

RAISBECK, LARA, RODRIGUEZ & RUEDA
MIEMBROS DE LA FIRMA
BAKER & MCKENZIE

CALLE 35 No. 7-25, Piso 4
TELÉFONO: (57-1) 332-2600
BOGOTÁ, D.C. COLOMBIA
WWW.BAKERINFO.COM
OFFICE.BOGOTA@BAKERNET.COM

120
112

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO.

DIVISION DE NUEVAS CREACIONES

E. _____ S. _____

SUPERINDUSTRIA Y COMERCIO

Radicación : 01023525 00000002

Folios: 230

Fecha (HUB): 2001-07-10 16:56:30

Trámite : 002 PATENTES D 1 REGISTRO/ 444 REPUESTA
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Re: Expediente No. 01.023.525.

Solicitud de Patente de Invención titulada:

"RECEPTORES DE INDUCTORES DE LA RESPUESTA
HIPERSENSIBLE Y USOS DE LOS MISMOS".

Solicitante: EDEN BIOSCIENCE CORPORATION.

RESPUESTA AL AUTO NOTIFICADO POR ESTADO No. 040
DE FECHA 10 DE MAYO DE 2001, OFICIO No. 1384

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá D.C., identificado tal como aparece al pie de mi firma en mi carácter de apoderado de la sociedad en referencia, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del término legal:

1. En atención a la primera observación del Examinador, adjunto el poder otorgado por Eden Bioscience Corporation a mi favor debidamente legalizado y oficialmente traducido al español.

- 2 -

2. En atención a la segunda observación del Examinador, me permito presentar un Petitorio Modificado en el cual indico el lugar de constitución de la sociedad solicitante.

3. En atención a la tercera observación del Examinador, adjunto el documento de cesión del derecho de patente otorgado por los inventores a Eden Bioscience Corporation., debidamente legalizado y traducido.

4. En atención a la última observación del Examinador, adjunto un (1) recibo por concepto de una (1) reivindicación de prioridad. De esta manera se completa el pago por concepto de reivindicación de prioridad sobre dos (2) documentos, ya que en el expediente ya radica un (1) pago por tal concepto.

5. Adicionalmente, me permito adjuntar los documentos sobre los cuales reivindica prioridad la presente solicitud, documentos US 60/250.710 y US 60/191.649, junto con la traducción de sus respectivos Capítulos Reivindicatorios.

Atentamente,



CARLOS R. OLARTE
C.C. No. 79.782.747 de Bogotá
T.P. 74.295

Anexos: Poder
Petitorio
Documento de Cesión
Recibo
Documentos de Prioridad (2)
Traducción del Capítulo Reivindicatorio de los Documentos de Prioridad (2)

128

114

PETITORIO

Espacio reservado para el adhesivo de radicación

FORMULARIO UNICO DE SOLICITUD DE PATENTE
2000-3

①

SOLICITUD DE :

☒ Patente de Invención.

☐ Patente de Modelo de Utilidad

②

SOLICITANTE
(71)

Nombre: EDEN BIOSCIENCE CORPORATION.

Dirección: 1816 North Creek Parkway N.
Bothell, Washington 98011-8205

Teléfono: 570-0010 Fax: 285-6908

E-Mail: _____

Domicilio: Estados Unidos de América

IDENTIFICACION

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Número _____

③

REPRESENTANTE
O APODERADO
(74)

Nombre: CARLOS R. OLARTE

Dirección: CALLE 35 No. 7-25 Piso 4

Teléfono: 570-0010 Fax: 285-6908

E-Mail: patents.bogota@bakernet.com

IDENTIFICACION

C.C. ☒ NIT ☐

C.E. ☐ Otro ☐

Número 79.782.747 TP 74.295

④

INVENTOR (ES)
(72)

Nombre: (1) XIAOLING SONG (2) HAO FAN
(3) ZHONG-MIN WEI

Dirección: (1) 14710 NE 179th Street, Apt. 75 Woodinville,
Washington 98072 USA.
(2) 19712 6th Drive S.E. Bothell, Washington 98012, USA (3) 8230 125th
Court Kirkland, Washington 98034 USA.

Teléfono: _____ Fax: _____

E-Mail: _____

Domicilio: Estados Unidos de América.

IDENTIFICACION

C.C. ☐ NIT ☐

C.E. ☐ Otro ☐

Número _____

⑤

Título (54)

RECEPTORES DE INDUCTORES DE LA RESPUESTA HIPERSENSIBLE Y USOS DE LOS
MISMOS.

⑥

Clasificación Internacional (51)

C07K14/415, C12N15/29

⑦

Prioridad

SI ☒ NO ☐

(33) País de Origen

US

US

(32) Fecha

Marzo 23, 2000

Diciembre 1, 2000

(31) Número de Solicitud

60/198,359

60/250,710

⑧

Para publicar a partir de la fecha de la presente solicitud a los:

6 meses ☐

12 meses ☐

18 meses ☐

Otro ☐

⑨

Comprobante de pago No.

Fecha:

Instrucciones para el diligenciamiento del presente formulario, ver reverso de esta página.

SUPERINDUSTRIA Y COMERCIO

NIT : 800176089-2 Radicacion : 01023525 00000002 Folios: 230

Fecha (RIB): 2001-07-10 16:56:30

Tramite : 002 PATENTES D 1 REGISTRO/ 444 REPUESTA

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

RECIBO OFICIAL DE CAJA : 01 - 33,956

FECHA : MAYO 30 DE 2001

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
JORGE LUIS GOMEZ	CONSIGNACION	BANCO POPULAR	050-00110-6	8151156	30/05/2001	17,412,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01	SOLICITUDES	
		82 INVOCACION DE UNA PRIORIDAD EN NU	100,000.00
		TOTAL :	\$ 100,000.00

SON CIN MIL PESOS

RESPONSABLE :

RECIBO DE CAJA APLICADO AL EXPEDIENTE No.

Carrera 13 No. 27-00 Pisos 5, 7 y 10
Conmutador 382 0840
Fax 350 5220
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

124
116

TRADUCCION OFICIAL No. 224/01 de un documento escrito en Inglés, el cual para su identificación se sella con el sello del Traductor. Esta es una Traducción Oficial efectuada el día 11 de junio de 2001 por la Sra. María Teresa Lara R., c.c. 41.568.055 de Bogotá, Traductora Oficial según Resolución No. 01016 del 2 de octubre de 1998, expedida por el Ministerio de Justicia.

=====

Poder otorgado por EDEN BIOSCIENCE CORPORATION para su representación en relación con asuntos de propiedad Industrial en Colombia. Certificación notarial suscrita por Ann M. Pierson, Notario Público del Estado de Washington, y legalizaciones subsiguientes.

EL PODER Y LA CERTIFICACIÓN NOTARIAL NO SE TRADUCEN POR ENCONTRARSE EN INGLÉS Y ESPAÑOL EN EL DOCUMENTO ORIGINAL.

[Sello notarial] ANN M. PIERSON

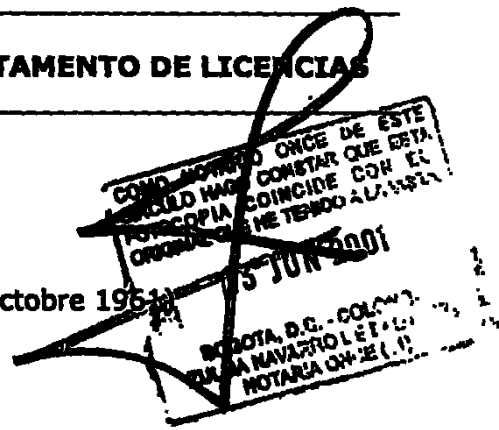
NOTARIO PUBLICO
LA COMISIÓN EXPIRA 7-1-03
ESTADO DE WASHINGTON

ESTADO DE WASHINGTON

DEPARTAMENTO DE LICENCIAS

APOSTILLE

(Convention de La Haye du 5 octobre 1954)



1. País: Estados Unidos de América

Este documento público

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINISTERIO DE JUSTICIA

125

117

2. ha sido firmado por **ANN M PIERSON**
3. actuando en calidad de **Notario Público**
4. lleva el sello/estampilla de **ANN M PIERSON**

CERTIFICADO

5. en Olympia, Washington
6. el día 10 de mayo de 2001
7. por Funcionaria de Autenticaciones **Lila Khan**
8. No. 500 - 4220
9. Sello/Estampilla
10. Firma

(Sello Oficial del
Departamento de Licencias
del Estado de Washington)

(Fdo.) Fred Stephens
Fred Stephens, Director
Departamento de Licencias
Olympia, Washington

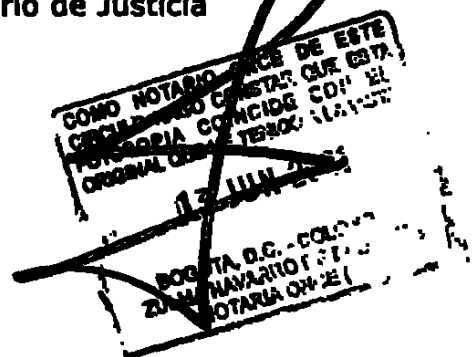
MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINISTERIO DE JUSTICIA

=====

La anterior es traducción fiel y completa del documento en referencia, de la cual se deja copia en este archivo para su confrontación.

Maria Teresa Lara R.

María Teresa Lara R.
c.c. 41.568.055 de Bogotá
Traductora Oficial según
Resolución No. 01016 del
2 de octubre de 1998, del
Ministerio de Justicia





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118

STATE OF WASHINGTON DEPARTMENT OF LICENSING

APOSTILLE

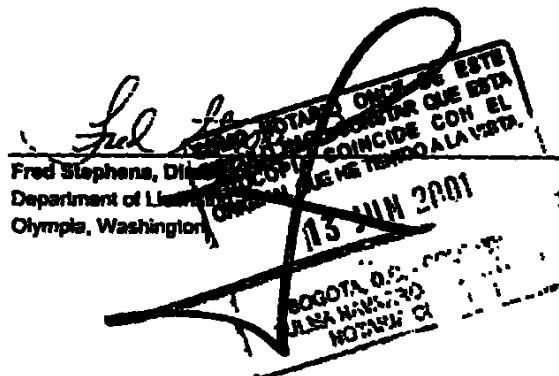
(Convention de La Haye du 5 octobre 1961)

1. Country: United States of America

This public document
2. has been signed by ANN M PIERSON
3. acting in the capacity of Notary Public
4. bears the seal/stamp of ANN M PIERSON

CERTIFIED

5. at Olympia, Washington 6. the Tenth Day of May, 2001
7. by Authenticating Officer Lila Khan
8. No. 500 - 4220
9. Seal/Stamp: 10. Signature:



Desarrollado
MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01616
DE MINJUSTICIA 224/01

[Signature]
BOGOTÁ, D.C. - COLOMBIA
13 JUN 2011
CÓMO NOTARIO ONCE DE ESTE
CIRCULO MASO COMENTA QUE ESTE
FOTOCOPIA COINCIDE CON EL
ORIGINAL QUE HE TENIDO EN VISTA

RAISBECK, LARA, RODRIGUEZ & RUEDA
MIEMBROS DE LA FIRMA
BAKER & MCKENZIE

CALLE 35 No. 7-25, Piso 4
TELÉFONO: (57-1) 332-2600
BOGOTÁ, D.C. COLOMBIA
WWW.BAKERINFO.COM
OFFICE.BOGOTA@BAKERNET.COM

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[SAMPLE]

POWER OF ATTORNEY

PODER

The undersigned. **EDEN BIOSCIENCE CORPORATION**

La abajo firmada.

(Name of Company)

EDEN BIOSCIENCE CORPORATION

located in (Street, address)
11816 North Creek Parkway N.,
(city, state, country)
Bothell, Washington 98011-8205 USA
declares that it hereby grants to

domiciliada en
11816 North Creek Parkway N.,
Bothell, Washington 98011-8205 .
declara por el presente otorgar a **EE.UU.**

CARLOS REINALDO OLARTE and/or

CARLOS REINALDO OLARTE and/or

a full and sufficient power of attorney to apply to the proper national officers and authorities for the issue of:

Patents of Invention, Patents for Utility Models, Industrial Designs, mergers, changes of name, assignments, filing and prosecutions, changes of address

to which end it empowers them to take all steps necessary before said authorities for the objects stated, to file petitions, prepare descriptions, make protests, declarations, appeals and objections, pay imposts, apply for certifications, receive documents and securities, abandon applications and collect refunds. To the said mandatories sufficient power is granted hereby to receive notifications for and on behalf of the Company, to confer powers on those who are to represent the company judicially and extrajudicially, to answer in court in the matter of all claims and demands that may be presented in connection with the patents, and to do everything that may be required, before administrative or judicial officers of any class giving them likewise power to substitute these presents and revoke such substitutions if necessary.

poder especial amplio y bastante para recabar de las oficinas y autoridades nacionales que correspondan, la obtención de :

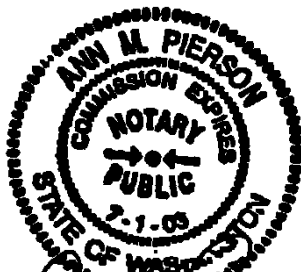
Patentes de Invención, Patentes de Modelo de Utilidad, Diseños Industriales, fusiones, cambios de nombre, cesiones, presentación y tramitación de oposiciones, cambios de dirección

a cuyo efecto los faculta para dar ante dichas autoridades, todos los pasos necesarios al objeto indicado, elevar solicitudes, formular descripciones, protestas, declaraciones, apelaciones y reclamos, oblar impuestos, solicitar testimonios, recibir documentos y valores, desistír y percibir. Se concede a los expresados mandatarios poder bastante para recibir notificaciones a nombre de la compañía, otorgar poderes a quienes vayan a representar a la compañía judicial y extrajudicialmente, responder en juicio a todas las reclamaciones o demandas que por motivo de las patentes se presentaren y hacer cuanto fuere necesario, ante las autoridades administrativas o judiciales de cualquier orden, dándoles asimismo facultad para sustituir el presente y en caso necesario revocar dichas sustituciones.

Done and subscribed in

On the day of

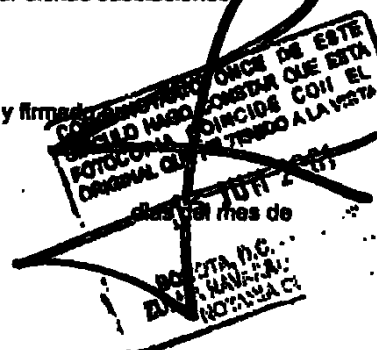
2001



Jerzy M. Butler, President
Signature of the person issuing the Power

Dado y firmado

A los



RAISBECK, LARA, RODRIGUEZ & RUEDA

Members of the Firm

BAKER & MCKENZIE

ATTORNEYS AT LAW

APARTADO AEREO 3748

TELEFONO: 285-1498

SANTAFE DE BOGOTA, D.C.

COLOMBIA

128
120

[SAMPLE]

NOTARIAL CERTIFICATION

CERTIFICACION NOTARIAL

n this (day) day of (Month) A los dias del mes de

8 , the undersigned Notary de 19 ,el suscrito Notario

CERTIFIES

DA FE

1. That (name of official signing power)

Jerry L. Butler

of legal age and domiciled in (Street, address)
(city, state, country)

Bothell, Washington, USA
set his hand on the foregoing document.

2. That the grantor is to me personally know and that he/she has
legal capacity to execute the instrument.

3. That the grantor has executed the foregoing document as

President (Position Held)

of the official person EDEN BIOSCIENCE CORPORATION

(name of the company)

4. That the judicial person EDEN BIOSCIENCE CORPORATION
(Name of the Company)

having its home office in (Street, address)
(address, city, state, country)

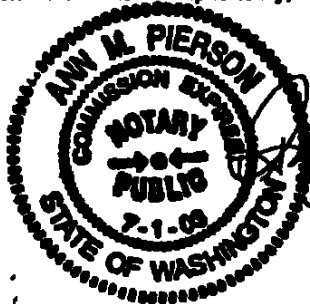
Bothell, Washington, USA
is duly organized, has present legal existence and that the purposes for
which the power of attorney is granted are within the scope of its corporate
purposes.

5. That the grantor has in fact the referred to authority and that his/her
representation is legal.

6. That I have based certifications 3, 4 and 5 above on the following authentic
documents for such purpose exhibited to me and which I mention specifically,
giving their dates and their origin or source:

(list sources)

Notary Public



1. Que Jerry L. Butler

mayor y domiciliado en

Bothell, Washington, EE.UU.

suscribió de su puño y letra el documento que antecede.

2. Que conozco al otorgante y que éste tiene capacidad legal
para el otorgamiento.

3. Que el otorgante ha suscrito el referido documento en su
carácter de

Presidente

de la persona jurídica

EDEN BIOSCIENCE CORPORATION

4. Que la persona jurídica

EDEN BIOSCIENCE CORPORATION

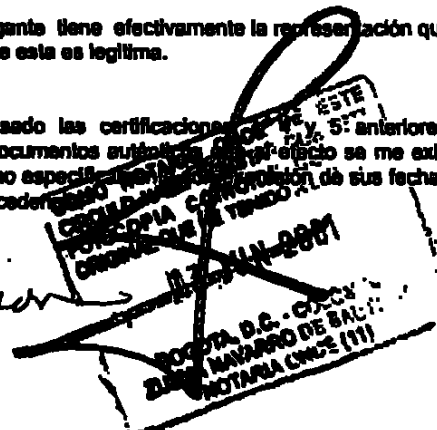
con sede en

Bothell, Washington, EE.UU.

está legalmente constituida, que tiene existencia legal actual y que
el acto para el cual se ha otorgado el poder está comprendido entre
los que constituyen su objeto social.

5. Que el otorgante tiene efectivamente la representación que
ostenta, y que esta es legítima.

6. Que he basado las certificaciones 3, 4 y 5 anteriores en los
siguientes documentos auténticos que a mi efecto se me exhibieron
que menciono específicamente dando fe de sus fechas y de su
origen o procedencia.



El Notario Público

Notarial Certification - 2

6.

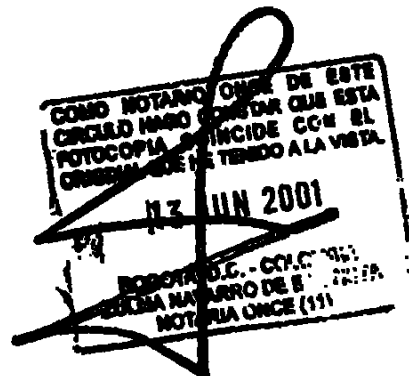
- Amended and Restated Articles of Incorporation dated November 13, 2000
- Third Amended and Restated Bylaws dated July 6, 2000
- Minutes of Board of Directors dated December 16, 1999

Acreditación notarial - 2

6.

- Artículos de incorporación modificados y reiterados del día trece de noviembre de dos mil
- Tercera directivas modificadas y reiteradas del día seis de julio de dos mil
- Libro actas del directorio del día dieciséis de diciembre de mil novecientos noventa y nueve

129
124



130
122

TRADUCCION OFICIAL No. 226/01 de un documento escrito en Inglés, el cual para su identificación se sella con el sello del Traductor. Esta es una Traducción Oficial efectuada el día 11 de junio de 2001 por la Sra. María Teresa Lara R., c.c. 41.568.055 de Bogotá, Traductora Oficial según Resolución No. 01016 del 2 de octubre de 1998, expedida por el Ministerio de Justicia.

= = = = =

Traspaso del invento "Receptores para Provocadores de Respuesta Hipersensible y Usos de los Mismos" a EDEN BIOSCIENCE CORPORATION. Certificación notarial suscrita por Jo Ann Whalen, Notario Público del Estado de Nueva York, y legalizaciones subsiguientes.

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINISTROS

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS
Oficina de Patentes y Marcas
SECRETARIO ASISTENTE Y COMISIONADO
DE PATENTES Y MARCAS
Washington, D.C. 20231

Octubre 30, 2000

PTAS

NIXON PEABODY LLP
MICHAEL L. GOLDMAN
CLINTON SQUARE
P.O. BOX 31051
ROCHESTER, NY 14603

101460287A

OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS
AVISO DE REGISTRO DE DOCUMENTO DE TRASPASO

El documento adjunto ha sido registrado por la División de Traspasos de la Oficina de Patentes y Marcas de los EE.UU. Una copia completa en microfilm se encuentra disponible en la Sala de Búsquedas de Traspasos bajo el Número de Rollo y Cuadro señalados a continuación.

Favor revisar toda la Información contenida en este aviso. La información contenida en este Aviso de Registro refleja los datos presentes en el Sistema de

131
123

Traspasos de Patentes y Marcas. En caso de que encuentren cualquier error o tengan cualquier pregunta en relación con este aviso, pueden comunicarse con el empleado cuyo nombre aparece en este aviso en el 703-308-9723. Favor enviar cualquier solicitud de corrección a: U.S. PATENT AND TRADEMARK OFFICE, Assignment Division, Box Assignments, CG-4, 1213 Jefferson Davis Hwy, Suite 320, Washington, D.C. 20231.

FECHA DE REGISTRO: 08/24/2000

ROLLO/CUADRO: 011062/0331
NÚMERO DE PÁGINAS: 4

MARIA TERESA LABA L.
TRADUCTORA OFICIAL
REGULACION No. 01010
DE MINISTROS

RESUMEN: TRASPASO DEL INTERÉS DEL CEDENTE (VER DOCUMENTO PARA DETALLES).

CEDENTE:

SONG, XIAOLING

FECHA DOC: 05/08/2000

CEDENTE:

FAN, HAO

FECHA DOC: 05/08/2000

CEDENTE:

WEI, ZHONG-MIN

FECHA DOC: 05/08/2000

CESIONARIA:

EDEN BIOSCIENCE CORPORATION
11816 NORTH CREEK PARKWAY N.
BOTHELL, WASHINGTON 98011-8205

NÚMERO DE SERIE: 60191649

FECHA PRESENTACIÓN: 03/23/2000

NÚMERO DE PATENTE:

FECHA EXPEDICIÓN:

REGISTRADO
Nixon Peabody LLP

NOV 6 2000

EXPED. 21829 / 60
DKT JB

AB?

124

TRASPAS

CONSIDERANDO, que los suscritos, **Xiaoling Song**, ciudadano de la República Popular China, residente en 14110 NE 179th Street, Apt. 75, Woodinville, Washington 98072, **Hao Fan**, ciudadano de la República Popular China, residente en 19712 6th Drive, S.E., Bothell, Washington 98012, y **Zhong-Min Wei**, ciudadano de los Estados Unidos, residente en 8230 125th Court, Kirkland, Washington 98034, hemos inventado ciertas mejoras nuevas y útiles en **RECEPTORES PARA PROVOCADORES DE RESPUESTA HIPERSENSIBLE Y USOS DE LOS MISMOS** para las cuales hemos elaborado una solicitud de Patente de Invención en los Estados Unidos, presentada bajo el No. de Serie de los EE.UU. **60/191,649** el **23 de marzo de 2000**;

Y CONSIDERANDO, que **Eden Bioscience Corporation**, con sede principal en 11816 North Creek Parkway N., Bothell, Washington 98011-8205, desea adquirir todo derecho, título e Interés en y sobre las citadas mejoras y la antedicha Solicitud:

AHORA, POR TANTO, por contraprestación válida y valiosa, incluido el salario o el pago por la realización de los Inventos, o los beneficios laborales, el recibo de la cual se reconoce por la presente, nosotros, los antedichos inventores, reconocemos por la presente que hemos vendido, cedido, traspasado y entregado, y por la presente vendemos, cedemos, traspasamos y entregamos, a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, todo derecho, título e Interés en todo el mundo en, sobre y en virtud de tales mejoras, y la citada solicitud y todas las divisiones, renovaciones y continuaciones de la misma, y todas las Patentes de Invención de los Estados Unidos que puedan otorgarse sobre la misma y todas las reexpediciones y prórrogas de la misma, y todos los derechos de prioridad

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bajo Convenciones Internacionales y las solicitudes de Patentes de Invención que puedan en un futuro presentarse para tales mejoras en cualquier país o países distintos de los Estados Unidos, y todas las Patentes de Invención que puedan otorgarse para dichas mejoras en cualquier país o países distintos de los Estados Unidos, y todas las prórrogas, renovaciones y reexpediciones de las mismas; y por la presente autorizamos y solicitamos al Comisionado de Patentes de los Estados Unidos, y a cualquier Funcionario de cualquier país o países distintos de los Estados Unidos, cuya función sea expedir patentes en virtud de solicitudes del tipo mencionado, expedir todas las Patentes de Invención para tales mejoras a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, de conformidad con los términos de este Instrumento.

Y POR LA PRESENTE pactamos y acordamos que le comunicaremos a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, cualesquiera hechos que lleguen a nuestro conocimiento con respecto a dichas mejoras y testificaremos en cualquier proceso judicial, firmaremos todos los documentos legales, otorgaremos todas las solicitudes de división, continuación y reexpedición, prestaremos todos los juramentos que correspondan y en general haremos todo lo posible por asistir a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, para obtener y hacer valer una protección de patente apropiada para tales mejoras en todos los países.

EN TESTIMONIO DE LO CUAL, firmo el presente y estampo aquí mi sello a los 8 días del mes de mayo de 2000.

(Fdo.) Xiaoling Song

MARIA TERESA LARA L.
TRADUCTORA OFICIAL
REGISTRADA EN EL
DE MEXICO

134
126

Mayo 18, 2001

Juro que esta es fiel copia del original.

h

(Fdo.) Jo Ann Whalen

[Sello] JO ANN WHALEN
Notario Público, Estado de Nueva York
Calificada en el Condado de Monroe
Certificado registrado en el Condado de Monroe
Reg. No. 5038037
La comisión expira el 1/17/2003

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01010
DE MINISTRIA

ESTADO DE WASHINGTON

CONDADO DE KING SS:

A los 8 días del mes de mayo de 2000, ante mí personalmente compareció el mencionado **Xiaoling Song**, a quien conozco personalmente como la persona que firmó el documento por su propia y libre voluntad para los propósitos allí señalados.

(Fdo.) Ann M. Pierson
Notario Público

[Sello] ANN M. PIERSON
NOTARIO PUBLICO
LA COMISION EXPIRA EL
7-1-03
ESTADO DE WASHINGTON

EN TESTIMONIO DE LO CUAL, firmo el presente y estampo aquí mi sello a los 8 días del mes de mayo de 2000.

(Fdo.) Hao Fan

135

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ESTADO DE WASHINGTON

CONDADO DE KING SS:

A los 8 días del mes de mayo de 2000, ante mí personalmente compareció el mencionado **Hao Fan**, a quien conozco personalmente como la persona que firmó el documento por su propia y libre voluntad para los propósitos allí señalados.

(Fdo.) Ann M. Pierson
Notario Público

[Sello] ANN M. PIERSON
NOTARIO PUBLICO
LA COMISION EXPIRA EL
7-1-03
ESTADO DE WASHINGTON

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE EJECUTIVA

EN TESTIMONIO DE LO CUAL, firmo el presente y estampo aquí mi sello a los 8 días del mes de mayo de 2000.

(Fdo.) Zhong-Min Wei

ESTADO DE WASHINGTON

CONDADO DE KING SS:

A los 8 días del mes de mayo de 2000, ante mí personalmente compareció el mencionado **Zhong-Min Wei**, a quien conozco personalmente como la persona que firmó el documento por su propia y libre voluntad para los propósitos allí señalados.

(Fdo.) Ann M. Pierson
Notario Público

[Sello] ANN M. PIERSON
NOTARIO PUBLICO
LA COMISION EXPIRA EL
7-1-03
ESTADO DE WASHINGTON

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ESTADO DE NUEVA YORK) ss.
CONDADO DE MONROE)
OFICINA DEL SECRETARIO DEL CONDADO

Yo, MAGGIE BROOKS, Secretaria del Condado de Monroe, del Tribunal de Condado en dicho Condado y del Tribunal Supremo, los cuales son ambos Tribunales de Registro, provistos de sello común, CERTIFICO POR EL PRESENTE, que

JO ANN WHALEN

ante quien manifiesta haber sido otorgado o tomado el Juramento, Declaración Jurada o Reconocimiento anterior, y quien lo certifica, era en el momento de otorgar o tomar el mismo una NOTARIO PUBLICO en y para el antedicho Estado, y estaba debidamente autorizada para tomarlo, y estaba autorizada por las leyes de este Estado para tomar y certificar el reconocimiento y prueba de escrituras que deban registrarse en este Estado; y que conozco bien su letra manuscrita y considero en verdad que su firma en el mismo es auténtica y que dicha funcionaria no está obligada por ley a tener un sello ni requiere tener una muestra de la Impresión del mismo registrada o depositada en esta oficina, o registrada o depositada en cualquier otro lugar.

En Fe de lo Cual, he colocado aquí mi firma y el sello oficial de dichos Tribunales a los 21 días del mes de mayo de 2001.

(Fdo.) Maggie Brooks

A No. 26286

Secretaria

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. País: Estados Unidos de América

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 61818
DE MONTREAL

137
129

Este documento público

2. ha sido firmado por **Maggie Brooks**
3. actuando en calidad de **Secretaria del Condado**
4. lleva el sello/estampilla del condado de **Monroe**

18

Certificado

5. en Albany, New York
6. el día 01 de junio de 2001
7. por Subsecretario de Estado Especial, Estado de Nueva York
8. No. A-77639E
9. Sello/Estampilla
10. Firma

(Gran Sello del
Estado de Nueva York)

(Fdo.) Joseph P. Brozowski

Joseph P. Brozowski
Subsecretario de Estado Especial

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINISTERIO DE JUSTICIA

=====

La anterior es traducción fiel y completa del documento en referencia, de la cual se deja copia en este archivo para su confrontación.

Maria Teresa Lara R.

