

Biosafety Clearing-House (BCH)

LIVING MODIFIED ORGANISM (LMO)


BCH-LMO-SCBD-116060-2

[? Decisions on the LMO ? Risk Assessments](#)

LAST UPDATED: 17 MAY 2021


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.



DP-Ø23211-2
Herbicide-tolerant and insect-protected maize

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

<https://bch.cbd.int/database/record?documentID=116060>


Name

Herbicide-tolerant and insect-protected maize

EN

Transformation event

DP23211

Unique identifier

DP-Ø23211-2

Developer(s)

- [ORGANIZATION: PIONEER HI-BRED INTERNATIONAL INC.](#) | [BCH-CON-SCBD-14931-2](#)

ORGANIZATION

Pioneer Hi-Bred International Inc.
Private sector (business and industry)
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50131, United States of America
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Website: www.pioneer.com/

Description

The maize (*Zea mays*) was modified for insect resistance and herbicide tolerance. To protect the maize against Western corn rootworm (*Diabrotica virgifera virgifera*), the maize expresses *Pseudomonas chlororaphis* insecticidal protein IPD072Aa and an RNA interference (RNAi) cassette. The IPD072Aa protein causes cells of the midgut epithelial cells to burst,

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disrupting the lining and leading to death. Coleoptera species are sensitive to the protein, while lepidopteran and hemipteran species are unaffected. The RNAi cassette expresses hairpin RNA (hpRNA), which target the western corn rootworm smooth septate junction protein 1 gene. Silencing of this gene compromises the integrity of the intestinal barrier formed by epithelial cells, leading to growth inhibition and death. For herbicide tolerance, the maize expresses *Streptomyces viridochromogenes* phosphinothricin acetyltransferase, which inactivates phosphinothricin, the active ingredient in glufosinate ammonium herbicides. The maize additionally contains *Escherichia coli* phosphomannose isomerase, which was a selectable marker during transformation.

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-246-6 ORGANISM | ZEA MAYS (MAIZE, CORN, MAIZE) |
Crops

Point of collection or acquisition of the recipient organism or parental organisms

