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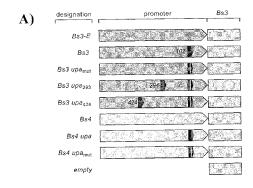
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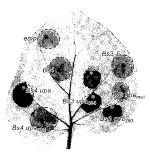
- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))
- (88) Date of publication of the international search report: 1 July 2010

(54) Title: PATHOGEN-INDUCIBLE PROMOTERS AND THEIR USE IN ENHANCING THE DISEASE RESISTANCE OF PLANTS

#### Figure 1







(57) Abstract: Methods for producing pathogen-inducible promoters for the expression of genes in plants are provided. The pathogen-inducible promoters are inducible by one, two, three, or more plant pathogens. Methods for producing R genes that are inducible in a plant by more than one plant pathogen are further provided. Additionally, provided are R genes and other nucleic acid molecules comprising the pathogen-inducible promoters and that are made by such methods as well as plants, plant parts, plant cells, seeds, and non-human host cells comprising the R genes and other nucleic acid molecules.





International application No PCT/US2009/063791

A. CLASSIFICATION OF SUBJECT MATTER INV. C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

#### **B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols) c12N - A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, PAJ, WPI Data

	Citation of document, with indication, where appropriate, or	the relevant passages	Relevant to claim No.
(	ROEMER PATRICK ET AL: "Plant recognition mediated by promo activation of the pepper Bs3 gene" SCIENCE (WASHINGTON D C), vol. 318, no. 5850, October 2007 (2007-10), pages XP002570742 ISSN: 0036-8075 figure 1D	ter resistance	1-4, 10-15, 39-44, 51-61
Special  A" docum consi E" earlier filing L" docum which citatic O" docum other	ther documents are listed in the continuation of Box C. categories of cited documents:  ent defining the general state of the art which is not dered to be of particular relevance document but published on or after the international date ent which may throw doubts on priority claim(s) or n is cited to establish the publication date of another on or other special reason (as specified) nent referring to an oral disclosure, use, exhibition or means ent published prior to the international filling date but than the priority date claimed	cited to understand the prin invention  "X" document of particular releve cannot be considered novel involve an inventive step wh  "Y" document of particular releve cannot be considered to inv document is combined with	er the international filing date onflict with the application but ciple or theory underlying the ance; the claimed invention or cannot be considered to nen the document is taken alone ance; the claimed invention volve an inventive step when the one or more other such docueing obvious to a person skilled me patent family
Date of the	March 2010	03/05/2010	

International application No.

PCT/US2009/063791

Box !	Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)	
1.	With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:	
	a. (means)	
	on paper	
	X in electronic form	
	b. (time)	
	X in the international application as filed	
	together with the international application in electronic form	
	subsequently to this Authority for the purpose of search	
i	Casesquarity to the retaining to the purpose of coale.	
2.	In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.	
3.	Additional comments:	ļ
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International application No PCT/US2009/063791

C(Continua	tion). DOCUMENTS CONSIDERED TO BE RELEVANT	PC1/US2009/063/91
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KAY SABINE ET AL: "A bacterial effector acts as a plant transcription factor and induces a cell size regulator" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, WASHINGTON, DC; US, vol. 318, no. 5850, 1 October 2007 (2007-10-01), pages 648-651, XP009125817 ISSN: 0036-8075 figure 3 page 651, left-hand column	
Α	MAROIS ERIC ET AL: "The Xanthomonas type III effector protein AvrBs3 modulates plant gene expression and induces cell hypertrophy in the susceptible host" July 2002 (2002-07), MOLECULAR PLANT-MICROBE INTERACTIONS, VOL. 15, NR. 7, PAGE(S) 637-646, XP002570743 ISSN: 0894-0282 the whole document	
	TINA JORDAN ET AL: "Physical delimitation of the pepper Bs3 resistance gene specifying recognition of the AvrBs3 protein from Xanthomonas campestris pv. vesicatoria" THEORETICAL AND APPLIED GENETICS; INTERNATIONAL JOURNAL OF PLANT BREEDING RESEARCH, SPRINGER, BERLIN, DE, vol. 113, no. 5, 28 July 2006 (2006-07-28), pages 895-905, XP019417763 ISSN: 1432-2242 page 902, right-hand column, paragraph 1	
X,P	WO 2009/042753 A1 (TWO BLADES FOUNDATION [US]; LAHAYE THOMAS [DE]; BONAS ULLA [DE]) 2 April 2009 (2009-04-02)  Bs3 promoter SEQID5 page 14, line 1 - line 4 page 14, line 15 - line 16 page 14, paragraph 2 page 17, paragraph 2 example 2 page 55; figure 4c; example 4 figure 1D  -/	1-4, 10-15, 39-44, 51-61