María Teresa Lara R.
c.c. 41.568.055 de Bogotá
Traductora Oficial según
Resolución No. 01016 del
2 de octubre de 1998, del
Ministerio de Justicia

Apostille

(Convention de La Haye du 5 Octobre 1961)

138
130

14

1. Country: United States of America

This public document

2. has been signed by Maggie Brooks

3. acting in the capacity of County Clerk

4. bears the seal/stamp of the county of Monroe

Certified

5. At Albany, New York 6. the 01st day of June 2001

7. by Special Deputy Secretary of State, State of New York

8. No. A-77639E

9. Seal/Stamp

10. Signature

Joseph P. Brozowski

Joseph P. Brozowski

Special Deputy Secretary of State

Descoloniz.
MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINJUSTICIA 226/01



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

131
139

OCTOBER 30, 2000

PTAS



101460287A

NIXON PEABODY LLP
MICHAEL L. GOLDMAN
CLINTON SQUARE
P.O. BOX 31051
ROCHESTER, NY 14603

70

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 08/24/2000

REEL/FRAME: 011062/0331
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
SONG, XIAOLING

DOC DATE: 05/08/2000

ASSIGNOR:
FAN, HAO

DOC DATE: 05/08/2000

ASSIGNOR:
WEI, ZHONG-MIN

DOC DATE: 05/08/2000

ASSIGNEE:
EDEN BIOSCIENCE CORPORATION
11816 NORTH CREEK PARKWAY N.
BOTHELL, WASHINGTON 98011-8205

SERIAL NUMBER: 60191649
PATENT NUMBER:

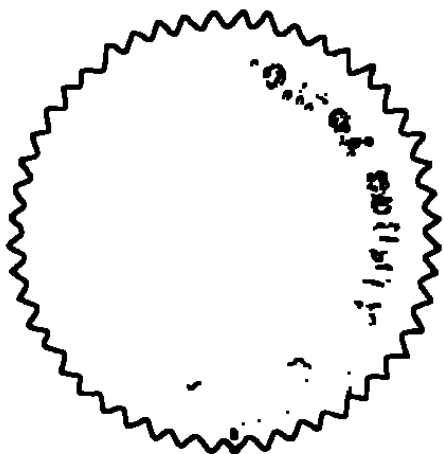
FILING DATE: 03/23/2000
ISSUE DATE:

ENTERED
Nixon Peabody LLP

NOV 6 2000

FILE 21829/60
DKT JB

STATE OF NEW YORK
COUNTY OF MONROE
OFFICE OF THE COUNTY CLERK



I, MAGGIE BROOKS, Clerk of the County of Monroe, of the County Court of said County, and of the Supreme Court, both being Courts of Record, having a common seal, DO CERTIFY, that

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John Whalen

before whom the annexed Oath, Affidavit, Acknowledgement purports to have been made or taken, and certified by her/him, was at the time of the making or taking thereof a NOTARY PUBLIC in and for said State, and was duly authorized to take the same, and was authorized by the laws of this State to take and certify the acknowledgement and proof of deeds to be recorded in this State; and I am well acquainted with his/her handwriting and verily believe her/his signature thereto is genuine and that such officer is not required by law to have a seal nor is she/he required to have a specimen impression thereof filed or deposited in this office, or recorded, filed or deposited in any other place.

In Witness Whereof, I have hereunto set my hand and the official seal of said Courts this 21st day of May 2001

Maggie Brooks

Maggie Brooks
Clerk

A N^o 26286

ASSIGNMENT

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10

WHEREAS, We, Xiaoling Song, a citizen of the People's Republic of China, residing at 14110 NE 179th Street, Apt. 75, Woodinville, Washington 98072, Hao Fan, a citizen of the People's Republic of China, residing 19712 6th Drive, S.E., Bothell, Washington 98012, and Zhong-Min Wei, a United States citizen, residing at 8230 125th Court, Kirkland, Washington 98034 have invented certain new and useful improvements in **RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF** for which we have executed an application for Letters Patent in the United States, filed as U. S. Serial No. 60/191,649 on March 23, 2000;

AND WHEREAS, EDEN Bioscience Corporation, with its principal place of business at 11816 North Creek Parkway N., Bothell, Washington 98011-8205, desires to acquire the entire right, title and interest in and to the said improvements and the said Application:

NOW, THEREFORE, for good and valuable consideration, including salary or payment for the making of inventions, or employee benefits, the receipt of which is hereby acknowledged, we, the said inventors, do hereby acknowledge that we have sold, assigned, transferred and set over, and by these presents do hereby sell, assign, transfer and set over, unto the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, the entire right, title and interest throughout the world in, to and under the said improvements, and the said application and all divisions, renewals and continuations thereof, and all Letters Patent of the United States which may be granted thereon and all reissues and extensions thereof, and all rights of priority under International Conventions and applications for Letters Patent which may hereafter be filed for said improvements in any country or countries foreign to the United States, and all Letters Patent which may be granted for said improvements in any country or countries foreign to the United States and all extensions, renewals and reissues thereof; and we hereby authorize and request the Commissioner of Patents of the United States, and any Official of any country or countries foreign to the United States, whose duty it is to issue patents on applications as aforesaid, to issue all Letters Patent for said improvements to the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, in accordance with the terms of this instrument.

AND WE HEREBY covenant and agree that we will communicate to the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, any facts known to us respecting said improvements and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing and reissue applications, make all rightful oaths and generally do everything possible to aid the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, to obtain and enforce proper patent protection for said improvements in all countries.

IN TESTIMONY WHEREOF, I hereunto set my hand and seal this 8th day of May, 2000.


Xiaoling Song

May 18, 2001

I swear this is a true copy of the original



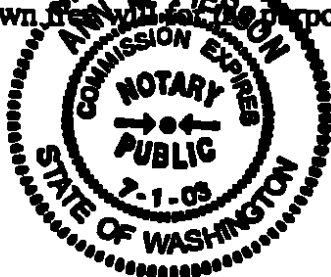
R392304.1
211229/60

JO ANN WHALEN
Notary Public, State of New York
Qualified in Monroe County
Certificate Filed in Monroe County
Reg. No. 6038037
Commission Expires 1/17/2003

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STATE OF WA
COUNTY OF King SS:

This 8th day of May, 2000, before me personally came the
above-named Xiaoling Song, to me personally known as the individual who executed the
same of his/her own free will for the purposes therein set forth.



[Signature]
Notary Public

IN TESTIMONY WHEREOF, I hereunto set my hand and seal this 8 day
of May, 2000.

[Signature]
Hao Fan

STATE OF WA
COUNTY OF King SS:

This 8th day of May, 2000, before me personally came the
above-named Hao Fan, to me personally known as the individual who executed the same of
his/her own free will for the purposes therein set forth.



[Signature]
Notary Public

IN TESTIMONY WHEREOF, I hereunto set my hand and seal this 8th day
of May, 2000.

[Signature]
Zhong-Min Wei

STATE OF WA
COUNTY OF King SS:

This 8th day of May, 2000, before me personally came the
above-named Zhong-Min Wei, to me personally known as the individual who executed the
same of his/her own free will for the purposes therein set forth.



[Signature]
Notary Public

✓F2
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TRADUCCION OFICIAL No. 225/01 de un documento escrito en Inglés, el cual para su identificación se sella con el sello del Traductor. Esta es una Traducción Oficial efectuada el día 11 de junio de 2001 por la Sra. María Teresa Lara R., c.c. 41.568.055 de Bogotá, Traductora Oficial según Resolución No. 01016 del 2 de octubre de 1998, expedida por el Ministerio de Justicia.

= = = = =

Traspaso del Invento "Receptores para Provocadores de Respuesta Hipersensible y Usos de los Mismos" a EDEN BIOSCIENCE CORPORATION. Certificación notarial suscrita por Jo Ann Whalen, Notario Público del Estado de Nueva York, y legalizaciones subsiguientes.

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINISTERIO DE JUSTICIA

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNID S
Oficina de Patentes y Marcas
SECRETARIO ASISTENTE Y COMISIONADO
DE PATENTES Y MARCAS
Washington, D.C. 20231

Abril 09, 2001

PTAS

NIXON PEABODY, L.L.P.
MICHAEL L. GOLDMAN
CLINTON SQUARE
P.O. BOX 31051
ROCHESTER, NY 14603

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OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS
AVISO DE REGISTRO DE DOCUMENTO DE TRASPASO

El documento adjunto ha sido registrado por la División de Traspasos de la Oficina de Patentes y Marcas de los EE.UU. Una copia completa en microfilm se encuentra disponible en la Sala de Búsquedas de Traspasos bajo el Número de Rollo y Cuadro señalados a continuación.

Favor revisar toda la Información contenida en este aviso. La Información contenida en este Aviso de Registro refleja los datos presentes en el Sistema de

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Traspasos de Patentes y Marcas. En caso de que encuentren cualquier error o tengan cualquier pregunta en relación con este aviso, pueden comunicarse con el empleado cuyo nombre aparece en este aviso en el 703-308-9723. Favor enviar cualquier solicitud de corrección a: U.S. PATENT AND TRADEMARK OFFICE, Assignment Division, Box Assignments, CG-4, 1213 Jefferson Davis Hwy, Suite 320, Washington, D.C. 20231.

FECHA DE REGISTRO: 01/22/2001

ROLLO/CUADRO: 011462/0806
NÚMERO DE PÁGINAS: 4

RESUMEN: TRASPASO DEL INTERÉS DEL CEDENTE (VER DOCUMENTO PARA DETALLES).

CEDENTE:

SONG, XIAOLING

FECHA DOC: 01/03/2001

CEDENTE:

FAN, HAO

FECHA DOC: 01/03/2001

CEDENTE:

WEI, ZHONG-MIN

FECHA DOC: 01/05/2001

CESIONARIA:

EDEN BIOSCIENCE CORPORATION
11816 NORTH CREEK PARKWAY N.
BOTHELL, WASHINGTON 98011-8205

NÚMERO DE SERIE: 60250710

FECHA PRESENTACIÓN: 12/01/2000

NÚMERO DE PATENTE:

FECHA EXPEDICIÓN:

REGISTRADO
Nixon Peabody LLP

ABR 13 2001

EXPED. 21829 / 61
DKT JB

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
REGOLUCION No. 01618
DE MINISTRIA

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TRASPAS

CONSIDERANDO, que los suscritos, **Xiaoling Song**, ciudadano de la República Popular China, residente en 14110 NE 179th Street, Apt. 75, Woodinville, Washington 98072, **Hao Fan**, ciudadano de la República Popular China, residente en 19712 6th Drive, S.E., Bothell, Washington 98012, y **Zhong-Min Wei**, ciudadano de los Estados Unidos, residente en 8230 125th Court, Kirkland, Washington 98034, hemos inventado ciertas mejoras nuevas y útiles en **RECEPTORES PARA PROVOCADORES DE RESPUESTA HIPERSENSIBLE Y USOS DE LOS MISMOS** para las cuales hemos elaborado una solicitud de Patente de Invención en los Estados Unidos, presentada bajo el No. de Serie de los EE.UU. **60/250,710** el **1 de diciembre de 2000**;

Y CONSIDERANDO, que **Eden Bioscience Corporation**, con sede principal en 11816 North Creek Parkway N., Bothell, Washington 98011-8205, desea adquirir todo derecho, título e interés en y sobre las citadas mejoras y la antedicha Solicitud:

AHORA, POR TANTO, por contraprestación válida y valiosa, Incluido el salario o el pago por la realización de los Inventos, o los beneficios laborales, el recibo de la cual se reconoce por la presente, nosotros, los antedichos Inventores, reconocemos por la presente que hemos vendido, cedido, traspasado y entregado, y por la presente vendemos, cedemos, traspasamos y entregamos, a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, todo derecho, título e Interés en todo el mundo en, sobre y en virtud de tales mejoras, y la citada solicitud y todas las divisiones, renovaciones y continuaciones de la misma, y todas las Patentes de Invención de los Estados Unidos que puedan otorgarse sobre la misma y todas las reexpediciones y prórrogas de la misma, y todos los derechos de prioridad

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
REGULACION No. 91016
DE MINISTROS

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bajo Convenciones Internacionales y las solicitudes de Patentes de Invención que puedan en un futuro presentarse para tales mejoras en cualquier país o países distintos de los Estados Unidos, y todas las Patentes de Invención que puedan otorgarse para dichas mejoras en cualquier país o países distintos de los Estados Unidos, y todas las prórrogas, renovaciones y reexpediciones de las mismas; y por la presente autorizamos y solicitamos al Comisionado de Patentes de los Estados Unidos, y a cualquier Funcionario de cualquier país o países distintos de los Estados Unidos, cuya función sea expedir patentes en virtud de solicitudes del tipo mencionado, expedir todas las Patentes de Invención para tales mejoras a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, de conformidad con los términos de este Instrumento.

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MARIA TERESA LARA R.
TRADUCTORA OFICIAL
REGISTRADA EN EL
DEPARTAMENTO DE JUSTICIA

Y POR LA PRESENTE pactamos y acordamos que le comunicaremos a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, cualesquiera hechos que lleguen a nuestro conocimiento con respecto a dichas mejoras y testificaremos en cualquier proceso judicial, firmaremos todos los documentos legales, otorgaremos todas las solicitudes de división, continuación y reexpedición, prestaremos todos los juramentos que correspondan y en general haremos todo lo posible por asistir a la citada **EDEN Bioscience Corporation**, sus sucesores, representantes legales y cesionarios, para obtener y hacer valer una protección de patente apropiada para tales mejoras en todos los países.

EN TESTIMONIO DE LO CUAL, firmo el presente y estampo aquí mi sello a los 3 días del mes de enero de 2001.

(Fdo.) Xiaoling Song

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ESTADO DE WASHINGTON

CONDADO DE KING SS:

A los 3 días del mes de enero de 2001, ante mí personalmente compareció el mencionado **Xiaoling Song**, a quien conozco personalmente como la persona que firmó el documento por su propia y libre voluntad para los propósitos allí señalados.

(Fdo.) Celia L. Moore
Notario Público

[Sello] CELIA L. MOORE
NOTARIO PUBLICO
LA COMISION EXPIRA EL
7-29-02
ESTADO DE WASHINGTON

MARIA TERESA LARA B.
"TRADUCTORA OFICIAL"
RESOLUCION No. 09018
DE MINISTRIA

EN TESTIMONIO DE LO CUAL, firmo el presente y estampo aquí mi sello a los 3 días del mes de enero de 2001.

(Fdo.) Hao Fan

ESTADO DE WASHINGTON

CONDADO DE KING SS:

A los 3 días del mes de enero de 2001, ante mí personalmente compareció el mencionado **Hao Fan**, a quien conozco personalmente como la persona que firmó el documento por su propia y libre voluntad para los propósitos allí señalados.

(Fdo.) Celia L. Moore
Notario Público

[Sello] CELIA L. MOORE
NOTARIO PUBLICO
LA COMISION EXPIRA EL
7-29-02
ESTADO DE WASHINGTON

147
140

EN TESTIMONIO DE LO CUAL, firmo el presente y estampo aquí mi sello a los 3 días del mes de enero de 2001.

(Fdo.) Zhong-Min Wei

ESTADO DE WASHINGTON

CONDADO DE KING SS:

A los 5 días del mes de enero de 2001, ante mí personalmente compareció el mencionado **Zhong-Min Wei**, a quien conozco personalmente como la persona que firmó el documento por su propia y libre voluntad para los propósitos allí señalados.

(Fdo.) Celia L. Moore
Notario Público

[Sello] CELIA L. MOORE
NOTARIO PUBLICO
LA COMISION EXPIRA EL
7-29-02
ESTADO DE WASHINGTON

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION MA. 87016
DE MINISTRIA

Mayo 18, 2001

Juro que esta es fiel copia del original.

(Fdo.) Jo Ann Whalen

[Sello] JO ANN WHALEN
Notario Público, Estado de Nueva York
Calificada en el Condado de Monroe
Certificado registrado en el Condado de Monroe
Reg. No. 5038037
La comisión expira el 1/17/2003

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141

ESTADO DE NUEVA YORK) ss.
CONDADO DE MONROE)
OFICINA DEL SECRETARIO DEL CONDADO

Yo, MAGGIE BROOKS, Secretaria del Condado de Monroe, del Tribunal de Condado en dicho Condado y del Tribunal Supremo, los cuales son ambos Tribunales de Registro, provistos de sello común, CERTIFICO POR EL PRESENTE, que

JO ANN WHALEN

ante quien manifiesta haber sido otorgado o tomado el Juramento, Declaración Jurada o Reconocimiento anterior, y quien lo certifica, era en el momento de otorgar o tomar el mismo una NOTARIO PUBLICO en y para el antedicho Estado, y estaba debidamente autorizada para tomarlo, y estaba autorizada por las leyes de este Estado para tomar y certificar el reconocimiento y prueba de escrituras que deban registrarse en este Estado; y que conozco bien su letra manuscrita y considero en verdad que su firma en el mismo es auténtica y que dicha funcionaria no está obligada por ley a tener un sello ni requiere tener una muestra de la Impresión del mismo registrada o depositada en esta oficina, o registrada o depositada en cualquier otro lugar.

En Fe de lo Cual, he colocado aquí mi firma y el sello oficial de dichos Tribunales a los 21 días del mes de mayo de 2001.

(Fdo.) Maggie Brooks

A No. 26285

Secretaría

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. País: Estados Unidos de América

MARIA TERESA LABA R.
TRADUCTORA OFICIAL
RESOLUCION No. 61016
DE MINISTERIO DE JUSTICIA

149
142

Este documento público

2. ha sido firmado por **Maggie Brooks**
3. actuando en calidad de **Secretaria del Condado**
4. lleva el sello/estampilla del condado de **Monroe**

22

Certificado

5. en Albany, New York
6. el día 01 de junio de 2001
7. por Subsecretario de Estado Especial, Estado de Nueva York
8. No. A-77640E
9. Sello/Estampilla
10. Firma

(Gran Sello del
Estado de Nueva York)

(Fdo.) Joseph P. Brozowski

Joseph P. Brozowski
Subsecretario de Estado Especial

MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01016
DE MINISTERIO DE JUSTICIA

= = = = =

La anterior es traducción fiel y completa del documento en referencia, de la cual se deja copia en este archivo para su confrontación.


María Teresa Lara R.
c.c. 41.568.055 de Bogotá
Traductora Oficial según
Resolución No. 01016 del
2 de octubre de 1998, del
Ministerio de Justicia

Apostille

(Convention de La Haye du 5 Octobre 1961)

150
143

1. Country: United States of America

This public document

2. has been signed by Maggie Brooks

3. acting in the capacity of County Clerk

4. bears the seal/stamp of the county of Monroe

Certified

5. At Albany, New York 6. the 01st day of June 2001

7. by Special Deputy Secretary of State, State of New York

8. No. A-77640E

9. Seal/Stamp

10. Signature

Joseph P. Brozowski

Joseph P. Brozowski

Special Deputy Secretary of State

Maria Teresa Lara R.
MARIA TERESA LARA R.
TRADUCTORA OFICIAL
RESOLUCION No. 01616
DE MINJUSTICIA

226/01



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

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APRIL 09, 2001

PTAS



101598098A

NIXON PEABODY, L.L.P.
MICHAEL L. GOLDMAN
CLINTON SQUARE
P.O. BOX 31051
ROCHESTER, NY 14603

UNITED STATES PATENT AND TRADEMARK OFFICE
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PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 01/22/2001

REEL/FRAME: 011462/0806
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
SONG, XIAOLING

DOC DATE: 01/03/2001

ASSIGNOR:
FAN, HAO

DOC DATE: 01/03/2001

ASSIGNOR:
WEI, ZHONG-MIN

DOC DATE: 01/05/2001

ASSIGNEE:
EDEN BIOSCIENCE CORPORATION
11816 NORTH CREEK PARKWAY N.
BOTHELL, WASHINGTON 98011-8205

SERIAL NUMBER: 60250710
PATENT NUMBER:

FILING DATE: 12/01/2000
ISSUE DATE:

ENTERED
Nixon Peabody LLP

APR 13 2001

FILE 20829/61
DKT 9/3

152
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ASSIGNMENT

WHEREAS, We, Xiaoling Song, a citizen of the People's Republic of China, residing at 14110 NE 179th Street, Apt. 75, Woodinville, Washington 98072, Hao Fan, a citizen of the People's Republic of China, residing 19712 6th Drive, S.E., Bothell, Washington 98012, and Zhong-Min Wei, a United States citizen, residing at 8230 125th Court, Kirkland, Washington 98034 have invented certain new and useful improvements in RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS AND USES THEREOF for which we have executed an application for Letters Patent in the United States, filed as U. S. Serial No. 60/250,710 on December 1, 2000;

AND WHEREAS, EDEN Bioscience Corporation, with its principal place of business at 11816 North Creek Parkway N., Bothell, Washington 98011-8205, desires to acquire the entire right, title and interest in and to the said improvements and the said Application:

NOW, THEREFORE, for good and valuable consideration, including salary or payment for the making of inventions, or employee benefits, the receipt of which is hereby acknowledged, we, the said inventors, do hereby acknowledge that we have sold, assigned, transferred and set over, and by these presents do hereby sell, assign, transfer and set over, unto the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, the entire right, title and interest throughout the world in, to and under the said improvements, and the said application and all divisions, renewals and continuations thereof, and all Letters Patent of the United States which may be granted thereon and all reissues and extensions thereof, and all rights of priority under International Conventions and applications for Letters Patent which may hereafter be filed for said improvements in any country or countries foreign to the United States, and all Letters Patent which may be granted for said improvements in any country or countries foreign to the United States and all extensions, renewals and reissues thereof; and we hereby authorize and request the Commissioner of Patents of the United States, and any Official of any country or countries foreign to the United States, whose duty it is to issue patents on applications as aforesaid, to issue all Letters Patent for said improvements to the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, in accordance with the terms of this instrument.

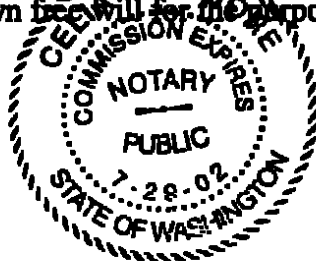
AND WE HEREBY covenant and agree that we will communicate to the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, any facts known to us respecting said improvements and testify in any legal proceeding, sign all lawful papers, execute all divisional, continuing and reissue applications, make all rightful oaths and generally do everything possible to aid the said EDEN Bioscience Corporation, its successors, legal representatives and assigns, to obtain and enforce proper patent protection for said improvements in all countries.

IN TESTIMONY WHEREOF, I hereunto set my hand and seal this 3rd day of January, 2001.

Xiaoling Song
Xiaoling Song

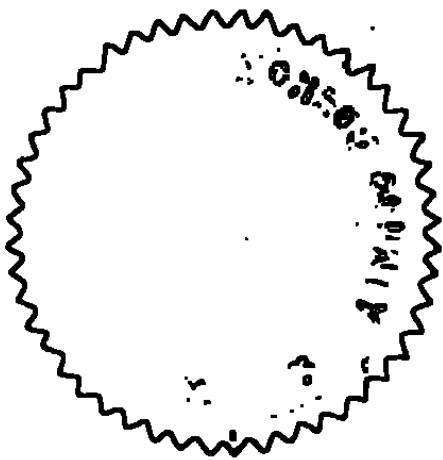
STATE OF Washington
COUNTY OF King SS:

This 3rd day of January, 2001, before me personally came the above-named Xiaoling Song, to ~~be~~ personally known as the individual who executed the same of his/her own free will for the purposes therein set forth.



William L. Thorne
Notary Public

STATE OF NEW YORK }
COUNTY OF MONROE } ss.
OFFICE OF THE COUNTY CLERK



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I, MAGGIE BROOKS, Clerk of the County of Monroe, of the County Court of said County, and of the Supreme Court, both being Courts of Record, having a common seal, DO CERTIFY, that

John Whalen
before whom the annexed Oath, Affidavit, Acknowledgement purports to have been made or taken, and certified by her/him, was at the time of the making or taking thereof a NOTARY PUBLIC in and for said State, and was duly authorized to take the same, and was authorized by the laws of this State to take and certify the acknowledgement and proof of deeds to be recorded in this State; and I am well acquainted with his/her handwriting and verily believe her/his signature thereto is genuine and that such officer is not required by law to have a seal nor is she/he required to have a specimen impression thereof filed or deposited in this office, or recorded, filed or deposited in any other place.

In Witness Whereof, I have hereunto set my hand and the official seal of said Courts this 21st day of May 2021.

Maggie Brooks

Maggie Brooks
Clerk

A N^o 26285

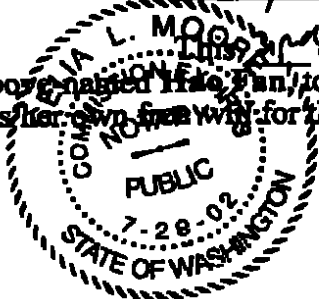
147 183

IN TESTIMONY WHEREOF, I hereunto set my hand and seal this ____ day
of 01/03/01, 2001.

Hao Fan
Hao Fan

STATE OF WASHINGTON
COUNTY OF KING SS:

This 19th day of JANUARY, 2001, before me personally came the
above-named Hao Fan, to me personally known as the individual who executed the same of
his/her own free will for the purposes therein set forth.



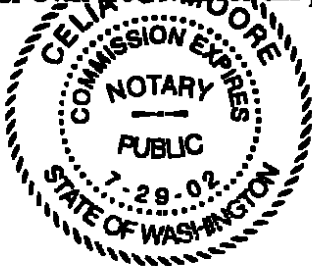
Celia L. Moore
Notary Public

IN TESTIMONY WHEREOF, I hereunto set my hand and seal this 5th day
of Jan, 2001.

Zhong-Min Wei
Zhong-Min Wei

STATE OF WASHINGTON
COUNTY OF KING SS:

This 5th day of JANUARY, 2001, before me personally came the
above-named Zhong-Min Wei, to me personally known as the individual who executed the
same of his/her own free will for the purposes therein set forth.



Celia L. Moore
Notary Public

May 18, 2001
I swear this is a true copy of the original.
Jo Ann Whalen

JO ANN WHALEN
Notary Public, State of New York
Qualified in Monroe County
Certificate Filed in Monroe County
Reg. No. 6038037
Commission Expires 1/17/2003

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Esta es una traducción parcial al idioma español de un documento escrito originalmente en el idioma inglés.

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LOS ESTADOS UNIDOS DE AMERICA

A TODOS AQUELLOS A QUIEN ESTE DOCUMENTO SE LES PRESENTE:

DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

10 **Oficina de Patentes y Marcas de los Estados Unidos**

Abril 17, 2001

15 ESTO ES PARA CERTIFICAR QUE LO ANEXADO A LA PRESENTE ES UNA
FIEL COPIA DE LOS EXPEDIENTES DE LA OFICINA DE PATENTES Y
MARCAS DE LOS ESTADOS UNIDOS DE AQUELLOS DOCUMENTOS DE LA
SOLICITUD DE PATENTE IDENTIFICADA A CONTINUACION QUE
REUNIERON LOS REQUERIMIENTOS PARA QUE SE LE CONCEDA UNA FECHA
20 DE PRESENTACION BAJO 35 USC (Código de los Estados Unidos)
111.

NO. DE SOLICITUD: 60/250,710

FECHA DE PRESENTACION: 1 de Diciembre de 2000

25

SELLO DE LA
OFICINA DE MARCAS Y PATENTES
DE LOS ESTADOS UNIDOS DE

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

[firma ilegible]

30

T. WALLACE
Oficial de Certificación

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REIVINDICACIONES:

1. Una proteína aislada la cual sirve como un receptor en plantas para inductores patógenos vegetales de respuesta hipersensible.

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2. Una proteína según la reivindicación 1, en donde el patógeno vegetal es seleccionado entre *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, y *Clavibacter*.

3. Una proteína según la reivindicación 2, en donde el patógeno vegetal es un patógeno *Erwinia*.

4. Una proteína según la reivindicación 3, en donde el patógeno vegetal es *Erwinia amylovora*.

5. Una proteína según la reivindicación 1, en donde la proteína proviene de un monocotiledóneo.

6. Una proteína según la reivindicación 5, en donde la proteína proviene del arroz.

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7. Una proteína según la reivindicación 1, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 6.

8. Una proteína según la reivindicación 1, en donde la proteína proviene de un dicotiledóneo.

9. Una proteína según la reivindicación 8, en donde la proteína proviene de la *Arabidopsis thaliana*.

10. Una proteína según la reivindicación 1, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 1.

11. Una proteína según la reivindicación 1, en donde la proteína es recombinante.

12. Una molécula de ácido nucleico aislada que codifica una proteína según la reivindicación 1.

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13. Una molécula de ácido nucleico según la reivindicación 12, en donde el patógeno vegetal es seleccionado entre *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, y *Clavibacter*.

14. Una molécula de ácido nucleico según la reivindicación 13, en donde el patógeno vegetal es un patógeno *Erwinia*.

15. Una molécula de ácido nucleico según la reivindicación 14, en donde el patógeno vegetal es *Erwinia amylovora*.

16. Una molécula de ácido nucleico según la reivindicación 12, en donde la proteína proviene de un monocotiledóneo.

17. Una molécula de ácido nucleico según la reivindicación 16, en donde la proteína proviene del arroz.

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18. Una molécula de ácido nucleico según la reivindicación 12, en donde la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6.

19. Una molécula de ácido nucleico según la reivindicación 12, en donde el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C.

20. Una molécula de ácido nucleico según la reivindicación 12, en donde el ácido nucleico tiene una secuencia nucleótida que comprende la SEQ. ID. No. 7.

21. Una molécula de ácido nucleico según la reivindicación 12, en donde la proteína proviene de un dicotiledóneo.

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22. Una molécula de ácido nucleico según la reivindicación 21, en donde la proteína proviene de la *Arabidopsis thaliana*.

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23. Una molécula de ácido nucleico según la reivindicación 12, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 1.

24. Una molécula de ácido nucleico según la reivindicación 12, en donde el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C.

25. Una molécula de ácido nucleico según la reivindicación 12, en donde el ácido nucleico tiene una secuencia nucleótida que comprende la SEQ. ID. No. 2.

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26. Una molécula de ácido nucleico según la reivindicación 12, en donde el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C.

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27. Una molécula de ácido nucleico según la reivindicación 12, en donde el ácido nucleico tiene una secuencia nucleótida que comprende la SEQ. ID. No. 3.

28. Una molécula de ácido nucleico antisentido según la reivindicación 12.

29. Un vector de expresión que contiene una molécula de ácido nucleico según la reivindicación 12 el cual es heterólogo al vector de expresión.

30. Un vector de expresión según la reivindicación 29, en donde la molécula del ácido nucleico es situada en

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el vector de expresión en orientación con sentido y un
correcto marco de lectura.

31. Un vector de expresión según la reivindicación 29, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido nucleico se hibridiza en la secuencia nucleótida de la

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SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

32. Un vector de expresión que contiene una molécula de ácido nucleico según la reivindicación 28 el cual es heterólogo al vector de expresión.

33. Una célula hospedera transgénica transformada con la molécula del ácido nucleico según la reivindicación 12.

34. Una célula hospedera transformada según la reivindicación 33, en donde la célula hospedera es seleccionada entre una célula vegetal y una célula bacteriana.

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35. Una célula hospedera según la reivindicación 33, en donde la molécula de ADN es transformada con un sistema de expresión.

