





Biosafety Clearing-House (BCH)

LIVING MODIFIED ORGANISM (LMO)

BCH-LMO-SCBD-109709-2

? Decisions on the LMO ? Risk Assessments

LAST UPDATED: 17 JUN 2022

Living Modified Organism identity

The image below identifies the LMO through its unique identifier, trade name and a link to this page of the BCH. Click on it to download a larger image on your computer. For help on how to use it go to the LMO quick-links page.

https://bch.cbd.int/database/record?documentID=109709



DAS-24236-5 × DAS-21Ø23-5 × SYN-IR1Ø2-7 × MON-88913-8 × DAS-8191Ø-7 Insect resistant, herbicide tolerant cotton



Read barcode or type above URL into internet browser to access information on this LMO in the Biosafety Clearing-House © SCBD 2012

Name

Insect resistant, herbicide tolerant cotton

ΕN

Transformation event

281-24-236 × 3006-210-23 × MON88913 × COT102 × DAS81910

Unique identifier

DAS-24236-5 × DAS-21Ø23-5 × SYN-IR1Ø2-7 × MON-88913-8 × DAS-8191Ø-7

Developer(s)

- ORGANIZATION: DOW AGROSCIENCES | BCH-CON-SCBD-14939-1

ORGANIZATION

Dow AgroSciences

Website: http://www.dowagro.com/homepage/index.htm

Description

The stacked cotton (*Gossypium hirsutum*) line was produced by cross breeding between each of the parental organisms for insect resistance and herbicide tolerance. For Lepidopteran resistance, the modified cotton expresses *Bacillus thuringiensis* Cry1Ac, Cry1F and Vegetative insecticidal protein 3A. For tolerance to glufosinate, the modified cotton expresses *Streptomyces viridochromogenes* phosphinothricin N-acetyltransferase and *Streptomyces hygroscopicus* phosphinothricin N-acetyltransferase. For tolerance to 2,4-dichlorophenoxyacetic acid, the cotton expresses *Delftia acidovorans* aryloxyalkanoate dioxygenase. For tolerance to glyphosate, the cotton expresses *Rhizobium radiobacter*

5-enolpyruvylshikimate-3-phosphate synthase. Additionally, an Escherichia coli hygromycin

ΕN

B phosphotransferase cassette is also present for hygromycin selection during transformation and breeding.

Recipient Organism or Parental Organisms

The term "Recipient organism" refers to an organism (either already modified or non-modified) that was subjected to genetic modification, whereas "Parental organisms" refers to those that were involved in cross breeding or cell fusion.

BCH-ORGA-SCBD-12080-6 ORGANISM GOSSYPIUM HIRSUTUM (COTTON)

Crops

BCH-LMO-SCBD-14938-7 LIVING MODIFIED ORGANISM DAS-21Ø23-5 - INSECT-RESISTANT COTTON

Resistance to diseases and pests (Insects, Lepidoptera (butterflies and moths))

BCH-LMO-SCBD-14940-7 LIVING MODIFIED ORGANISM | DAS-24236-5 - INSECT-RESISTANT COTTON

Dow AgroSciences | Resistance to diseases and pests (Insects, Lepidoptera (butterflies and moths))

BCH-LMO-SCBD-15168-16 LIVING MODIFIED ORGANISM | MON-88913-8 - ROUNDUP READY™ FLEX™ COTTON |

Resistance to herbicides - Glyphosate

BCH-LMO-SCBD-14992-8 LIVING MODIFIED ORGANISM SYN-IR1Ø2-7 - VIPCOT™ COTTON

Resistance to antibiotics - Hygromycin Resistance to diseases and pests - Insects - Lepidoptera (butterflies and moths) Selectable marker genes and reporter genes

BCH-LMO-SCBD-108872-2 LIVING MODIFIED ORGANISM DAS-8191Ø-7 - COTTON MODIFIED FOR

HERBICIDE TOLERANCE

XXXX | Resistance to herbicides (Glufosinate), Tolerance to 2,4-Dichlorophenoxyacetic acid