International application No PCT/US2009/063791

0/0	No. DOCUMENTS	PCT/US2009/063791		
C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
T	KAY SABINE ET AL: "Detailed analysis of the DNA recognition motifs of the Xanthomonas type III effectors AvrBs3 and AvrBs3 Delta rep16" PLANT JOURNAL, vol. 59, no. 6, September 2009 (2009-09), pages 859-871, XP002570744 ISSN: 0960-7412 page 860, left-hand column figure 3 figure 6			
T	BOCH JENS ET AL: "Breaking the Code of DNA Binding Specificity of TAL-Type III Effectors" SCIENCE (WASHINGTON D C), vol. 326, no. 5959, December 2009 (2009-12), pages 1509-1512, XP002570745 ISSN: 0036-8075 the whole document			
Т	ROEMER PATRICK ET AL: "A single plant resistance gene promoter engineered to recognize multiple TAL effectors from disparate pathogens" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 106, no. 48, December 2009 (2009-12), pages 20526-20531, XP002570746 ISSN: 0027-8424 page 20530, left-hand column; figure 4			
T	ROEMER PATRICK ET AL: "Recognition of AvrBs3-Like Proteins Is Mediated by Specific Binding to Promoters of Matching Pepper Bs3 Alleles" August 2009 (2009-08), PLANT PHYSIOLOGY (ROCKVILLE), VOL. 150, NR. 4, PAGE(S) 1697-1712, XP002570747 ISSN: 0032-0889 the whole document figure 10			

# International application No. PCT/US2009/063791

## INTERNATIONAL SEARCH REPORT

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)
This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows:
see additional sheet.
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  1-4, 39-44(completely); 10-15, 51-61(partially)
The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.  The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.  No protest accompanied the payment of additional search fees.

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4, 39-44(completely); 10-15, 51-61(partially)

Method for making a pathogen inducible promoter comprising producing a nucleic acid molecule comprising one upa box, said upa box binding to a specific TAL effector; wherein the upa box comprises a sequence selected from the group of SEQIDs 17,18,20,22,24,28-33,35-48; a pathogen inducible promoter produced by said method and an expression cassette comprising said upa box and an operably linked R gene; method fo making an R gene, by fusing a upa box containing promoter to an R gene; said method wherein said upa box is selected from the group of SEQIDs 17,18,20,22,24,28-33,35-48, wherein said R gene product is Bs3; a transfomed plant or seed comprising an R gene produced by said method.

2. claims: 5-9, 16-38, 45-50(completely); 10-15, 51-61(partially)

Method for making a pathogen inducible promoter comprising producing a nucleic acid molecule comprising at least two upa boxes, said upa boxes binding different specific TAL effectors; wherein the upa box comprises a sequence selected from the group of SEQIDs 17,18,20,22,24,28-33,35-48; a pathogen inducible promoter produced by said method and an expression cassette comprising said upa box and an operably linked R gene; method for making a promoter inducible by two or more different pathogens by introducing at least two upa boxes known to bind different TAL efectors from different pathogens, a pthogen inducible promoter produced by said method; transfomed plant cellas, seeds and host cells comprising said construct; method for making an R gene, by fusing at least two upa box containing promoter to an R gene; said method wherein said upa box is selected from the group of SEQIDs 17,18,20,22,24,28-33,35-48, wherein said R gene product is Bs3; a transfomed plant or seed comprising an R gene produced by said method.

#### 3. claims: 62-69

Method comprising i) exposing a pant to a TAL effector, ii) identifying TAL induced genes, iii) obtaining the promoter sequence of said genes, iv) comparing the promoters and identifying a upa box, v) assaying for upa box activity, and vi) identifying the upa boxes having upa box activity; said method comprising applying said TAL effector to the plant; further comprising applying cycloheximide before step ii); said method wherein the plant is rice and wherein the TAL effector is either PthXo1, PthXo6, or PthXo7.

Information on patent family members

International application No
PCT/US2009/063791

Patent document cited in search report	Publication	Patent family	Publication
	date	member(s)	date
WO 2009042753 A1	02-04-2009	AR 068568 A1 US 2009133158 A1	18-11-2009 21-05-2009