel test (because the data is not distributed as the normal probability distribution).

(2) Information concerning vector

1) Name and origin

The vector is PHP 28181A, which is linear DNA fragment. This linear DNA fragment PHP28181A is a fragment that a plasmid PHP28181 which was produced from the plasmid pUC19 derived from E. coli (*Escherichia coli*) is cleaved with restriction enzymes of *Hin*d III(1) and *Not* I(2,113) (Figure 2, p. 39).

2) Properties

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

The number of base pairs of the linear DNA fragment PHP28181A is 2,112 bp, and its nucleotide sequence is shown in p. 10 of Attachment 1 (Confidential and non-disclosed.

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

There is no nucleotide sequence having any specific functions for the linear DNA fragment PHP28181A.

Outer skeleton region of the plasmid PHP28181 contains the antibiotic ampicillin tolerance gene *bla*(Ap^R) (Sutcliffe, 1978; Yanisch-Perron, *et al.*, 1985). The gene serves as a marker for selecting microorganisms containing the transforming plasmid when the vector is propagated in the microorganism. It was confirmed that the antibiotic tolerance gene is not introduced in the recipient organism.

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

There is no data for infectivity for the vector.

(3) Method of preparing living modified organisms

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

Nucleic acid composition of the linear DNA fragment PHP28181A as transformed is shown in Figure 2 (p. 39).

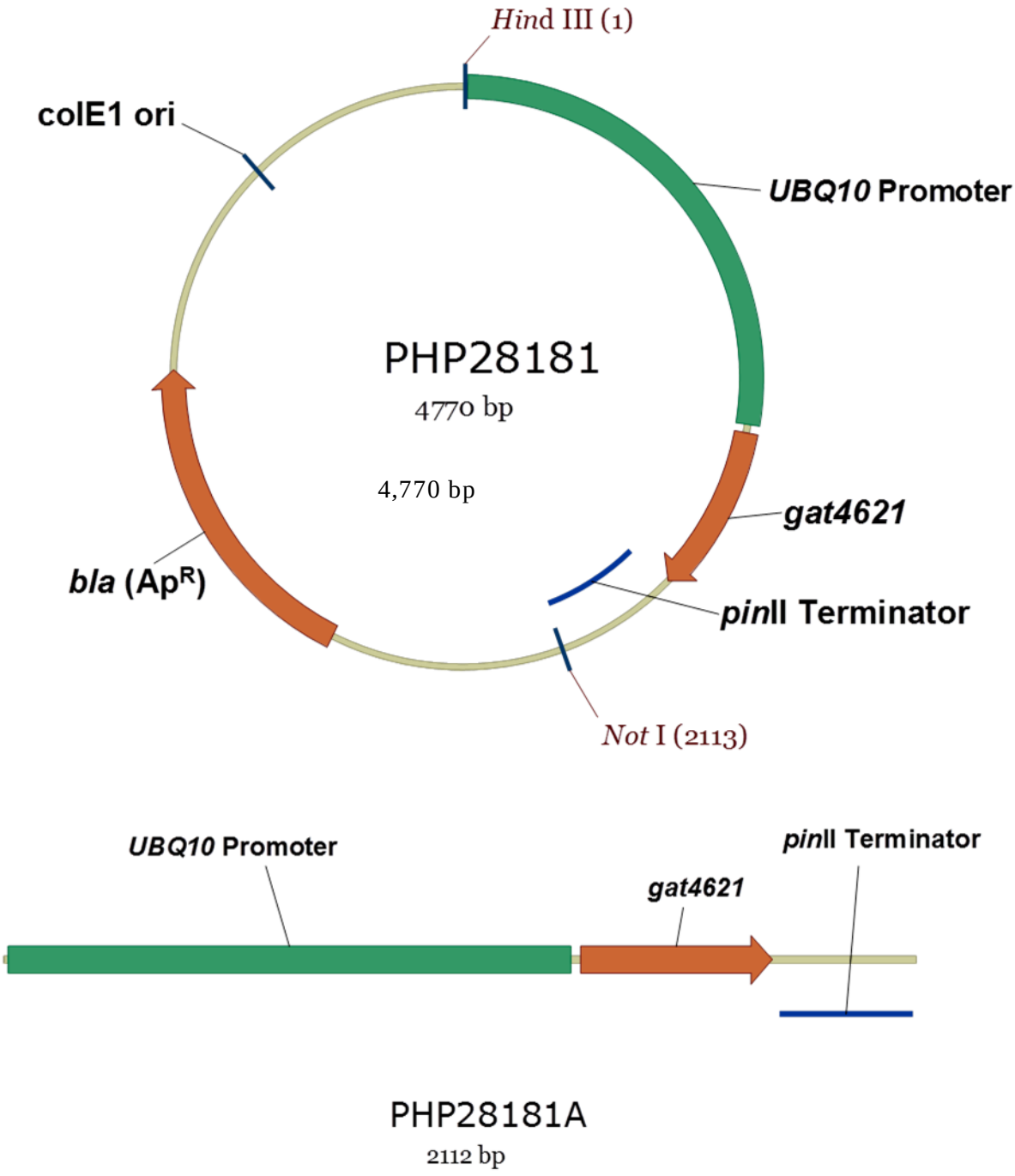

Figure 2 Composition of donor nucleic acid and cleavage site with restriction enzymes in the plasmid PHP28181 and the linear DNA fragment PHP28181A

Top of the figure: Plasmid PHP28181.

Bottom of the figure: Linear DNA fragment PHP28181A which was cleaved with the restriction enzymes of *Hin*d III and *Not* I.

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

The linear DNA fragment PHP28181A was transferred by particle gun method.

3) Processes of rearing living modified organisms

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

The embryo was cultured in a medium in which the herbicide glyphosate for 4 weeks to select a grown embryo tolerant to the herbicide glyphosate (T0 generation).

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

—

(c) Process of rearing and pedigree trees of the following lines; cells to which the nucleic acid was transferred, the line in which the state of existence of replication products of transferred nucleic acid was confirmed, the line subjected to isolated field tests; and the line used for collection of other necessary information for assessment of Adverse Effects on Biological Diversity

Each generation used for confirmation is shown in Figure 3 (p. 40; Confidential and non-disclosed) of the following figure of rearing process. The range to be approved in the present application (-) is T2 or later generations.