Zea mays line PHR03 EN

Characteristics of the modification process

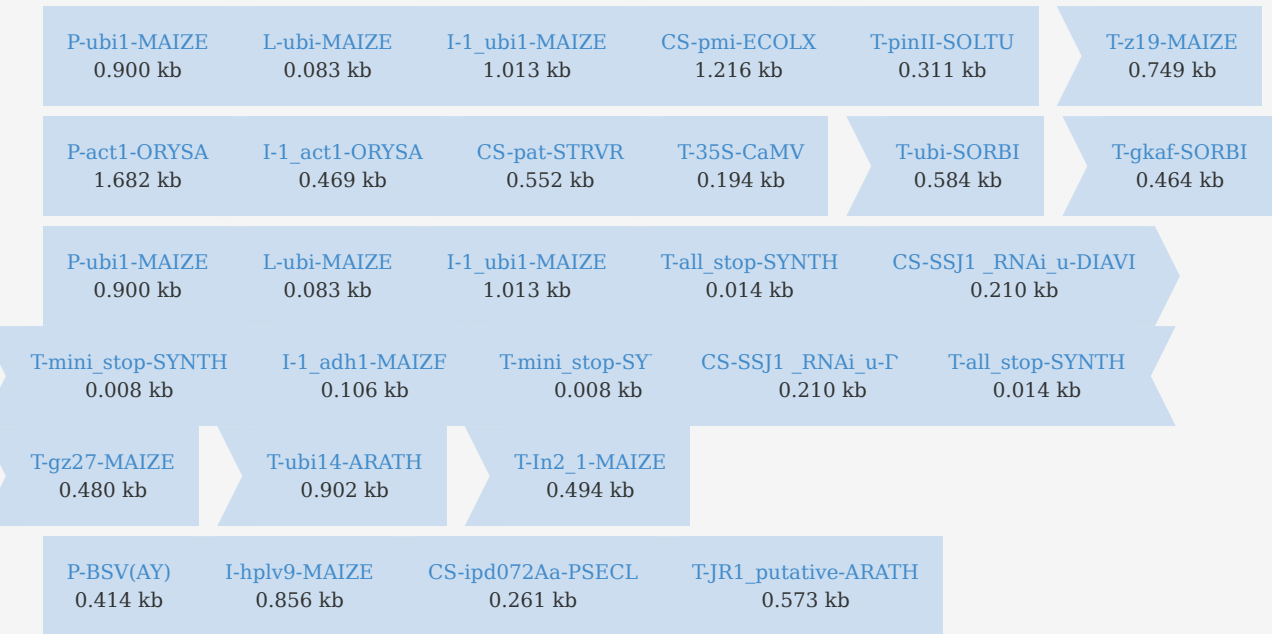
Vector

PHP74643 (see notes related to genetic elements below) EN

Techniques used for the modification

Agrobacterium-mediated DNA transfer

Genetic elements construct



Introduced or modified genetic element(s)

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) |

Promoter

[BCH-GENE-SCBD-103927-2](#) UBIQUITIN 5' UNTRANSLATED REGION | (MAIZE, CORN) |

Leader

[BCH-GENE-SCBD-103627-5](#) UBIQUITIN INTRON 1 | (MAIZE, CORN) |

Intron

[BCH-GENE-SCBD-15003-7](#) PHOSPHOMANNOSE ISOMERASE GENE | (BACTERIA) |

Protein coding sequence | Mannose tolerance, Selectable marker genes and reporter genes

[BCH-GENE-SCBD-100367-4](#) PROTEINASE INHIBITOR II GENE TERMINATOR | (POTATO) |

Terminator

[BCH-GENE-SCBD-116046-1](#) 19-KDA ZEIN GENE TERMINATOR - ZEA MAYS - MAIZE, CORN, MAIZE |

[BCH-GENE-SCBD-100364-5](#) RICE ACTIN 1 GENE PROMOTER | (RICE) |

Promoter

[BCH-GENE-SCBD-100355-6](#) RICE ACTIN 1, INTRON | (RICE) |

Intron

[BCH-GENE-SCBD-15002-4](#) PHOSPHINOTHRICIN N-ACETYLTRANSFERASE GENE |

Protein coding sequence | Resistance to herbicides (Glufosinate)

[BCH-GENE-SCBD-100290-6](#) CAMV 35S TERMINATOR |

Terminator

[BCH-GENE-SCBD-116047-2](#) UBIQUITIN TERMINATOR - SORGHUM BICOLOR - SORGHUM |

[BCH-GENE-SCBD-116048-1](#) ALL-STOP CODON SEQUENCE |

[BCH-GENE-SCBD-116049-1](#) MINI-STOP CODON SEQUENCE |

[BCH-GENE-SCBD-116053-1](#) SMOOTH SEPTATE JUNCTION PROTEIN 1 - DIABROTICA VIRGIFERA
VIRGIFERA - WESTERN CORN ROOTWORM, DIAVI |

[BCH-GENE-SCBD-104337-2](#) ALCOHOL DEHYDROGENASE 1, INTRON 1 | (MAIZE, CORN) |

Intron

[BCH-GENE-SCBD-116051-1](#) 27-KDA GAMMA ZEIN TERMINATOR - ZEA MAYS - MAIZE, CORN, MAIZE |

[BCH-GENE-SCBD-116050-1](#) UBIQUITIN 14 TERMINATOR - ARABIDOPSIS THALIANA - THALE CRESS,
MOUSE-EAR CRESS, ARABIDOPSIS, ARATH |

[BCH-GENE-SCBD-105058-3](#) IN2-1 TERMINATOR | (MAIZE, CORN) |

Terminator

[BCH-GENE-SCBD-116056-2](#) BANANA STREAK VIRUS (ACUMINATA YUNNAN) PROMOTER - BANANA
STREAK VIRUS ACUMINATA YUNNAN - BSV(AY) |