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36. Una célula hospedera según la reivindicación 33, en donde tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido

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nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

37. Una célula hospedera transformada con una molécula de ácido nucleico según la reivindicación 28.

38. Una planta transgénica transformada con la molécula de ADN de la reivindicación 12.

39. Una planta transgénica según la reivindicación 38, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino,

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manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

40. Una planta transgénica según la reivindicación 38, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

41. Una planta transgénica según la reivindicación 38, en donde la planta es un monocotiledóneo.

42. Una planta transgénica según la reivindicación 38, en donde la planta proviene de un dicotiledóneo.

43. Una planta transgénica según la reivindicación 38, en donde tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de,

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formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

44. Una planta transgénica transformada con una molécula de ácido nucleico según la reivindicación 28.

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45. Una semilla de planta transgénica transformada con la molécula de ADN de la reivindicación 12.

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46. Una planta transgénica según la reivindicación 45 en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

47. Una planta transgénica según la reivindicación 45, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

48. Una planta transgénica según la reivindicación 45, en donde la planta es un monocotiledóneo.

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49. Una planta transgénica según la reivindicación 45, en donde la planta proviene de un dicotiledóneo.

50. Una planta transgénica según la reivindicación 45, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido

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nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

51. Una semilla de planta transgénica transformada con una molécula de ácido nucleico según la reivindicación 28.

52. Un método para identificar agentes que tiene como objetivo células vegetales, el cual comprende:

formar una mezcla de reacción que comprende una proteína según la reivindicación 1 y un agente candidato; evaluar la mezcla de reacción para unión entre la proteína y el agente candidato; y

identificar compuestos candidatos los cuales se unen a la proteína en la mezcla de reacción como agentes objetivo de células vegetales.

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53. Un método según la reivindicación 52, en donde la proteína proviene de un monocotiledóneo.

54. Un método según la reivindicación 53, en donde la proteína proviene del arroz.

55. Un método según la reivindicación 52, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 6.

56. Un método según la reivindicación 52, en donde la proteína proviene de un dicotiledóneo.

57. Un método según la reivindicación 56, en donde la proteína proviene de la *Arabidopsis thaliana*.

58. Un método según la reivindicación 52, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 1.

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59. Un método para identificar agentes que tiene como objetivo células vegetales, el cual comprende:

formar una mezcla de reacción que comprende una célula hospedera transformada con una molécula de ácido nucleico según la reivindicación 12 y un agente candidato;

evaluar la mezcla de reacción para unión entre la proteína producida por la célula hospedera y el agente candidato; y

identificar compuestos candidatos los cuales se unen a la proteína producida por la célula hospedera en la mezcla de reacción como agentes objetivo de células vegetales.

60. Un método según la reivindicación 59, en donde la proteína proviene de un monocotiledóneo.

61. Un método según la reivindicación 60, en donde la proteína proviene del arroz.

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62. Un método según la reivindicación 59, en donde la proteína proviene de un dicotiledóneo.

63. Un método según la reivindicación 62, en donde la proteína proviene de la *Arabidopsis thaliana*.

64. Un método según la reivindicación 59, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido

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nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

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65. Un método para mejorar la receptividad de la planta al tratamiento con inductores de la respuesta hipersensible, el cual comprende:

proporcionar una planta transgénica o una semilla de planta transgénica transformada con la molécula de ácido nucleico según la reivindicación 12.

66. Un método según la reivindicación 65, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza

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en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

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67. Un método según la reivindicación 65, en donde una planta transgénica es proporcionada.

68. Un método según la reivindicación 65, en donde una semilla de planta transgénica es proporcionada y dicho método además comprende:

sembrar las semillas de las plantas bajo condiciones que sean efectivas para que las plantas crezcan a partir de las semillas sembradas.

69. Un método según la reivindicación 65, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

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70. Un método según la reivindicación 65, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

71. Un método según la reivindicación 65, en donde el tratamiento con inductores de la respuesta hipersensible es para impartir resistencia a la enfermedad.

72. Un método según la reivindicación 65, en donde el tratamiento con inductores de la respuesta hipersensible es para mejorar el crecimiento de las plantas.

73. Un método según la reivindicación 65, en donde el tratamiento con inductores de la respuesta hipersensible es para controlar insectos.

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74. Un método según la reivindicación 65, en donde el tratamiento con inductores de la respuesta hipersensible es para impartir tolerancia al estrés.

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75. Un método según la reivindicación 65, en donde la planta transgénica o semilla de planta es adicionalmente transformada con un segundo ácido nucleico que codifica un inductor de la respuesta hipersensible, en donde la expresión del segundo ácido nucleico surte efecto sobre el tratamiento con inductores de la respuesta hipersensible.

76. Un método según la reivindicación 65, en donde el tratamiento con inductores de la respuesta hipersensible comprende:

aplicar un inductor de la respuesta hipersensible a la planta o semilla de la planta.

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77. Un método según la reivindicación 76, en donde el inductor de la respuesta hipersensible es aplicado de manera aislada.

78. Un método para impartir resistencia a la enfermedad, mejorar el crecimiento, controlar insectos, y/o impartir resistencia al estrés, el cual comprende:

proporcionar una planta transgénica o semilla de planta transgénica transformada con una construcción de ADN efectiva para silenciar la expresión de una molécula de ácido nucleico según la reivindicación 12.

79. Un método según la reivindicación 78, en donde la proteína proviene de un monocotiledóneo.

80. Un método según la reivindicación 79, en donde la proteína proviene del arroz.

81. Un método según la reivindicación 78, en donde la proteína proviene de un dicotiledóneo.

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82. Un método según la reivindicación 81, en donde la proteína proviene de la *Arabidopsis thaliana*.

83. Un método según la reivindicación 78, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido nucleico

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se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; 6 (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

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84. Un método según la reivindicación 78, en donde la planta transgénica es proporcionada.

85. Un método según la reivindicación 78, en donde una semilla de planta transgénica es proporcionada y dicho método además comprende:

sembrar las semillas de las plantas bajo condiciones que sean efectivas para que las plantas crezcan a partir de las semillas sembradas.

86. Un método según la reivindicación 78, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa,

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batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

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87. Un método según la reivindicación 78, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

88. Un método según la reivindicación 78, en donde la planta transgénica o semilla de planta es adicionalmente transformada con un segundo ácido nucleico que codifica un inductor de la respuesta hipersensible, en donde la expresión del segundo ácido nucleico surte efecto sobre el tratamiento con inductores de la respuesta hipersensible.

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89. Un método según la reivindicación 78 que además comprende:

aplicar un inductor de la respuesta hipersensible a una planta o semilla de planta.

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90. Un método según la reivindicación 89 en donde el inductor de la respuesta hipersensible es aplicado de manera aislada.

91. Un método según la reivindicación 78, en donde resistencia a la enfermedad es impartida a las plantas.

92. Un método según la reivindicación 78, en donde un crecimiento mejorado de las plantas es impartido a las mismas.

93. Un método según la reivindicación 78, en donde un controlar insectos es impartido a las plantas.

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94. Un método según la reivindicación 78, en donde una tolerancia al estrés es impartida.

95. Un método según la reivindicación 78, en donde la construcción de ADN es una molécula de ácido nucleico antisentido a una molécula de ácido nucleico que codifica un receptor en plantas para inductores de la respuesta hipersensible de patógenos vegetales.

96. Un método según la reivindicación 78, en donde la construcción de ADN se puede transcribir a un primer ácido nucleico que codifica un receptor en plantas para inductores de la respuesta hipersensible de patógenos vegetales acoplados a un segundo ácido nucleico que codifica el complemento invertido del primer ácido nucleico.

97. Un método para impartir resistencia a la enfermedad, mejorar el crecimiento, controlar insectos,

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y/o impartir resistencia al estrés en plantas, el cual comprende:

proporcionar una planta transgénica o semilla de planta transgénica transformada con una molécula de ácido nucleico según la reivindicación 12.

98. Un método según la reivindicación 97, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 ó 8 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ.

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ID. No. 3; (6) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 6; (7) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 7 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; ó (8) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 7.

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99. Un método según la reivindicación 97, en donde la planta transgénica es proporcionada.

100. Un método según la reivindicación 97, en donde una semilla de planta transgénica es proporcionada y dicho método además comprende:

sembrar las semillas de las plantas bajo condiciones que sean efectivas para que las plantas crezcan a partir de las semillas sembradas.

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101. Un método según la reivindicación 97, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

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102. Un método según la reivindicación 97, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

103. Un método según la reivindicación 97, en donde resistencia a la enfermedad es impartida.

104. Un método según la reivindicación 97, en donde un crecimiento vegetal es mejorado.

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105. Un método según la reivindicación 97, en donde insectos son controlados.

106. Un método según la reivindicación 97, en donde una tolerancia al estrés es impartida.

107. Un método según la reivindicación 97, en donde la proteína proviene de un monocotiledóneo.

108. Un método según la reivindicación 107, en donde la proteína proviene del arroz.

109. Un método según la reivindicación 97, en donde la proteína proviene de un dicotiledóneo.

110. Un método según la reivindicación 97, en donde la proteína proviene de la *Arabidopsis thaliana*.

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME;

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 17, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/250,710

FILING DATE: DECEMBER 01, 2000



By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

REQUEST FORM FOR A PROVISIONAL APPLICATION
FOR PATENT UNDER 37 CFR 1.53(c)

Docket No.: 21829/61 (EBC-004)

[+]

Assistant Commissioner for Patents
Washington, D.C. 20231
Box: Provisional Patent Application

This is a Request for filing a PROVISIONAL APPLICATION FOR PATENT under
37 CFR 1.53(c), entitled **RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICTORS**
AND USES THEREOF by the following named inventors:

Full name of first inventor: Xiaoling Song
Residence: 14110 NE 179th Street, Apt. 75, Woodinville, Washington 98072
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Full name of third inventor: Zhong-Min Wei
Residence: 8230 125th Court, Kirkland, Washington 98034
Citizenship: People's Republic of China
Post Office Address: same

Enclosed are:

- ☒ Specification, claims and abstract (56 pages)
- ☒ Drawings (8 sheets)
- ☒ Applicants claim small entity status. (See 37 CFR 1.27.)
- ☐ Combined Declaration and Power of Attorney (pages)
- ☒ A self-addressed, prepaid postcard for acknowledging receipt.
- ☐ Other: [list other enclosures]
- ☒ A check in the amount of \$75.00 to cover the provisional filing fee is enclosed.

The invention was made by an agency of the United States Government or under a contract
with an agency of the United States Government:

- ☒ No.
- ☐ Yes, the name of the U.S. Government agency and the Government contract number are:

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TITLE: **RECEPTORS FOR HYPERSENSITIVE RESPONSE
ELICITORS AND USES THEREOF**

INVENTORS: **Xinellng Song, Hao Fan, and Zhong-Min Wei**

DOCKET NO.: **21829/61 (EBC-004)**

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PROVISIONAL APPLICATION

RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICTORS
AND USES THEREOF

FIELD OF THE INVENTION

The present invention relates to receptors for hypersensitive response elicitors and uses thereof.

BACKGROUND OF THE INVENTION

Plants have evolved a complex array of biochemical pathways that enable them to recognize and respond to environmental signals, including pathogen infection. There are two major types of interactions between a pathogen and plant -- compatible and incompatible. When a pathogen and a plant are compatible, disease generally occurs. If a pathogen and a plant are incompatible, the plant is usually resistant to that particular pathogen. In an incompatible interaction, a plant will restrict pathogen proliferation by causing localized necrosis, or death of tissues, to a small zone surrounding the site of infection. This reaction by the plant is defined as the hypersensitive response ("HR") (Kiraly, Z. "Defenses Triggered by the Invader: Hypersensitivity," *Plant Disease: An Advanced Treatise* 5:201-224 J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York (1980); (Klement "Hypersensitivity," *Phytopathogenic Prokaryotes* 2:149-177, M.S. Mount and G. H. Lacy, eds. Academic Press, New York (1982)). The localized cell death not only contains the infecting pathogen from spreading further but also leads to a systemic resistance preventing subsequent infections by other pathogens. Therefore, HR is a common form of plant resistance to diseases caused by bacteria, fungi, nematodes, and viruses.

A set of genes designated as *hrp* (Hypersensitive Response and Pathogenicity) is responsible for the elicitation of the HR by pathogenic bacteria including *Erwinia* spp, *Pseudomonas* spp, *Xanthomonas* spp, and *Ralstonia solanacearum* (Willis et al. "*hrp* Genes of Phytopathogenic Bacteria," *Mol. Plant-Microbe Interact.* 4:132-138 (1991), Bonas, U. "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: *Current topics in Microbiology and Immunology*, Vol. 192, *Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms*. J. L. Dangl, ed. Springer-Verlag, Berlin (1994); Alfano et al., "Bacterial Pathogens in

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- Plants: Life Up Against the Wall," *Plant Cell* 8:1683-98 (1996). Typically, there are multiple *hrp* genes clustered in a 30-40 kb DNA. Mutation in any one of the *hrp* genes will result in the loss of bacterial pathogenicity in host plants and the HR in non-host plants. On the basis of genetic and biochemical characterization, the
- 5 function of the *hrp* genes can be classified into three groups: 1) structural genes encoding extracellularly located HR elicitors, for example harpin of *Erwinia amylovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992)); 2) secretion genes encoding a secretory apparatus for exporting HR elicitors and other proteins from the
- 10 bacterial cytoplasm to the cell surface or extracellular space (Van Gijsegem et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," *Trends Microbiol.* 1:175-180 (1993); He et al., "Pseudomonas syringae pv. *Syringae* harpin_{psa}: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255 (1993); Wei et al., "HrpI
- 15 of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," *J. Bacteriol.* 175:7985-67 (1993), Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific *Petunia* Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J.* 13:543-53 (1994), Galan et al., "Cross-talk between Bacterial Pathogens and their Host Cells,"
- 20 *Ann. Rev. Cell Dev. Biol.* 12:221-55 (1996); Bogdanove et al., "*Erwinia amylovora* Secretes Harpin via a Type III Pathway and Contains a Homolog of yopN of *Yersinia*," *J. Bacteriol.* 178:1720-30 (1996); Bogdanove et al., "Homology and Functional Similarity of a *hrp*-linked Pathogenicity Operon, *dspEF*, of *Erwinia amylovora* and the *avrE* locus of *Pseudomonas syringae* pathovar tomato," *Proc. Natl*
- 25 *Acad. Sci. USA* 95:1325-30 (1998)); and 3) regulatory genes that control the expression of *hrp* genes (Wei, Z. M., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992); Wei et al., "*hrpL* Activates *Erwinia amylovora hrp* Genes in Response to Environmental Stimuli," *J. Bacteriol.* 174:1875-82 (1995); Xiao et al., "A Single
- 30 Promoter Sequence Recognized by a Newly Identified Alternate Sigma Factor Directs Expression of Pathogenicity and Host Range Determinants in *Pseudomonas syringae*," *J. Bacteriol.* 176:3089-91 (1994); Kim et al., "The *hrpA* and *hrpC* Operons

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of *Erwinia amylovora* Encode Components of a Type III Pathway that Secretes Harpin," *J. Bacteriol.* 179:1690-97 (1997); Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," *J. Bacteriol.* 180:5203-10 (1998); Wengelnik et al., "HrpG, A Key hrp
5 Regulatory Protein of *Xanthomonas campestris* pv. *Vesicatoria* is Homologous to Two Component Response Regulators," *Mol. Plant-Microbe Interact.* 9:704-12 (1996)). Because of their role in interactions between plants and microbes, *hrp* genes have been a focus for bacterial pathogenicity and plant defense studies.

In addition to the local defense response, HR also activates the defense
10 system in uninfected parts of the same plant. This results in a general systemic resistance to a secondary infection termed Systemic Acquired Resistance ("SAR") (Ross, R. F. "Systemic Acquired Resistance Induced by Localized Virus Infections in Plants," *Virology* 14:340-58 (1961); Malamy et al., "Salicylic Acid and Plant Disease Resistance," *Plant J.* 2:643-654 (1990)). SAR confers long-lasting systemic disease
15 resistance against a broad spectrum of pathogens and is associated with the expression of a certain set of genes (Ward et al. "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," *Plant Cell* 3:1085-94 (1991)). SAR is an important component of the disease resistance of plants and has long been of interest, because the potential of inducing the plant to protect itself could
20 significantly reduce or eliminate the need for chemical pesticides. SAR can be induced by biotic (microbes) and abiotic (chemical) agents (Gorlach et al. "Benzothiadiazole, a Novel Class of Inducers of Systemic Acquired Resistance, Activates Gene Expression and Disease Resistance in Wheat," *Plant Cell* 8:629-43 (1996)). Historically, weak virulent pathogens were used as a biotic inducing agent
25 for SAR. Non-virulent plant growth promotion bacteria (PGPR) were also reported to be able to induce resistance of some plants against various diseases. Biotic agent-induced SAR has been the subject of much research, especially in the late 70s and early 80s. Only very limited success was achieved, however, due to: 1) inconsistency of the performance of living organisms in different environmental conditions;
30 2) considerable concerns regarding the unpredictable consequences of the intentional introduction of weakly virulent pathogens into the environment; and 3) the technical complication of applying a living microorganism into a variety of environmental

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conditions. To overcome the limitations of using living organisms to induce SAR, scientists have long been looking for an HR elicitor derived from a pathogen for SAR induction. With the advancement of molecular biology, the first proteinaceous HR elicitor with broad host spectrum was isolated in 1992 from *Erwinia amylovora*, a pathogenic bacterium causing fire blight in apple and pear. The HR elicitor was named "harpin". It consists of 403 amino acids with a molecular weight about 40 kDa. The harpin protein is heat-stable and glycine-rich with no cysteine. The gene encoding the harpin protein is contained in a 1.3 kB DNA fragment located in the middle of the *hrp* gene cluster. Harpin is secreted into the extracellular space and is very sensitive to proteinase digestion. Since the first harpin was isolated from *Erwinia amylovora*, several harpin or harpin-like proteins have been isolated from other major groups of plant pathogenic bacteria. In addition to the harpin of *Erwinia amylovora*, the following harpin or harpin-like proteins have been isolated and characterized: HrpN of *Erwinia chrysanthemi*, *Erwinia carotovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science*, 257:85 (1992)), and *Erwinia stewartii*; HrpZ of *Pseudomonas syringae* (He et al. "Pseudomonas syringae pv. Syringae harpin_{ps}: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255 (1993)), PopA of *Ralstonia solanacearum*, (Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J* 13:543-53 (1994)); HrpW of *Erwinia amylovora* (Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," *J. Bacteriol.* 180:5203-10 (1998)), and *Pseudomonas syringae*. All of the currently described harpin or harpin-like proteins share common characteristics. They are heat-stable and glycine-rich proteins with no cysteine amino acid residue, are very sensitive to digestion by proteinases, and elicit the HR and induce resistance in many plants against many diseases. Based on their shared biochemical and biophysical characteristics as well as biological functions, these HR elicitors from different pathogenic bacteria belong to a new protein family -- i.e. the harpin protein family. The described characteristics, especially their ability to induce HR in a broad range of plants, distinguish the harpin protein family from other host specific

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proteinaceous HR elicitors, for example elicitors from *Phytophthora* spp (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," *Eur. J. Plant Path.* 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitor-Induced Systemic Acquired Resistance in Tobacco," *Plant Physiol* 110:365-76 (1996)) or avirulence proteins (such as Avr9) from *Cladosporium fulvum*, which are only able to elicit the HR in a specific variety or species of a plant.

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In nature, when certain bacterial infections occur, harpin protein is expressed and then secreted by the bacteria, signaling the plant to mount a defense against the infection. Harpin serves as a signal to activate plant defense and other physiological systems, which include SAR, growth enhancement, and resistance to certain insect damage.

The current understanding of critical plant molecules that may have a significant role in interacting with elicitors and then triggering a sequential signal transduction cascade is described as follows.

Interaction of Plant Resistance Genes (R) and Pathogen Avirulence Genes (avr)

The concept of gene-for-gene interaction is that "for each gene determining resistance (R gene) in the host, there is a corresponding gene determining avirulence in the pathogen (avr gene)". In this model, pathogen avirulence genes generate a specific ligand molecule, called an elicitor. Only plants carrying the matching resistance gene respond to this elicitor and invoke the HR. In the past few years, several disease-resistance, R genes, have been cloned and sequenced. It was expected that R genes might encode components involved in signal recognition or signal transduction pathways that ultimately lead to defense responses. The cloned R genes could be grouped into four classes: (1) cytoplasmic protein kinase; (2) protein kinases with an extracellular domain; (3) cytoplasmic proteins with a region of leucine-rich repeats and a nucleotide-binding site; and (4) proteins with a region of leucine-rich repeats that appear to encode extracellular proteins. (Review in Bent, A.F. "Plant Disease Resistance Genes: Function Meets Structure," *Plant Cell* 8:1757-71 (1996); Baker B., et al., "Signaling in Plant-Microbe Interactions," *Science* 276:726-33 (1997)). The first R gene cloned, Pto, encodes a serine/threonine protein

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kinase. The protein product of Pto directly interacts with the cognate avirulence gene protein, AvrPro, which has been demonstrated in a yeast two-hybrid system. It was shown that only co-existence of both AvrPro and Pto proteins could elicit HR in plants (Tang et al., "Initiation of Plant Disease Resistance by Physical Interaction of AvrPto and Pto kinase," Science 274:2060-63 (1996); Scofield et al., "Molecular Basis of Gene-for-Gene Specificity in Bacterial Speck Disease of Tomato," Science 274:2063-65 (1996); Zhou et al., "The Pto kinase Conferring Resistance to Tomato Bacterial Speck Disease Interacts with Proteins that Bind a cis-element of Pathogenesis-related Genes," EMBO J. 16:3207-18 (1997)). The results from cloned R genes support the view that plant-pathogen interactions involve protein-protein interactions. Syringolide, a water-soluble, low-molecular-weight elicitor, triggers a defense response in soybean cultivars carrying the Rpg4 disease-resistance gene. A 34-KDa protein has been isolated from soybean and is considered to be the physiological active syringolide receptor (Ji et al., "Characterization of a 34-kDa Soybean Binding Protein for the syringolide Elicitors," Proc. Natl. Acad. Sci. USA 95:3306-11 (1998)).

Putative Binding Factor of Elicitin

Elicitins are a family of small proteins secreted by *Phytophthora* species that have a high degree of homology. Pure elicins alone can cause a hypersensitive response, a local cell death, and trigger systemic acquired resistance in tobacco and other plants (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path. 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," Plant Physiol 110:365-76 (1996)). However, the spectrum of HR elicitation and induced systemic resistance in plants is much narrower than that achieved by harpin family elicitors. Like harpin, elicins induce a series of metabolic events in tobacco cells, including the accumulation of phytoalexins, ethylene production, transmembrane electrolyte leakage, H₂O₂ accumulation, and expression of plant defense related genes (Yu L, et al., "Elicitins from *Phytophthora* and Basic Resistance in Tobacco," Proc. Natl. (1995); Keller et al., "Pathogen-Induced Elicitin Production in Transgenic Tobacco Generates a Hypersensitive Response and

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Nonspecific Disease Resistance," *The Plant Cell* 11:223-35 (1999)). A putative receptor-like binding factor has been identified in tobacco plasma membrane, which has a specific high-affinity to the cryptogein, one member of the elicitin family (Wendehenne, et al., "Evidence for Specific, High-Affinity Binding Sites for a Proteinaceous Elicitor in Tobacco Plasma Membrane," *FEBS Letters* 374:203-207 (1995)). Recently, it was found that 2 basic elicitins (i.e. cryptogein and cinnamomin) and two acidic elicitins (i.e. capsicein and parasiticein) were able to interact with the same binding sites on tobacco plasma membranes (Bourque et al., "Comparison of Binding Properties and Early Biological Effects of Elicitins in Tobacco Cells," *Plant Physiol.* 118:1317-26 (1998)). However, the gene of the receptor-like factor has not been isolated.

Putative Binding Factor of Glycoprotein Elicitors

15 A 42 kDa glycoprotein elicitor has been isolated from *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f. sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," *Mol. Plant Microbe Interact.* 4:19-27 (1991)). An oligopeptide of 13 amino acids within the glycoprotein ("Pep-13") was able to induce a response in
20 plants like that achieved by the full glycoprotein. A high affinity-binding pattern has been observed in parsley microsomal membranes with an isotope labeled oligopeptide. There are estimated to be about 1600 to 2900 binding sites per cell with evidence indicating that a low abundant protein receptor of the Pep-13 is localized in the plasma membrane (Nurnberger et al., "High Affinity Binding of a Fungal
25 Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Responses," *Cell* 78:449-60 (1994)).

Harpin Protein Binding Factors

30 Harpin proteins, which elicit HR in a variety of different nonhost plants, have been isolated from plant pathogens (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"

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Science 257:85 (1992)). A family of harpin proteins has been identified from plant bacterial pathogens. All of them have similar biological activities. It is well documented that harpin protein can induce plants to produce active oxygen, change ion flux, lead to local cell death, and induce systemic acquired resistance ("SAR") (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992); He et al., "*Pseudomonas syringae* pv. *syringae* Harpin_{ps}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-66 (1993); Baker, C.J., et al., "Harpin, an Elicitor of the Hypersensitive Response in Tobacco Caused by *Erwinia amylovora*, Elicits Active Oxygen Production in Suspension Cells," *Plant Physiol.* 102:1341-44 (1993)). No harpin protein binding factor has been isolated so far. It was reported that an amphipathic protein, named HRAP, isolated from sweet pepper could dissociate harpin_{ps} in multimeric form (*hrpZ* from *Pseudomonas syringae*). The biological activity of the HRAP is believed to be its ability to intensify harpin_{ps}-mediated hypersensitive response. HRAP protein does not bind to harpin_{ps} directly (Chen et al., "An Amphipathic Protein from Sweet Pepper can Dissociate Harpin_{ps} Multimeric Forms and Intensify the Harpin_{ps}-Mediated Hypersensitive Response," *Physiological & Molecular Pathology* 52:139-49 (1998)). Using a fluorochrome tagged antibody to harpin to examine the interaction of harpin_{ps} and tobacco suspension cells, it was found that harpin_{ps} interacted with the cultured cells, but not with protoplasts with the cell walls being digested and removed. It was interpreted that harpin_{ps} was localized in the outer portion of the plant cell, probably on the cell wall. However, it was not ruled out that the binding factor was located on the plasma membrane.

25 The present invention seeks to identify receptors for hypersensitive response elicitor proteins or polypeptides and uses of such receptors.

SUMMARY OF THE INVENTION

30 The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also disclosed are nucleic acid molecules encoding such receptors as well as expression

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vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules.

5 The protein of the present invention can be used in conjunction with a method of identifying agents targeting plant cells by forming a reaction mixture including the protein and a candidate agent, evaluating the reaction mixture for binding between the protein and the candidate agent, and identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

10 The nucleic acid molecule of the present invention can be used in a method of identifying agents targeting plant cells by forming a reaction mixture including a cell transformed with the nucleic acid molecule of the present invention and a candidate agent, evaluating the reaction mixture for binding between protein produced by the host cell and candidate agent, and identifying candidate compounds which bind to the protein or the host cell in the reaction mixture as plant cell targeting agents.

15 Another aspect of the present invention relates to a method of enhancing a plant's receptivity to treatment with hypersensitive response elicitors by providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule of the present invention.

20 The present invention is also directed to a method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants by providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule encoding a receptor in accordance with the present invention.

25 The discovery of the present invention has great significance. This putative receptor protein can be used as a novel way to screen for new inducers of plant resistance against insect, disease, and stress, and of growth enhancement. This protein is the first step toward the understanding of the harpin induced signal transduction pathway in plants. Further studies of this pathway will provide more possible targets for new plant vaccine and growth enhancement products development. In addition, this protein can serve as an anchor providing a new way to target anything to the plant cells.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a yeast two-hybrid screening with the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) and a schematic representation of the interaction between harpin and a cDNA encoded protein. Harpin is fused to LexA protein which contains a DNA binding domain ("BD"). The cDNA encoded protein is fused to the GAL4 transcription activation domain ("AD"). This interaction targets the activation domain to two different LexA-dependent promoters with consequent activation of the transcription of the *HIS3* and *lacZ* reporter genes.

Figures 2A-B show that the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) is a good yeast two-hybrid bait. Reporter genes were not expressed in yeast strain L40 containing plasmids expressing the LexA - harpin fusion in combination with plasmids expressing GAL4 activation domain alone, or fused to unrelated protein. Therefore, harpin is not autoactive in this yeast two-hybrid system. In addition, reporter genes were not expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-harpin fusion in combination with plasmids expressing LexA alone, or fused to unrelated protein. Figure 2A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 2B shows a synthetic minimal ("SD") media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figures 3A-B show the interaction between HrBP1 (hypersensitive response elicitor binding protein 1) and a hypersensitive response elicitor (i.e. harpin) is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-HrBP1 fusion in combination with plasmids expressing LexA fused to hypersensitive response elicitor (i.e. harpin), but were not expressed in combination with LexA alone, or LexA fused to unrelated proteins. Figure 3A is a β -galactosidase assay where the blue color indicates the expression of *lacZ* reporter gene. Figure 3B is an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

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Figures 4A-B show the interaction of HrBP1 and a hypersensitive response elicitor (i.e. harpin) in another orientation. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the LexA - HrBP1 fusion in combination with plasmids expressing GAL4 activation domain fused to harpin, but were not expressed in combination with GAL4 activation domain alone, or GAL4 activation domain fused to unrelated proteins. Therefore, interaction between harpin and HrBP1 is specific. Figure 4A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 4B shows an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figure 5 shows the nucleic acid sequence of HrBP1 cDNA.

Figure 6 shows the deduced amino acid sequence of HrBP1.

Figure 7 shows the gene structure of HrBP1 and a schematic representation of the exons and introns of the HrBP1 gene. When comparing the HrBP1 cDNA sequence with the *Arabidopsis thaliana* genomic DNA sequence published in a public database, four exons and three introns were discovered.