(Confidential and non-disclosed)

Figure 3 Rearing process for the modified oilseed rape

(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

Segregation ratio of *gat4621* gene was tested with tolerance to the herbicide glyphosate, using T1F2 and T3F3 generations of the modified oilseed rape (Attachment 3; Confidential and non-disclosed). As the result, as shown in Table 17 (p. 41), the ratio of presence or absence of the tolerance to the herbicide glyphosate was 3:1, as an expected value. Since the *gat4621* gene is shown to be stably transferred in line with mendelian inheritance, it was considered that the replication product of transferred nucleic acid exists on the genome of chromosome of oilseed rape.

Table 17 Separation ratio of *gat4621* gene using the tolerance to the herbicide glyphosate as an index

Generations	Total number of individuals	Expected value (3:1)		Measured value		χ^2 value
		Number of tolerated individuals	Number of sensitive individuals	Number of tolerated (living) individuals	Number of sensitive (fired) individuals	
T1F2	75	56.25	18.75	54	21	0.360
T3F3	99	74.25	24.75	72	27	0.273

Condition for test: Cultivate the modified oilseed rapes at T1F2 and T3F3 generations in greenhouse. At 14 days (T1F2 generation) or 10 days (T3F3 generation) after seeding, 2.7 kg a.e./ha (4 times of maximum amount in use registered as agrichemicals in US and Canada) of the herbicide glyphosate was sprayed. At 7 days after spraying, whether or not the tolerance is present was evaluated.

Statistical analysis: Chi square text. Rejection limit of null hypothesis at 5% level is 3.84.

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

As the result of southern blotting analysis using leaves at T2, T3, T3F2, T3F3 and F1*[2] generation, it was confirmed that one copy of full length gene expression cassette was transferred in either generation, and the transferred gene was stably transferred (Attachment 4; Confidential and non-disclosed).

3) Positional relationship in the case of multiple copies existing in chromosome

—

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

In order to confirm the expression stability of GAT4621 protein in the modified oilseed rape, analyses were performed by a test of spraying herbicide and ELISA method (Attachment 5; Confidential and non-disclosed).

Test of spraying herbicide

As the result of the test of spraying the herbicide glyphosate using T2 and T3 generations, it was confirmed that the traits of the tolerance to the herbicide provided for the modified oilseed rape was stably inherited (Table 18, p. 42).

Table 18 Tolerance to spraying the herbicide

Modified oilseed rape		Non-modified oilseed rape [3)]
T2 generations [1)]	7.9 ± 0.4 (7 - 8)	1.0 ± 0
T3 generation [2)]	8.8 ± 0.4 (8 - 9)	

Mean value±Standard deviation (minimum value – maximum value).
1) n=8. 2) n=64. 3) 1822 lines, n=8.
Condition for test: 2.7 kg a.e./ha (4 times of maximum amount in use registered as agrichemicals in US and Canada) of the herbicide glyphosate was sprayed to 3- to 4-leaf stage. At 14 days (T2 generation) or 11 days (T3 generation), the tolerance was evaluated according to the following 1 to 9 scales.

Scale: 1 = Fired.
2 = Severe bleaching (chlorosis), severe necrotic spot, severe inhibition for growth.
3 = Severe bleaching, severe necrotic spot, inhibition for growth.
4 = Severe bleaching, middle firing (necrosis), inhibition for growth.
5 = Middle to severe bleaching, no necrosis, slight inhibition for growth.
6 = Slight to middle bleaching, but recovered, no necrosis, few inhibition for growth.
7 = Slight bleaching, but completely recovered, no necrosis and no inhibition for growth.
8 = Slight bleaching, but completely recovered, no necrosis and no inhibition for growth.
9 = Healthy.

ELISA analysis

As the result of measuring expressed protein by ELISA method using leaves of T3 and T3F1 generations, it was confirmed that the GAT4621 protein was produced at several generations (Table 19, p. 43).

Table 19 Expressed amount of the GAT4621 protein in the leaf

(ng/mg dried weight)

Modified oilseed rape		Non-modified oilseed rape [3]
T3 generation [1]	9.1 (7.0 - 11)	Not Detected[4]
T3F1 generation [2]	8.8 ± 0.91 (7.4 - 10)	

Mean value±Standard deviation (minimum value – maximum value).

1) n=2. Cultivate 10 individuals at T3 generation, collect leaves at 24 days after seeding, and each 5 individuals were collected as 2 samples.

2) n=7. The modified oilseed rape at T3 generation and the non-modified oilseed rape 7 lines (except 1822 lines. See Figure 3, p. 40; Confidential and non-disclosed) are bred each other to produce 7 lines of hybrids (T3F1 generation). Cultivate 16 individuals of each hybrid, collect leaves in each hybrid at 24 days after seeding, and each 16 individuals were collected as 7 samples in total.

3) 1822 lines, 4474 lines, 4082×3932 lines and 5536×3932 lines. n=4.

4) Lower limit of detection: 0.29 ng/mg dried weight.

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

Since the transferred nucleic acid does not contain any transmittable sequences, there is no possibility to transfer it to wild animals or wild plants via virus infection and other route.

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

Methods of detection and identification:

Real time quantitative PCR analysis using the following primer pair (Attachment 6; Confidential and non-disclosed).

- Primer pair specific to the modified oilseed rape: a gene to be inserted and its 5' end of a boundary region of genome of oilseed rape were amplified (

Figure 4, p. 44; Table 4, p. 24 of Attachment 6; Confidential and non-disclosed)

- Endogenous gene primer pair (control): Endogenous *FatA* gene of oilseed rape was amplified (Table 2, p. 23 of Attachment 6; Confidential and non-disclosed

The size of the amplified product in case of using the specific primer pair is 84 bp, and those in case using the endogenous gene primer pair is 151 bp.

The amplified product is confirmed by the endogenous gene primer pair in any of the non-modified oilseed rape and the modified oilseed rape. In the case of the specific

primer pair, only the modified oilseed rape can be detected in case of using the specific primer pair. Accordingly, the modified oilseed rape can be identified by using both primer pairs.

Sensitivity (Genome DNA of the modified oilseed rape / Genome DNA of oilseed rape × 100):

- Lower limit of quantification: 0.08 %
- Lower limit of detection: 0.04 %

Reliability:

As the result of analyzing each 2 repeat at 2 portions using the modified oilseed rape at T5 generation, it was confirmed that the reproducibility was obtained by the present method.