[BCH-GENE-SCBD-116052-1](#) PREDICTED CALMODULIN 5 GENE INTRON - ZEA MAYS - MAIZE, CORN,
MAIZE |

[BCH-GENE-SCBD-116057-2](#) INSECTICIDAL PROTEIN IPD072AA - PSEUDOMONAS CHLORORAPHIS -
PSECL |

Resistance to diseases and pests - Insects - Coleoptera (beetles) - Western corn rootworm (Diabrotica virgifera)

[BCH-GENE-SCBD-116054-1](#) PUTATIVE GENE OF THE MANNOSE-BINDING PROTEIN SUPERFAMILY
TERMINATOR - ARABIDOPSIS THALIANA - THALE CRESS, MOUSE-EAR CRESS, ARABIDOPSIS, ARATH |

[BCH-GENE-SCBD-116062-1](#) GAMMA KAFARIN TERMINATOR - SORGHUM BICOLOR - SORGHUM |

Notes regarding the genetic elements present in this LMO

The modified maize contains four gene cassettes: *Escherichia coli* phosphomannose isomerase (*pmi*), *Streptomyces viridochromogenes* phosphinothricin N-acetyltransferase (*pat*), *Diabrotica virgifera virgifera* smooth septate junction protein 1 double stranded RNA (DvSSJ1-dsRNA) and *Pseudomonas chlororaphis* insecticidal protein ipd072Aa (*ipd072Aa*).

Transcription of *pmi* is under control of a *Zea mays* (maize) ubiquitin promoter and a *Solanum tuberosum* proteinase inhibitor II gene terminator. The transcript is also expected to initially include a 5' untranslated region (5' UTR) and intron 1 of the maize ubiquitin at the 5' end of the *pmi* coding sequence. These sequences are not expected to be translated, but enhance transcription. A maize 19-kDA zein terminator is also present after the proteinase inhibitor II terminator and acts to prevent transcriptional interference between gene cassettes.

Transcription of *pat* is under control of an *Oryza sativa* (rice) actin 1 promoter and a *Cauliflower mosaic virus* (CaMV) terminator. A rice actin 1 intron is included at the 5' end of the *pat* to enhance expression of the coding sequence. *Sorghum bicolor* ubiquitin and gamma-kafarin terminators are also present after the CaMV terminator, which acts to prevent transcriptional interference between gene cassettes.

Transcription of the RNA interference (RNAi) cassette (DvSSJ1-dsRNA) is under control of a maize ubiquitin promoter and maize 27-kDA gamma zein terminator. From 5' to 3', the transcript is expected to contain: maize 5' UTR, maize ubiquitin intron 1, (sense) synthetic all-stop codon sequence, (sense) *DvSSJ1*, (sense) mini-stop codon sequence, maize alcohol dehydrogenase 1 intron, (anti-sense) mini-stop codon sequence, (anti-sense) *DvSSJ1* and (anti-sense) synthetic all-stop codon sequence. The maize ubiquitin 5' UTR and intron promote high levels of transcription. After transcription, the sense and anti-sense regions base pair to form a double stranded structure (hairpin RNA; hpRNA) with the alcohol dehydrogenase sequence acting as a connecting loop. Translation is not expected to occur for the hpRNA because of RNAi processing within the host plant (see Additional information section below) and the all-stop and mini-stop synthetic sequences, which would terminate translation before any protein is produced. An *Arabidopsis thaliana* ubiquitin 14 and a maize In2-1 terminator are also present after the 27-kDA gamma zein terminator to prevent transcriptional interference between gene cassettes.

Transcription of the *ipd072Aa* is under control of a *Banana streak virus* promoter and an *Arabidopsis thaliana* putative mannose-binding protein superfamily terminator. A maize predicted calmodulin 5 gene intron was included at the 5' end of the *ipd072Aa* coding sequence to enhance expression.

