

Biosafety Clearing-House (BCH)

LIVING MODIFIED ORGANISM (LMO)


BCH-LMO-SCBD-260914-1

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

LAST UPDATED: 04 JUL 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.



DP-915635-4
Borer-resistant, herbicide-tolerant 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=260914>


Name

Borer-resistant, herbicide-tolerant maize

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Transformation event

DP915635

Unique identifier

DP-915635-4

Developer(s)

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

ORGANIZATION

Pioneer Hi-Bred International Inc.
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Description

The maize was modified through a site-specific transformation protocol for insect resistance and herbicide tolerance. For resistance to Western corn rootworm (*Diabrotica virgifera*), the maize expresses *Ophioderma pendulum* insecticidal protein IPD079Ea, which has a pore-forming mode of action against feeding larvae. The protein binds receptors in the insect's

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midgut which are different from the receptors that Bt toxins interact with, allowing the maize to overcome Bt-resistance in Coleoptera pests. For tolerance to glufosinate, the maize expresses *Streptomyces viridochromogenes* phosphinothricin N-acetyltransferase, which inactivates the herbicidal compound through acetylation. In addition, the maize also contains an *Escherichia coli* phosphomannose isomerase cassette, which allows for modified plants to use mannose as a carbon source and thus is a selectable marker during transformation. The transformation protocol involved two steps to achieve a site specific integration into the maize genome. In the first step, four plasmids were introduced by microparticle bombardment, which introduced recombination sites at a specific location using transiently expressed CRISPR/Cas9. In the second step, Agrobacterium-mediated transformation was used to introduce the final gene cassettes through a recombination with sequences present in the first insertion site. More information regarding the two-step site-specific transformation is provided below.

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

Maize variety PHR03 EN

Characteristics of the modification process

Vector

PHP73878 and PHP83175 EN

Techniques used for the modification

Agrobacterium-mediated DNA transfer
Biolistic / Particle gun
Gene editing (e.g. CRISPR-Cas, etc.)

Genetic elements construct



CS-ipd079ea-OPHPE
1.440 kb

T-sci_1b-SORBI
0.953 kb

T-gz27-MAIZE
0.460 kb

T-In2_1-MAIZE
0.940 kb

O-frt1-YEAST
0.048 kb

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-260877-1 FLIPPASE RECOMBINASE RECOGNITION TARGETS | SACCHAROMYCES CEREVISIAE (YEAST, YEASX) |

Recognition sequence

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-103069-3 LOXP RECOMBINATION SITE |

recombination site

BCH-GENE-SCBD-116047-2 UBIQUITIN TERMINATOR - SORGHUM BICOLOR - SORGHUM |

BCH-GENE-SCBD-116062-1 GAMMA KAFARIN TERMINATOR - SORGHUM BICOLOR - SORGHUM |

BCH-GENE-SCBD-260878-1 RCC3 ENHANCER | SORGHUM BICOLOR (SORGHUM) |

Enhancer

BCH-GENE-SCBD-116052-1 PREDICTED CALMODULIN 5 GENE INTRON - ZEA MAYS - MAIZE, CORN, MAIZE |

BCH-GENE-SCBD-260880-1 PCOA PROMOTER | ZEA MAYS (MAIZE, CORN, MAIZE) |

Promoter

BCH-GENE-SCBD-260899-1 INSECTICIDAL PROTEIN IPD079EA | OPHIODERMA PENDULUM (OLD WORLD ADDER'S-TONGUE, DAUN RAMBU, OPHPE) |

Protein coding sequence | Resistance to diseases and pests (Insects, Coleoptera (beetles), Western corn rootworm (Diabrotica virgifera))

BCH-GENE-SCBD-260900-2 SUBTILISIN-CHYMOTRYPSIN INHIBITOR 1B TERMINATOR | SORGHUM BICOLOR (SORGHUM) |

Terminator

BCH-GENE-SCBD-116051-1 27-KDA GAMMA ZEIN TERMINATOR - ZEA MAYS - MAIZE, CORN, MAIZE |

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

Terminator

Notes regarding the genetic elements present in this LMO

The modified maize contains three gene cassettes: *Escherichia coli* phosphomannose isomerase (*pmi*); *Streptomyces viridochromogenes* phosphinothricin N-acetyltransferase (*pat*) and *Ophioderma pendulum* insecticidal protein IPD079Ea (*ipd079ea*).