Figure 8 shows a Northern blot where the translated region of HrBP1 cDNA (855 bp) was used as a probe in the Northern analysis of both total RNA and Poly A⁺ RNA from *Arabidopsis thaliana*. One single band with an apparent molecular weight of about 1.1 Kb was detected.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated receptors for hypersensitive response elicitor proteins or polypeptides. Also disclosed are DNA molecules encoding such receptors as well as expression systems, host cells, and plants containing such molecules. Uses of the receptors themselves and the DNA molecules encoding them are disclosed. The receptor for a hypersensitive response elicitor from a plant pathogen can be from a monocot or a dicot. One example of such a receptor is that found in *Arabidopsis thaliana* which has the amino acid sequence of SEQ. ID. No. 1 as follows:

Met Ala Thr Ser Ser Thr Phe Ser Ser Leu Leu Pro Ser Pro Pro Ala
1 5 10 15

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Leu Leu Ser Asp His Arg Ser Pro Pro Pro Ser Ile Arg Tyr Ser Phe
20 25 30

5 Ser Pro Leu Thr Thr Pro Lys Ser Ser Arg Leu Gly Phe Thr Val Pro
35 40 45

Glu Lys Arg Asn Leu Ala Ala Asn Ser Ser Leu Val Glu Val Ser Ile
50 55 60

10 Gly Gly Glu Ser Asp Pro Pro Pro Ser Ser Ser Gly Ser Gly Gly Asp
65 70 75 80

15 Asp Lys Gln Ile Ala Leu Leu Lys Leu Lys Leu Leu Ser Val Val Ser
85 90 95

Gly Leu Asn Arg Gly Leu Val Ala Ser Val Asp Asp Leu Glu Arg Ala
100 105 110

20 Glu Val Ala Ala Lys Glu Leu Glu Thr Ala Gly Gly Pro Val Asp Leu
115 120 125

Thr Asp Asp Leu Asp Lys Leu Gln Gly Lys Trp Arg Leu Leu Tyr Ser
130 135 140

25 Ser Ala Phe Ser Ser Arg Ser Leu Gly Gly Ser Arg Pro Gly Leu Pro
145 150 155 160

30 Thr Gly Arg Leu Ile Pro Val Thr Leu Gly Gln Val Phe Gln Arg Ile
165 170 175

Asp Val Phe Ser Lys Asp Phe Asp Asn Ile Ala Glu Val Glu Leu Gly
180 185 190

35 Ala Pro Trp Pro Phe Pro Pro Leu Glu Ala Thr Ala Thr Leu Ala His
195 200 205

Lys Phe Glu Leu Leu Gly Thr Cys Lys Ile Lys Ile Thr Phe Glu Lys
210 215 220

40 Thr Thr Val Lys Thr Ser Gly Asn Leu Ser Gln Ile Pro Pro Phe Asp
225 230 235 240

45 Ile Pro Arg Leu Pro Asp Ser Phe Arg Pro Ser Ser Asn Pro Gly Thr
245 250 255

Gly Asp Phe Glu Val Thr Tyr Val Asp Asp Thr Met Arg Ile Thr Arg
260 265 270

50 Gly Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ala
275 280

This protein, known as HrBP1p, is encoded by a cDNA molecule having SEQ. ID.

No. 2 as follows:

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tttttctctt tcaacaatgg cgaattcttc tactttctcg tcaactactac attcaccacc 60
agctcttctt tccgaccacc gttctcttcc accatccatc agatactect ttttctcctt 120
aactactcca aaatcgtctc gtttgggttt cactgtaccg gagaagagaa acctcgtcgc 180
taattcgtct ctogttgaag tatccattgg cggagaaagt gaccaccacc catcctcctc 240

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5 tggatcagga ggagacgaca agcaaatgac attactcaaa ctcaaatlac ttagtgtagt 300
 ttcgggatta aacagaggac ttgtggcgag tggtgatgat ttagaagag ctgaagtggc 360
 tgctaaagaa cttgaacctg ctgggggacc ggttgattta acgatgac tcgataagct 420
 tcaagggaaa tggaggctgt tgtatagtag tgcgttctct tctcgtctct taggtggtag 480
 5 cgcctcgtgt ctacctactg gacgtttgat cctgttact cttggccagg tgtttcnacg 540
 gattgatgtg tttagcaag attttgataa catagcagag gtgaatttag gagcccttg 600
 gccatttcog ccattagaag ccactgogac attggcacac aagtttgaac tcttaggcac 660
 ttgcaagac aagataacat ttgagaaaac aactgtgaag acatcgggaa acttctcga 720
 gattcctcog tttgatctc cgggcttcc cgcagtttc agaccatcgt caaaccttg 780
 10 aactggggat ttcgaagtta cctatgttga tgataccatg cgcataactc gggggacag 840
 aggtgaactt aggttatctg tcatgtctta attctcaag ctttgacatg taagatata 900
 taataacttt ctgcttgatg cgtctcatg agttttgtac aatcatgtg aactatata 960
 tgcgctttat aagtaantga gtgtcttgtt caatgaatca 1000

15 The genomic DNA molecule containing the receptor encoding cDNA
 molecule of SEQ. ID. No. 2 has SEQ. ID. No. 3 as follows:

20 aattagaana attacaacc aacatctagt tagaatattt aatttgcaac aatgtctctg 60
 agtatagtga aaaaaataga agatcgata tcgaatagta cgtatagat catctagatc 120
 cattcgaaact aacgtctact tttcttttcc agcatlaaca tgtagcttgc cattagcatt 180
 tacatgttgc aataacaca aattgggaaa ttgaagact aaaaaacctt gtacagcaga 240
 tggttttaa cgtgattcca tggacacaa cgaannacgg cagaactaag ccaaaaacg 300
 tcaactaagc atatcaagc ttttaattga agcctaatat aacacaaagt ggttatccat 360
 aatctgttct taatctcttg cgttagttat cttttcatta ttgcanttc gcaattctat 420
 25 attcttatat tcaacttgt tcttcttcca aattgtaatt atatctacat cgtcttagct 480
 tgaccattat agtccagta ccaagttctc ttcttaactt taatctcagc tactattctc 540
 atactgtaa tatcttttgc tcaccaaca tatatttoga accaaactgc taaggttta 600
 tcataaattg cgttcttagc cacacaattt tgcagttcca accattaat gccacaaat 660
 ttggacgatt tcttaagaca agagaacat agcaaccua ccttattgat taatatgaa 720
 30 atgtctccat aaaaactgga gatttcccc aataaagaga acacggcaaa tgttcaogta 780
 atctcaaga tgaatgttta attttttctt tcagaaaaa acaaaaaac ttaactcaat 840
 atagacaact agaatggata ccaactaagc aaagaantt caaagaca atatatattg 900
 gatatgaagt tacattattt tcaacttta tatactacta aaagctcaa aatttgttct 960
 aaaaatgat ccaatcaat ggaaggtatg aatgtcatat gactaaaga gaaaacaca 1020
 35 cctgtatata agtatggat cgtctgctc cagagtga caaacacga tgtgggtctt 1080
 tattgggcca tacttaattg gaaaaggag aaaaaaatt gggcaatgtc tatggtcga 1140
 atttatatgt tttaactca taatatcaat atttaattt atatatgtg gtcttaatt 1200
 agtattatct acatagatta aatcaaatg actgcatatg gtccataata atacaacca 1260
 40 agcaattta aattttgtg cacaacga catcattta ctcaaaagt aatattgaat 1320
 ttggtttac cacaacga tgcgctca taacccgtg tgtttctca atcaataat 1380
 aattaagtc ttcttctct tcttctctc taataattat ctactctct tcttttttt 1440
 ttctcttca acaatggga cttctctac ttctctga ctactacct caccaccag 1500
 tcttcttcc gaccacgtt ctctctacc atccataga tactctttt ctccottac 1560

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tactccaaaa tegtctcgtt tgggtttcac tgtaccggag aagagaaaac tegtctctaa 1620
 ttcgtctctc gttgaagtat ccattggcgg agaaagtac coaccaecat catcatcgg 1680
 atcaggagga gaagacaagc aaattgcatt actcaactc aaattacttg tgagtctgat 1740
 tcaaaccaat cgggtgaatt ataagaaatt ggtttcgttt ctttgggaatt agggcttata 1800
 ttaotgttaa gattcgatta tagagtgaat tttgggaaga tttttcagat ttgatttgtg 1860
 atgtgttgtg ttgtgagaaa ttgcagagtg tagtttcggg attaaacga ggacttgttg 1920
 cgagtgttga tgatttagaa agagctgaag tggctgctaa agaacttga actgctgggg 1980
 gacoggttga ttaaccgat gatcttgata agcttcaag gaaatggagg ctgttgtata 2040
 gtagtgcgtt ctcttctcgg tcttttagtg gttagcgtcc tggctctacct actggacgtt 2100
 tgatccctgt tactcttggc caggtaatc ttgaatcatt gctctgtttt taccogtcaa 2160
 gattloggtt ttccggtaag ttgttgagga gtttatgtgc atggtctagt ctatctcaa 2220
 tagtcttctc tgagttagaa tggggotgag cttaagaatct agctttctga ggttacaatt 2280
 tggtaattgc atctatact cgaagcaaa cttttttcta tattgtcaag ttatgtgtg 2340
 cggctctgtc tatcattggt agtctttgtt gagcttgaat ggtgaggagc ttagaatcta 2400
 gcaatgtcat ctactctta atcatttttt tctatattgc caagtattg tgtaoggtct 2460
 tagtcaatca tctttattct tggttgagtc tgaatggta tgagcttaga atctagcttt 2520
 ctttggttta aatttggcaa agaaccatac ctgaaatcgg agaaagcaaa cttctttaat 2580
 attatctott gtttctgaat cattaaaca ggtgtttcaa cggattgatg tgtttagcaa 2640
 agattttgat aacatagcag aggtggaaat agygacctt tggccatttc cggcattaga 2700
 agccactgcy acattggcac acaagtttga actcttaggt ttgcatttcc ctttctctca 2760
 ttaagtttat cgaattgtgt aagagcaaaa taacttatct gtatctttga catttatggg 2820
 gaaaacagyc acttgcaaga tcaagataac atttgagaaa acaactgtga agacatcggg 2880
 aaacttgctc cagattcttc cgtttgata: cccgaggctt cccgacagtt tcagaccatc 2940
 gtcaaacctt ggaacttggg atttogaag: tacctatgtt gatgatacca tgcgcataac 3000
 tccgctgggac agaggtgaac ttagggtatc cgtcattgct taattctcaa agctttgaca 3060
 tgtaaagata aataaatact ttctgcttga tgcagctcca tgagttttgt acaaatcatg 3120
 tgaacatata aatgoccttt ataagtaat gagtgtcttg ttcaatgaat catatgaag 3180
 aatttgtatg actcagaaa ttggacatg atatagacct tcaaatttt gcaacctcta 3240
 atgtgagata ttagtgaatt tttcttaggt tggtagagag aacggatttg caaaaaata 3300
 togaaggtca atgattaca gcaaaacct atcttgatga ttcaaatat agagttaaca 3360
 agcaagatg agacaatctt atacgagaga gctaaaaaa atggattcca aatccagcaa 3420
 gtacaaaaat cgcagaaaat aagatgaac caacttaaa cagagatgtt ccctttocct 3480
 tcttgtcacc accgatctcg aatgcttgc acotctgaa taacaacaa accaaccaca 3540
 tgtaaagcaa ttaccaagt acaatccgg tataatgaac tgatctatgt tctatgcaco 3600
 ttgataggac gctgcgaaa gtgottgcag otttgacact gaagcttcaa aacaatcttc 3660
 ttogtggctc tagcctgtta acaagattca caagatgtat ctcagtocaa aactgagact 3720
 attgguatgt ctgtttcttc acagctcact tccaaatct tactataat ggttccttaa 3780
 aactacctca tttaactaa cttagacctaa ttcaactga aaaaacatc aatgatgat 3840
 aatcaatgtt accttttgt ggaagacagg cttagtctga ccaccataac cagattgttt 3900
 accgtctaaa cgaaccttc cttgagcagc aagactgtct ttaccttct tgtattgggt 3960
 aaccttgtgc aaagta:gtc ttttgcattc ctgttctta cagtaagtgt tctttgtctt 4020
 tggaaatgtc accttcaaaa ttcataaat caaaatgaa tcactcacac acatacaaaa 4080
 tcaagagact ttaaggtta atcaaatat aacatcatt tagattgaaa acctttatga 4140
 tagatctgaa aaacaataca ataatcaat caaccatgta ttgttgttct tcaagctcaa 4200

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cgaaacttttac aaatttccaaa atccacatcga aagagagagaa acaattttacc atttttcgcgt 4260

Hypersensitive response elicitors recognized by the receptors of the present invention are able to elicit local necrosis in plant tissue contacted by the elicitor.

Examples of suitable bacterial sources of hypersensitive response elicitor polypeptides or proteins include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* is disclosed in U.S. Patent No. 5,850,015 and U.S. Patent No. 6,001,959, which are hereby incorporated by reference. This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* and its encoding DNA molecule has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has a glycine content of greater than 21% and contains substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in U.S. Patent No. 5,849,868 to Beer and Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85-88 (1992), which are hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the

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hypersensitive response elicitor derived from *Pseudomonas syringae* and its encoding DNA molecule is found in U.S. Patent Nos. 5,708,139 and 5,858,786 and He et al., "*Pseudomonas syringae* pv. *syringae* Harpin_{PM}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-66 (1993), which are hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J.* 13:543-533 (1994), which is hereby incorporated by reference. This protein has 344 amino acids, a molecular weight of 33.2 kDa, and a pI of 4.16, is heat stable and glycine rich (20.6%).

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *glycines* has a partial amino acid sequence corresponding to SEQ. ID. No. 4 as follows:

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1 5 10 15
Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20 25

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. *glycines*. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes a partial amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

Met Asp Ser Ile Gly Asn Asn Phe Ser Asn
1 5 10

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

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subsp. *carotovora* Strain Ecc71 Overexpress *hrp N_{EC}* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," *MPMI*, 9(7):565-73 (1996), which is hereby incorporated by reference. This protein has 356 amino acids, a molecular weight of 35.6 kDa, and a pI of 5.82 and is heat stable and glycine rich (21.3%).

5 The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," *8th Int'l. Cong. Molec. Plant-Microbe Interact.*, July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," *Ann. Mtg. Am. Phytopath. Soc.*, July 27-31, 1996,
10 which are hereby incorporated by reference.

 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamoni*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most
15 Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," *Molec. Plant-Microbe Interact.*, 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," *Eur. J. Biochem.*, 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in
20 Tobacco, by Isolates of *Phytophthora parasitica*," *Plant Path.*, 41:298-307 (1992), Baillreul et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," *Plant J.*, 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco
25 and Other Plants," *Eur. J. Plant Path.*, 102:181-92 (1996), which are hereby incorporated by reference. These hypersensitive response elicitors from *Phytophthora* are called elicitins. All known elicitins have 98 amino acids and show >66% sequence identity. They can be classified into two groups, the basic elicitins and the acidic elicitins, based on the physicochemical properties. This classification also
30 corresponds to differences in the elicitins' ability to elicit HR-like symptoms. Basic elicitins are 100 times more effective than the acidic ones in causing leaf necrosis on tobacco plants.

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The hypersensitive response elicitor from Gram positive bacteria like *Clavibacter michiganensis* is described in WO 99/11133, which is hereby incorporated by reference.

5 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response using conditions under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

10 Turning again to the receptor of the present invention for such hypersensitive response elicitors, fragments of the above receptor protein are encompassed by the method of the present invention. In addition, fragments of full length receptor proteins from other plants can also be utilized.

15 Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known receptor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for receptor activity according to the procedure described above.

20 As an alternative, fragments of a receptor protein can be produced by digestion of a full-length receptor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave receptor proteins at different sites based on the amino acid sequence of the receptor protein. Some of the fragments that result from proteolysis may be active receptors.

25 In another approach, based on knowledge of the primary structure of the receptor protein, fragments of the receptor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

30 Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the receptor being produced. Alternatively, subjecting a full length receptor to high temperatures and

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pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

5 Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a tag or other sequence for ease of synthesis, purification, or identification of the polypeptide.

10 Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. No. 2 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing with the SSC buffer at 37°C; and preferably in a hybridization buffer
15 comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

The receptor of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional
20 techniques. Typically, the receptor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the receptor protein of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the receptor protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, or chemical
25 treatment, and the homogenate is centrifuged to remove bacterial debris. The cell lysate can be further purified by conventionally utilized chromatography procedures. The supernatant is then subjected to heat treatment and the protein is separated by centrifugation. The supernatant fraction containing the receptor protein is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to
30 separate the receptor protein. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

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The DNA molecule encoding the receptor protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host

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cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

10 Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and
15 accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes.
20 Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably
25 promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote
30 transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a

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number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*,
5 and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which
10 inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

15 Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong"
20 transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage
25 lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the receptor protein has been cloned into an expression system, it is ready to be incorporated into a host cell.
30 Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

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One aspect of the present invention involves enhancing a plant's receptivity to treatment with a hypersensitive response elicitor by providing a transgenic plant or transgenic plant seed, transformed with a nucleic acid molecule encoding a receptor protein for a hypersensitive response elicitor. It has been found that hypersensitive response elicitors are useful in imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance in a variety of plants. In view of the receptor of the present invention's interaction with such elicitors, it is expected that these beneficial effects would be enhanced by carrying out such elicitor treatments with plants transformed with the receptor encoding gene of the present invention.

Transgenic plants containing a gene encoding a receptor in accordance with the present invention can be prepared according to techniques well known in the art.

A vector containing the receptor encoding gene described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, *Mol. Gen. Genetics*, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., *Nature*, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g.,

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dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies.

- 5 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

- The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are
10 electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

- Another method of introducing the DNA molecule into plant cells is to
15 infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration
20 medium without antibiotics at 25-28°C.

- Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized
25 only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

- Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A.*
30 *rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

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After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds.

These elicitor treatment methods can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed transformed with a receptor gene in accordance with

the present invention under conditions effective for the elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance.

Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to
5 impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart resistance to stress.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance in
10 the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed both a DNA molecule encoding a receptor in accordance with the present invention and with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. The plant is grown under conditions effective to permit that
15 DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to
20 impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress.

The embodiment where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor or 2) application of
25 bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these
30 embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

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The hypersensitive response elicitor treatment can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, 5 barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable 10 ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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With regard to the use of hypersensitive response elicitors in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion 15 number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

20 The method of imparting pathogen resistance to plants is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants 25 *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide to enhance plant growth, various forms of plant growth enhancement or 30 promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced,

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increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, there is significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

The use of hypersensitive response elicitors for insect control encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

Elicitor treatment is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

Hypersensitive response elicitor treatment is also useful in imparting resistance to plants against environmental stress. Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide,

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NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients).

The application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with a hypersensitive response elicitor, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated with an elicitor, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or to impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include (NH₄)₂NO₃. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. In addition, the hypersensitive response elicitor

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can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

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5 In the alternative technique involving the use of transgenic plants and transgenic seeds encoding a hypersensitive response elicitor encoding gene, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art as described above.

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10 In another embodiment, the present invention relates to a DNA construct which is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors. An example of such a construct would be an antisense DNA molecule of the DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3. Alternatively, the DNA construct can have a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3 (or a portion thereof) and its complementary strand and is used to generate a single transcript with an inverted repeat (i.e. a double-stranded) RNA. This transcript as well as the above-discussed antisense nucleic acid molecule can be used to induce silencing of a nucleic acid molecule encoding a receptor for a hypersensitive response elicitor.

20 Sensing the hypersensitive response elicitor by the receptor is the very first step of the signal transduction pathway in plants which eventually leads to disease resistance, growth enhancement, insect control, and stress resistance. Silencing the receptor provides a powerful tool to find and study the downstream components of this pathway. Additionally, the receptor could be a negative regulator of such plant signal transduction pathway. Silencing of the receptor will impart to plants the ability to resist disease and stress, control insects, and enhance growth without hypersensitive response elicitor treatment.

EXAMPLES

30 Example 1 - Materials and Methods

The laboratory technique used in the following example is straight forward. All DNA manipulations described here followed conventional protocols

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(Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory (1989); Ausubel, et al., "Current Protocols in Molecular Biology," John Wiley (1987), which are hereby incorporated by reference). The plasmids and microorganisms described herein, used for making the present invention, were
5 obtained from commercial sources, or from the authors of previous publications. Sequences were analyzed with Clone Manager 5 (Scientific & Educational Software, Durham, North Carolina).

Yeast strain L40 was grown in YPD or in different minimal synthetic dropout selection media at 30°C. *E.coli* strains DH5α and HB101 were grown in LB
10 at 37°C.

The yeast Two-Hybrid system is based on the fact that many eukaryotic transcription factors are composed of physically separable, functionally independent DNA-binding domain (DNA-BD) and activation domain (AD). Both the DNA-BD and the AD are required to activate a gene. When physically separated by
15 recombinant DNA technology and expressed in the same host cell, the DNA-BD and the AD do not interact directly with each other and thus cannot activate the responsive gene (Ma & Ptashne, "Converting a Eukaryotic Transcriptional Inhibitor into an Activator," *Cell* 55:443 (1988) and Brent & Ptashne, "A Eukaryotic Transcriptional Activator Bearing the DNA Specificity of a Prokaryotic Repressor," *Cell* 43:729
20 (1985), which are hereby incorporated by reference). But if the DNA-BD and the AD are brought into close physical proximity in the promoter region, the transcriptional activation function will be restored. Therefore, the yeast *Saccharomyces cerevisiae* and the Two-Hybrid system have become essential genetic tools for studying the macromolecular interactions.

25 In the Two-Hybrid system utilized here, the DNA-BD, encoded in the bait vector pVJL11 (Jullien-Flores, V., "Bridging Ral GTPase to Rho Pathways. RLIP76, a Ral Effector with CDC42/Rac GTPase-activating Protein Activity," *J. Biol. Chem.* 27:22473 (1995), which is hereby incorporated by reference), is the prokaryotic LexA protein, and the activation domain, encoded in the prey vector
30 pGAD 10 or pGAD GH (Clontech; Hannon, GJ., "Isolation of the Rb-related p130 Through its Interaction with CDK2 and Cyclins," *Genes Dev.* 7:2378 (1993), which is hereby incorporated by reference) is derived from the yeast GAL4 protein. pVJL11

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also has a *TRP1* marker, and the pGAD a *LEU2* marker. An interaction between the bait protein (fused to the DNA-BD) and a library-encoded protein (fused to the AD) creates a novel transcriptional activator with binding affinity for LexA operators. The yeast host L40 {MATa his3D200 trp1-901 leu2-3, 112 ade2 LYS2::(*lexAop*)₄-HIS3 URA3::(*lexAop*)₁-lacZ} harbors two reporter genes, *lacZ* and *HIS3*, which contain upstream LexA binding site. The *HIS3* nutritional reporter provides a sensitive growth selection that can identify a single positive transformant out of several million candidate clones. The expression of the reporter genes indicates interaction between a candidate protein and the bait protein. See Figure 1.

10 *Erwinia amylovora* harpin was used as the bait protein to screen the *Arabidopsis thaliana* MATCHMAKER cDNA library cloned in the pGAD 10 vector (Clontech, laboratories, Inc., Palo Alto, California). One cDNA library encoded protein was identified as a strong harpin interacting protein and, thus, a putative harpin receptor. The present invention reports the nucleic acid sequence and the
15 deduced amino acid sequence of this cDNA.

Example 2

HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11. PCR was carried out using the 1.3 Kb harpin fragment (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992), which is hereby incorporated by reference) as a template to amplify the harpin encoding region. A Bam HI site was added to the 5' end of the coding sequence, and a Sal I site to the 3' end. A Bam HI and Sal I digested PCR fragment was ligated with the bait vector pVJL11 digested with the same restriction enzymes. pVJL11 has a *TRP1* marker to be selected in yeast and an Amp resistance marker to be selected in *E. coli*. The plasmid DNA was amplified in *E. coli* strain DH5α. When tested in the Two-Hybrid system with empty prey vector pGAD GH and several unrelated proteins, HrpN didn't show auto-
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30 activation or nonspecific interaction with unrelated proteins, as shown in Figure 2.

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Example 3

HrpN-pVJL 11 was transformed into yeast strain L40 by a lithium acetate (LiAc)-mediated method (Ito et al., "Transformation of Intact Yeast Cells Treated with Alkali Cations," *J. Bacteriol.* 153:163 (1983) and Vojtek et al., "Mammalian Ras Interacts Directly with the Serine/Threonine Kinase Raf.," *Cell* 74:205 (1993), which are hereby incorporated by reference). The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for harpin interacting proteins. Approximately 6.8 million primary library transformants were plated onto plates lacking histidine, leucine, and tryptophan. A total of 148 colonies grew on the histidine dropout plates, 55 of which stained positive when tested for expression of β -galactosidase. After three rounds of selection on synthetic minimal (SD) media plates lacking leucine, tryptophan, and histidine, and confirming by the expression of the second reporter gene *lacZ* using a β -galactosidase assay, 47 colonies seemed to be strong interacting candidates.

Example 4

Plasmid DNA was extracted from the 47 independent yeast colonies and shuttled into *E. coli* strain HB101, which carries the *leuB* mutation. Therefore, the prey plasmid (cDNA-pGAD 10) was selected for on minimal nutrient plates since pGAD 10 bears the *LEU2* marker.

The 47 independently rescued prey plasmids purified from *E. coli* were re-tested in the yeast two-hybrid system with harpin as bait. They were also tested against unrelated protein. 20 turned out to be strong specific interaction candidates again. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The sequence reactions were performed using the PE Prism BigDye™ dye terminator reaction kit. The sequencing gel was run in Thatagen (Bothell, WA)

One of the eight plasmids, which had the longest cDNA insert of 1kb, was used for further analysis. When co-transformed into yeast strain L40, it was shown to be negative with empty bait and unrelated proteins in the Two-Hybrid

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system, indicating the specificity of the interaction between harpin and this receptor candidate. See Figure 3.

Example 5

The longest cDNA insert, HrBP1, was subcloned into the Bam HI and SalI sites of the bait vector pVJL 11. This construct didn't show auto-activation of the reporter genes, nor interaction with unrelated proteins in the yeast Two-Hybrid system. However, the expression of the reporter genes was activated when L40 was co-transformed with HrBP1-pVJL11 and HrpN-pGAD GH, indicating the specific interaction between HrBP1p (the protein encoded by HrBP1) and harpin. See Figure 4.

Example 6

Total RNA was extracted from two-week-old *Arabidopsis thaliana* using QIAGEN RNeasy plant mini kit (Qiagen, Inc., Valencia, California). Poly A⁺ RNA was further purified from the total RNA with a QIAGEN Oligotex column (Qiagen, Inc., Valencia, California). A Northern blot was carried out using the translated region of HrBP1 as a probe. One single species with an apparent molecular weight of about 1.1 Kb was detected from both total RNA and Poly A⁺ RNA. See Figure 8. Therefore, the longest cDNA of HrBP1 from the yeast two-hybrid screen seems to be the full-length cDNA. The integrity of the 5' of cDNA was further confirmed by a primer extension assay.

As described, a yeast Two-Hybrid system was used to screen for harpin interacting proteins. HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11, which has a *TRP1* marker. The *lexA* harpin fusion protein is expressed from this construct in yeast. The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for hypersensitive response elicitor interacting proteins. Out of the 6.8 million independent colonies screened, 148 grew on SD media plates lacking leucine, tryptophan, and histidine. After three rounds of selection on a -His plate and confirmation by a β -galactosidase assay, 47 colonies seemed to be strongly interacting

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candidates. The prey plasmids from the 47 candidate colonies were rescued and shuttled into *E. coli*.

5 The 47 independent prey plasmids purified from *E. coli* were re-tested in the yeast Two-hybrid system with harpin as bait. They were also tested against
10 unrelated protein: LexA-lamin. 20 turned out to have a strong specific interaction. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The longest sequence was 1,000 bp, which was named HrBP1. It was mapped to *Arabidopsis thaliana* genomic DNA, chromosome 3, P1 clone MLM24 (Nakamura, "Structural Analysis of *Arabidopsis thaliana* chromosome 3," Direct submission to the DDBJ/EMBL/GenBank databases (1998), which is hereby incorporated by reference). Four exons and three introns were discovered (See Figure 7). Exon 4 includes a 130 bp non-translated 3' region. The in-frame open reading frame from the first methionine encodes a polypeptide (named HrBP1p) of 284 amino acids. The predicted molecular weight of HrBP1p is
15 30454.3 and pI is 5.72. There is no apparent hydrophobic trans-membrane domain in this polypeptide. The HrBP1-AD fusion prey was negative with empty bait and unrelated proteins in the yeast 2-H system, indicating the specificity of the interaction between harpin and this receptor candidate. When being put in the opposite orientation, i.e. HrBP1p fused with the DNA-BD and harpin with the AD, they still
20 specifically interacted with each other.

HrBP1 has no significant sequence similarity with sequences deposited in major sequence database accessible with the Blast search program. Therefore, HrBP1p is a novel protein.

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Example 7

The HrBP1 cDNA was subcloned into the Nde I and Sal I sites of the vector pET-28a (Novagen, Madison, WI). The his-tagged HrBP1p recombinant protein was purified with Ni-NTA resin (QIAGEN Inc., Valencia, CA) according to the manual provided by the manufacturer. The HrBP1 cDNA was also subcloned into the BamH I and Sal I sites of the vector pGEX-4T-1 (Amersham Pharmacia Biotech, Piscataway, NJ). The GST-tagged HrBP1p recombinant protein was purified by Glutathione Sepharose™ 4B (Amersham Pharmacia Biotech, Piscataway NJ) according to the manufacturer's instructions.

Example 8

Anti-sense HrBP1, which is complementary to SEQ. ID. No. 2, was sub-cloned into binary vector pPZP212, under the control of the NOS promotor. *Arabidopsis thaliana* plants were transformed with this construct using *Agrobacterium*-mediated transformation. The *Agrobacterium tumefaciens* strain used was GV3101 (C58C1 Rif^r) pMP90 (Gmr). *Arabidopsis* plants were also transformed with a construct, which had an inverted repeat consisting of bases 3-650 of the HrBP1 cDNA (i.e. SEQ. ID. No. 2) and the complementary sequence of bases 3-500 of HrBP1 cDNA. This construct generated a double-stranded mRNA in transformed plants.

Both anti-sense and double-stranded approaches were intended to silence the expression of HrBP1. Some of the transformed *Arabidopsis* plants had spontaneous necrosis on their leaves. They were also very diseased and developmentally retarded.

Example 9

The HrBP1 homologue from rice, R6, was clone by yeast two hybrid screening. However, it is not a full-length cDNA; there is some sequence information missing from the 5' end. The partial sequence of HrBP1-like cDNA from

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At protein level: Identities = 151/203 (74.4%), Positives = 177/203 (87.2%), Gaps = 2/203 (0%)

15 R6-p : 61 VYSSAFSSHTLGGSRPGPPTGRLLPITLQGVFORIDVSKDFONTIVDVELGAPWPLPFVE
+YSSAFSSR+LGGSRPG PTGRL+P+TLQGVFORIDV SKDFONTI +VELGAPWP PP+K
H-RFP1b: 142 LYSSAFSSRSLGGSRPGLPTGRLIPVTLQGVFORIDVSKDFONTIAEVELGAPWPFPLE

R6-p : 181 TYLDGDTNITRGDGELEVFVIS 203
TY+D RITRGDGELEVFVI+
HrBP1p: 262 TYVDUTMRITRGDGELEVFVIA 284

At DNA level: Identities = 397/610 (65%) (dots indicate identical bases)

[illegible]

R6 181 ggtgtacagcagcgcgcttctcgttcgaggcgcg-cggcggcagccgcccgcccgccgcac
HrBP1 423 .t.....t.....t.t.t.t.t.t.t.t.t.t.t.t.t.t.t.t.