Characteristics of the modification process

Vector

pAGM281; pMYC3006; pCOT-1; PV-GHGT35 and pDAB4468

ΕN

Techniques used for the modification

Cross breeding

Genetic elements construct

P-ubi1-MAIZE I-1 ubi1-MAIZE CS-pat-STRVR T-orf25-RHIRD CS-cry1F-BACTU 0.000 kb 0.000 kb0.000 kb 0.000 kb 0.000 kbP-4ocs delta mas-SYNTH 0.000 kb P-4ocs delta mas-SYNTH CS-pat-STRVR T-orf25-RHIRD P-ubi1-MAIZE CS-cry1Ac-BA 0.000 kb 0.000 kb0.000 kb0.000 kb 0.000 kbT-nos-RHIR CS-hpt-EC P-ubiAt3 0.250 kb 1.030 k 1.720 kb T-nos-RHIRD P-Act2 CS-Vip3A-BACTU 1.410 kb 2.370 kb 0.250 kb

L -TSF1 L-35s-CMoVb I-TSF1 TP-ctp2-ARATH CS-CP4epsps-RHIRD P-TSF1 1.039 kb 0.000 kb0.045 kb 0.621 kb 0.277 kb 1.367 kb T-rbcS E9-PEA 0.652 kb P-ACT8-ARATH L-35S-CaMV L-ACT8-ARATH I-ACT8-ARATH TP-ctp2-ARATH 1.174 kb 0.000 kb0.140 kb 0.472 kb 0.227 kb CS-CP4epsps-RHIRD T-rbcS E9-PEA 1.367 kb $0.642~\mathrm{kb}$ CS-aad12-DELAC E-rb7 mar-TOBAC P-ubi10-ARATH T-ORF23-RHIRD 1.170 kb 1.320 kb 0.880 kb 0.460 kbCS-bar-STRHY T-ORF1-RHIRD P-CsVMV 0.520 kb 0.550 kb 0.700 kb

Introduced or modified genetic element(s)

Intron

Transit signal

Some of these genetic elements may be present as fragments or truncated forms. Please see notes below, where applicable.

BCH-GENE-SCBD-100362-7 UBIQUITIN GENE PROMOTER | (MAIZE, CORN) BCH-GENE-SCBD-103627-5 UBIQUITIN INTRON 1 | (MAIZE, CORN) BCH-GENE-SCBD-15002-4 PHOSPHINOTHRICIN N-ACETYLTRANSFERASE GENE Protein coding sequence | Resistance to herbicides (Glufosinate) BCH-GENE-SCBD-100363-5 ORF25 POLYA TERMINATOR SEQUENCE Terminator BCH-GENE-SCBD-14987-8 CRY1F | BACILLUS THURINGIENSIS - BT, BACILLUS, BACTU Protein coding sequence | Resistance to diseases and pests (Insects, Lepidoptera (butterflies and moths)) BCH-GENE-SCBD-103900-3 40CSΔMAS2' PROMOTER Promoter BCH-GENE-SCBD-14986-6 CRY1AC | BACILLUS THURINGIENSIS - BT, BACILLUS, BACTU Protein coding sequence | Resistance to diseases and pests (Insects, Lepidoptera (butterflies and moths)) BCH-GENE-SCBD-105196-2 FMV 35S ENHANCER Leader BCH-GENE-SCBD-103903-1 ELONGATION FACTOR EF-1ALPHA PROMOTER | (THALE CRESS) BCH-GENE-SCBD-103904-1 ELONGATION FACTOR EF-1ALPHA LEADER | (THALE CRESS) BCH-GENE-SCBD-103905-1 ELONGATION FACTOR EF-1ALPHA INTRON 1 | (THALE CRESS)

BCH-GENE-SCBD-100365-6 CHLOROPLAST TRANSIT PEPTIDE 2 | (THALE CRESS)

BCH-GENE-SCBD-14979-7 5-ENOLPYRUVYLSHIKIMATE-3-PHOSPHATE SYNTHASE GENE | Protein coding sequence | Resistance to herbicides (Glyphosate)

BCH-GENE-SCBD-101877-5 RBCS-E9 GENE TERMINATOR | (GARDEN PEA)

Terminator

BCH-GENE-SCBD-105197-2 CAMV 35S ENHANCER

Leader

BCH-GENE-SCBD-103907-2 ACTIN 8 PROMOTER | (THALE CRESS)

Promoter

BCH-GENE-SCBD-103908-3 ACTIN 8 LEADER SEQUENCE | (THALE CRESS)

Leader Sequence

BCH-GENE-SCBD-104517-1 ACTIN 2 GENE PROMOTER | (THALE CRESS)

Promotei

BCH-GENE-SCBD-14990-5 VEGETATIVE INSECTICIDAL PROTEIN 3A | BACILLUS THURINGIENSIS - BT,

BACILLUS, BACTU

Protein coding sequence | Resistance to diseases and pests (Insects, Lepidoptera (butterflies and moths))