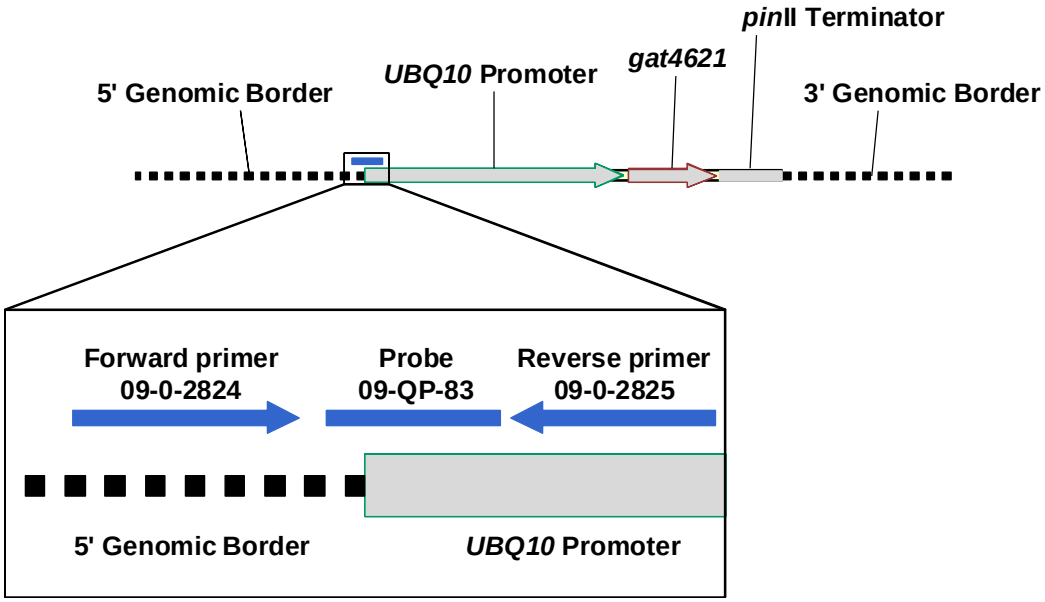

Figure 4 Portion which was amplified by the real time quantitative PCR analysis

The gene to be inserted and its 5' end of the boundary region of genome of oilseed rape were amplified by the primer pairs specific to the modified oilseed rape (Forward primer and Reverse primer).
The dotted line indicates the genome of oilseed rape.

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

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

The trait which was provided by the expression of *gat4621* gene is a tolerance to the herbicide glyphosate (Table 18, p. 42).

2) With respect to the physiological or ecological characteristics listed below, presence or absence of difference between genetically modified agricultural products and the taxonomic species to which the recipient organism belongs, and the degree of difference, if present

In 2011, isolated field tests were conducted at Utsunomiya office of DuPont Kabushiki Kaisha, the difference between the modified oilseed rape and the taxonomic species to which the recipient organism belongs was reviewed using the following a to g as an index (Attachment 7; Confidential and non-disclosed). For the test, F1*5 generation as the modified oilseed rape and 5536×1822 lines as the non-modified oilseed rape were used.

The investigation for e. Yield and f. Crossability was conducted in North America.

(a) Morphological and growth characteristics

Germination rate, date of uniformity of germination, color of leaf, period of flowering, mature period, plant height, number of primarily branches, aboveground weight, plant type, number of flowers on main stem, pod length, grain color, and whether or not the size of seed is aligned were investigated (p. 7 to 12 of Attachment 7; Confidential and non-disclosed). As the result, the germination rate, the number of primarily branches, the aboveground weight and the pod length were statistically significantly lowered, compared with those in the non-modified oilseed rape (Table 20, p. 46). The date of uniformity of germination was later by 1 day, than those in the non-modified oilseed rape. There was no difference in the remaining items to be investigated between the modified oilseed rape and the non-modified oilseed rape.

Table 20 Result of investigation of the morphological and growth characteristics

Items	Non-modified oilseed rape		Modified oilseed rape		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
Germination rate (%) [1)]	84.8	—	77.8	—	0.04662*
Date of uniformity of germination	April 27	—	April 28	—	—
Color of leaf	Greenish yellow	—	Greenish yellow	—	—
Period of flowering	June 4	—	June 4	—	—
Mature period	August 9	—	August 9	—	—
Plant height (cm) [2)]	200.4	168.8 - 232.0	212.8	181.1 - 244.5	0.1962
Number of primarily branches[2)]	9.7	9.0 - 10.4	8.8	8.0 - 9.5	0.03395 *
Aboveground weight (g) [2)]	644.0	324.0 - 964.0	506.9	184.8 - 829.1	0.01256 *
Plant type [2)]	I	—	I	—	—
Number of flowers on main stem [2)]	105.9	95.7 - 114.4	100.5	89.0 - 109.8	0.2774
Pod length (cm) [3)]	7.49	7.25 - 7.72	6.87	6.64 - 7.11	0.006905 *
Grain color [4)]	Gray-brown	—	Gray-brown	—	—
Whether or not the size of seed is alighned [4)]	Middle	—	Middle	—	—

1) Seed 270 grains. Statistical analysis: Fisher's exact test.
2) Modified oilseed rape n=36, Non-modified oilseed rape n=35. Statistical analysis: Linear mixed model.
3) n=18. Statistical analysis: Linear mixed model.
4) n=3.
* Statistically significance (P<0.05).

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

Cold tolerance at the early stage of growth:

Degree of cold damage at the early stage of growth was visually determined (p. 14 of Attachment 7; Confidential and non-disclosed). As the result, no statistically significance ($P<0.05$) was not observed with the non-modified oilseed rape, in any data to be investigated (Table 21, p. 47).

Table 21 Degree of cold damage at the early stage of growth

Date to be investigated	Non-modified oilseed rape (n=23)		Modified oilseed rape (n=22)		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
14 day after	6.3	5.5 - 7.1	6.4	5.6 - 7.2	0.5696
27 day after	6.1	5.3 - 6.9	6.2	5.3 - 7.0	0.8566

Seed it at November 11, and breed for 14 days in greenhouse, then transfer to an open space, and visually observe and determine an area of leaf showing wilt, decoloring and necrosis, at 14 days (December 9) and 27 days (December 22) thereafter (Degree of damage: 1=No effect, 2=1 to 20%, 3=21 to 40%, 4=41 to 60%, 5=61 to 80%, 6=81 to 99%, 7=100%).
Statistical analysis: Linear mixed model.