R6 **301** ggacttcgacaacatcgctcgatgtcggagctcggcgcccatggcgctgcgcgcgggtgga
45 **HrBP1** **543** A...T..T..T.....GCG...G...G...AT...A...C...T.....AT.T.....AT.A...

50 R6 421 attcgacaagacgacgggtgaagacgaaggggaaacctgtcccagctgccgcgctggagt
HFBP1 663 attg.....atc.....tcc.....tc....

[illegible]

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Example 10

5 A Northern blot using an RNA probe of bases 651-855 of the HrBP1 coding region picked up a band with a size similar to HrBP1 in many species such as rice, tobacco, citrus, cotton, grass, pansy, pepper, potato, tomato, wheat, and corn.

	Rice	Yes
	Tobacco	Yes
10	Citrus	Yes
	Cotton	Yes
	Grass	Yes
	Pansy	Yes
	Pepper	Yes
15	Potato	Yes
	Tomato	Yes
	Wheat	Yes
	Corn	Yes
	Geranium	?
20	soy bean	?
	sunflower	?
	strawberry	?

Sequence of HrBP1 fragment used as the Northern probe (SEQ. ID. No. 8):

25 GATCAAGATAACATTTGAGAAAACAACCTGTGAAGACATCGGAAACTTGTGCGAGATTCTCCGTTTGATATCCCG
AGGCTTCCCGACAGTTTCAGACCATCGTCAAAACCTGGAACTGGGGATTTCGAAAGTTACCTATGTTGATGATACCA
TGCGCATAACTCGCGGGGACAGAGGTGAACCTTAGGGTATTCGTCAATTGCTTAA

Example 11

35 A transgenic approach was used for functional analysis of HrBP1. *Arabidopsis* plants were transformed with an HrBP1 anti-sense construct or a construct that generates double stranded RNA of an HrBP1 fragment (sense strand of HrBP1 coding region bases 4-650 + anti-sense strand of HrBP1 coding region bases

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4-500). The double stranded RNA method was found to be more efficient in silencing the HrBP1 gene. In the T1 generation, four lines showed spontaneous HR-mimic lesion. The most severe line was developmentally retarded, looked very sick, and didn't produce seeds. However, this phenotype did not appear in the T2 and T3 generations and, therefore, was not genetically inheritable. T2 and T3 generations of HrBP1 "knockout" *Arabidopsis* plants were more resistant to *Pseudomonas syringae* DC3000 than wild type plants. T2 plants were also more sensitive to harpin protein in an HR assay than wild type plants. Preliminary data also showed that HrBP1 "knockout" lines bolted earlier than wild type plants and were bigger as well. HrBP1 seemed like a negative regulator in *Arabidopsis*.

HrBP1 was over-expressed in tobacco plants under the control of an NOS promoter or a GST promoter. Three high expression lines (2 NOS lines and 1 GST line) were chosen for further studies in the T2 generation. When infiltrated with purified harpin, the transgenic lines developed HR much faster than wild type plants, which is consistent with a previous experiment in which His-tagged HrBP1 increased tobacco's sensitivity to harpin protein. The HrBP1 over-expressing lines were taller than wild type Xanthi NN plants and were also more resistant to TMV as measured by both disease spot size and spot number. Spot size was more significant: the diameter of the spot on transgenic plants was over 30% less than that on the wild type plants.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated protein which serves as a receptor in plants for plant pathogen hypersensitive response elicitors.

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2. A protein according to claim 1, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and *Clavibacter*.

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3. A protein according to claim 2, wherein the plant pathogen is an *Erwinia* pathogen.

4. A protein according to claim 3, wherein the plant pathogen is *Erwinia amylovora*.

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5. A protein according to claim 1, wherein the protein is from a monocot.

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6. A protein according to claim 5, wherein the protein is from rice.

7. A protein according to claim 1, wherein the protein has an amino acid sequence of SEQ. ID. No. 6.

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8. A protein according to claim 1, wherein the protein is from a dicot.

9. A protein according to claim 8, wherein the protein is from *Arabidopsis thaliana*.

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10. A protein according to claim 1, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

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11. A protein according to claim 1, wherein the protein is recombinant.
- 5 12. An isolated nucleic acid molecule encoding a protein according to claim 1.
- 10 13. A nucleic acid molecule according to claim 12, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and *Clavibacter*.
14. A nucleic acid molecule according to claim 13, wherein the plant pathogen is an *Erwinia* pathogen.
- 15 15. A nucleic acid molecule according to claim 14, wherein the plant pathogen is *Erwinia amylovora*.
16. A nucleic acid molecule according to claim 12, wherein the protein is from a monocot.
- 20 17. A nucleic acid molecule according to claim 16, wherein the protein is from rice.
18. A nucleic acid molecule according to claim 12, wherein the protein has an amino acid sequence of SEQ. ID. No. 6.
- 25 19. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C.
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20. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence of SEQ. ID. No. 7.

21. A nucleic acid molecule according to claim 12, wherein the protein is from a dicot.

22. A nucleic acid molecule according to claim 21, wherein the protein is from *Arabidopsis thaliana*.

23. A nucleic acid molecule according to claim 12, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

24. A nucleic acid molecule according to claim 12, wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

25. A nucleic acid molecule according to claim 12, wherein the nucleic acid has a nucleotide sequence of SEQ. ID. No. 2.

26. A nucleic acid according to claim 12, wherein the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

27. A nucleic acid according to claim 12, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 3.

28. An antisense nucleic acid molecule to the nucleic acid according to claim 12.

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29. An expression vector containing a nucleic acid molecule according to claim 12 which is heterologous to the expression vector.

30. An expression vector according to claim 29, wherein the
5 nucleic acid molecule is positioned in the expression vector in sense orientation and correct reading frame.

31. An expression vector according to claim 29, wherein either:
10 (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising
15 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature
20 of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

32. An expression vector containing a nucleic acid molecule according to claim 28 which is heterologous to the expression vector.

25 33. A transgenic host cell transformed with the nucleic acid molecule according to claim 12.

34. A host cell transformed according to claim 33, wherein the host
cell is selected from the group consisting of a plant cell and a bacterial cell.

30 35. A host cell according to claim 33, wherein the DNA molecule is transformed with an expression system.

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36. A host cell according to claim 33, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

37. A host cell transformed with a nucleic acid molecule according to claim 28.

38. A transgenic plant transformed with the DNA molecule of claim 12.

39. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

40. A transgenic plant according to claim 38, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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41. A transgenic plant according to claim 38, wherein the plant is a monocot.

5 42. A transgenic plant according to claim 38, wherein the plant is from a dicot.

43. A transgenic plant according to claim 38, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid
10 hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising
15 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature
20 of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

44. A transgenic plant transformed with a nucleic acid molecule according to claim 28.

25 45. A transgenic plant seed transformed with the DNA molecule of claim 12.

46. A transgenic plant seed according to claim 45, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive,
30 cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber,

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apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

47. A transgenic plant seed according to claim 45, wherein the
5 plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*,
petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

48. A transgenic plant seed according to claim 45, wherein the
10 plant is a monocot.

49. A transgenic plant seed according to claim 45, wherein the
plant is a dicot.

50. A transgenic plant seed according to claim 45, wherein either:
15 (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid
hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent
conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M
SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide
20 sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence
of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising
20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the
nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has
an amino acid sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the
25 nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization
buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature
of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

51. A transgenic plant seed transformed with a nucleic acid
30 molecule according to claim 28.

52. A method of identifying agents targeting plant cells
comprising:

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forming a reaction mixture comprising a protein according to claim 1 and a candidate agent;

evaluating the reaction mixture for binding between the protein and the candidate agent; and

5 identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

53. A method according to claim 52, wherein the protein is from a monocot.

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54. A method according to claim 53, wherein the protein is from rice.

55. A method according to claim 52, wherein the protein has an amino acid sequence of SEQ. ID. No. 6.

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56. A method according to claim 52, wherein the protein is from a dicot.

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57. A method according to claim 56, wherein the protein is from *Arabidopsis thaliana*.

58. A method according to claim 52, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

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59. A method of identifying agents targeting plant cells comprising:

forming a reaction mixture comprising a host cell transformed with a nucleic acid molecule according to claim 12 and a candidate agent;

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evaluating the reaction mixture for binding between protein produced by the host cell and the candidate agent; and

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identifying candidate compounds which bind to the protein
produced by the host cell in the reaction mixture as plant cell targeting agents.

- 5 60. A method according to claim 59, wherein the protein is from a
monocot.
61. A method according to claim 60, wherein the protein is from
rice.
- 10 62. A method according to claim 59, wherein the protein is from a
dicot.
63. A method according to claim 62, wherein the protein is from
Arabidopsis thaliana.
- 15 64. A method according to claim 59, wherein either: (1) the
protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes
to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a
hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at
20 a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ.
ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3
under stringent conditions of a hybridization buffer comprising 20% formamide in
0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid
comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid
25 sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide
sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer
comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of
42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.
- 30 65. A method of enhancing plant receptivity to treatment with
hypersensitive response elicitors comprising:
 providing a transgenic plant or transgenic plant seed
transformed with the nucleic acid molecule according to claim 12.

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5 66. A method according to claim 65, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid
10 comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

15 67. A method according to claim 65, wherein a transgenic plant is provided.

20 68. A method according to claim 65, wherein a transgenic plant seed is provided and said method further comprises:
planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

25 69. A method according to claim 65, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato,
30 sorghum, and sugarcane.

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70. A method according to claim 65, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

5 71. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting disease resistance.

72. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for enhancing plant growth.

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73. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for controlling insects.

15 74. A method according to claim 65, wherein the hypersensitive response elicitor treatment is for imparting stress tolerance.

75. A method according to claim 65, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects the hypersensitive response elicitor treatment.

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76. A method according to claim 65, wherein the hypersensitive response elicitor treatment comprises:
applying a hypersensitive response elicitor to the plant or plant seed.

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77. A method according to claim 76, wherein the hypersensitive response elicitor is applied in isolated form.

30 78. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:

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providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule according to claim 12.

5 79. A method according to claim 78, wherein the protein is from a monocot.

10 80. A method according to claim 79, wherein the protein is from rice.

81. A method according to claim 78, wherein the protein is from a dicot.

15 82. A method according to claim 81, wherein the protein is from *Arabidopsis thaliana*.

20 83. A method according to claim 78, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid
25 comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

30 84. A method according to claim 78, wherein a transgenic plant is provided.

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85. A method according to claim 78, wherein a transgenic plant seed is provided and said method further comprises:

planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

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86. A method according to claim 78, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

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87. A method according to claim 78, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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88. A method according to claim 78, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects a hypersensitive response elicitor treatment.

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89. A method according to claim 78 further comprising:
applying a hypersensitive response elicitor to the plant or plant seed.

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90. A method according to claim 89, wherein the hypersensitive response elicitor is applied in isolated form.

91. A method according to claim 78, wherein disease resistance is imparted to plants.

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92. A method according to claim 78, wherein enhanced growth is imparted to plants.

93. A method according to claim 78, wherein insect control is imparted to plants.

94. A method according to claim 78, wherein stress resistance is imparted to plants.

95. A method according to claim 78, wherein the DNA construct is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors.

96. A method according to claim 78, wherein the DNA construct is transcribable to a first nucleic acid encoding a receptor in plants for plant pathogen hypersensitive response elicitors coupled to a second nucleic acid encoding the inverted complement of the first nucleic acid.

97. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:
providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 12.

98. A method according to claim 97, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. Nos. 2 or 8 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3; (6) the protein has an amino acid

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sequence of SEQ. ID. No. 6; (7) the nucleic acid hybridizes to the nucleotide sequence of SEQ. ID. No. 7 under stringent conditions of hybridization buffer comprising 20% formamide in 0.9 M saline/0.09M SSC buffer at a temperature of 42°C; or (8) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 7.

5

99. A method according to claim 97, wherein a transgenic plant is provided.

100. A method according to claim 97, wherein a transgenic plant seed is provided and said method further comprises:
planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

101. A method according to claim 97, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

102. A method according to claim 97, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saltpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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103. A method according to claim 97, wherein disease resistance is imparted.

104. A method according to claim 97, wherein plant growth is enhanced.

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105. A method according to claim 97, wherein insects are controlled.

5 106. A method according to claim 97, wherein stress tolerance is imparted.

107. A method according to claim 97, wherein the protein is from a monocot.

10 108. A method according to claim 107, wherein the protein is from rice.

109. A method according to claim 97, wherein the protein is from a dicot.

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110. A method according to claim 97, wherein the protein is from *Arabidopsis thaliana*.

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ABSTRACT OF THE DISCLOSURE

The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also
5 disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules. Both the protein and nucleic acid can be used to identify agents targeting plant cells and to enhance a plant's receptivity to treatment with a hypersensitive response elicitor.

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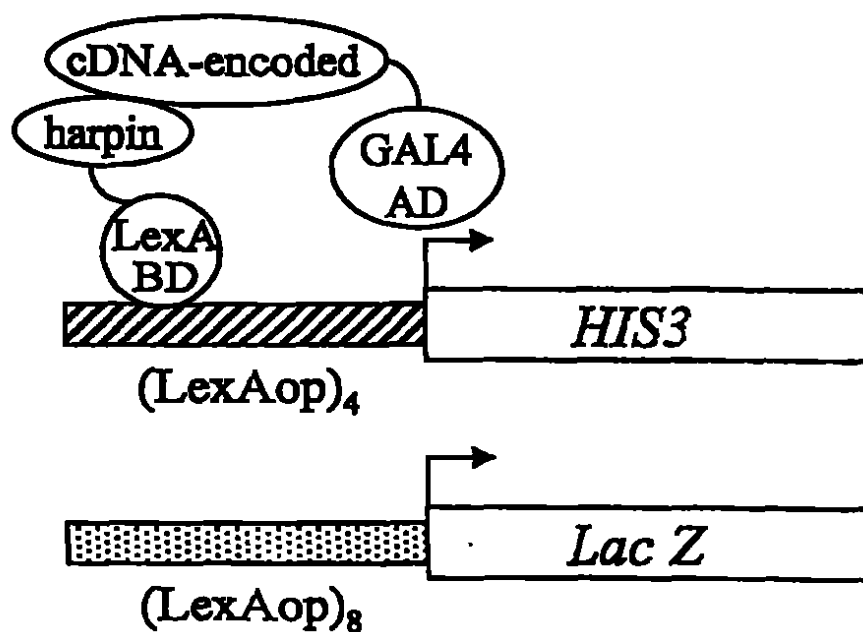
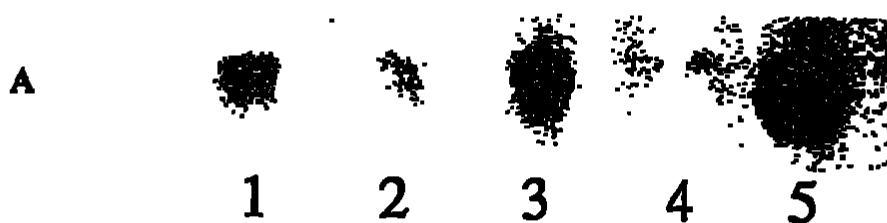


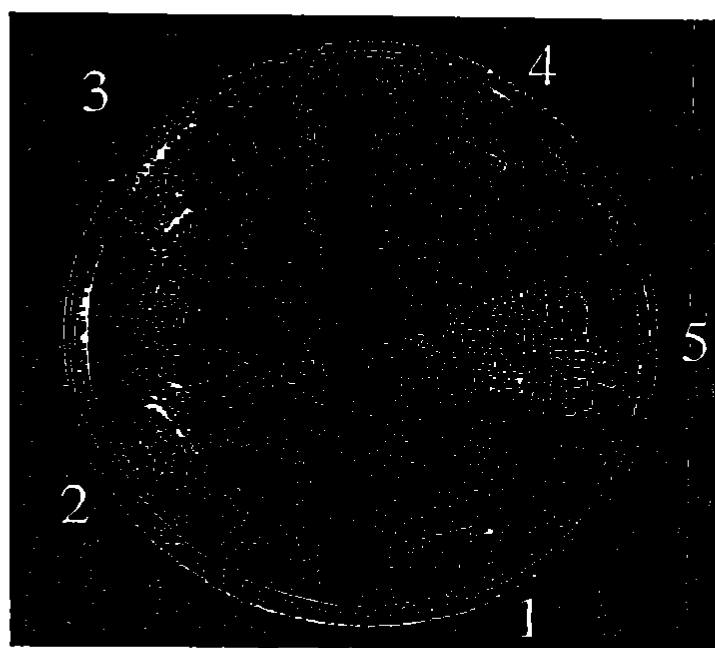
Figure 1

249
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B



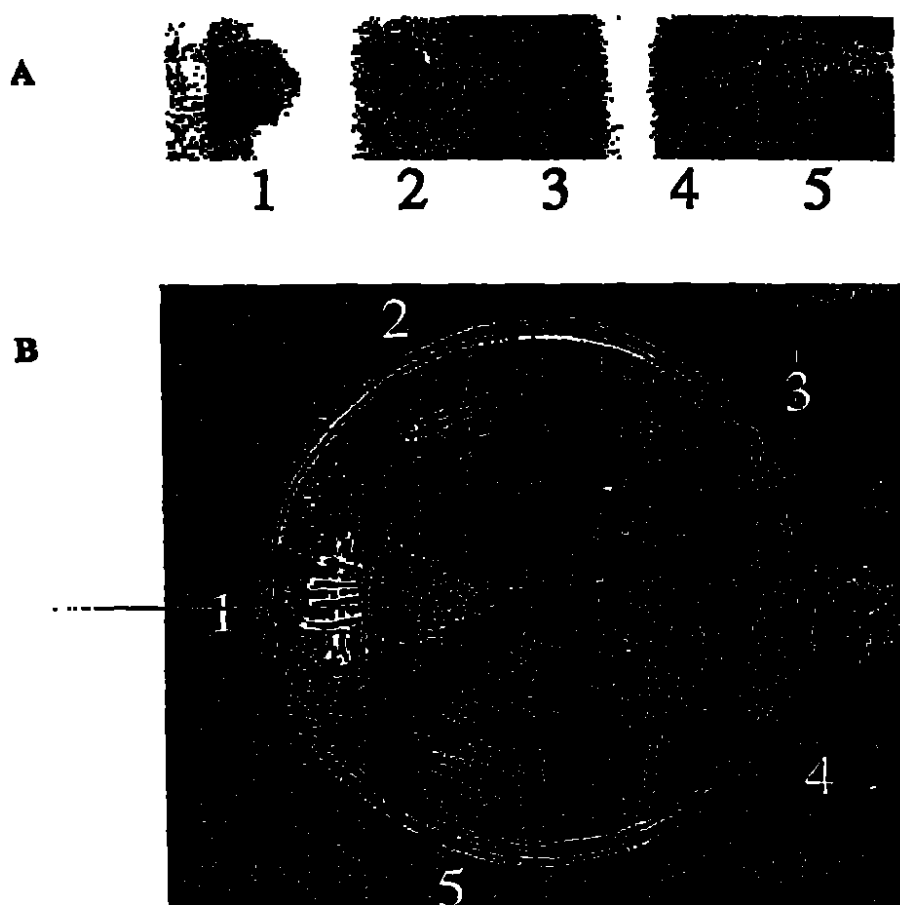
	Bait	Prey
1	harpin	empty vector
2	harpin	unrelated protein
3	empty vector	harpin
4	unrelated protein	harpin
5	(positive control)	

Figure 2

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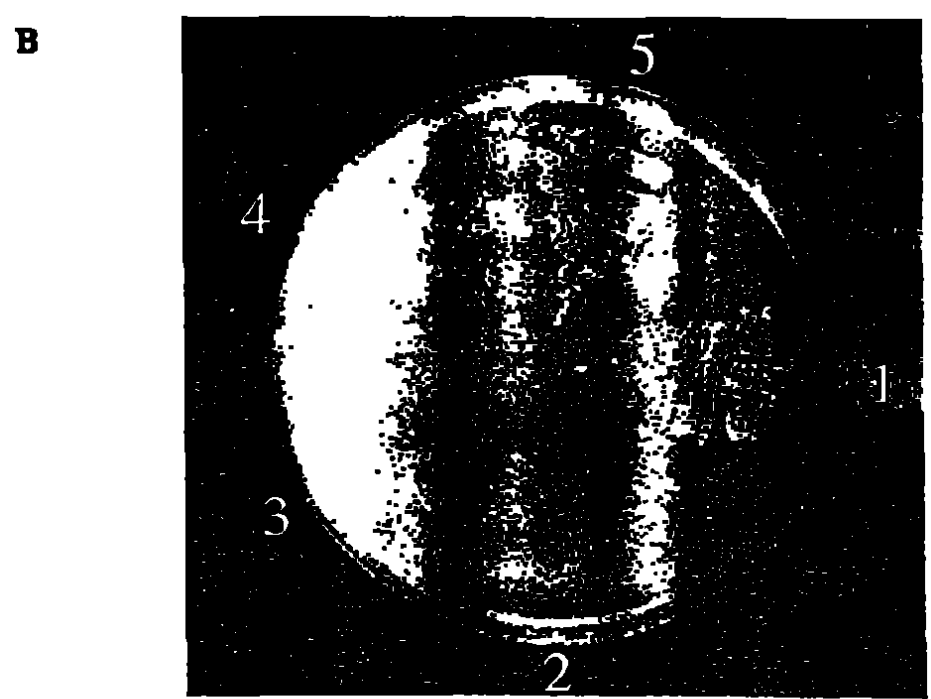
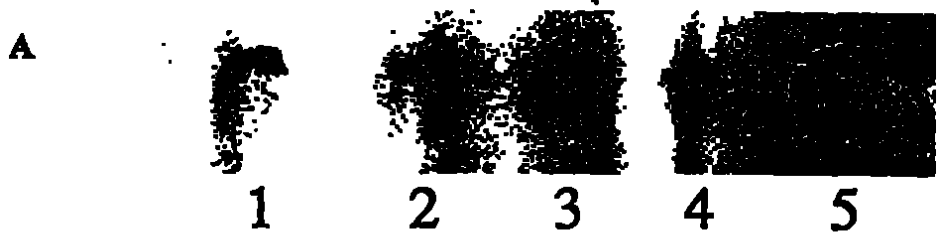
	Bait	Prey
1	harpin	HrBP1
2	unrelated protein 1	HrBP1
3	unrelated protein 2	HrBP1
4	empty vector	HrBP1
5	(positive control)	

Figure 3

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	Bait	Prey
1	HrBP1	harpin
2	HrBP1	empty vector
3	HrBP1	unrelated protein 1
4	HrBP1	unrelated protein2
5	(positive control)	

Figure 4

001021-0140208

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246

02 Jul 1999

Sequence Data

Page 1

Molecule: HrBP1, 1000 bps DNA
Description: cDNA sequence of HrBP1
File Name: fig5.cm5, dated 04 Jun 1999
Printed: 1-1000 bps (Full), format Single Strand

ACT

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61 agctcttctt tccgaccacc gttctctctc accatccatc agatactcct tttctccctt
121 aactactoca aaatcgtctc gtttgggttt caotgtacog gagaagagaa acctcgctgc
181 caattcgtct ctcgttgaag tatccattgg cggagaaggt gaccaccac catcatcatc
241 tggatcagga ggagacgaca agcaaatgac attactcaa ctcaaattac ttagtgtagt
301 ttogggatta aacagaggac ttgtggcgag tgttgatgat ttagaagag ctgaagtggc
361 tgctaaagaa cttgaactg ctgggggacc ggttgattta accgatgac ttgataagct
421 tcaagggaaa tggaggctgt tgtatagtag tgcgttctct tctcgtctt taggtggtag
481 cegtccctgg ctacctactg gacgtttgat cccgtttact ottggcgaag tgtttcaacg
541 gattgatgtg tttagcaaaag attttgataa catagcagag gtggaattag gagcccttg
601 gccatttcog ccattagaag ccactggcac attggcacac aagtttgaa tcttaggcac
661 ttgcaagatc aagataacat ttgagaaaac aactgtgaag acatcgggaa acttgctgca
721 gattcctcog tttgatatcc cgaggcttcc cgacagtttc agaccatcgt caaacctgg
781 aactggggat ttcgaaagta cctatgttga tgataccatg cgcataactc gggggacag
841 aggtgaactt aggttattcg tcattgctta attctcaag ctttgacatg taaagatana
901 taataacttt ctgcttgatg cagtctcatg agttttgtac aaatcatgtg aacatataaa
961 tgcgctttat aagtaaatga gtgtcttgtt caatgaatca
```

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Figure 5

253
247

02 Jul 1999

Sequence Data

Page 1

Molecule: 4+47+14R-p, 284 aas Protein
Description: ORF from 4+47+14R
File Name: fig6.cm5, dated 04 Jun 1999
Printed: 1-284 aas (Full), format Single Strand

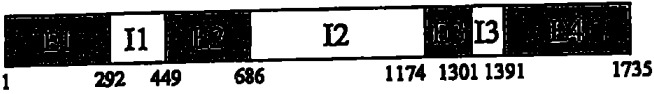
253

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1  mntstfssl lpsppalled hrapppsiry sfsplttks srlgftvpek rmlaansslv
61  evsiggesdp ppssegaggd dkqiallklk llsvvsglnr glvasvddle rasvaakela
121 taggpvdlt dldklqgkwr llyssafssr slggrpglp tgrlipvtlg qvfqridvls
181 kdfdniaeva lgapwfppl eatatlahkf ellgtckiki tfekttvkts galsqippfd
241 iprlpdsfrp smupgtgdfv vtyvddturi trgdrgelrv fvla
```

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Figure 6

Figure 7



Gene structure of HrBP1

E Exon I Intron

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Total RNA
Poly-A⁺ RNA

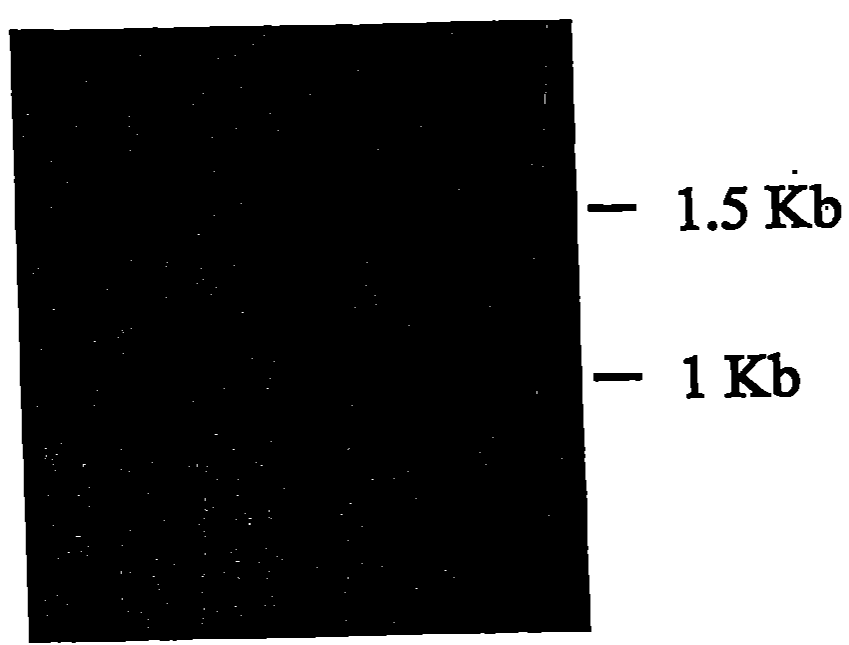


Figure 8

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Esta es una traducción parcial al idioma español de un documento escrito originalmente en el idioma inglés.

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DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

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Oficina de Patentes y Marcas de los Estados Unidos

Abril 19, 2001

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NO. DE SOLICITUD: 60/191,649

FECHA DE PRESENTACION: 23 de Marzo de 2000

25

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OFICINA DE MARCAS Y PATENTES
DE LOS ESTADOS UNIDOS DE

Por la Autoridad del
COMISIONADO DE PATENTES Y MARCAS

[firma ilegible]

30

N. WILLIAMS
Oficial de Certificación

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REIVINDICACIONES:

1. Una proteína aislada la cual sirve como un receptor en plantas para inductores patógenos vegetales de respuesta hipersensible.

2. Una proteína según la reivindicación 1, en donde el patógeno vegetal es seleccionado entre *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, y *Clavibacter*.

3. Una proteína según la reivindicación 2, en donde el patógeno vegetal es un patógeno *Erwinia*.

4. Una proteína según la reivindicación 3, en donde el patógeno vegetal es *Erwinia amylovora*.

5. Una proteína según la reivindicación 1, en donde la proteína proviene de un monocotiledóneo.

6. Una proteína según la reivindicación 1, en donde la proteína proviene de un dicotiledóneo.

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7. Una proteína según la reivindicación 8, en donde la proteína proviene de la *Arabidopsis thaliana*.

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8. Una proteína según la reivindicación 1, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 1.

9. Una proteína según la reivindicación 1, en donde la proteína es recombinante.

10. Una molécula de ácido nucleico aislada que codifica una proteína según la reivindicación 1.

11. Una molécula de ácido nucleico según la reivindicación 10, en donde el patógeno vegetal es seleccionado entre *Erwinia*, *Pseudomonas*, *Xanthamonas*, *Phytophthora*, y *Clavibacter*.

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12. Una molécula de ácido nucleico según la reivindicación 11, en donde el patógeno vegetal es un patógeno *Erwinia*.

13. Una molécula de ácido nucleico según la reivindicación 12, en donde el patógeno vegetal es *Erwinia amylovora*.

14. Una molécula de ácido nucleico según la reivindicación 10, en donde la proteína proviene de un monocotiledóneo.

15. Una molécula de ácido nucleico según la reivindicación 10, en donde la proteína proviene de un dicotiledóneo.

16. Una molécula de ácido nucleico según la reivindicación 15, en donde la proteína proviene de la *Arabidopsis thaliana*.

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17. Una molécula de ácido nucleico según la reivindicación 10, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 1.

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18. Una molécula de ácido nucleico según la reivindicación 10, en donde el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C.

19. Una molécula de ácido nucleico según la reivindicación 10, en donde el ácido nucleico tiene una secuencia nucleótida que comprende la SEQ. ID. No. 2.

20. Una molécula de ácido nucleico según la reivindicación 10, en donde el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C.

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21. Una molécula de ácido nucleico según la reivindicación 10, en donde el ácido nucleico tiene una secuencia nucleótida que comprende la SEQ. ID. No. 3.

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22. Una molécula de ácido nucleico antisentido según la reivindicación 10.