BCH-GENE-SCBD-100269-8 NOPALINE SYNTHASE GENE TERMINATOR

Terminator

BCH-GENE-SCBD-104795-4 RB7 MATRIX ATTACHMENT REGION | (TOBACCO PLANT)

Enhancer

BCH-GENE-SCBD-104802-5 POLYUBIQUITIN10 GENE PROMOTER | (THALE CRESS)

Promoter

BCH-GENE-SCBD-104805-2 ARYLOXYALKANOATE DIOXYGENASE GENE

Protein coding sequence | Resistance to herbicides

BCH-GENE-SCBD-104806-3 ORF23 3' UNTRANSLATED REGION

Terminator

BCH-GENE-SCBD-101900-6 CSVMV PROMOTER | CASSAVA VEIN MOSAIC VIRUS (CASSAVA VEIN MOSAIC

VIRUS, CVMV, CSVMV)

Promoter

BCH-GENE-SCBD-14972-12 PHOSPHINOTHRICIN N-ACETYLTRANSFERASE GENE

Protein coding sequence | Resistance to herbicides (Glufosinate)

BCH-GENE-SCBD-104807-2 ORF1 3' UNTRANSLATED REGION

Terminator

BCH-GENE-SCBD-14991-8 HYGROMYCIN B PHOSPHOTRANSFERASE GENE | (BACTERIA)

Protein coding sequence | Resistance to antibiotics (Hygromycin), Selectable marker genes and reporter genes

BCH-GENE-SCBD-101874-2 UBIQUITIN GENE 3 PROMOTER | (THALE CRESS)

Promoter

BCH-GENE-SCBD-103909-2 ACTIN 8 INTRON 1 | (THALE CRESS)

Intron

Notes regarding the genetic elements present in this LMO

DNA insert from DAS-24236-5 vector pAGM281

The DNA insert from 281-24-236 contains two gene cassettes: *Bacillus thuringiensis* var. *aizawai cry1F* and *Streptomyces viridochromogenes* phosphinothricin N-acetyltransferase

ΕN

(pat).

Transcription of *pat* is under control of a *Zea mays* ubiquitin promoter and an *Agrobacterium tumefaciens* open reading frame 25 (ORF25) terminator. The transcript is expected to initially contain a *Z. mays* ubiquitin intron 1 at the 5' end. The intron enhances the expression of *pat*.

Transcription of cry1F is under control of a synthetic 4ocs Δ Mas2' promoter and the same ORF25 terminator as the pat expression cassette. The cassette is in the reverse orientation to utilize the same terminator.

Note:

- Transcription is expected to occur at elevated and constitutive levels due to the promoters.
- The coding sequences of *pat* and *cry1F* have been optimized for expression in plants
- Cry1F is a chimeric, full-length δ-endotoxin comprised of the core toxin of Cry1F and nontoxic portions of *B. thuringiensis* Cry1Ca3 and Cry1Ab1 proteins, which form the C-terminal end of the protein and are removed during the activation of the Cry1F protein.

DNA insert from DAS-21Ø23-5 vector pMYC3006

The DNA insert from 3006-210-23 contains two gene cassettes: *Bacillus thuringiensis cry1Ac* and *Streptomyces viridochromogenes* phosphinothricin N-acetyltransferase (*pat*).

Transcription of *pat* is under control of a synthetic 4ocsΔMas2' promoter and an *Agrobacterium tumefaciens* open reading frame 25 (ORF25) terminator. Transcription of *cry1Ac* is under control of a *Zea mays* ubiquitin promoter and the same ORF25 terminator as the *pat* expression cassette.

Note:

- The ORF25 terminator serves as the terminator for both gene expression cassettes. The *cry1Ac* cassette is in the reverse orientation to utilize the same terminator.
- The coding sequences of *cry1Ac* and *pat* have been optimized for expression in plant cells.
- Transcription is expected to occur at elevated and constitutive levels due to the promoters.
- Southern blot analysis demonstrated that a single, intact DNA insertion was present in the parental genome. The analysis also indicated that no vector backbone sequences were integrated during transformation.

DNA insert from SYN-IR1Ø2-7 vector pCOT-1

The DNA insert from COT102 contains two gene cassettes: *Escherichia coli* hygromycin B phosphotransferase (*hph*) and *Bacillus thuringiensis vegetative insecticidal protein 3A*.