High temperature tolerance at the early stage of growth:

Germination rate and living rate, bolting ratio, plant height, and aboveground dried weight at 27 days after seeding were investigated (p. 15 to 16 of Attachment 7; Confidential and non-disclosed). As the result, no statistically significance ($P<0.05$) with the non-modified oilseed rape was observed in any items to be investigated (Table 22, p. 47).

Table 22 Result of investigation for the high temperature tolerance at the early stage of growth

Items	Non-modified oilseed rape		Modified oilseed rape		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
Germination rate (%) [1)]	80.0	—	73.0	—	0.06754
Living rate (%) [2)]	94.4	—	94.4	—	1.000
Bolting ratio (%) [2)]	0.0	—	0.0	—	1.000
Plant height (cm) [3)]	24.02	21.93 - 26.11	24.72	22.64 - 26.80	0.5707
Aboveground dried weight (mg) [3)]	4099	2971 - 5228	3914	2787 - 5042	0.7730

Seed at an open space on July 22, and cultivate for 4 weeks.
1) Seed 270 grains of each line. Statistical analysis: Fisher's exact test.
2) n=90. Statistical analysis: Fisher's exact test.
3) Modified oilseed rape: n=22, Non-modified oilseed rape: n=24. Statistical analysis: Linear mixed model.

c Wintering ability and summering ability of the mature plant

As the result of investigating a degree of firing at the harvest period, no statistical significance with the non-modified oilseed rape is observed (Table 23, p. 48; p. 7 to 12 of Attachment 7; Confidential and non-disclosed).

Table 23 Result of investigation of degree of firing

Item	Non-modified oilseed rape		Modified oilseed rape		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
Degree of motality	3.4	2.7 - 4.3	3.9	3.1 - 4.9	0.3159

At August during the harvest period, degree of browning and yellowing at its stem (main stem, branched stem and flower stem) was visually determined (Degree of browning and yellowing: 1=All green (no browning and no yellowing), 2=1 to 20%, 3=21 to 40%, 4=41 to 60%, 5=61 to 80%, 6=81 to 99%, 7=Firing). Modified oilseed rape: n=36, Non-modified oilseed rape: n=35. Statistical analysis: Linear mixed model.

(d) Fertility and pollen size

Dyeing ratio of pollen by iode and potassium iodide liquid and long diameter thereof were investigated (p. 21 of Attachment 7; Confidential and non-disclosed). As the result, all of the pollen which was treated with iode and potassium iodide solution was stained therewith, and any abnormality of the pollen was not observed. Accordingly, it was considered that it had fertility. No statistical significance ($P<0.05$) in the long diameter thereof was observed with the non-modified oilseed rape (Table 24, p. 48).

Table 24 Result of investigation of long diameter of pollen

Items	Non-modified oilseed rape		Modified oilseed rape		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
Long diameter (μm)	41.38	39.81 - 42.85	40.94	39.32 - 42.43	0.2266

n=36. Statistical analysis: Linear mixed model.

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

Production of the seed:

As the result of investigating pod shooting rate, total pod shooting number, number of seeds per pod and weight of a fifty grains, the pod shooting rate and the total pod shooting number were statistically significantly lowered in comparison with the non-modified oilseed rape ($P<0.05$) (Table 25, p. 49; p. 7 to 12 of Attachment; Confidential and non-disclosed).

Table 25 Result of investigation of production of the seed

Items	Non-modified oilseed rape		Modified oilseed rape		P value (*: Statistical significance P<0.05)
	Mean value	Confidence interval	Mean value	Confidence interval	
Pod shooting rate (%) [1)]	65.6	58.9 - 71.7	46.9	39.9 - 54.0	<0.0001 *
Total pod shooting number [2)]	1347	775.6 - 1918	951.6	377.1 - 1526	0.0002109 *
Number of seed per pod [3)]	30.7	29.7 - 31.6	30.4	29.4 - 31.4	0.5390
Weight of 50 grains (mg) [4)]	172.1	165.1 - 179.1	177.7	170.8 - 184.7	0.2053

1) Modified oilseed rape n=36, Non-modified oilseed rape n=35. Statistical analysis: Generalized linear mixed model in which the probability distribution is arranged to be the binomial distribution and logistic link function is used.
2) Modified oilseed rape n=36, Non-modified oilseed rape n=35. Statistical analysis: Linear mixed model.
3) n=18. Statistical analysis: Linear mixed model.
4) n=9. Statistical analysis: Linear mixed model.

In the field test which was conducted in North America, no statistical significance (P value<0.05) in the seed is observed (Table 26, p. 49).

Table 26 Yield of the seed in the field test in North America

(kg/ha)

	Non-modified oilseed rape [1)]	Modified oilseed rape [1)]	Conventional actual value of non-modified oilseed rape [2)]
Mean value	1830	1860	118 - 6630
Minimum value - Maximum value	575 - 2960	404 - 3190	
Confidence interval	1090 - 2560	1130 - 2600	
P value		0.620	

1) Condition for cultivation: 2010, 7 fields in North America (2 places in Saskatchewan and 1 place in Manitoba in Canada, and 3 places in North Dakoda and 1 place in Washington, US). Each 4 repeated. n=28.
Modified oilseed rape: F1*[5] generation (Figure 3, p. 40; Confidential and non-disclosed).
Non-modified oilseed rape: 5536×1822 lines.
Statistical analysis: Linear mixed model.
2) Cultivate at 2008 and 2009.
Condition for cultivation: •2008, 5 fields in North America (Albata, Manitoba and Ontario in Canada, and North Dakota and Washington in US). Each 3 repeated. n=15. 3 lines of non-modified oilseed rape (46A65, 45H72 and 45H73).
• 2009, 5 fields in North America (3 places in Manitoba, Canada, and North Dakota and Washington in US). Each 4 repeated. n=20. 4 lines of non-modified oilseed rape (46H02, 46A65, 44A89 and 45H73).

Shedding habit:

As the result of investigating the shedding habit, no statistical significance is observed with the non-modified oilseed rape (Table 27, p. 50; p. 7 to 12 of Attachment 7; Confidential and non-disclosed).