23. Un vector de expresión que contiene una molécula de ácido nucleico según la reivindicación 10 el cual es heterólogo al vector de expresión.

24. Un vector de expresión según la reivindicación 23, en donde la molécula del ácido nucleico es situada en el vector de expresión en orientación con sentido y un correcto marco de lectura.

25. Un vector de expresión según la reivindicación 23, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la

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SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3;

26. Un vector de expresión que contiene una molécula de ácido nucleico según la reivindicación 22 el cual es heterólogo al vector de expresión.

27. Una célula hospedera transgénica transformada con la molécula del ácido nucleico según la reivindicación 10.

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28. Una célula hospedera transformada según la reivindicación 27, en donde la célula hospedera es seleccionada entre una célula vegetal y una célula bacteriana.

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29. Una célula hospedera según la reivindicación 27, en donde la molécula de ADN es transformada con un sistema de expresión.

30. Una célula hospedera según la reivindicación 27, en donde tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de

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salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ.

ID. No. 3.

31. Una célula hospedera transformada con una molécula de ácido nucleico según la reivindicación 22.

32. Una planta transgénica transformada con la molécula de ADN de la reivindicación 10.

33. Una planta transgénica según la reivindicación 32, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

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34. Una planta transgénica según la reivindicación 32, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

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35. Una planta transgénica según la reivindicación 32, en donde la planta es un monocotiledóneo.

36. Una planta transgénica según la reivindicación 32, en donde la planta proviene de un dicotiledóneo.

37. Una planta transgénica según la reivindicación 32, en donde tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo

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condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3.

38. Una planta transgénica transformada con una molécula de ácido nucleico según la reivindicación 22.

39. Una semilla de planta transgénica transformada con la molécula de ADN de la reivindicación 10.

40. Una planta transgénica según la reivindicación 39 en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

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41. Una planta transgénica según la reivindicación 39, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

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42. Una planta transgénica según la reivindicación 39, en donde la planta es un monocotiledóneo.

43. Una planta transgénica según la reivindicación 39, en donde la planta proviene de un dicotiledóneo.

44. Una planta transgénica según la reivindicación 39, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza

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en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3.

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45. Una semilla de planta transgénica transformada con una molécula de ácido nucleico según la reivindicación 22.

46. Un método para identificar agentes que tiene como objetivo células vegetales, el cual comprende:

formar una mezcla de reacción que comprende una proteína según la reivindicación 1 y un agente candidato;

evaluar la mezcla de reacción para unión entre la proteína y el agente candidato; y

identificar compuestos candidatos los cuales se unen a la proteína en la mezcla de reacción como agentes objetivo de células vegetales.

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47. Un método según la reivindicación 46, en donde la proteína proviene de un monocotiledóneo.

48. Un método según la reivindicación 46, en donde la proteína proviene de un dicotiledóneo.

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49. Un método según la reivindicación 48, en donde la proteína proviene de la *Arabidopsis thaliana*.

50. Un método según la reivindicación 49, en donde la proteína tiene una secuencia de aminoácidos parcial de la SEQ. ID. No. 1.

51. Un método para identificar agentes que tiene como objetivo células vegetales, el cual comprende:

formar una mezcla de reacción que comprende una célula hospedera transformada con una molécula de ácido nucleico según la reivindicación 10 y un agente candidato;

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evaluar la mezcla de reacción para unión entre la proteína producida por la célula hospedera y el agente candidato; y

identificar compuestos candidatos los cuales se unen a la proteína producida por la célula hospedera en la mezcla de reacción como agentes objetivo de células vegetales.

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52. Un método según la reivindicación 51, en donde la proteína proviene de un monocotiledóneo.

53. Un método según la reivindicación 51, en donde la proteína proviene de un dicotiledóneo.

54. Un método según la reivindicación 53, en donde la proteína proviene de la *Arabidopsis thaliana*.

55. Un método según la reivindicación 51, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo

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condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3.

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56. Un método para mejorar la receptividad de la planta al tratamiento con inductores de la respuesta hipersensible, el cual comprende:

proporcionar una planta transgénica o una semilla de planta transgénica transformada con la molécula de ácido nucleico según la reivindicación 10.

57. Un método según la reivindicación 56, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos

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de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 3.

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58. Un método según la reivindicación 56, en donde una planta transgénica es proporcionada.

59. Un método según la reivindicación 56, en donde una semilla de planta transgénica es proporcionada y dicho método además comprende:

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sembrar las semillas de las plantas bajo condiciones que sean efectivas para que las plantas crezcan a partir de las semillas sembradas.

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60. Un método según la reivindicación 56, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana, pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

61. Un método según la reivindicación 56, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

62. Un método según la reivindicación 56, en donde el tratamiento con inductores de la respuesta

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hipersensible es para impartir resistencia a la enfermedad.

63. Un método según la reivindicación 56, en donde el tratamiento con inductores de la respuesta hipersensible es para mejorar el crecimiento de las plantas.

64. Un método según la reivindicación 56, en donde el tratamiento con inductores de la respuesta hipersensible es para controlar insectos.

65. Un método según la reivindicación 56, en donde el tratamiento con inductores de la respuesta hipersensible es para impartir tolerancia al estrés.

66. Un método según la reivindicación 56, en donde la planta transgénica o semilla de planta es adicionalmente transformada con un segundo ácido nucleico que codifica un inductor de la respuesta hipersensible, en donde la expresión del segundo ácido nucleico surge

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efecto sobre el tratamiento con inductores de la respuesta hipersensible.

67. Un método según la reivindicación 56, en donde el tratamiento con inductores de la respuesta hipersensible comprende:

aplicar un inductor de la respuesta hipersensible a la planta o semilla de la planta.

68. Un método según la reivindicación 67, en donde el inductor de la respuesta hipersensible es aplicado de manera aislada.

69. Un método para impartir resistencia a la enfermedad, mejorar el crecimiento, controlar insectos, y/o impartir resistencia al estrés, el cual comprende:

proporcionar una planta transgénica o semilla de planta transgénica transformada con una construcción de ADN efectiva para silenciar la expresión de una molécula de ácido nucleico según la reivindicación 10.

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70. Un método según la reivindicación 69, en donde la proteína proviene de un monocotiledóneo.

71. Un método según la reivindicación 69, en donde la proteína proviene de un dicotiledóneo.

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72. Un método según la reivindicación 71, en donde la proteína proviene de la *Arabidopsis thaliana*.

73. Un método según la reivindicación 69, en donde, tanto: (1) la proteína tiene una secuencia de aminoácidos de la SEQ. ID. No. 1; (2) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 2 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de salina/0.09M SSC a una temperatura de 42°C; (3) el ácido nucleico comprende una secuencia nucleótida de la SEQ. ID. No. 2; (4) el ácido nucleico se hibridiza en la secuencia nucleótida de la SEQ. ID. No. 3 bajo condiciones rigurosas de amortiguador de hibridización que comprende 20% de formamida en buffer de 0.9M de

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salina/0.09M SSC a una temperatura de 42°C; (5) el ácido nucleico comprende una secuencia nucleótida de la SEQ.

ID. No. 3.

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74. Un método según la reivindicación 69, en donde la planta transgénica es proporcionada.

75. Un método según la reivindicación 69, en donde una semilla de planta transgénica es proporcionada y dicho método además comprende:

sembrar las semillas de las plantas bajo condiciones que sean efectivas para que las plantas crezcan a partir de las semillas sembradas.

76. Un método según la reivindicación 69, en donde la planta es seleccionada entre alfalfa, arroz, trigo, cebada, centeno, algodón, girasol, maní, maíz, papa, batata, achicoria, lechuga, escarola, repollo, col de Bruselas, remolacha, chiviría, coliflor, brócoli, nabo, rábano, espinaca, cebolla, ajo, berenjena, pimienta, apio, zanahoria, calabaza, calabacín, pepino, manzana,

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pera, melón, cítrico, fresa, uva, frambuesa, piña, soja, tabaco, tomate, sorgo, y caña de azúcar.

77. Un método según la reivindicación 69, en donde la planta es seleccionada entre *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonio, flor de fuego, crisantemo, clavel, y cinnia.

78. Un método según la reivindicación 69, en donde la planta transgénica o semilla de planta es adicionalmente transformada con un segundo ácido nucleico que codifica un inductor de la respuesta hipersensible, en donde la expresión del segundo ácido nucleico surte efecto sobre el tratamiento con inductores de la respuesta hipersensible.

79. Un método según la reivindicación 69 que además comprende:

aplicar un inductor de la respuesta hipersensible a una planta o semilla de planta.

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80. Un método según la reivindicación 79 en donde el inductor de la respuesta hipersensible es aplicado de manera aislada.

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81. Un método según la reivindicación 69, en donde resistencia a la enfermedad es impartida a las plantas.

82. Un método según la reivindicación 69, en donde un crecimiento mejorado de las plantas es impartido a las mismas.

83. Un método según la reivindicación 69, en donde un controlar insectos es impartido a las plantas.

84. Un método según la reivindicación 69, en donde una tolerancia al estrés es impartida.

85. Un método según la reivindicación 69, en donde la construcción de ADN es una molécula de ácido nucleico antisentido a una molécula de ácido nucleico que codifica

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un receptor en plantas para inductores de la respuesta hipersensible de patógenos vegetales.

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86. Un método según la reivindicación 69, en donde la construcción de ADN se puede transcribir a un primer ácido nucleico que codifica un receptor en plantas para inductores de la respuesta hipersensible de patógenos vegetales acoplados a un segundo ácido nucleico que codifica el complemento invertido del primer ácido nucleico.

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THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

April 19, 2001

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/191,649

FILING DATE: March 23, 2000



By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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N. WILLIAMS

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JCS53 U.S. PTO

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
REQUEST FORM FOR A PROVISIONAL APPLICATION
FOR PATENT UNDER 37 CFR 1.53(e)

Docket No.: 21829/60 (EBC-004)

[+]

Assistant Commissioner for Patents
Washington, D.C. 20231
Box: Provisional Patent Application

This is a Request for filing a PROVISIONAL APPLICATION FOR PATENT under
37 CFR 1.53(e), entitled RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS
AND USES THEREOF by the following named inventors:

Full name of first inventor: Xiaoling Song
Residence: 14110 NE 179th Street, Apt. 75, Woodinville, Washington 98072
Citizenship: People's Republic of China
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Citizenship: People's Republic of China
Post Office Address: same

Full name of third inventor: Zhong-Min Wei
Residence: 8230 125th Court, Kirkland, Washington 98034
Citizenship: People's Republic of China
Post Office Address: same

Enclosed are:

- ☒ Specification, claims and abstract (49 pages)
- ☒ Drawings (8 sheets)
- ☐ Verified statement claiming small entity status
- ☐ Combined Declaration and Power of Attorney (pages)
- ☒ A self-addressed, prepaid postcard for acknowledging receipt.
- ☐ Other: [list other enclosures]
- ☒ A check in the amount of \$75.00 to cover the provisional filing fee is enclosed.

The invention was made by an agency of the United States Government or under a contract
with an agency of the United States Government:

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

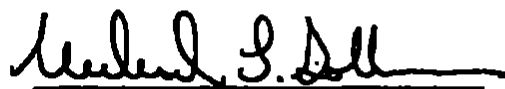
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Date: March 23, 2000



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EXPRESS MAIL CERTIFICATE

DOCKET NO.: 21829/60 (EBC-004)
APPLICANTS: Xiaoling Song, Hao Fan, and Zhong-Min Wei
TITLE: RECEPTORS FOR HYPERSENSITIVE RESPONSE
ELICITORS AND USES THEREOF

Certificate is attached to the Request Form for a Provisional Patent Application for Patent Under 37 CFR 1.53(b)(2) Transmittal Form (2 pages) of the above-identified application.

"EXPRESS MAIL" NUMBER: EL434484431US
DATE OF DEPOSIT: March 23, 2000

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Assistant Commissioner for Patents, Washington, D.C. 20231, Box: Provisional Patent Application.

Jo Ann Whalen
(Typed or Printed Name of Person Mailing
Paper of Fee)

Jo Ann Whalen
(Signature of Person Mailing Paper of Fee)

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EXPRESS MAIL CERTIFICATE

DOCKET NO.: 21829/60 (EBC-004)
APPLICANTS: Xiaoling Song, Hao Fan, and Zhong-Min Wei
TITLE: RECEPTORS FOR HYPERSENSITIVE RESPONSE
ELICITORS AND USES THEREOF

Certificate is attached to the Provisional Patent Application (49 pages) and
Sequence Listing (5 pages) of the above-identified application.

"EXPRESS MAIL" NUMBER: EL434484431US
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Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the
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Jo Ann Whalen
(Typed or Printed Name of Person Mailing
Paper of Fee)

Jo Ann Whalen
(Signature of Person Mailing Paper of Fee)

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EXPRESS MAIL CERTIFICATE

DOCKET NO.: 21829/60 (EBC-004)
APPLICANTS: Xiaoling Song, Hao Fan, and Zhong-Min Wei
TITLE: RECEPTORS FOR HYPERSENSITIVE RESPONSE
ELICITORS AND USES THEREOF

Certificate is attached to the Informal Drawings (8 pages) of the above-identified application.

"EXPRESS MAIL" NUMBER: EL434484431US
DATE OF DEPOSIT: March 23, 2000

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In Ann Whalen
(Typed or Printed Name of Person Mailing
Paper of Fee)

In Ann Whalen
(Signature of Person Mailing Paper of Fee)

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TITLE: **RECEPTORS FOR HYPERSENSITIVE RESPONSE
ELICITORS AND USES THEREOF**

INVENTORS: **Xiaoling Song, Hao Fan, and Zhong-Min Wei**

DOCKET NO.: **21829/60 (EBC-004)**

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PROVISIONAL APPLICATION

1352993.1

RECEPTORS FOR HYPERSENSITIVE RESPONSE ELICITORS
AND USES THEREOF

FIELD OF THE INVENTION

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The present invention relates to receptors for hypersensitive response elicitors and uses thereof.

BACKGROUND OF THE INVENTION

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Plants have evolved a complex array of biochemical pathways that enable them to recognize and respond to environmental signals, including pathogen infection. There are two major types of interactions between a pathogen and plant — compatible and incompatible. When a pathogen and a plant are compatible, disease generally occurs. If a pathogen and a plant are incompatible, the plant is usually resistant to that particular pathogen. In an incompatible interaction, a plant will restrict pathogen proliferation by causing localized necrosis, or death of tissues, to a small zone surrounding the site of infection. This reaction by the plant is defined as the hypersensitive response ("HR") (Kiraly, Z. "Defenses Triggered by the Invader: Hypersensitivity," Plant Disease: An Advanced Treatise 5:201-224 J. G. Horsfall and E. B. Cowling, eds. Academic Press, New York (1980); (Klement "Hypersensitivity," Phytopathogenic Prokaryotes 2:149-177, M.S. Mount and G. H. Lacy, eds. Academic Press, New York (1982)). The localized cell death not only contains the infecting pathogen from spreading further but also leads to a systemic resistance preventing subsequent infections by other pathogens. Therefore, HR is a common form of plant resistance to diseases caused by bacteria, fungi, nematodes, and viruses.

A set of genes designated as *hrp* (Hypersensitive Response and Pathogenicity) is responsible for the elicitation of the HR by pathogenic bacteria including *Erwinia* spp, *Pseudomonas* spp, *Xanthomonas* spp, and *Ralstonia solanacearum* (Willis et al. "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991), Bonas, U. "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current topics in Microbiology and Immunology, Vol. 192, Bacterial Pathogenesis of Plants and Animals: Molecular and Cellular Mechanisms. J. L. Dangl, ed. Springer-Verlag, Berlin (1994); Alfano et al., "Bacterial Pathogens in

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- Plants: Life Up Against the Wall," *Plant Cell* 8:1683-98 (1996). Typically, there are multiple *hrp* genes clustered in a 30-40 kb DNA. Mutation in any one of the *hrp* genes will result in the loss of bacterial pathogenicity in host plants and the HR in non-host plants. On the basis of genetic and biochemical characterization, the
- 5 function of the *hrp* genes can be classified into three groups: 1) structural genes encoding extracellularly located HR elicitors, for example harpin of *Erwinia amylovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992)); 2) secretion genes encoding a secretory apparatus for exporting HR elicitors and other proteins from the
- 10 bacterial cytoplasm to the cell surface or extracellular space (Van Gijsegem et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," *Trends Microbiol.* 1:175-180 (1993); He et al., "*Pseudomonas syringae* pv. *Syringae* harpin_{ps}: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255 (1993); Wei et al., "HrpI
- 15 of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," *J. Bacteriol.* 175:7985-67 (1993), Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific *Petunia* Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J.* 13:543-53 (1994), Galan et al., "Cross-talk between Bacterial Pathogens and their Host Cells,"
- 20 *Ann. Rev. Cell Dev. Biol.* 12:221-55 (1996); Bogdanove et al., "*Erwinia amylovora* Secretes Harpin via a Type III Pathway and Contains a Homolog of yopN of *Yersinia*," *J. Bacteriol.* 178:1720-30 (1996); Bogdanove et al., "Homology and Functional Similarity of a *hrp*-linked Pathogenicity Operon, *dspEF*, of *Erwinia amylovora* and the *avrE* locus of *Pseudomonas syringae* pathovar tomato," *Proc Natl*
- 25 *Acad Sci USA* 95:1325-30 (1998)); and 3) regulatory genes that control the expression of *hrp* genes (Wei, Z. M., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992); Wei et al., "*hrpL* Activates *Erwinia amylovora hrp* Genes in Response to Environmental Stimuli," *J. Bacteriol.* 174:1875-82 (1995); Xiao et al., "A Single
- 30 Promoter Sequence Recognized by a Newly Identified Alternate Sigma Factor Directs Expression of Pathogenicity and Host Range Determinants in *Pseudomonas syringae*," *J. Bacteriol.* 176:3089-91 (1994); Kim et al., "The *hrpA* and *hrpC* Operons

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of *Erwinia amylovora* Encode Components of a Type III Pathway that Secretes Harpin," *J. Bacteriol.* 179:1690-97 (1997); Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," *J. Bacteriol.* 180:5203-10 (1998); Wengelnik et al., "HrpG, A Key hrp
5 Regulatory Protein of *Xanthomonas campestris* pv. *Vesicatoria* is Homologous to Two Component Response Regulators," *Mol. Plant-Microbe Interact.* 9:704-12 (1996)). Because of their role in interactions between plants and microbes, *hrp* genes have been a focus for bacterial pathogenicity and plant defense studies.

In addition to the local defense response, HR also activates the defense
10 system in uninfected parts of the same plant. This results in a general systemic resistance to a secondary infection termed Systemic Acquired Resistance ("SAR") (Ross, R. F. "Systemic Acquired Resistance Induced by Localized Virus Infections in Plants," *Virology* 14:340-58 (1961); Malamy et al., "Salicylic Acid and Plant Disease Resistance," *Plant J.* 2:643-654 (1990)). SAR confers long-lasting systemic disease
15 resistance against a broad spectrum of pathogens and is associated with the expression of a certain set of genes (Ward et al. "Coordinate Gene Activity in Response to Agents that Induce Systemic Acquired Resistance," *Plant Cell* 3:1085-94 (1991)). SAR is an important component of the disease resistance of plants and has long been of interest, because the potential of inducing the plant to protect itself could
20 significantly reduce or eliminate the need for chemical pesticides. SAR can be induced by biotic (microbes) and abiotic (chemical) agents (Gorlach et al. "Benzothiadiazole, a Novel Class of Inducers of Systemic Acquired Resistance, Activates Gene Expression and Disease Resistance in Wheat," *Plant Cell* 8:629-43 (1996)). Historically, weak virulent pathogens were used as a biotic inducing agent
25 for SAR. Non-virulent plant growth promotion bacteria (PGPR) were also reported to be able to induce resistance of some plants against various diseases. Biotic agent-induced SAR has been the subject of much research, especially in the late 70s and early 80s. Only very limited success was achieved, however, due to: 1) inconsistency of the performance of living organisms in different environmental conditions;
30 2) considerable concerns regarding the unpredictable consequences of the intentional introduction of weakly virulent pathogens into the environment; and 3) the technical complication of applying a living microorganism into a variety of environmental

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conditions. To overcome the limitations of using living organisms to induce SAR, scientists have long been looking for an HR elicitor derived from a pathogen for SAR induction. With the advancement of molecular biology, the first proteinaceous HR elicitor with broad host spectrum was isolated in 1992 from *Erwinia amylovora*, a pathogenic bacterium causing fire blight in apple and pear. The HR elicitor was named "harpin". It consists of 403 amino acids with a molecular weight about 40 kDa. The harpin protein is heat-stable and glycine-rich with no cysteine. The gene encoding the harpin protein is contained in a 1.3 kB DNA fragment located in the middle of the *hrp* gene cluster. Harpin is secreted into the extracellular space and is very sensitive to proteinase digestion. Since the first harpin was isolated from *Erwinia amylovora*, several harpin or harpin-like proteins have been isolated from other major groups of plant pathogenic bacteria. In addition to the harpin of *Erwinia amylovora*, the following harpin or harpin-like proteins have been isolated and characterized: HrpN of *Erwinia chrysanthemi*, *Erwinia carotovora* (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science*, 257:85 (1992)), and *Erwinia stewartii*; HrpZ of *Pseudomonas syringae* (He et al, "Pseudomonas syringae pv. Syringae harpin_{ms}: A Protein that is Secreted Via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255 (1993)), PopA of *Ralstonia solanacearum*, (Arlat et al. "PopA1, a Protein which Induces a Hypersensitive-Like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J.* 13:543-53 (1994)); HrpW of *Erwinia amylovora* (Kim et al., "HrpW of *Erwinia amylovora*, a New Harpin that Contains a Domain Homologous to Pectate Lyases of a Distinct Class," *J. Bacteriol.* 180:5203-10 (1998)), and *Pseudomonas syringae*. All of the currently described harpin or harpin-like proteins share common characteristics. They are heat-stable and glycine-rich proteins with no cysteine amino acid residue, are very sensitive to digestion by proteinases, and elicit the HR and induce resistance in many plants against many diseases. Based on their shared biochemical and biophysical characteristics as well as biological functions, these HR elicitors from different pathogenic bacteria belong to a new protein family -- i.e. the harpin protein family. The described characteristics, especially their ability to induce HR in a broad range of plants, distinguish the harpin protein family from other host specific

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proteinaceous HR elicitors, for example elicitors from *Phytophthora* spp (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," *Eur. J. Plant Path.* 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," *Plant Physiol* 110:365-76 (1996)) or avirulence proteins (such as Avr9) from *Cladosporium fulvum*, which are only able to elicit the HR in a specific variety or species of a plant.

In nature, when certain bacterial infections occur, harpin protein is expressed and then secreted by the bacteria, signaling the plant to mount a defense against the infection. Harpin serves as a signal to activate plant defense and other physiological systems, which include SAR, growth enhancement, and resistance to certain insect damage.

The current understanding of critical plant molecules that may have a significant role in interacting with elicitors and then triggering a sequential signal transduction cascade is described as follows.

Interaction of Plant Resistance Genes (R) and Pathogen Avirulence Genes (avr)

The concept of gene-for-gene interaction is that "for each gene determining resistance (R gene) in the host, there is a corresponding gene determining avirulence in the pathogen (avr gene)". In this model, pathogen avirulence genes generate a specific ligand molecule, called an elicitor. Only plants carrying the matching resistance gene respond to this elicitor and invoke the HR. In the past few years, several disease-resistance, R genes, have been cloned and sequenced. It was expected that R genes might encode components involved in signal recognition or signal transduction pathways that ultimately lead to defense responses. The cloned R genes could be grouped into four classes: (1) cytoplasmic protein kinase; (2) protein kinases with an extracellular domain; (3) cytoplasmic proteins with a region of leucine-rich repeats and a nucleotide-binding site; and (4) proteins with a region of leucine-rich repeats that appear to encode extracellular proteins. (Review in Bent, A.F. "Plant Disease Resistance Genes: Function Meets Structure," *Plant Cell* 8:1757-71 (1996); Baker B., et al., "Signaling in Plant-Microbe Interactions," *Science* 276:726-33 (1997)). The first R gene cloned, Pto, encodes a serine/threonine protein

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kinase. The protein product of Pto directly interacts with the cognate avirulence gene protein, AvrPro, which has been demonstrated in a yeast two-hybrid system. It was shown that only co-existence of both AvrPro and Pto proteins could elicit HR in plants (Tang et al., "Initiation of Plant Disease Resistance by Physical Interaction of AvrPto and Pto kinase," *Science* 274:2060-63 (1996); Scofield et al., "Molecular Basis of Gene-for-Gene Specificity in Bacterial Speck Disease of Tomato," *Science* 274:2063-65 (1996); Zhou et al., "The Pto kinase Conferring Resistance to Tomato Bacterial Speck Disease Interacts with Proteins that Bind a cis-element of Pathogenesis-related Genes," *EMBO J.* 16:3207-18 (1997)). The results from cloned R genes support the view that plant-pathogen interactions involve protein-protein interactions. Syringolide, a water-soluble, low-molecular-weight elicitor, triggers a defense response in soybean cultivars carrying the Rpg4 disease-resistance gene. A 34-KDa protein has been isolated from soybean and is considered to be the physiological active syringolide receptor (Ji et al., "Characterization of a 34-kDa Soybean Binding Protein for the syringolide Elicitors," *Proc. Natl. Acad. Sci. USA* 95:3306-11 (1998)).

Putative Binding Factor of Elicitin

Elicitins are a family of small proteins secreted by *Phytophthora* species that have a high degree of homology. Pure elicins alone can cause a hypersensitive response, a local cell death, and trigger systemic acquired resistance in tobacco and other plants (Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," *Ann. J. Plant Path.* 102:181-92 (1996); Keller, et al. "Physiological and Molecular Characteristics of Elicitin-Induced Systemic Acquired Resistance in Tobacco," *Plant Physiol* 110:365-76 (1996)). However, the spectrum of HR elicitation and induced systemic resistance in plants is much narrower than that achieved by harpin family elicitors. Like harpin, elicins induce a series of metabolic events in tobacco cells, including the accumulation of phytoalexins, ethylene production, transmembrane electrolyte leakage, H₂O₂ accumulation, and expression of plant defense related genes (Yu L, et al., "Elicitins from *Phytophthora* and Basic Resistance in Tobacco," *Proc. Natl.* (1995); Keller et al., "Pathogen-Induced Elicitin Production in Transgenic Tobacco Generates a Hypersensitive Response and

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Nonspecific Disease Resistance," The Plant Cell 11:223-35 (1999)). A putative receptor-like binding factor has been identified in tobacco plasma membrane, which has a specific high-affinity to the cryptogin, one member of the elicitin family (Wendehenne, et al., "Evidence for Specific, High-Affinity Binding Sites for a Proteinaceous Elicitor in Tobacco Plasma Membrane," FEBS Letters 374:203-207 (1995)). Recently, it was found that 2 basic elicitins (i.e. cryptogin and cinnamomin) and two acidic elicitins (i.e. capsicein and parasiticein) were able to interact with the same binding sites on tobacco plasma membranes (Bourque et al., "Comparison of Binding Properties and Early Biological Effects of Elicitins in Tobacco Cells," Plant Physiol. 118:1317-26 (1998)). However, the gene of the receptor-like factor has not been isolated.

Putative Binding Factor of Glycoprotein Elicitors

A 42 kDa glycoprotein elicitor has been isolated from *Phytophthora megasperma* (Parker et al., "An Extracellular Glycoprotein from *Phytophthora megasperma* f. sp. *glycinea* Elicits Phytoalexin Synthesis in Cultured Parsley Cells and Protoplasts," Mol. Plant Microbe Interact. 4:19-27 (1991)). An oligopeptide of 13 amino acids within the glycoprotein ("Pep-13") was able to induce a response in plants like that achieved by the full glycoprotein. A high affinity-binding pattern has been observed in parsley microsomal membranes with an isotope labeled oligopeptide. There are estimated to be about 1600 to 2900 binding sites per cell with evidence indicating that a low abundant protein receptor of the Pep-13 is localized in the plasma membrane (Numberger et al., "High Affinity Binding of a Fungal Oligopeptide Elicitor to Parsley Plasma Membranes Triggers Multiple Defense Responses," Cell 78:449-60 (1994)).

Harpin Protein Binding Factors

Harpin proteins, which elicit HR in a variety of different nonhost plants, have been isolated from plant pathogens (Wei et al. "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"

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257:85 (1992)). A family of harpin proteins has been identified from plant pathogens. All of them have similar biological activities. It is well known that harpin protein can induce plants to produce active oxygen, change gene expression, lead to local cell death, and induce systemic acquired resistance ("SAR") (He et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992); He et al., "*Pseudomonas syringae* Harpin_{psa}: A Protein that is Secreted via the Hrp Pathway and Elicits a Hypersensitive Response in Plants," *Cell* 73:1255-66 (1993); Baker, C.J., "Harpin, an Elicitor of the Hypersensitive Response in Tobacco Caused by *Erwinia amylovora*, Elicits Active Oxygen Production in Suspension Cells," *Plant Cell* 5:1341-44 (1993)). No harpin protein binding factor has been isolated so far. It is reported that an amphipathic protein, named HRAP, isolated from sweet pepper, could dissociate harpin_{psa} in multimeric form (*hrpZ* from *Pseudomonas syringae*). The biological activity of the HRAP is believed to be its ability to intensify harpin_{psa}-mediated hypersensitive response. HRAP protein does not bind to harpin_{psa} (He et al., "An Amphipathic Protein from Sweet Pepper can Dissociate Harpin_{psa} Multimeric Forms and Intensify the Harpin_{psa}-Mediated Hypersensitive Response," *Physiological & Molecular Pathology* 52:139-49 (1998)). Using a harpin_{psa}-tagged antibody to harpin to examine the interaction of harpin_{psa} and suspension cells, it was found that harpin_{psa} interacted with the cultured cells, but not with protoplasts with the cell walls being digested and removed. It was concluded that harpin_{psa} was localized in the outer portion of the plant cell, probably on the cell membrane. However, it was not ruled out that the binding factor was located on the cell membrane.

The present invention seeks to identify receptors for hypersensitive response elicitor proteins or polypeptides and uses of such receptors.

SUMMARY OF THE INVENTION

The present invention is directed to an isolated protein which serves as a receptor for harpin in plants for a plant pathogen hypersensitive response elicitor. Also included are nucleic acid molecules encoding such receptors as well as expression

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vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules.

5 The protein of the present invention can be used in conjunction with a method of identifying agents targeting plant cells by forming a reaction mixture including the protein and a candidate agent, evaluating the reaction mixture for binding between the protein and the candidate agent, and identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

10 The nucleic acid molecule of the present invention can be used in a method of identifying agents targeting plant cells by forming a reaction mixture including a cell transformed with the nucleic acid molecule of the present invention and a candidate agent, evaluating the reaction mixture for binding between protein produced by the host cell and candidate agent, and identifying candidate compounds which bind to the protein or the host cell in the reaction mixture as plant cell targeting agents.