Transcription of *hph* is under control of the *Arabidopsis thaliana* ubiquitin 3 promoter and the *Agrobacterium tumefaciens* nopaline synthase (*nos*) terminator. The gene cassette is present in the counterclockwise orientation.

The expression of *Bacillus thuringiensis* vegetative insecticidal protein 3A (*vip3A*) is under transcriptional control of the *A. thaliana* actin 2 promoter and the *nos* terminator.

Note:

- The coding sequence of the *vip3A* was altered for optimal expression in plant cells.
- Transcription is expected to occur at elevated and constitutive levels due to the promoters.
- Southern blot analysis indicated the incorporation of a single copy of the transgenes without the integration of the vector backbone sequences.
- Southern blot analysis confirmed the expression of the proteins.

DNA insert from MON-88913-8 vector PV-GHGT35

The DNA insert from MON88913 contains two codon-optimised *Agrobacterium tumefaciens* 5-enolpyruvylshikimate-3-phosphate synthase (*epsps*) cassettes:

- 1. the first *epsps* coding sequence under the regulation of a chimeric transcriptional promoter (*Figwort mosaic virus* 34S promoter enhancer and *Arabidopsis thaliana* elongation factor EF-1 alpha (*tsf1*) promoter), *tsf1* leader and intron sequences, an *A. thaliana* chloroplast transit peptide 2 sequence and a *Pisum sativum* ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) E9 transcript termination and polyadenylation sequence (T-E9).
- 2. the second *epsps* coding sequence regulated by another chimeric transcriptional promoter (*Cauliflower mosaic virus* 35S enhancer and *A. thaliana* actin 8 (*act8*) promoter), *act8* leader and intron sequences, *A. thaliana* chloroplast targeting peptide 2 and T-E9.

High levels of transcription are expected from both cassettes due to the presence of viral enhancer sequences. The EPSPS protein is expected to accumulate in the chloroplast due to the transit signal peptide.

Note:

The promoter for both genetic constructs are chimeric promoters containing viral enhancer sequences. Thus, the size of the promoters (Elongation factor 1 alpha and Actin 8) in the 'Genetic elements construct' reflects the size of chimeric promoters (FMV 35S enhancer + Elongation factor 1 alpha promoter; CaMV 35S enhancer + Actin 8 promoter).

DNA insert from DAS-8191Ø-7 vector DAB4468

The DNA insert from DAS81910 contains two gene cassettes: *Delftia acidovorans* aryloxyalkanoate dioxygenase (*aad-12*) and *Streptomyces hygroscopicus* phosphinothricin Nacetyltransferase (*pat*).

Transcription of *aad-12* is under control of the *Arabidopsis thaliana* polyubiquitin 10 promoter and the *Agrobacterium tumefaciens* open reading frame 23 3' untranslated region.

Transcription of *pat* is under control of the Cassava Vein Mosaic Virus promoter and the *A. tumefaciens* ORF1 3' untranslated region.

Note:

· Transcription is expected to occur at elevated and constitutive levels due to the

promoters.

• Southern blot analysis indicated a single, intact insertion of *aad-12* and *pat* into the parental genome without the integration of the vector backbone sequences.

For more information, kindly refer to the parental LMO records.

LMO characteristics

Modified traits

Resistance to diseases and pests

Insects

Lepidoptera (butterflies and moths)

Resistance to herbicides

Glufosinate

Glyphosate

Tolerance to 2,4-Dichlorophenoxyacetic acid

Common use(s) of the LMO

Fiber/textile

Detection method(s)

External link(s)

? DAS-21Ø23-5 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) (JRC) [English]

? DAS-24236-5 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) (JRC) [English]

? DAS-8191Ø-7 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) (<code>JRC</code>) [<code>English</code>]

? MON-88913-8 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) (JRC) [English]

? SYN-IR1Ø2-7 - EU Reference Laboratory for GM Food and Feed (EURL-GMFF) (JRC) [English]

Additional Information

Other relevant website addresses and/or attached documents

? Diagrama de breeding comercial de Algodón con evento apilado DAS.docx [English]

? EUginius - 3006-210-23 x 281-24-236 x COT102 x MON88913 x DAS81910 [English]

BCH-LMO-SCBD-109709-2

Further Information

Questions about the Cartagena Protocol on Biosafety or the operation of the Biosafety Clearing-House may be directed to the Secretariat of the Convention on Biological Diversity.

Secretariat of the Convention on Biological Diversity

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