Table 27 Result of investigation of the shedding habit

Items	Non-modified oilseed rape		Modified oilseed rape		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
Shedding habit (%)	3.05	1.64 - 5.60	4.59	2.49 - 8.31	0.3519

Modified oilseed rape n=36, Non-modified oilseed rape n=35.
Statistical analysis: Generalized linear mixed model in which the probability distribution is arranged to be the binomial distribution and logistic link function is used.

Dormancy and germination rate:

Seed the seed at the time of harvest and investigate the germination rate. As the result, no statistical significance ($P<0.05$) was observed with the non-modified oilseed rape (Table 28, p. 50; p. 13 of Attachment 7; Confidential and non-disclosed).

Table 28 Germination rate of the seed just after harvesting

Items	Non-modified oilseed rape	Modified oilseed rape	P value
Germination rate (%)	86.7	89.7	0.312

Seed 300 grains of each line.
Statistical analysis: Fisher's exact test.

(f) Crossability

Under the condition of installing an insect-proof net, seeds were harvested from the non-modified oilseed rape which was cultivated in neighboring with the modified oilseed rape to investigate the crossability using the tolerance to glyphosate as an index (p. 22 of Attachment 7; Confidential and non-disclosed). As the result, 3 grains (0.2%) of cross seed were observed among 1,269 grains of seeds as harvested.

In addition, in the field of California, US, the crossability from the modified oilseed rape to the neighboring non-modified oilseed rape was 0.869%, and no statistical significance ($P<0.05$) is observed with the crossability between the non-modified oilseed rapes (0.824%) (Table 29, p. 51; Confidential and non-disclosed). The crossability from the modified oilseed rape to the non-modified oilseed rape was not above the crossability 0.7 to 21% of the oilseed rape which was conventionally reported (Staniland *et al.*, 2000; Anderson and de Vicente, 2010).

Table 29 Crossability in US

Distance from the parent pollen (m)	Oilseed rape used as the parent pollen	Mean value(%)*	Confidence interval(%)	P value
0.5 (Neighboring)	Modified	0.869	0.000 - 3.060	0.9696
	Non-modified	0.824	0.000 - 3.015	
1.0	Modified	0.072	0.000 - 0.198	0.7312
	Non-modified	0.096	0.000 - 0.221	
5.0	Modified	0.046	0.000 - 0.106	0.2828
	Non-modified	0.009	0.000 - 0.068	

Condition for cultivation: During 2010 to 2011, cultivate pollinated strain (non-modified oilseed rape) around the parent pollen. The modified oilseed rape at T5 generation, or the non-modified oilseed rape having tolerance to the herbicide acetolactic acid synthase inhibitor were used as the parent pollen. 3 repeated.

Method for confirming the crossability: seeds were collected from 16 places with regard to each distance among the pollinated strain which was bred at 0.5 m (neighboring), 1.0 m and 5.0 m from the parent pollen (p. 19 of Attachment 8; Confidential and non-disclosed). Seed total 144,000 grains of the seed per 1 line and determine according to the crossability by the presence or absence of the tolerance to the herbicide.

Statistical analysis: Linear mixed model.

* Least mean square.

(g) Productivity of harmful substances

The productivity was examined by the second crop test, the plow-in test and the soil microflora test.

Succeeding crop test:

By cultivating radish plant, which is a crop to be tested, in the soil in which the modified oilseed rape and the non-modified oilseed rape were cultivated and investigating the germination rate and the dried weight, the productivity of substance which was secreted from the root and affected on other plant was reviewed (p. 17 to 18 of Attachment 7; Confidential and non-disclosed).

As the result, no statistical significance ($P<0.05$) in the germination rate between both soils was observed (Table 30, p. 52).

While no statistical significance in the dried weight was observed, the variation of the obtained values is large and the lower limit of the confidential interval is less than 0. Accordingly, an additional test was conducted. As the result, its variation becomes less, the lower limit of the confidential interval is larger than 0 and no statistical significance ($P<0.05$) was observed (Table 30, p. 52).

Table 30 Germination rate and dried weight of radish plant in the second crop test

Items		Soil after cultivating the non-modified oilseed rape		Soil after cultivating the modified oilseed rape		P value
		Mean value	Confidence interval	Mean value	Confidence interval	
Germination rate (%) [1]		94.7	—	98.7	—	0.3665
Dried weight (mg)	Intial [2]	204.9	−53.62 - 463.5	233.6	−24.94 - 492.1	0.7642
	Additional test [3]	212.4	185.0 - 239.7	214.1	186.4 - 241.7	0.9213

1) Seed 75 grains of each line. Statistical analysis: Fisher's exact test.
2) Soil after cultivating the modified oilseed rape: n=26, Soil after cultivating the non-modified oilseed rape: n=27.
Statistical analysis: Linear mixed model.
3) Soil after cultivating the modified oilseed rape: n=24, Soil after cultivating the non-modified oilseed rape: n=27.
Statistical analysis: Linear mixed model.

Plow-in test:

By cultivating radish plant, which is a crop to be tested, in a soil in which the stem leaves of the modified oilseed rape and the non-modified oilseed rape were added to the cultivated soil and investigating its germination rate and dried weight, the productivity of substances which contain within the plant body and affected on other plant was examined (p. 19 of Attachment 7; Confidential and non-disclosed). As the result, no statistical significance ($P<0.05$) in any items to be tested between both soils was observed (Table 31, p. 53).

Table 31 Germination rate and dried weight of radish plant in the plow-in test

Items	Soil of plow-in for non-modified oilseed rape		Soil in plow-in for modified oilseed rape		P value
	Mean value	Confidence interval	Mean value	Confidence interval	
Germination rate (%) 1)	90.7	—	89.3	—	1.000
Dried weight (mg) 2)	213.0	163.8 - 262.3	196.3	147.0 - 245.7	0.5518

1) Seed 75 grains of each line. Statistical analysis: Fisher's exact test.
2) Soil of plow-in test for the modified oilseed rape: n=24, Soil of plow-in test for the non-modified oilseed rape; n=24.
Statistical analysis: Linear mixed model.

Soil microflora test:

By measuring number of microorganisms (bacterial count, actinomycetal count and mycotical count) in the soil after cultivating the oilseed rape, the productivity of substances which were secreted from the root and affected on the soil microorganism was examined (p. 20 of Attachment 7; Attachment 9; Confidential and non-disclosed). As the result, no statistical significance ($P<0.05$) in any microorganisms between both soils was observed (Table 32, p. 53).