15 Another aspect of the present invention relates to a method of enhancing a plant's receptivity to treatment with hypersensitive response elicitors by providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule of the present invention.

20 The present invention is also directed to a method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants by providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule encoding a receptor in accordance with the present invention.

25 The discovery of the present invention has great significance. This putative receptor protein can be used as a novel way to screen for new inducers of plant resistance against insect, disease, and stress, and of growth enhancement. This protein is the first step toward the understanding of the harpin induced signal transduction pathway in plants. Further studies of this pathway will provide more possible targets for new plant vaccine and growth enhancement products development. In addition, this protein can serve as an anchor providing a new way to target anything to the plant cells.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a yeast two-hybrid screening with the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) and a schematic representation of the interaction between harpin and a cDNA encoded protein. Harpin is fused to LexA protein which contains a DNA binding domain ("BD"). The cDNA encoded protein is fused to the GAL4 transcription activation domain ("AD"). This interaction targets the activation domain to two different LexA-dependent promoters with consequent activation of the transcription of the *HIS3* and *lacZ* reporter genes.

Figures 2A-B show that the *Erwinia amylovora* hypersensitive response elicitor (i.e. harpin) is a good yeast two-hybrid bait. Reporter genes were not expressed in yeast strain L40 containing plasmids expressing the LexA - harpin fusion in combination with plasmids expressing GAL4 activation domain alone, or fused to unrelated protein. Therefore, harpin is not autoactive in this yeast two-hybrid system. In addition, reporter genes were not expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-harpin fusion in combination with plasmids expressing LexA alone, or fused to unrelated protein. Figure 2A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 2B shows a synthetic minimal ("SD") media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figures 3A-B show the interaction between HrBP1 (hypersensitive response elicitor binding protein 1) and a hypersensitive response elicitor (i.e. harpin) is specific. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the GAL4 activation domain-HrBP1 fusion in combination with plasmids expressing LexA fused to hypersensitive response elicitor (i.e. harpin), but were not expressed in combination with LexA alone, or LexA fused to unrelated proteins. Figure 3A is a β -galactosidase assay where the blue color indicates the expression of *lacZ* reporter gene. Figure 3B is an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

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Figures 4A-B show the interaction of HrBP1 and a hypersensitive response elicitor (i.e. harpin) in another orientation. Reporter genes were expressed in yeast strain L40 containing plasmids expressing the LexA - HrBP1 fusion in combination with plasmids expressing GAL4 activation domain fused to harpin, but were not expressed in combination with GAL4 activation domain alone, or GAL4 activation domain fused to unrelated proteins. Therefore, interaction between harpin and HrBP1 is specific. Figure 4A shows a β -galactosidase assay where blue color indicates the expression of *lacZ* reporter gene. Figure 4B shows an SD media plate which lacks leucine, tryptophan, and histidine. Growth on such a plate indicates the expression of the *HIS3* reporter gene.

Figure 5 shows the nucleic acid sequence of HrBP1 cDNA.

Figure 6 shows the deduced amino acid sequence of HrBP1.

Figure 7 shows the gene structure of HrBP1 and a schematic representation of the exons and introns of the HrBP1 gene. When comparing the HrBP1 cDNA sequence with the *Arabidopsis thaliana* genomic DNA sequence published in a public database, four exons and three introns were discovered.

Figure 8 shows a Northern blot where the translated region of HrBP1 cDNA (855 bp) was used as a probe in the Northern analysis of both total RNA and Poly A⁺ RNA from *Arabidopsis thaliana*. One single band with an apparent molecular weight of about 1.1 Kb was detected.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to isolated receptors for hypersensitive response elicitor proteins or polypeptides. Also disclosed are DNA molecules encoding such receptors as well as expression systems, host cells, and plants containing such molecules. Uses of the receptors themselves and the DNA molecules encoding them are disclosed. The receptor for a hypersensitive response elicitor from a plant pathogen can be from a monocot or a dicot. One example of such a receptor is that found in *Arabidopsis thaliana* which has the amino acid sequence of SEQ. ID. No. 1 as follows:

Met Ala Thr Ser Ser Thr Phe Ser Ser Leu Leu Pro Ser Pro Pro Ala
1 5 10 15

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Leu Leu Ser Asp His Arg Ser Pro Pro Pro Ser Ile Arg Tyr Ser Phe
20 25 30

5 Ser Pro Leu Thr Thr Pro Lys Ser Ser Arg Leu Gly Phe Thr Val Pro
35 40 45

Glu Lys Arg Asn Leu Ala Ala Asn Ser Ser Leu Val Glu Val Ser Ile
50 55 60

10 Gly Gly Glu Ser Asp Pro Pro Pro Ser Ser Ser Gly Ser Gly Gly Asp
65 70 75 80

15 Asp Lys Gln Ile Ala Leu Leu Lys Leu Lys Leu Leu Ser Val Val Ser
85 90 95

Gly Leu Asn Arg Gly Leu Val Ala Ser Val Asp Asp Leu Glu Arg Ala
100 105 110

20 Glu Val Ala Ala Lys Glu Leu Glu Thr Ala Gly Gly Pro Val Asp Leu
115 120 125

Thr Asp Asp Leu Asp Lys Leu Gln Gly Lys Trp Arg Leu Leu Tyr Ser
130 135 140

25 Ser Ala Phe Ser Ser Arg Ser Leu Gly Gly Ser Arg Pro Gly Leu Pro
145 150 155 160

30 Thr Gly Arg Leu Ile Pro Val Thr Leu Gly Gln Val Phe Gln Arg Ile
165 170 175

Asp Val Phe Ser Lys Asp Phe Asp Asn Ile Ala Glu Val Glu Leu Gly
180 185 190

35 Ala Pro Trp Pro Phe Pro Pro Leu Glu Ala Thr Ala Thr Leu Ala His
195 200 205

Lys Phe Glu Leu Leu Gly Thr Cys Lys Ile Lys Ile Thr Phe Glu Lys
210 215 220

40 Thr Thr Val Lys Thr Ser Gly Asn Leu Ser Gln Ile Pro Pro Phe Asp
225 230 235 240

Ile Pro Arg Leu Pro Asp Ser Phe Arg Pro Ser Ser Asn Pro Gly Thr
245 250 255

Gly Asp Phe Glu Val Thr Tyr Val Asp Asp Thr Met Arg Ile Thr Arg
260 265 270

50 Gly Asp Arg Gly Glu Leu Arg Val Phe Val Ile Ala
275 280

This protein, known as HrBP1p, is encoded by a cDNA molecule having SEQ. ID.
No. 2 as follows:

55

tttttccttc tcaacantgg cgactttcttc tactttctctg tcaactactac cttcaccacc 60
agctctttott tccgaccacc gttctctctcc accatccatc agatactoct tttctccctt 120
aactactcca aaatcgtctc gtttgggttt cactgtaccg gagaagagaa acctcgtctc 180
taattcgtct ctogttgaag tatccattgg cygagaaagt gacccaccac catcatcacc 240.

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5 tggatcagga ggagacgaca agcaaatgac attactcmaa ctcaaatlac ttagtgtagt 300
ttcgggatta aacagaggac ttgtggcgag tgttgatgat ttgaaagag ctgaagtggc 360
tgctaaagaa cttgaaactg ctgggggacc ggttgattta accgatgac ttgataagct 420
tcaagggaaa tggaggctgt tgtatagtag tgogttctct tctcgtctct taggtggtag 480
cgtctcgtgt ctacctactg gacgtttgat cctgttact cttggccagc tgtttcaacy 540
gattgatgtg tttagcaaac attttgataa catagcagag gtggaattag gagcccttg 600
gccatttcog ccattagaag ccactgogac attggcacac aagtttgaac tottaggcac 660
ttgcaagac aagataacat ttgagaaaac aactgtgaag acatcgggaa aottgtcgc 720
gattcctcog tttgatatcc cgaggtcttc cgacagtttc agaccatcgt caaaccttg 780
10 aactggggat ttogaagtta cctatgttga tgataaatg cgcataactc gggggagac 840
aggtgaactt aggttatcog tcattgttta attctcaag ctttgacatg taagataaa 900
taataacttt ctgcttgatg cagtctcgt agttttgtac aatcatgtg aacatataa 960
tgcgctttat aagtaaatga gtgtcttggt caatgaatca 1000

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15 The genomic DNA molecule containing the receptor encoding cDNA molecule of SEQ. ID. No. 2 has SEQ. ID. No. 3 as follows:

20 aattagaana attcaaaccc aacatctagt tagaatattt aatttgcac aatgtcttcg 60
agtatagtga aaaaaataga agatcgaata tgaatagta cgtatagaat catctagatc 120
cattcgaact aacgtctact tttcttttcc agcattaaca tgtagcttgt cattagcatt 180
tacatgttgc aataaacca aattgggaaa ttgaagact aaaaaacctt gtacagcaga 240
tggtttaaca cgtggattca tggacacaaa cagaacccg cagaactaag cacaaaaacg 300
tcaactaagc atatacaagc ttttaagtca agcctaatat aaacacagc ggttatccat 360
aactctgtct taatctcttg cagtagttat cttttcatta ttgcacatc gcaattctat 420
25 attcttatac ttcaacttgt tcttcttcca aattgtaatt atatatcat cgtcttaget 480
tgaccattat agctccagta ccaagttctc ttcttaactt taatatcagc tactattctc 540
atactgtaaa tatcttttgt tcacacacaa tatatttoga accaaactgc taanagctta 600
tcataaatg cagttctagc cacacaaatt tgcagttcca accattaaat gccacaaaat 660
ttggacgatt tcttaagaca agaaagacat agcaaccaa ccttattgat taatatgaa 720
30 atgtctccat aaaaotggga gatttcccca aataaagaga acacggcaaa tgttcacgta 780
atotocaaga tgaatgttta attttttctt tcagaaaaaa acaaaaaaac ttanctcaat 840
atagacaaact agaatggata ccaactaagc aaagaaatt caaagacaa atatatattg 900
gatatgaagt taacttattt tcaacttta tatactacta aaagcctaaa aatttgttct 960
aaatgatata ccaataaat ggaaggcatg aatgtcatat gactaaaaga gaaaaacaa 1020
35 cctgtatata agtattgat catgctgct ccagtgaca aaacatacga tgtggtctt 1080
tattgggcca tacttaaatg gaaaaggag aaaaaaatt gggcaatgtc tatggtoga 1140
atttatatgt tttacatcaa taanctaat atttaattt atatatgtg gtcttaactc 1200
agtattatct aatagatta aatcaaatg actgcatatg gtccataata atacaacca 1260
agcaattaa aattttgttg cacaaaacya cttcatttta ctcaaaaag aatatgaat 1320
40 ttogtttaag cacacacgta tacgcgctaa taaccctgg tgettctca atacataat 1380
aattaaagtc ttctctctct tcttctctc tacaattat ctactctctc tegtttttt 1440
ttccttctca acaatggoga cttctctac tttctcgtca ctactacct caccacacg 1500
tcttcttcc gaccacgtt ctctccacac atccatcaga tactctttt ctcccttaac 1560

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tactccaaaa tcyctctogtt tgggttttcaa tgtaccggag aagagaaaac tcyctctotaa 1620
 ttcgtctctc gttgaagtat ccattggcgg agaaagtac ccccccacat catcatctgy 1680
 atcaggagga gacgacaagc aaattgcatt actcaaacctc aaattacttg tgagtctgat 1740
 tcaaaccant cggtgaaatt ataagaaatt ggtttcgttt ctttggaaat agggtttata 1800
 ttactgttaa gattcgatta tagagtgaat tttgggaaga tttttcagat ttgatttgtg 1860
 atgtgttgtg ttgtgagaaa ttgcagagtg tagtttcggg attaaacaga ggacttbtgy 1920
 ogagtgttga tgatttagaa agagctgaag tggctgttaa agaacttga actgctgggy 1980
 gaccggttga tttaacogat gatcttgata agcttcnagg gaaatggagg ctgttgtata 2040
 gtagtgcgtt ctcttctcgg tcttttagtg gtagcgttoo tggctacat actggacgtt 2100
 tgatccctgt tactcttggc caggttaattc ttgaatcatt gctctgtttt taccogtcaa 2160
 gattcogttt ttccggtaag ttgttgaggg gtttatgtgc atggtctagt ctatcatcaa 2220
 tagtcttgtc tgagtttgaa tggggctgag ctaagaatct agctttctga ggttacaatt 2280
 tggtaantgtc atcttatact cgaagcaaa cttttttcta tattgtcaag tttatgtgta 2340
 cggctctggtc tatcattggt agtctttgtt gagcttgaat ggtgaggagc ttagaatcta 2400
 gcaatgtcat ctactcetta atcatttttt tctatattgc caagtctatg tgtacggtct 2460
 tagtcaatca tctttattct tggttgagtt tgaatggta tgagcttaga atctagcttt 2520
 ctttggttta aatttggcaa agaacctac ctgaatcggc agaaagcaaa cttctttant 2580
 attatctott gttttgaaat cattaaaaa ggtgtttcaa cggattgatg tgttttagca 2640
 agattttgat aacatagcag aggtggant aggagocctc tggccatttc ogccattaga 2700
 agccactgcy acattggcac caagtttga actcttaggt ttgcatttcc cttctctca 2760
 ttaagtttat cgaattgtgt aagagcaaaa taacttatct gtatotttga catttatggy 2820
 gaaaacgggc acttgcaaga tcaagataac atttgagaaa acaactgtga agacatcgyy 2880
 aaacttgtcy cagattctc cgtttgatat cccgaggctt cccgacagtt tcagaccatc 2940
 gtcaaacctt ggaactgggy atttcgaagt tacctatgtt gatgatacca tgcgontaac 3000
 tgcgggygac agaggtgaa ttaggytatt cytcattgtt taattctcaa agctttgca 3060
 tgaagata aataaact tctgtctga tgcagtctca tgagtttgt acaaatctg 3120
 tgaacatata autgcgttt ataagtaant gagtgtcttg ttcaatgaat catatgaag 3180
 aatttgtatg actcagaaa ttggacaatg atatagacot tccaaatttt gcacctcta 3240
 atgtgagata ttagtattt tttcttaggt tggtagagag aacggatttg caaaaaata 3300
 tcaaggtca atgattaaac gcaaaacct atcttgatga ttcaaatat agagttaaca 3360
 agcaagatg agacaatctt atacyagaga gtaaaacaa atggattcca atccagcaa 3420
 gtacaaaaat cgcagaaat aagatgaac caacttaaa cagagatgtt ccttttccct 3480
 tcttgtcacc acgatctcy aaatgcttg acccttgaaa taacaacaa accaacacaa 3540
 tgaagcaaa ttacaaagt acaaatccgy tataatgaac tgatctatgt tctatgaaco 3600
 ttgataggac gctgcgaaa gtgcttgag ctttgacact gaagcctcaa acaatcttc 3660
 ttogtggctc tagcotgta acagattca caagatgtat otcagtcaa aactgagact 3720
 attggaatgt ctgtttctc acagctcaat tccaaattc tactataaat ggttccctaa 3780
 aactacctca tttcaactaa ctgaacctaa ttcaactga aaaaacatc aatgcattat 3840
 aatcaatgtt acccttttgt ggaagacag cttagtctga ccaccataac cagattgttt 3900
 acggtcataa cgaactttc cttgagcagc agactgtct ttacccttct tgtattggyt 3960
 aaccttgtgc aaagtatgt ttttgcatc ctgttctta cagtaagtgt tctttgtctt 4020
 tggaaatgtt accctcaaaa ttcataaat caaaatga tcaactcac acatacaaaa 4080
 tcaagagact ttttaagtta atcaaaatc aaacatctt tagattgaa acttttatga 4140
 tagatctgaa aaacaatca ataantcaat caacctgta ttgttgtctt tcaagtcna 4200

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cgacatttac aaattccaaa atcacatoga aagagaagaa acaatttacc attttogcgt 4260

Hypersensitive response elicitors recognized by the receptors of the present invention are able to elicit local necrosis in plant tissue contacted by the
5 elicitor.

Examples of suitable bacterial sources of hypersensitive response elicitor polypeptides or proteins include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solanacearum*, *Xanthomonas campestris*, and mixtures thereof).
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An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.
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The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* is disclosed in U.S. Patent No. 5,850,015 and U.S. Patent No. 6,001,959, which are hereby incorporated by reference. This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine.
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The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* and its encoding DNA molecule has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has a glycine content of greater than 21% and contains substantially no cysteine. The
25 hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in U.S. Patent No. 5,849,868 to Beer and Wei, Z.-M., et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85-88 (1992), which are hereby incorporated by reference.

30 The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the

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hypersensitive response elicitor derived from *Pseudomonas syringae* and its encoding DNA molecule is found in U.S. Patent Nos. 5,708,139 and 5,858,786 and He et al., "*Pseudomonas syringae* pv. *syringae* Harpin_{PM}: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," *Cell* 73:1255-66 (1993), which are hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," *EMBO J.* 13:543-533 (1994), which is hereby incorporated by reference. This protein has 344 amino acids, a molecular weight of 33.2 kDa, and a pI of 4.16, is heat stable and glycine rich (20.6%).

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *glycines* has a partial amino acid sequence corresponding to SEQ. ID. No. 4 as follows:

Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Val	Ala	Ile	Ile	Ala	Ile	Leu	Ala
1				5					10					15	
Ala	Ile	Ala	Leu	Pro	Ala	Tyr	Gln	Asp	Tyr						
			20						25						

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. *glycines*. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes a partial amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

Met	Asp	Ser	Ile	Gly	Asn	Asn	Phe	Ser	Asn
1				5					10

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

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subsp. *carotovora* Strain Ecc71 Overexpress *hrp N₂₂₂* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," *MPML*, 9(7):565-73 (1996), which is hereby incorporated by reference. This protein has 356 amino acids, a molecular weight of 35.6 kDa, and a pI of 5.82 and is heat stable and glycine rich (21.3%).

5 The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996,
10 which are hereby incorporated by reference.

 Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most
15 Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in
20 Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path., 41:298-307 (1992), Baillreul et al., "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco
25 and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference. These hypersensitive response elicitors from *Phytophthora* are called elicitins. All known elicitins have 98 amino acids and show >66% sequence identity. They can be classified into two groups, the basic elicitins and the acidic elicitins, based on the physicochemical properties. This classification also
30 corresponds to differences in the elicitins' ability to elicit HR-like symptoms. Basic elicitins are 100 times more effective than the acidic ones in causing leaf necrosis on tobacco plants.

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The hypersensitive response elicitor from Gram positive bacteria like *Clavibacter michiganensis* is described in WO 99/11133, which is hereby incorporated by reference.

5 The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response using conditions under which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

10 Turning again to the receptor of the present invention for such hypersensitive response elicitors, fragments of the above receptor protein are encompassed by the method of the present invention. In addition, fragments of full length receptor proteins from other plants can also be utilized.

15 Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known receptor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for receptor activity according to the procedure described above.

20 As an alternative, fragments of a receptor protein can be produced by digestion of a full-length receptor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave receptor proteins at different sites based on the amino acid sequence of the receptor protein. Some of the fragments that result from proteolysis may be active receptors.

25 In another approach, based on knowledge of the primary structure of the receptor protein, fragments of the receptor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

30 Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the receptor being produced. Alternatively, subjecting a full length receptor to high temperatures and

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pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

5 Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a tag or other sequence for ease of synthesis, purification, or identification of the polypeptide.

10 Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of 50 continuous bases of SEQ. ID. No. 2 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing with the SSC buffer at 37°C; and preferably in a hybridization buffer
15 comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

20 The receptor of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the receptor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the receptor protein of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the receptor protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, or chemical
25 treatment, and the homogenate is centrifuged to remove bacterial debris. The cell lysate can be further purified by conventionally utilized chromatography procedures. The supernatant is then subjected to heat treatment and the protein is separated by centrifugation. The supernatant fraction containing the receptor protein is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to
30 separate the receptor protein. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

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The DNA molecule encoding the receptor protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted
5 into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form
10 of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as
15 vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19,
20 pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by
25 reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York
30 (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host

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number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*,
5 and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which
10 inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene
15 transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong"
20 transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage
25 lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the receptor protein has been cloned into an expression system, it is ready to be incorporated into a host cell.
30 Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

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One aspect of the present invention involves enhancing a plant's receptivity to treatment with a hypersensitive response elicitor by providing a transgenic plant or transgenic plant seed, transformed with a nucleic acid molecule encoding a receptor protein for a hypersensitive response elicitor. It has been found
5 that hypersensitive response elicitors are useful in imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance in a variety of plants. In view of the receptor of the present invention's interaction with such elicitors, it is expected that these beneficial effects would be enhanced by carrying out such elicitor treatments with plants transformed with the
10 receptor encoding gene of the present invention.

Transgenic plants containing a gene encoding a receptor in accordance with the present invention can be prepared according to techniques well known in the art.

A vector containing the receptor encoding gene described above can be
15 microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, *Mol. Gen. Genetics*, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., *Nature*, 296:72-74 (1982), which is hereby incorporated by reference.

20 Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to
25 Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA.
30 Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g.,

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dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies.

- 5 Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

- 10 The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

- 15 Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

- 20 *Agrobacterium* is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

- 25 Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

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After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedures. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds.

These elicitor treatment methods can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed transformed with a receptor gene in accordance with

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the present invention under conditions effective for the elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance.

Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to
5 impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart resistance to stress.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance in
10 the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed both a DNA molecule encoding a receptor in accordance with the present invention and with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. The plant is grown under conditions effective to permit that
15 DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to
20 impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart resistance to stress.

The embodiment where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor or 2) application of
25 bacteria which do not cause disease and are transformed with a gene encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seeds cells. In these
30 embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

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5 The hypersensitive response elicitor treatment can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, *petunia*, *pelargonium*, poinsettia, chrysanthemum, carnation, and zinnia.

15 With regard to the use of hypersensitive response elicitors in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

20 The method of imparting pathogen resistance to plants is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants *Pseudomonas solanacearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthomonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi: *Fusarium oxysporum* and *Phytophthora infestans*.

30 With regard to the use of the hypersensitive response elicitor protein or polypeptide to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced,

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5 increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, there is significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

10 The use of hypersensitive response elicitors for insect control encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

20 Elicitor treatment is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, tomato pinworm, and maggots. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

30 Hypersensitive response elicitor treatment is also useful in imparting resistance to plants against environmental stress. Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air pollution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide,

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NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macromutrients).

5 The application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant
10 seeds or propagules (e.g., cuttings), the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with a hypersensitive response elicitor, the seeds can be
15 planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated with an elicitor, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or to impart stress resistance.

20 The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the elicitor can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds contains a
25 hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematocide, and mixtures thereof. Suitable fertilizers include (NH₄)₂NO₃. An example of a suitable insecticide is
30 Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. In addition, the hypersensitive response elicitor

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can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative technique involving the use of transgenic plants and transgenic seeds encoding a hypersensitive response elicitor encoding gene, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art as described above.

In another embodiment, the present invention relates to a DNA construct which is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors. An example of such a construct would be an antisense DNA molecule of the DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3. Alternatively, the DNA construct can have a DNA molecule having the nucleotide sequence of SEQ. ID. Nos. 2 or 3 (or a portion thereof) and its complementary strand and is used to generate a single transcript with an inverted repeat (i.e. a double-stranded) RNA. This transcript as well as the above-discussed antisense nucleic acid molecule can be used to induce silencing of a nucleic acid molecule encoding a receptor for a hypersensitive response elicitor.

Sensing the hypersensitive response elicitor by the receptor is the very first step of the signal transduction pathway in plants which eventually leads to disease resistance, growth enhancement, insect control, and stress resistance. Silencing the receptor provides a powerful tool to find and study the downstream components of this pathway. Additionally, the receptor could be a negative regulator of such plant signal transduction pathway. Silencing of the receptor will impart to plants the ability to resist disease and stress, control insects, and enhance growth without hypersensitive response elicitor treatment.

EXAMPLES

Example 1 - Materials and Methods

The laboratory technique used in the following example is straight forward. All DNA manipulations described here followed conventional protocols

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(Sambrook et al., "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory (1989); Ausubel, et al., "Current Protocols in Molecular Biology," John Wiley (1987), which are hereby incorporated by reference). The plasmids and microorganisms described herein, used for making the present invention, were
5 obtained from commercial sources, or from the authors of previous publications. Sequences were analyzed with Clone Manager 5 (Scientific & Educational Software, Durham, North Carolina).

Yeast strain L40 was grown in YPD or in different minimal synthetic dropout selection media at 30°C. *E.coli* strains DH5α and HB101 were grown in LB
10 at 37°C.

The yeast Two-Hybrid system is based on the fact that many eukaryotic transcription factors are composed of physically separable, functionally independent DNA-binding domain (DNA-BD) and activation domain (AD). Both the DNA-BD and the AD are required to activate a gene. When physically separated by
15 recombinant DNA technology and expressed in the same host cell, the DNA-BD and the AD do not interact directly with each other and thus cannot activate the responsive gene (Ma & Ptashne, "Converting a Eukaryotic Transcriptional Inhibitor into an Activator," *Cell* 55:443 (1988) and Brent & Ptashne, "A Eukaryotic Transcriptional Activator Bearing the DNA Specificity of a Prokaryotic Repressor," *Cell* 43:729
20 (1985), which are hereby incorporated by reference). But if the DNA-BD and the AD are brought into close physical proximity in the promoter region, the transcriptional activation function will be restored. Therefore, the yeast *Saccharomyces cerevisiae* and the Two-Hybrid system have become essential genetic tools for studying the macromolecular interactions.

25 In the Two-Hybrid system utilized here, the DNA-BD, encoded in the bait vector pVJL11 (Jullien-Flores, V., "Bridging Ral GTPase to Rho Pathways. RLIP76, a Ral Effector with CDC42/Rac GTPase-activating Protein Activity," *J. Biol. Chem.* 27:22473 (1995), which is hereby incorporated by reference), is the prokaryotic LexA protein, and the activation domain, encoded in the prey vector
30 pGAD 10 or pGAD GH (Clontech; Hannon, GJ., "Isolation of the Rb-related p130 Through its Interaction with CDK2 and Cyclins," *Genes Dev.* 7:2378 (1993), which is hereby incorporated by reference) is derived from the yeast GAL4 protein. pVJL11

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also has a *TRP1* marker, and the pGAD a *LEU2* marker. An interaction between the bait protein (fused to the DNA-BD) and a library-encoded protein (fused to the AD) creates a novel transcriptional activator with binding affinity for LexA operators. The yeast host L40 {MATa his3D200 trp1-901 leu2-3, 112 ade2 LYS2::(*lexAop*)₄-HIS3

5 URA3::(*lexAop*)₈-*lacZ*} harbors two reporter genes, *lacZ* and *HIS3*, which contain upstream LexA binding site. The *HIS3* nutritional reporter provides a sensitive growth selection that can identify a single positive transformant out of several million candidate clones. The expression of the reporter genes indicates interaction between a candidate protein and the bait protein. See Figure 1.

10 *Erwinia amylovora* harpin was used as the bait protein to screen the *Arabidopsis thaliana* MATCHMAKER cDNA library cloned in the pGAD 10 vector (Clontech, laboratories, Inc., Palo Alto, California). One cDNA library encoded protein was identified as a strong harpin interacting protein and, thus, a putative harpin receptor. The present invention reports the nucleic acid sequence and the

15 deduced amino acid sequence of this cDNA.

Example 2

HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid

20 bait vector pVJL11. PCR was carried out using the 1.3 Kb harpin fragment (Wei et al., "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," *Science* 257:85 (1992), which is hereby incorporated by reference) as a template to amplify the harpin encoding region. A Bam HI site was added to the 5' end of the coding sequence, and a Sal I site to the 3' end. A Bam HI

25 and Sal I digested PCR fragment was ligated with the bait vector pVJL11 digested with the same restriction enzymes. pVJL11 has a *TRP1* marker to be selected in yeast and an Amp resistance marker to be selected in *E. coli*. The plasmid DNA was amplified in *E. coli* strain DH5α. When tested in the Two-Hybrid system with empty prey vector pGAD GH and several unrelated proteins, HrpN didn't show auto-

30 activation or nonspecific interaction with unrelated proteins, as shown in Figure 2.

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Example 3

HrpN-pVJL 11 was transformed into yeast strain L40 by a lithium acetate (LiAc)-mediated method (Ito et al., "Transformation of Intact Yeast Cells Treated with Alkali Cations," *J. Bacteriol.* 153:163 (1983) and Vojtek et al., "Mammalian Ras Interacts Directly with the Serine/Threonine Kinase Raf," *Cell* 74:205 (1993), which are hereby incorporated by reference). The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for harpin interacting proteins. Approximately 6.8 million primary library transformants were plated onto plates lacking histidine, leucine, and tryptophan. A total of 148 colonies grew on the histidine dropout plates, 55 of which stained positive when tested for expression of β -galactosidase. After three rounds of selection on synthetic minimal (SD) media plates lacking leucine, tryptophan, and histidine, and confirming by the expression of the second reporter gene *lacZ* using a β -galactosidase assay, 47 colonies seemed to be strong interacting candidates.

Example 4

Plasmid DNA was extracted from the 47 independent yeast colonies and shuttled into *E. coli* strain HB101, which carries the *leuB* mutation. Therefore, the prey plasmid (cDNA-pGAD 10) was selected for on minimal nutrient plates since pGAD 10 bears the *LEU2* marker.

The 47 independently rescued prey plasmids purified from *E. coli* were re-tested in the yeast two-hybrid system with harpin as bait. They were also tested against unrelated protein. 20 turned out to be strong specific interaction candidates again. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The sequence reactions were performed using the PE Prism BigDye™ dye terminator reaction kit. The sequencing gel was run in Thetagen (Bothell, WA)

One of the eight plasmids, which had the longest cDNA insert of 1kb, was used for further analysis. When co-transformed into yeast strain L40, it was shown to be negative with empty bait and unrelated proteins in the Two-Hybrid

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system, indicating the specificity of the interaction between harpin and this receptor candidate. See Figure 3.

Example 5

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The longest cDNA insert, HrBP1, was subcloned into the Bam HI and SalI sites of the bait vector pVJL 11. This construct didn't show auto-activation of the reporter genes, nor interaction with unrelated proteins in the yeast Two-Hybrid system. However, the expression of the reporter genes was activated when L40 was co-transformed with HrBP1-pVJL11 and HrpN-pGAD GH, indicating the specific interaction between HrBP1p (the protein encoded by HrBP1) and harpin. See Figure 4.