Note:

- DP23211 maize was created through two subsequent transformations:
 - First transformation: particle bombardment of PHP56614 with two additional plasmids (PHP21139 and PHP31729). PHP21139 and PHP31729 were not integrated, but transiently express WUS and ODP2 proteins to improve regeneration of maize plants
 - Second transformation: Agrobacterium-mediated transformation with PHP74643, subsequently resulting in site-specific recombination
- For information on the backbone sequences or removed genetic elements, kindly refer to the attached documents. Non-integrated or removed sequences include: WUS, ODP2, I-Crel,

NPTII, DsRED2 and FLP.

- Next generation sequences (Southern-by-sequencing) confirmed the plants contained a single insertion, the intended genetic elements and no unintended insertions (such as antibiotic resistance from plasmid backbones)
- *In silico* analysis indicated that the 210 basepair region of the *DvSSJ1* targeted by the RNAi construct is specific to corn rootworms in the *Diabrotica* genus, Chrysomelidae family and Coleoptera order.

LMO characteristics

Modified traits

Resistance to diseases and pests

Insects

Coleoptera (beetles)

Western corn rootworm (*Diabrotica virgifera*)

Resistance to herbicides

Glufosinate

Selectable marker genes and reporter genes

Other

Tolerance to mannose

Common use(s) of the LMO

Food

Feed

Detection method(s)

Additional Information

See attached documents in 'Additional information' section for information on expression within maize plant tissues.

IPD072Aa is 86 amino acids and ~10 kDa in weight. The protein is expressed in all tissues with the highest accumulation being in root tissues. The lowest accumulations are in grain and pollen tissues.

PMI is 391 amino acids and ~43 kDa in weight. The protein is expressed in all tissues.

PAT is 183 amino acids ~21 kDa in weight. The protein can be detected in most tissues, but it below the limit of detection in leaf tissues of mature plants (for the techniques used in the reference documents).

The DvSSJ1-dsRNA had highest expression in leaf and root tissues and lowest expression in grain and pollen tissues.

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Additional Information

Additional Information

dsRNA construct from DP23211

The dsRNA/hpRNA sequence silences the *dvssj1* gene in western corn rootworm (i.e. not an endogenous maize gene). When pest ingests DP23211 tissue, midgut epithelial cells take up DvSSJ1 hpRNA, which then trigger an RNAi response due to the double stranded nature of the molecule. The hpRNA is processed into small interfering RNA (siRNA) and complex with host proteins to form an RNA-induced silencing complex. This complex binds to mRNA containing complementary sequences and results in cleavage and degradation of the mRNA. Silencing of *DvSSJ1* mRNA in the midgut epithelial cells results in decreased translation of the DvSSJ1 protein. This protein is part of the smooth septate junction protein complex, a type of occluding junction found in invertebrate epithelial cells that is involved in physically connecting adjacent epithelial cells to create the intestinal barrier and are important in regulating invertebrate gut homeostasis. The *dvssj1* gene is an ortholog of the *Drosophila melanogaster* snakeskin gene, the protein of which is a critical component of the SSJ protein complex. Reduction of DVSSJ1 protein in disrupts the SSJ protein complex and leads to loss of barrier integrity, larval growth inhibition and mortality.

While dsRNA processing into siRNA will occur in the plant, intact dsRNA would likely be present as well. There is evidence to suggest that the DvSSJ1 hpRNA *in planta* is the functional RNA form to control western corn rootworm.

Other relevant website addresses and/or attached documents

? [Euginius - DP23211 maize](#) (*English*)

? [WO2019209700A1 - Maize event DP-023211-2 and methods for detection thereof.pdf](#) (*English*)

? [Food Standards Australia New Zealand - Safety assessment - Application A1202 - Food derived from herbicide-tolerant and insect-protected corn line DP23211.docx](#) (*English*)

? [APHIS - 20-20301p - DP23211 maize \[Large file - 1202 pages \(26.6 MB\) - link to PDF\]](#) (*English*)

? [Agronomic and compositional assessment of genetically modified DP23211 maize for corn rootworm control.pdf](#) (*English*)

[BCH-LMO-SCBD-116060-2](#)

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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