Table 32 Result of the soil microflora test

Items	Soil after cultivating the non-modified oilseed rape		Soil after cultivating the modified oilseed rape		P value
	Mean value	Standard deviation	Mean value	Standard deviation	
Bacteria count ($\times 10^7$)	3.8	1.1	4.0	1.3	0.88
Actinomycetal count ($\times 10^6$)	6.3	1.4	5.6	1.5	0.69
Mycotical count ($\times 10^4$)	4.4	4.2	6.9	3.5	0.55

3 repeated, mean value of those obtained from 5 petri dishes for 1 repeated. n=3.
Method for culturing: Dilution plate technique.
Statistical analysis: Corresponding t test.
Number of microorganisms: Number of forming the colony / 1 g dried soil.

3. Information concerning use and the like of living modified organisms

(1) Content of the use and the like

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

(2) Method of the use and the like

—

(3) Method for collecting information after stating the Type 1 use by a person to be approved

—

(4) Countermeasure for preventing Adverse Effects on Biological Diversity in the case of possibility of Adverse Effects on Biological Diversity

See Plan for Emergency Measure.

(5) Result of use and the like in similar environment to the environment in which the use in the laboratory and the like or the Type 1 Use is planned

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(6) Information concerning the use and the like in overseas

Statuses of applying the modified oilseed rape in overseas and Japan are shown in Table 33 and Table 34 (p. 55).

Table 33 Status of applying in overseas

Applied countries	Purpose	Date of applying and/or approving	Destination for applying
Canada	Utilization as environmental stability and as feed Utilization as food	Approved at May 2012 Approved at May 2012	Canadian Food Inspection Agency (CFIA) Health Canada (HC)
US	Utilization as food and feed Cultivation	Finish for confirmation at May 2012 Approved at July 2013	US Food and Drug Administration (FDA) US Department of Agriculture (USDA)
EU	Imported	Applied at May 2012	European Food Safety Authority (EFSA)
Mexico	Imported	Approved at July 2012	New Mexico Department of Health (DOH)
China	Imported	Applied at June 2012	Ministry of Agriculture of the People's Republic of China (MOA)
Australia and New Zealand	Imported	Applied at June 2013	Food Standards Australia New Zealand (FSANZ)

Table 34 Status of applying in Japan

Purpose	Date for applying and approving	Destination for applying
Type 1 Use (Cultivation, storage, transportation, disposal and acts incidental to them in the isolated field) [1)]	Approved at April 2011	Ministry of Agriculture, Forestry and Fisheries of Japan and Ministry of the Environment
Safety as food [2)]	Applied at October 2013	Ministry of Health, Labour and Welfare
Safety as feed [3)]	Applied at October 2013	Ministry of Agriculture, Forestry and Fisheries of Japan

1) Act on the Conservation and Sustainable Use of Biological Diversity through Regulations on the Use of Living Modified Organisms (Act No. 97 of 2003).
2) Food Sanitation Act (Act No. 233 of 1947).
3) Act Concerning Safety Assurance and Quality Improvement of Feed (Act No. 35, 1953).

II. Assessment of each item on Adverse Effects on Biological Diversity

The recipient organism oilseed rape has the long-term of safe use in Japan. In the assessment of Adverse Effects on Biological Diversity, based on Part III of Supplement list of Procedure for assessment of Adverse Effects on Biological Diversity, any possibilities producing any effects were discussed, in comparison of the modified oilseed rape and the non-modified oilseed rape.

1. Competitiveness

(1) Identification of wild animals and wild plants that has a possibility to be affected

Oilseed rape grows wild on dry river beds, along railway, and around ports which is considered due to falling down at transporting, from Hokkaido to Kyushu (National Institute for Environmental Studies, 2011; Shimizu et al., 2008; Nakai, 2003; Ministry of Agriculture, Forestry and Fisheries of Japan, 2010a). However, oilseed rape will be gradually replaced with perennial herb and timber, unless the rape is grown in places at which disturbance is periodically occurred (cliff, wayside, river bed and the like) (OECD, 1997). While the seed of the oilseed rape has been imported for long term, there is no report that the oilseed rape affects on the maintenance of individual and individual group of wild animals and wild plants in Japan.

In the investigation around Kashima port in Ibaraki Prefecture, the oilseed rape tolerance to the herbicide glyphosate was only grown in the conventional place to be grown for the non-modified oilseed rape (National Institute for Agro-Environmental Sciences, 2007). In addition, since under a condition on which other plant community is widely present and competitiveness is occurred, the growth of oilseed rape cannot be observed or it is eliminated for extremely short period if observed, it has been believed that the oilseed rape does not expand its area to be grown by riding other plants, even if the oilseed rape is invaded in a circumferential community (Ministry of Agriculture, Forestry and Fishries of Japan, 2009). Therefore, even if the modified oilseed rape is grown, it has been considered that the region to be grown thereof is not expanded by riding other plants by competitiveness (Ministry of Agriculture, Forestry and Fishries of Japan, 2009).

Various properties relating to the priority in the competitiveness of the modified oilseed rape (morphological and growth characteristics, cold tolerance or high-temperature tolerance at the early stage of growth, wintering ability and summering ability of the mature plant, production of the seed, shedding habit, and dormancy and germination rate) were evaluated (I. 2(6)2), p. 45). As the result, the date of uniformity of germination is later by 1 day in comparison with the non-modified oilseed rape, and the germination rate, the primarily branches, the aboveground weight, the pod length, the pod shooting rate and the total pod shooting number were statistically significantly lowered in comparison with the non-modified oilseed rape. However, since there is no difference in other items to be tested including the germination rate in the investigation of the high-temperature tolerance at the early stage of growth, the germination rate of the seed just after harvesting, the yield of the seed in the field test at North America, and the like, no result improving the competitiveness was observed.

While the modified oilseed rape is provided with the tolerance to the herbicide glyphosate, it can be hardly considered that the trait of the tolerance to the herbicide improves the priority in the competitiveness of the modified oilseed rape under the natural environment in which herbicides are not normally sprayed. In addition, there is no report that *gat4621* gene which was transferred into the modified oilseed rape and the tolerance to the herbicide glyphosate decrease the competitiveness.

As mentioned above, no wild animals and wild plants which have any possibility on which the competitiveness was affected were identified.

