UN environment

Convention on Biological Diversity

Biosafety Clearing-House (BCH)

LIVING MODIFIED ORGANISM (LMO) BCH-LMO-SCBD-115759-2

? Decisions on the LMO ? Risk Assessments

LAST UPDATED: 18 NOV 2020

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=115759

CBD

ESF-DAR58-3
Blight-tolerant Darling 58 American Chestnut

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

Name

Blight-tolerant Darling 58 American Chestnut EN

Transformation event

Darling 58

Unique identifier

ESF-DAR58-3

Developer(s)

- ORGANIZATION: STATE UNIVERSITY OF NEW YORK COLLEGE OF ENVIRONMENTAL SCIENCE AND FORESTRY | BCH-CON-SCBD-115754-1

ORGANIZATION

State University of New York College of Environmental Science and Forestry
Academic or research institute
American Chestnut Research and Restoration Project
1 Forestry Drive
Syracuse, New York
12310, United States of America
Phone: +1 315-470-6744
Email: chestnut@esf.edu
Website: https://www.esf.edu/chestnut/

Description

The Darling 58 American chestnut (*Castanea dentata*) was modified for blight resistance EN through the inclusion of wheat (*Triticum aestivum*) oxalate oxidase. The enzyme detoxifies oxalic acid produced by the chestnut blight fungus (*Cryphonectria parasitica*) and allows the tree to tolerate infection. However, the tree is not resistant to blight infection. The modified tree additionally contains an *Escherichia coli* neomycin phosphotransferase II cassette for kanamycin selection 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-115755-1 ORGANISM | CASTANEA DENTATA - AMERICAN CHESTNUT |

Characteristics of the modification process

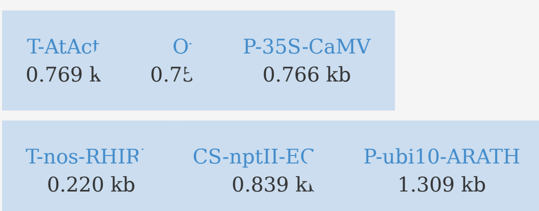
Vector

p35S-OxO 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-100287-7 CAMV 35S PROMOTER |

Promoter

BCH-GENE-SCBD-115756-1 OXALATE OXIDASE - TRITICUM AESTIVUM - WHEAT |

Resistance to diseases and pests - Fungi

BCH-GENE-SCBD-115757-1 ACTIN 2 TERMINATOR - ARABIDOPSIS THALIANA - THALE CRESS, MOUSE-EAR CRESS, ARABIDOPSIS, ARATH |

BCH-GENE-SCBD-15001-5 NEOMYCIN PHOSPHOTRANSFERASE II | (BACTERIA) |

Protein coding sequence | Resistance to antibiotics (Kanamycin)

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

Terminator

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

Promoter

Notes regarding the genetic elements present in this LMO

Transgene expression: EN

The p35S-OxO vector contains two gene cassettes: *Triticum aestivum* oxalate oxidase (OxO) and *Escherichia coli* neomycin phosphotransferase II (*nptII*). Both cassettes are in a counterclockwise orientation.

Expression of OxO is under control of the *Cauliflower mosaic virus* (CaMV) 35S promoter and the *Arabidopsis thaliana* actin II terminator. The CaMV promoter achieves high levels of transcription.

Expression of *nptII* is under control of an *A. thaliana* ubiquitin 10 promoter and the *Agrobacterium tumefaciens* nopaline synthase terminator. The ubiquitin 10 promoter achieves high levels of expression throughout the tissues of the plant.

Note:
- qPCR analysis indicated the integration of a single copy for OxO in the American chestnut genome.
- Sequencing of the flanking sequences at the insertion site indicated the T-DNA was inserted into a non-coding sequence of Chromosome 7 .
- qPCR analysis indicated transcription of OxO.
- Histochemical staining indicated OxO activity from Darling 58 tisues.

LMO characteristics

Modified traits

Resistance to diseases and pests
  Fungi
Resistance to antibiotics
  Kanamycin
Selectable marker genes and reporter genes

Common use(s) of the LMO

Research
Other (Restoration)

Detection method(s)

Additional Information

For DNA (PCR) detection methods, kindly refer to the APHIS submission document. EN

Additional Information

Additional Information

The American chestnut is critically endangered due to the introduction of the chestnut blight fungus (*Cryphonectria parasitica* (Murr.) Barr) from Asia. The disease was first discovered in the Bronx Zoological Park (New York, USA). The pathogen infects the trees through the stem through wounds or cracks in the bark. Symptoms of the disease include the formation of crankers, mycelial growth in the inner bark and appearance of orange fruiting bodies, which disperse spores by the wind. The fungus can also persist as a saprophyte. In addition to chestnut blight, the wild-type trees are also susceptible to root rot caused by *Phytophthora cinnamomi*.

The fungus invades the tree by secreting oxalic acid into kill living tissue. Oxalic acid acidifies host tissues to toxic levels, chelates calcium ions from cell wall pectin, suppresses reactive oxygen burst and promotes programmed cell death. The pathogen does not exclusively rely on oxalic acid to infect the tree. Thus, in trees containing a transgene or endogenous OxO gene, the fungal damage is lessened and the lifecycle is more saprophytic (not necrotrophic).

Other relevant website addresses and/or attached documents

? APHIS - Petition for determination of nonregulated status for blight-tolerant darling 58 american chestnut.pdf *(English)*

? Transgenic American Chestnuts Do Not Inhibit Germination of Native Seeds or Colonization of Mycorrhizal Fungi.pdf *(English)*

? Developing Blight-Tolerant American Chestnut Trees.pdf *(English)*

? Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen.pdf *(English)*

BCH-LMO-SCBD-115759-2

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
413 rue Saint-Jacques, suite 800
Montreal, Québec, H2Y 1N9
Canada
Fax: +1 514 288-6588
Email: secretariat@cbd.int