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Example 6

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Total RNA was extracted from two-week-old *Arabidopsis thaliana* using QIAGEN RNeasy plant mini kit (Qiagen, Inc., Valencia, California). Poly A⁺ RNA was further purified from the total RNA with a QIAGEN Oligotex column (Qiagen, Inc., Valencia, California). A Northern blot was carried out using the translated region of HrBP1 as a probe. One single species with an apparent molecular weight of about 1.1 Kb was detected from both total RNA and Poly A⁺ RNA. See Figure 8. Therefore, the longest cDNA of HrBP1 from the yeast two-hybrid screen seems to be the full-length cDNA. The integrity of the 5' of cDNA was further confirmed by a primer extension assay.

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As described, a yeast Two-Hybrid system was used to screen for harpin interacting proteins. HrpN of *Erwinia amylovora* was subcloned into the yeast Two-Hybrid bait vector pVJL11, which has a *TRP1* marker. The *lexA* harpin fusion protein is expressed from this construct in yeast. The *Arabidopsis thaliana* MATCHMAKER cDNA library (Clontech Laboratories, Inc., Palo Alto, CA) was screened for hypersensitive response elicitor interacting proteins. Out of the 6.8 million independent colonies screened, 148 grew on SD media plates lacking leucine, tryptophan, and histidine. After three rounds of selection on a -His plate and confirmation by a β -galactosidase assay, 47 colonies seemed to be strongly interacting

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candidates. The prey plasmids from the 47 candidate colonies were rescued and shuttled into *E. coli*.

The 47 independent prey plasmids purified from *E. coli* were re-tested in the yeast Two-hybrid system with harpin as bait. They were also tested against
5 unrelated protein: LexA-lamin. 20 turned out to have a strong specific interaction. Sequencing analysis showed that the 20 independent cDNA clones were actually from the same gene with different integrity at the 5' end. The longest sequence was 1,000 bp, which was named HrBP1. It was mapped to *Arabidopsis thaliana* genomic DNA,
10 chromosome 3, P1 clone MLM24 (Nakamura, "Structural Analysis of *Arabidopsis thaliana* chromosome 3," Direct submission to the DDBJ/EMBL/GenBank databases (1998), which is hereby incorporated by reference). Four exons and three introns were discovered (See Figure 7). Exon 4 includes a 130 bp non-translated 3' region. The in-frame open reading frame from the first methionine encodes a polypeptide
15 (named HrBP1p) of 284 amino acids. The predicted molecular weight of HrBP1p is 30454.3 and pI is 5.72. There is no apparent hydrophobic trans-membrane domain in this polypeptide. The HrBP1-AD fusion prey was negative with empty bait and unrelated proteins in the yeast 2-H system, indicating the specificity of the interaction between harpin and this receptor candidate. When being put in the opposite orientation, i.e. HrBP1p fused with the DNA-BD and harpin with the AD, they still
20 specifically interacted with each other.

HrBP1 has no significant sequence similarity with sequences deposited in major sequence database accessible with the Blast search program. Therefore, HrBP1p is a novel protein.

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Example 7

The HrBP1 cDNA was subcloned into the Nde I and Sal I sites of the vector pET-28a (Novagen, Madison, WI). The his-tagged HrBP1p recombinant protein was purified with Ni-NTA resin (QIAGEN Inc., Valencia, CA) according to the manual provided by the manufacturer. The HrBP1 cDNA was also subcloned into the BamH I and Sal I sites of the vector pGEX-4T-1 (Amersham Pharmacia Biotech, Piscataway, NJ). The GST-tagged HrBP1p recombinant protein was purified by Glutathione Sepharose™ 4B (Amersham Pharmacia Biotech, Piscataway NJ) according to the manufacturer's instructions.

Example 8

Anti-sense HrBP1, which is complementary to SEQ. ID. No. 2, was sub-cloned into binary vector pPZP212, under the control of the NOS promoter. *Arabidopsis thaliana* plants were transformed with this construct using Agrobacterium-mediated transformation. The *Agrobacterium tumefaciens* strain used was GV3101 (CS8C1 Rfr) pMP90 (Gmr). *Arabidopsis* plants were also transformed with a construct, which had an inverted repeat consisting of bases 3-650 of the HrBP1 cDNA (i.e. SEQ. ID. No. 2) and the complementary sequence of bases 3-500 of HrBP1 cDNA. This construct generated a double-stranded mRNA in transformed plants.

Both anti-sense and double-stranded approaches were intended to silence the expression of HrBP1. Some of the transformed *Arabidopsis* plants had spontaneous necrosis on their leaves. They were also very diseased and developmentally retarded.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

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WHAT IS CLAIMED:

1. An isolated protein which serves as a receptor in plants for plant pathogen hypersensitive response elicitors.

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2. A protein according to claim 1, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and *Clavibacter*.

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3. A protein according to claim 2, wherein the plant pathogen is an *Erwinia* pathogen.

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4. A protein according to claim 3, wherein the plant pathogen is *Erwinia amylovora*.

5. A protein according to claim 1, wherein the protein is from a monocot.

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6. A protein according to claim 1, wherein the protein is from a dicot.

7. A protein according to claim 6, wherein the protein is from *Arabidopsis thaliana*.

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8. A protein according to claim 1, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

9. A protein according to claim 1, wherein the protein is recombinant.

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10. An isolated nucleic acid molecule encoding a protein according to claim 1.

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11. A nucleic acid molecule according to claim 10, wherein the plant pathogen is selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and *Clavibacter*.

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12. A nucleic acid molecule according to claim 11, wherein the plant pathogen is an *Erwinia* pathogen.

10 13. A nucleic acid molecule according to claim 12, wherein the plant pathogen is *Erwinia amylovora*.

14. A nucleic acid molecule according to claim 10, wherein the protein is from a monocot.

15 15. A nucleic acid molecule according to claim 10, wherein the protein is from a dicot.

16. A nucleic acid molecule according to claim 15, wherein the protein is from *Arabidopsis thaliana*.

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17. A nucleic acid molecule according to claim 10, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

25 18. A nucleic acid molecule according to claim 10, wherein the nucleic acid hybridizes to nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

30 19. A nucleic acid molecule according to claim 10, wherein the nucleic acid has a nucleotide sequence of SEQ. ID. No. 2.

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20. A nucleic acid according to claim 10, wherein the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C.

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21. A nucleic acid according to claim 10, wherein the nucleic acid has a nucleotide sequence comprising SEQ. ID. No. 3.

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22. An antisense nucleic acid molecule to the nucleic acid according to claim 10.

23. An expression vector containing a nucleic acid molecule according to claim 10 which is heterologous to the expression vector.

24. An expression vector according to claim 23, wherein the nucleic acid molecule is positioned in the expression vector in sense orientation and correct reading frame.

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25. An expression vector according to claim 23, wherein either:
(1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3.

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26. An expression vector containing a nucleic acid molecule according to claim 22 which is heterologous to the expression vector.

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27. A transgenic host cell transformed with the nucleic acid molecule according to claim 10.

28. A host cell transformed according to claim 27, wherein the host cell is selected from the group consisting of a plant cell and a bacterial cell.

29. A host cell according to claim 27, wherein the DNA molecule is transformed with an expression system.

30. A host cell according to claim 27, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3.

31. A host cell transformed with a nucleic acid molecule according to claim 22.

32. A transgenic plant transformed with the DNA molecule of claim 10.

33. A transgenic plant according to claim 32, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

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34. A transgenic plant according to claim 32, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saltpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

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35. A transgenic plant according to claim 32, wherein the plant is a monocot.

36. A transgenic plant according to claim 32, wherein the plant is from a dicot.

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37. A transgenic plant according to claim 32, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3.

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38. A transgenic plant transformed with a nucleic acid molecule according to claim 22.

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39. A transgenic plant seed transformed with the DNA molecule of claim 10.

40. A transgenic plant seed according to claim 39, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber,

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apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco,
tomato, sorghum, and sugarcane.

41. A transgenic plant seed according to claim 39, wherein the
plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*,
petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

42. A transgenic plant seed according to claim 39, wherein the
plant is a monocot.

43. A transgenic plant seed according to claim 39, wherein the
plant is a dicot.

44. A transgenic plant seed according to claim 39, wherein either:
(1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid
hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a
hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at
a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ.
ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3
under stringent conditions of a hybridization buffer comprising 20% formamide in
0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid
comprises a nucleotide sequence of SEQ. ID. No. 3.

45. A transgenic plant seed transformed with a nucleic acid
molecule according to claim 22.

46. A method of identifying agents targeting plant cells
comprising:

forming a reaction mixture comprising a protein according to
claim 1 and a candidate agent;
evaluating the reaction mixture for binding between the protein
and the candidate agent; and

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identifying candidate compounds which bind to the protein in the reaction mixture as plant cell targeting agents.

5 47. A method according to claim 46, wherein the protein is from a monocot.

48. A method according to claim 46, wherein the protein is from a dicot.

10 49. A method according to claim 48, wherein the protein is from *Arabidopsis thaliana*.

15 50. A method according to claim 49, wherein the protein has an amino acid sequence of SEQ. ID. No. 1.

51. A method of identifying agents targeting plant cells comprising:

forming a reaction mixture comprising a host cell transformed with a nucleic acid molecule according to claim 10 and a candidate agent;

20 evaluating the reaction mixture for binding between protein produced by the host cell and the candidate agent; and

identifying candidate compounds which bind to the protein or the host cell in the reaction mixture as plant cell targeting agents.

25 52. A method according to claim 51, wherein the protein is from a monocot.

53. A method according to claim 51, wherein the protein is from a dicot.

30 54. A method according to claim 53, wherein the protein is from *Arabidopsis thaliana*.

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55. A method according to claim 51, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3.

56. A method of enhancing plant receptivity to treatment with hypersensitive response elicitors comprising:
providing a transgenic plant or transgenic plant seed transformed with the nucleic acid molecule according to claim 10.

57. A method according to claim 56, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3.

58. A method according to claim 56, wherein a transgenic plant is provided.

59. A method according to claim 56, wherein a transgenic plant seed is provided and said method further comprises:

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planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

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60. A method according to claim 56, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

61. A method according to claim 56, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

62. A method according to claim 56, wherein the hypersensitive response elicitor treatment is for imparting disease resistance.

63. A method according to claim 56, wherein the hypersensitive response elicitor treatment is for enhancing plant growth.

64. A method according to claim 56, wherein the hypersensitive response elicitor treatment is for controlling insects.

65. A method according to claim 56, wherein the hypersensitive response elicitor treatment is for imparting stress tolerance.

66. A method according to claim 56, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects the hypersensitive response elicitor treatment.

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67. A method according to claim 56, wherein the hypersensitive response elicitor treatment comprises:
applying a hypersensitive response elicitor to the plant or plant seed.

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68. A method according to claim 67, wherein the hypersensitive response elicitor is applied in isolated form.

69. A method of imparting disease resistance, enhancing growth, controlling insects, and/or imparting stress resistance to plants comprising:
providing a transgenic plant or transgenic plant seed transformed with a DNA construct effective to silence expression of a nucleic acid molecule according to claim 10.

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70. A method according to claim 69, wherein the protein is from a monocot.

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71. A method according to claim 69, wherein the protein is from a dicot.

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72. A method according to claim 71, wherein the protein is from *Arabidopsis thaliana*.

73. A method according to claim 69, wherein either: (1) the protein has an amino acid sequence of SEQ. ID. No. 1; (2) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 2 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; (3) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 2; (4) the nucleic acid hybridizes to a nucleotide sequence of SEQ. ID. No. 3 under stringent conditions of a hybridization buffer comprising 20% formamide in 0.9M saline/0.09M SSC buffer at a temperature of 42°C; or (5) the nucleic acid comprises a nucleotide sequence of SEQ. ID. No. 3.

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74. A method according to claim 69, wherein a transgenic plant is provided.

5 75. A method according to claim 69, wherein a transgenic plant seed is provided and said method further comprises:
planting the plant seeds under conditions effective for plants to grow from the planted plant seeds.

10 76. A method according to claim 69, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple,
15 pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20 77. A method according to claim 69, wherein the plant is selected from the group consisting of *Arabidopsis thaliana*, *Saltpavilla*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25 78. A method according to claim 69, wherein the transgenic plant or plant seed is further transformed with a second nucleic acid encoding a hypersensitive response elicitor, wherein expression of the second nucleic acid effects a hypersensitive response elicitor treatment.

30 79. A method according to claim 69 further comprising:
applying a hypersensitive response elicitor to the plant or plant seed.

80. A method according to claim 79, wherein the hypersensitive response elicitor is applied in isolated form.

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81. A method according to claim 69, wherein disease resistance is imparted to plants.

5 82. A method according to claim 69, wherein enhanced growth is imparted to plants.

83. A method according to claim 69, wherein insect control is imparted to plants.

10 84. A method according to claim 69, wherein stress resistance is imparted to plants.

15 85. A method according to claim 69, wherein the DNA construct is an antisense nucleic acid molecule to a nucleic acid molecule encoding a receptor in plants for plant pathogen hypersensitive response elicitors..

20 86. A method according to claim 69, wherein the DNA construct is transcribable to a first nucleic acid encoding a receptor in plants for plant pathogen hypersensitive response elicitors coupled to a second nucleic acid encoding the inverted complement of the first nucleic acid.

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ABSTRACT OF THE DISCLOSURE

The present invention is directed to an isolated protein which serves as a receptor in plants for a plant pathogen hypersensitive response elicitor. Also disclosed are nucleic acid molecules encoding such receptors as well as expression vectors, host cells, transgenic plants, and transgenic plant seeds containing such nucleic acid molecules. Both the protein and nucleic acid can be used to identify agents targeting plant cells and to enhance a plant's receptivity to treatment with a hypersensitive response elicitor.

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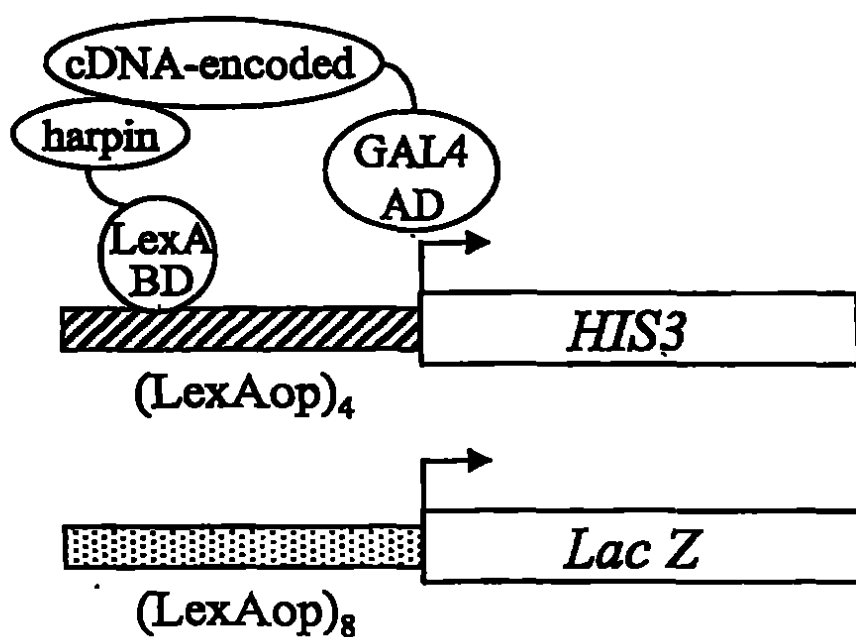
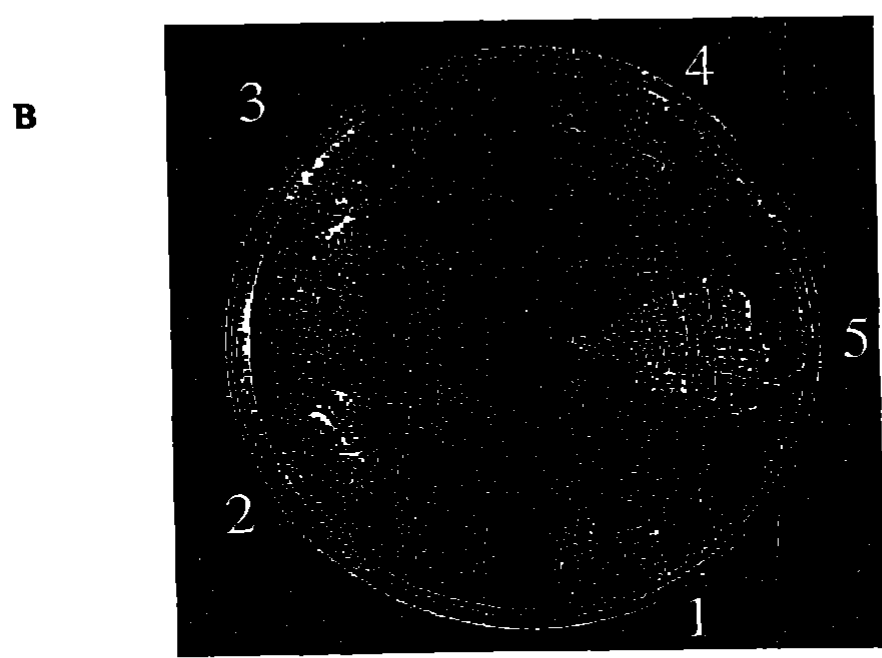


Figure 1

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	Bait	Prey
1	harpin	empty vector
2	harpin	unrelated protein
3	empty vector	harpin
4	unrelated protein	harpin
5	(positive control)	

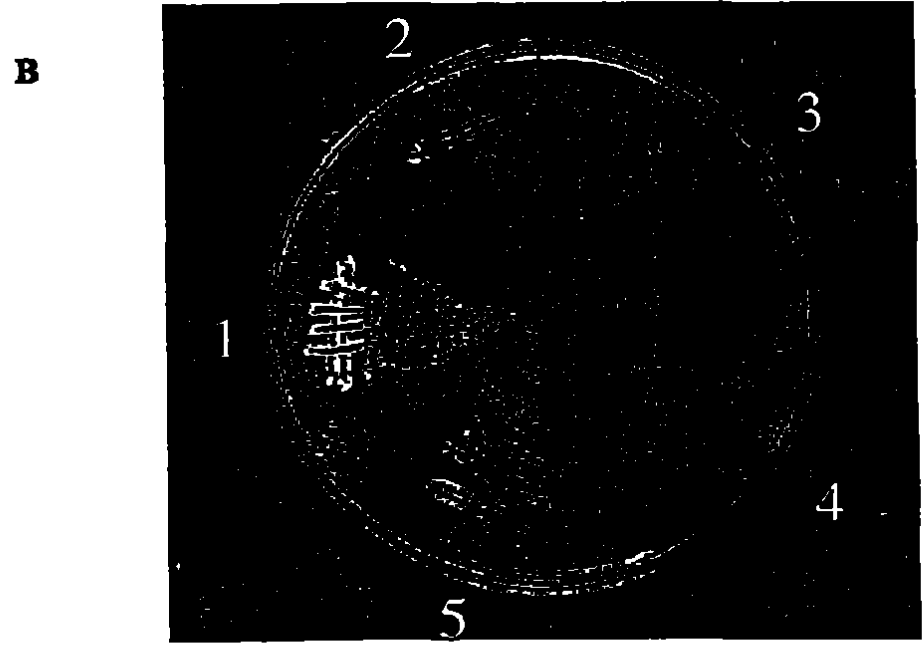
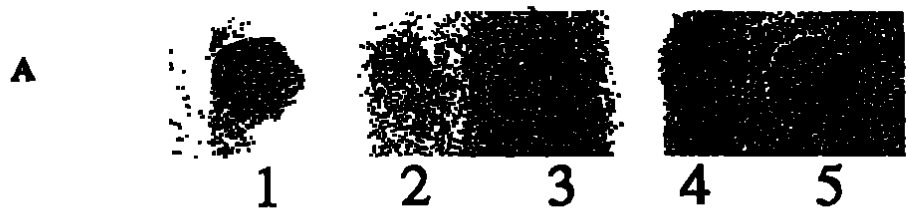
Figure 2

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	Bait	Prey
1	harpin	HrBP1
2	unrelated protein 1	HrBP1
3	unrelated protein 2	HrBP1
4	empty vector	HrBP1
5	(positive control)	

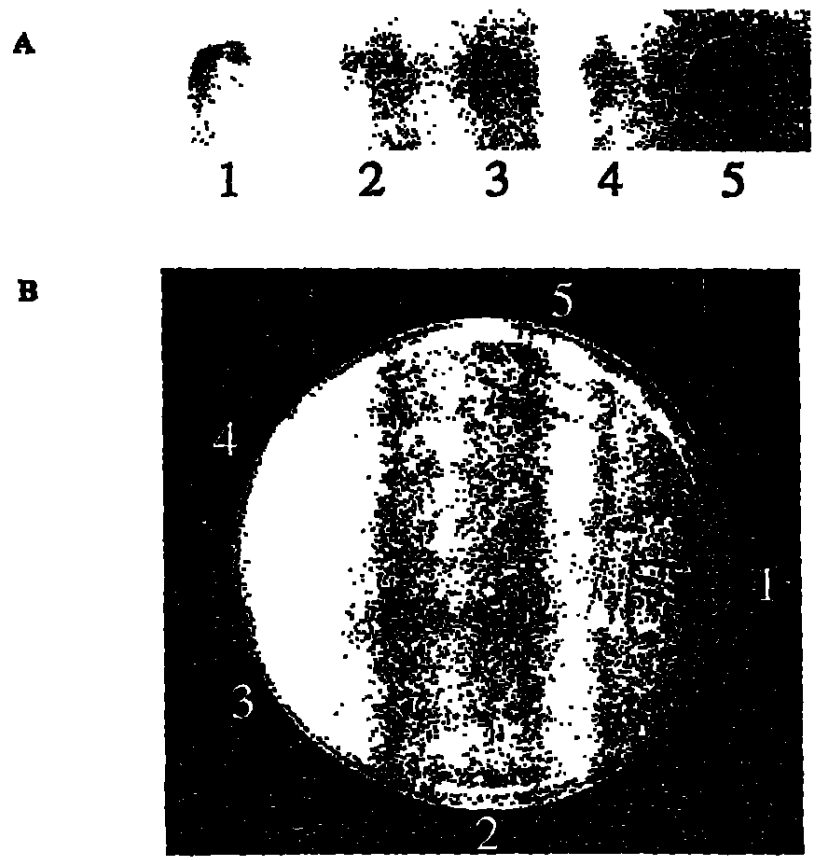
Figure 3

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	Bait	Prey
1	HrBP1	harpin
2	HrBP1	empty vector
3	HrBP1	unrelated protein 1
4	HrBP1	unrelated protein2
5	(positive control)	

Figure 4

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02 Jul 1999

Sequence Data

Page 1

Molecule: HrBP1, 1000 bps DNA
Description: cDNA sequence of HrBP1
File Name: fig5.cm5, dated 04 Jun 1999
Printed: 1-1000 bps (Full), format Single Strand

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61 agctctctctt tccgaccacc gttctctctcc accatccatc agatactcct tttctccttc
121 aactactcoa aaatcgtctc gtttgggttt cactgtacog gagaagagaa acctcgtctgc
181 taattcgtct ctcgttgaa gttccattgg cggagaagt gaoccaccac catcatcatc
241 tggatcagga ggagacgaca agcaaatgac attactcaaa ctcaaatcac ttagtgtagt
301 ttccgggatta aacagaggac ttgtggcgag tggtgatgat ttagaagag ctgaagtggc
361 tgcataagaa cttgaaactg ctgggggacc ggttgattta accgatgac ttgataagct
421 tcaaggga aaagggtgtg tgatatagtag tggtttctct tctcgtctct taggtggtag
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541 gattgatgtg tttagcaaa gattttgata catagcagag gtggaattag gagcccttg
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661 ttgcaagatc aagataacat ttgagaaaac aactgtgaag acatcgggaa acttgctgca
721 gattcctcog tttgatatcc cagggcttcc cgcaggttcc agaccatcgt caaacctgg
781 aactggggat ttgaaagtta cctatgttga tgataccatg cgcataactc gggggacag
841 aggtgaactt agggatattcgc tcattgctta attctcaag ctttgacatg taagataaa
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961 tgcgctttat aagtaaatga gtgtcttgtt caatgaatca
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Figure 5

003230-1649

345

336

1-22

02 Jul 1999

Sequence Data

Page 1

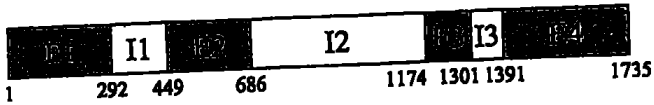
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Description: ORF from 4+47+14R
File Name: fig6.cm5, dated 04 Jun 1999
Printed: 1-284 aas (Full), format Single Strand

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121 taggpvdlt dldklqgkwr llyasafaar elggsrpgip tgrlipvtlg qvfqridvfe
181 kdfdniaeve lgapwppppl eatatlahkf ellgtckiki tfekttvkts galsqippfd
241 iprlpdsfrp sanpgtgdfv vtyvddtwri trgdrgelrv fvia
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Figure 6

601-1649-032300

Figure 7



Gene structure of HrBP1

E Exon I Intron

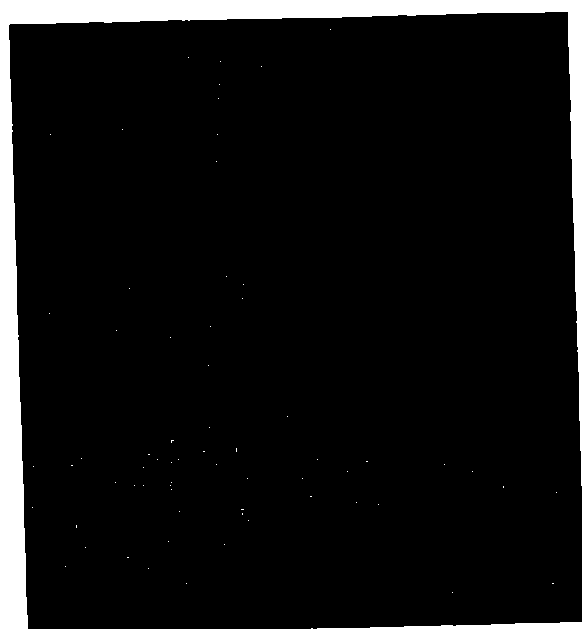
228

CP
228

000220-64 109

Total RNA

Poly-A⁺ RNA



— 1.5 Kb

— 1 Kb

Figure 8

338

229

2do. EXAMEN DE FORMA PATENTE DE INVENCION

Folio 349 **339**

Radicación no 01-23525

Concepto Técnico no 198

Clasificación Internacional de Patentes CO7K14 /415 C12 N15/29

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486 se determinó que esta solicitud de Patente de Invención:

- | | |
|--|--|
| SI ☒ NO ☐ | Contiene un resumen de la invención (Art.26-e). |
| SI ☒ NO ☐ | Contiene el título o nombre de la invención (Art.27-d). |
| SI ☒ NO ☐ | Contiene la descripción de la invención (Art.26-b). |
| SI ☒ NO ☐ | NO ES APLICABLE ☐ Contiene los dibujos necesarios para comprender la invención (Art.26-d) |
| SI ☒ NO ☐ | Contiene una o más reivindicaciones. (Art.26-c). |
| SI ☐ NO ☐ | NO ES APLICABLE ☒ Han sido desarrollados los productos y/o procedimientos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen. (Art. 26- h). |
| SI ☐ NO ☐ | NO. ES APLICABLE ☒ Los productos y/o procedimientos han sido obtenidos o desarrollados a partir de los conocimientos tradicionales de las comunidades indígenas, afroamericanas, o locales de los países miembros, de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes.(Art.26-i). |
| SI ☒ NO ☐ | La invención se refiere a un producto relativo a un material biológico. (Art.26-J). |
| SI ☒ NO ☐ | La prioridad coincide con la materia reivindicada. |
| SI ☒ NO ☐ | CUMPLE LOS REQUISITOS TÉCNICOS |

OBSERVACIONES Y OTROS: SI ☒ NO ☐ ver hoja(s) anexa(s)

EXAMINADOR TÉCNICO
202044

Bogotá D. C. Enero 23 de 2003.

Carrera 13 No. 27-00 Piso 5 y 10
Conmutador: 334 12 21-2-3-4
Fax: 281 31 25 e-mail: info@sic.gov.co
Santa Fe de Bogotá D.C. - Colombia

340

Folio 350

Concepto técnico no.198

OBSERVACIONES

- Anexa los documentos mediante los cuales reivindica prioridad No. 60/250,710 folios 188-256 y 60/191,649 folios 282-348.
- Anexa las traducciones respectivas de las prioridades presentadas. Folios 154-187 y 254 -281.
- Indica el lugar de constitución de la sociedad solicitante. Folios 128 y 129.
- Anexa documento mediante el cual se confiere el poder al representante legal, junto con la persona jurídica del solicitante. Folio 127.
- Anexa el comprobante de pago por presentación de la prioridad. Folio 123.

 **EXAMINADOR TECNICO**
202044

Bogotá, Enero 23 de 2003.

Folio 351

2020
Bogotá DC

Doctor(a)
CARLOS R. OLARTE
Apoderado(a)

REFERENCIA:	Radicación No.	00-23525
	Trámite	002
	Evento	001
	Actuación	416
	Oficio No.	217
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la misma Decisión.

Cúmplase

Dado en Bogotá DC, a los

17 4 FEB 2003


ALIX CARMENZA CESPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

202044

DIVISION DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 01-023525

**EL EXTRACTO DE LA PRESENTE SOLICITUD DE PATENTE DE INVENCION
FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No 529, DE
FECHA 27 DE JUNIO DE 2003, EL TERMINO HABIL SEÑALADO POR LA LEY
EXPIRA EL 24 DE SEPTIEMBRE DE 2003.**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI **NO** **X**

Bogotá D.C., Diciembre de 2003

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: 1-23525

EDICTO No. 1301

**LA SECRETARÍA GENERAL AD-HOC
HACE SABER:**

343

Que esta Superintendencia profirió Resolución No. 37170 de 30-DIC-2003. Que el contenido de la parte resolutive es como sigue: E Superintendente de Industria y Comercio.

RESUELVE:

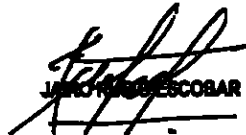
ARTICULO PRIMERO: Declarar abandonada la solicitud de privilegio de patente de invención contenida en el expediente de la referencia.

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución al doctor Carlos R. Ojeda, o a quien haga sus veces, quien actúa como apoderado de Eden Bioscience Corporation, entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá, D.C., a los

30 DIC. 2003

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,


JAIRO RIVAS ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc 22-ENE-2004  Esta edicto se desflja hoy 04-FEB-2004 a las cinco (5:00 p.m.) 
Secretaría General Ad-Hoc
EDICTO No. 1301