(2) Assessment of the specific content for the effect

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(3) Assessment of easiness occurring the effect

—

(4) Determination of whether Adverse Effects on Biological Diversity is possibly occurred

As mentioned above, it was judged that the modified oilseed rape does not have any possibility occurring the Adverse Effects on Biological Diversity by competitiveness.

2. Productivity of harmful substances

(1) Identification of wild animals and wild plants that has a possibility to be affected

The conventional seed of oilseed rape contains erucic acid and glucosinolate which are considered to be harmful to animals (OGTR, 2008). The 1822 line which was used for the recipient organism of the modified oilseed rape is a so-called canola in which contents of both substances were reduced by breed improvement.

There is no report that the GAT4621 protein which is produced by the modified oilseed rape is a harmful substance, and the identity with the known allergen has not been observed (I.2.(1).2).(2).b, p.5). In addition, while the herbicide glyphosate is converted into *N*-acetyl glyphosate by the GAT4621 protein which is produced by the modified oilseed rape, there is no report that this product is a harmful substance.

While the contents of *N*-acetyl amino acids were increased in the modified oilseed rape, *N*-acetyl amino acids are contained in various plants and there was no report relating to any effect of these on insects. Several items to be analyzed in the aboveground plant body and the root were statistically significantly observed between the modified oilseed rape and the non-modified oilseed rape. However, upon considering that no constant trend in increasing and/or decreasing of amino acids and free amino acids in the seed, the aboveground plant body and the root as a whole is observed, and the like, it was considered that the increasing *N*-acetyl amino acids does not any biologically significant effect on the amino acid pools of the recipient organism. Further,

the modified oilseed rape in the feeding test does not cause any adverse effects on the growth of the imported cabbageworm and no difference in the degree of feeding damage between the modified oilseed rape and the non-modified oilseed rape was observed in 34 field tests in total. According to these facts, it was considered that the increasing of the content of *N*-acetyl amino acids in the modified oilseed rape has a low possibility of affecting on insects. Based on the result in each toxicity test, it was considered that these *N*-acetyl amino acids do not affect an adverse effect to animal health (I.2.(1).2).(3)., p. 5).

While the second crop test, the plow-in test and the soil microflora test were actually conducted, no result indicating that the productivity of any harmful substances in the modified oilseed rape is increased was observed (I.2.(6).2).g, p. 52).

As mentioned above, no wild animals and wild plants which have a possibility affected by the productivity of harmful substance was identified.

(2) Assessment of the specific content for the effect

—

(3) Assessment of easiness occurring the effect

—

(4) Determination of whether Adverse Effects on Biological Diversity is possibly occurred

As mentioned above, it was judged that the modified oilseed rape does not have any possibility occurring the Adverse Effects on Biological Diversity by the productivity of harmful substances.

3. Crossability

(1) Identification of wild animals and wild plants that has a possibility to be affected

Since there are no domestic species in Japan among relative species which has been reported to be naturally hybridizable with oilseed rape (FitzJohn *et al*, 2007; OECD, 1997; OGTR, 2008), no domestic wild animals and wild plants in Japan which have a possibility to be affected were identified.

While the living species grown in Japan among the relative species which have been reported to be naturally hybridizable with oilseed rape (FitzJohn *et al*, 2007; OECD, 1997; OGTR, 2008) are *B. juncea*, *B. nigra, B. rapa*, *H. incana*, *R. raphanistrum* and *S. arvensis*, these alien species which were already naturalized through cultivation and the like (The Ecological Society of Japan, 2003; Shimizu et al., 2008; Nakai, 2003 and Ishida, 2004).

(2) Assessment of the specific content for the effect

—

(3) Assessment of easiness occurring the effect

—

(4) Determination of whether Adverse Effects on Biological Diversity is possibly occurred

As mentioned above, it was judged that the modified oilseed rape does not have any possibility occurring the Adverse Effect on Biological Diversity by the crossability.

4. Other characteristics

The following possibilities (i) and (ii) were considered for an effect on Biological Diversity, in the case that the relative species listed in II.3.(1) (p. 58) is formed into a hybrid with the modified oilseed rape by the hybridization.

(i) A possibility that the hybrid caused by the hybridization is dominated by the competitiveness to rid individual group of other wild plant species

(ii) A possibility that the inserted gene acts as a burden to decrease of individual groups of hybrids, as the result, it affects on the maintenance of the individual group of wild animals and wild plants including insects which are rared depending on the relative species

Firstly, the possibility, that the hybrid caused by the hybridization is dominated by the competitiveness to rid individual group of other wild plant species, was examined.

Originally, the crossability of oilseed rape with these relative species is low. In addition, in the investigation of the pollen and the crossability, any results were not obtained that the crossability of the modified oilseed rape is increased with those in the conventional oilseed rape (I.2.(6).(2).d. and f., p. 48 and 51). Accordingly, it is considered that a crossability between the modified oilseed rape and the aforementioned relative species is also low similar to those in the conventional oilseed rape.

The hybrid caused by the hybridization between the conventional oilseed rape and the aforementioned relative species is low in the living rate, the germination ability of pollen, the production of the seed and the like and the priority of the formed hybrid in the competitiveness is low. Accordingly, a possibility of prioritizing the hybrid under the natural condition is low. Also for the modified oilseed rape, no result increasing the priority in the competitiveness is obtained (I.2.(6).(2), p. 45). In addition, it can be hardly considered that the trait of tolerance to the herbicide glyphosate increased the priority in the competitiveness under the natural environment in which herbicides are not normally sprayed.

Actually, only 1 or 2 individuals of a hybrid between a gene-modified oilseed rape tolerance to a herbicide which has been imported in Japan and *B. rapa* are intermittently confirmed along with river beds, and no result indicating a trend of expanding distribution of the hybrid is obtained (National Institute of Environmental

Studies, 2011). In addition, in the investigation at the port, the growth of the gene-modified oilseed rape was limited in the almost same range for 3 years, and no hybrid individual with *B. juncea* or *B. rapa* was found, indicating a low possibility of spreading the introduced gene into relative species which are hybridizable (Ministry of Agriculture, Forestry and Fishries of Japan, 2010a).

Therefore, the possibility that the hybrid formed by the hybridization between the modified oilseed rape and the relative species dominate by the competitiveness, and the possibility of becoming the hybrid as dominating species are low.

Next, the possibility that the inserted gene act as a burden to decrease of individual groups of hybrids, as the result, it affects on the maintenance of the individual group of wild animals and wild plants including insects which are rared depending on the relative species was examined.