The *pmi* coding sequence was inserted near an endogenous promoter and thus is still expected to have sufficient activity for expression. Transcription is terminated by a *Solanum tuberosum* proteinase inhibitor II (*pinII*) terminator. A second terminator, maize 19-kDa zein gene terminator, was included to prevent transcription beyond the gene cassette (limits read through/'leaky' expression of the adjacent gene cassette).

The *pat* coding sequence is under control of an *Oryza sativa* actin promoter and *Cauliflower mosaic virus* 35S terminator. The promoter contains an intron from the rice actin gene for enhanced expression. Two additional terminators, *Sorghum bicolor* ubiquitin and gamma-kafarin, are included to isolate the gene cassette, preventing transcription beyond the gene cassette (limits read through/'leaky' expression of the adjacent gene cassette).

The *ipd079ea* coding sequence under control of a maize PCOa promoter and *S. bicolor* subtilisin-chymotrypsin inhibitor 1B terminator. The promoter is enhanced by *S. bicolor* RCc3 enhancers and promotes root-specific expression. An additional two terminators, maize 27-kDa gamma zein and In2-1, were included to prevent transcription beyond the gene cassette.

Note:

- Sequencing analysis indicated that the maize contains a single, intact insertion of the expected sequences into chromosome 1 of the maize genome. The sequences were not rearranged or truncated. The analysis also indicated that PHP73878 and PHP83175 vector backbone sequences were absent.
- Sequencing analysis indicated that PHP70605, PHP21139 and PHP21875 were absent from the maize genome (also see below).

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Transformation of the maize:

The transformation of the maize (site-specific integration) was performed in two steps to insert the transgene cassettes into chromosome 1 in a controlled manner.

1. The first transformation (microparticle bombardment)

- PHP73878 (for integration into maize genome)
 - Contains sequences for CRISPR/Cas9 mediated recombination, *loxP* site for *Cre* recombination, FRT1 site for FLP recombination, *nptII*, *pinII* terminator and FRT site.
- PHP70605 (facilitates recombination; not inserted into the maize genome)
 - Contains T3 promoter, maize *ubi* promoter, nuclear locating signal from SV40, *cas9* exon 1, intron 1 from potato *LS1*, *cas9* exon 2, nuclear locating signal from *A. tumefaciens virD2*, *pinII* terminator, maize *polIII* U6 promoter, guide RNA (gRNA) for Cas9 and maize *polIII* U6
- PHP21139 (aids in regeneration during tissue culture; not inserted into genome)
 - Contains maize *Wuschel 2 (wus2)*

- PHP21875 (aids in the regeneration of plants *in vitro*; not inserted into genome)
 - Contains coding optimized ovule development protein 2 (*odp2*)
 - Following microparticle bombardment, the gRNA and Cas9 are transiently expressed. The gRNA targets sequences on chromosome 1 (which are also present in PHP73878) to cause a site specific integration of PHP73878 into chromosome 1.
 - Plants were regenerated using tissue culture and selected for using kanamycin (from the introduced *E. coli* neomycin phosphotransferase II cassette).
2. The second transformation (*Agrobacterium tumefaciens*-mediated)
- PHP83175:
 - Integrated into genome: *pmi*, *pat* and *ipd079ea*.
 - Not integrated into genome (transiently expressed): *wus2*, *odp2* and yeast flippase.
 - Following introduction of the plasmid into host cells by *Agrobacterium tumefaciens*, expression of flippase directs a recombination between the FRT sites in PHP73878 and PHP83175, resulting in the replacement of the *nptII* cassette with *pmi*, *pat* and *ipd079ea* cassettes. Transient expression of *wus2* and *odp2* facilitate the regeneration and tissue culture of transformed plants.

For more information, kindly refer to the documents attached in the 'Additional information' section of this record.

LMO characteristics

Modified traits

Resistance to diseases and pests

Insects

Coleoptera (beetles)

Western corn rootworm (*Diabrotica virgifera*)

Resistance to herbicides

Glufosinate

Changes in physiology and/or production

Mannose metabolism

Selectable marker genes and reporter genes

Common use(s) of the LMO

Feed

Food

Additional Information

Other relevant website addresses and/or attached documents

? [Euginius - DP915635](#) [English]

- ? [Complex Trait Loci in Maize Enabled by CRISPR-Cas9 Mediated Gene Insertion.pdf](#) [*English*]
- ? [Anses - BIOT2021SA0116.pdf](#) [*French*]
- ? [US20210381000A1 - Maize event DP-915635-4 and methods for detection thereof.pdf](#) [*English*]
- ? [?????????????????????????????????????.pdf](#) []

BCH-LMO-SCBD-260914-1

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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