There is no report that the maintenance of wild animals and wild plants including insects, which are rared depending on the relative species by occurring the hybrid in which the conventional oilseed rape and the relative species are hybridized, were affected. In addition, the possibilities that the gene providing the tolerance to the herbicide, which was inserted into the modified oilseed rape, acts as a burden and that it affects on the priority of the hybrid in the competitiveness are low. Based on these facts, the possibilities that the individual group of hybrids is decreased than those in the conventional status and that, as the result, it affects on the individual group of the relative species are low. Accordingly, it was considered that the inserted gene does not affect on the maintenance of the individual group of wild animals and wild plants including insects which are rared depending on the relative species.

As mentioned above, it was judged that a possibility causing the Adverse Effects on Biological Diversity by the hybridization between the modified oilseed rape and the relative species is low.

III. Comprehensive assessment of the Adverse Effects on Biological Diversity

The species of oilseed rape to which the recipient organism belongs grows wild on dry river beds, along railway, and around ports from Hokkaido to Kyushu. However, oilseed rape will be gradually replaced with perennial herb and timber, unless the rape is grown in places at which disturbance is periodically occurred. While the seed of the oilseed rape has been imported for long term, there is no report that the oilseed rape affects on the maintenance of individual and individual group of wild animals and wild plants in Japan. In addition, it is not considered that, if the gene-modified oilseed rape is grown, the range to be grown is expanded by riding the other plants by competitiveness.

Various properties relating to the competitiveness of the modified oilseed rape were evaluated. As the result, since the properties were statistically significantly low or does not have any difference in comparison with those in the non-modified oilseed rape, no result increasing the priority in the competitiveness is observed.

While the modified oilseed rape is provided with the tolerance to the herbicide glyphosate, it can be hardly considered that the trait of the tolerance to the herbicide increases the priority in the competitiveness of the modified oilseed rape under the natural environment in which herbicides are not normally sprayed.

Therefore, it was judged that the modified oilseed rape does not have any possibility occurring the Effects on Biological Diversity by the competitiveness.

The conventional seed of oilseed rape contains erucic acid and glycosinolate which are considered to be harmful to animals. The line which was used for the recipient organism of the modified oilseed rape is a so-called canola in which contents of both substances were reduced by breed improvement, and it is considered that the line does not any effect on wild animals.

There is no report that the GAT4621 protein which is produced by the modified oilseed rape is a harmful substance, and no identity with the known allergen is observed.

While the contents of *N*-acetyl amino acids were increased in the modified oilseed rape, *N*-acetyl amino acids are contained in various plants and there was no report relating to any effect of these on insects. Several items to be analyzed in the aboveground plant body and the root were statistically significantly observed between the modified oilseed rape and the non-modified oilseed rape. However, upon considering that no constant trend in increasing and/or decreasing of amino acids and free amino acids in the seed, the aboveground plant body and the root as a whole is observed, and the like, it was considered that the increasing *N*-acetyl amino acids does not any biologically significant effect on the amino acid pools of the recipient organism. In addition, in the feeding test and the field test, no adverse effect to insects by the modified oilseed rape was observed. According to these facts, it was considered that the increasing of the content of *N*-acetyl amino acids in the modified oilseed rape has a low possibility affecting on insects. Based on the result in each toxicity test, it was considered that these *N*-acetyl amino acids do not affect an adverse effect to animal health.

While the second crop test, the plow-in test and the soil microflora test were actually conducted, no result indicating that the productivity of any harmful substances in the modified oilseed rape is increased was observed.

Therefore, it was judged that the modified oilseed rape does not have a possibility occurring the Adverse Effects on Biological Diversity by the productivity of the harmful substance.

Since there are no domestic species in Japan among relative species which has been reported to be naturally hybridizable with oilseed rape, no domestic wild animals and wild plants in Japan which have a possibility to be affected were identified.

While the living species grown in Japan among the relative species which have been reported to be naturally hybridizable with oilseed rape are *B. juncea*, *B. nigra, B. rapa*, *H. incana*, *R. raphanistrum* and *S. arvensis*, either of these is alien species, and these are not identified as the domestic wild plant in Japan which has any possibility as affected.

Therefore, it was judged that the modified oilseed rape does not have any possibility occurring the Adverse Effects on Biological Diversity by its crossability.

For the effect on the Biological Diversity in the case of the relative species is formed into a hybrid with the modified oilseed rape by the hybridization, two possibilities of: (i) a possibility that the hybrid caused by the hybridization is dominated by the competitiveness to rid individual group of other wild plant species; and (ii) a possibility that the inserted gene acts as a burden to decrease of individual groups of hybrids, as the result, it affects on the maintenance of the individual group of wild animals and wild plants including insects which are rared depending on the relative species, were considered.

Originally, the crossability of oilseed rape with these relative species is low. In addition, any results were not obtained that the crossability of the modified oilseed rape is increased with those in the conventional oilseed rape. Accordingly, it is considered that a crossability between the modified oilseed rape and the aforementioned relative species is also low similar to those in the conventional oilseed rape. The hybrid caused by the hybridization between the conventional oilseed rape and the aforementioned relative species is low in the living rate, the germination ability of pollen, the production of the seed and the like. In addition, it can be hardly considered that the trait of tolerance to the herbicide glyphosate increased the priority in the competitiveness under the natural environment in which herbicides are not normally sprayed. Accordingly, the possibility that the hybrid formed by the hybridization between the modified oilseed rape and the relative species is dominated by the competitiveness, and the possibility of becoming the hybrid as dominating species are low.

It is considered that the possibilities that the individual group of hybrids is decreased than those in the conventional status and that, as the result, it affects on the individual group of the relative species are low, by the inserted gene. Accordingly, it was considered that the inserted gene does not affect on the maintenance of the individual group of wild animals and wild plants including insects which are rared depending on the relative species.

Therefore, it was judged that a possibility causing the Adverse Effects on Biological Diversity by the hybridization between the modified oilseed rape and the relative species is low

As mentioned above, as the comprehensive assessment, it was concluded that there is no possibility occurring the Effect on Biological Diversity in Japan, in the case that the modified oilseed rape was used according to the Type 1 Use Regulation.

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List of attached materials

Confidential and non-disclosed

5

Attached materials 1 to 8: Confidential and non-disclosed