

**(12) PATENT
(19) AUSTRALIAN PATENT OFFICE**

**(11) Application No. AU 199860431 B2
(10) Patent No. 748088**

(54) Title
Enhancement of growth in plants

(51)⁷ International Patent Classification(s)
C12N 015/03 A61K 039/00
A01H 004/00 C12N 015/05

(21) Application No: **199860431**

(22) Application Date: **1998.01.27**

(87) WIPO No: **WO98/32844**

(30) Priority Data

(31) Number **60/036048** (32) Date **1997.01.27** (33) Country **US**

(43) Publication Date : **1998.08.18**

(43) Publication Journal Date : **1998.10.01**

(44) Accepted Journal Date : **2002.05.30**

(71) Applicant(s)
Cornell Research Foundation, Inc.

(72) Inventor(s)
Dewen Qiu; Zhong-Min Wei; Steven V. Beer

(74) Agent/Attorney
PHILLIPS ORMONDE and FITZPATRICK, 367 Collins Street, MELBOURNE VIC 3000

(56) Related Art
**714512
56935/98
SCIENCE V257 P85-88**



60431/98

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/03, 15/05, A01N 13/00, A61K 39/00, 1/11, 1/15, A01H 4/00		A1	(11) International Publication Number: WO 98/32844
(21) International Application Number: PCT/US98/01507		(43) International Publication Date: 30 July 1998 (30.07.98)	
(22) International Filing Date: 27 January 1998 (27.01.98)			
(30) Priority Data: 60/036,048 27 January 1997 (27.01.97) US			
(71) Applicant: CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(72) Inventors: QIU, Dewen; 17815 8th Avenue, N.E., Seattle, WA 98155 (US). WEI, Zhong-Min; 8230 N.E. 125th Court, Kirkland, WA 98034 (US). BEER, Steven, V.; 211 Hudson Street, Ithaca, NY 14850 (US).			
(74) Agents: GOLDMAN, Michael, L. et al.; Nixon, Hargrave, Devans & Doyle LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).			

(54) Title: ENHANCEMENT OF GROWTH IN PLANTS

(57) Abstract

The present invention relates to a method of enhancing growth of plants. This involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants produced from the plant seed. Alternatively, transgenic plants or transgenic plant seeds transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to enhance plant growth.

IP AUSTRALIA
10 DEC 1998

ENHANCEMENT OF GROWTH IN PLANTS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/036,048, filed January 27, 1997.

5 This invention was made with support from the U.S. Government under USDA NRI Competitive Research Grant No. 91-37303-6430.

FIELD OF THE INVENTION

10 The present invention relates to the enhancement of growth in plants.

BACKGROUND OF THE INVENTION

15 The improvement of plant growth by the application of organic fertilizers has been known and carried out for centuries (H. Marschner, "Mineral Nutrition of Higher Plants," Academic Press: New York pg. 674 (1986)). Modern man has developed a complex 20 inorganic fertilizer production system to produce an easy product that growers and farmers can apply to soils or growing crops to improve performance by way of growth enhancement. Plant size, coloration, maturation, and yield may all be improved by the application of 25 fertilizer products. Inorganic fertilizers include such commonly applied chemicals as ammonium nitrate. Organic fertilizers may include animal manures and composted lawn debris, among many other sources.

30 In most recent years, researchers have sought to improve plant growth through the use of biological products. Insect and disease control agents such as Beauveria bassiana and Trichoderma harizatum have been registered for the control of insect and disease problems and thereby indirectly improve plant growth and 35 performance (Fravel et al., "Formulation of

- 2 -

Microorganisms to Control Plant Diseases," Formulation of Microbial Biopesticides, Beneficial Microorganisms, and Nematodes, H.D. Burges, ed. Chapman and Hall: London (1996).

5 There is some indication of direct plant growth enhancement by way of microbial application or microbial by-products. Nodulating bacteria have been added to seeds of leguminous crops when introduced to a new site (Weaver et al., "Rhizobium," Methods of Soil Analysis,
10 Part 2, Chemical and Microbiological Properties, 2nd ed., American Society of Agronomy: Madison (1982)). These bacteria may improve the nodulation efficiency of the plant and thereby improve the plant's ability to convert free nitrogen into a usable form, a process called
15 nitrogen fixation. Non-leguminous crops do not, as a rule, benefit from such treatment. Added bacteria such as *Rhizobium* directly parasitize the root hairs, then begin a mutualistic relationship by providing benefit to the plant while receiving protection and sustenance.

20 Mycorrhizal fungi have also been recognized as necessary microorganisms for optional growth of many crops, especially conifers in nutrient-depleted soils. Mechanisms including biosynthesis of plant hormones (Frankenberger et al., "Biosynthesis of Indole-3-Acetic
25 Acid by the Pine Ectomycorrhizal Fungus *Pisolithus tinctorius*," Appl. Environ. Microbiol. 53:2908-13 (1987)), increased uptake of minerals (Harley et al., "The Uptake of Phosphate by Excised Mycorrhizal Roots of Beech," New Phytologist 49:388-97 (1950) and Harley et
30 al., "The Uptake of Phosphate by Excised Mycorrhizal Roots of Beech. IV. The Effect of Oxygen Concentration Upon Host and Fungus," New Phytologist 52:124-32 (1953)), and water (A.B. Hatch, "The Physical Basis of Mycotrophy in *Pinus*," Black Rock Forest Bull. No. 6, 168 pp. (1937))
35 have been postulated. Mycorrhizal fungi have not

achieved the common frequency of use that nodulating bacteria have due to variable and inconsistent results with any given mycorrhizal strain and the difficulty of study of the organisms.

5 Plant growth-promoting rhizobacteria ("PGPR") have been recognized in recent years for improving plant growth and development. Hypothetical mechanisms range from direct influences (e.g., increased nutrient uptake) to indirect mechanisms (e.g., pathogen displacement).

10 Growth enhancement by application of a PGPR generally refers to inoculation with a live bacterium to the root system and achieving improved growth through bacterium-produced hormonal effects, siderophores, or by prevention of disease through antibiotic production, or competition.

15 In all of the above cases, the result is effected through root colonization, sometimes through the application of seed coatings. There is limited information to suggest that some PGPR strains may be direct growth promoters that enhance root elongation under gnotobiotic conditions (Anderson et al., "Responses of Bean to Root Colonization With *Pseudomonas putida* in a Hydroponic System," *Phytopathology* 75:992-95 (1985), Lifshitz et al., "Growth Promotion of Canola (rapeseed) Seedlings by a Strain of *Pseudomonas putida* Under Gnotobiotic Conditions," *Can. J. Microbiol.* 33:390-95 (1987), Young et al., "PGPR: Is There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," *Promoting Rhizobacteria: Progress and Prospects*, Second International Workshop on Plant Growth-promoting

20 33:390-95 (1987), Young et al., "PGPR: Is There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," *Promoting Rhizobacteria: Progress and Prospects*, Second International Workshop on Plant Growth-promoting

25 33:390-95 (1987), Young et al., "PGPR: Is There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," *Promoting Rhizobacteria: Progress and Prospects*, Second International Workshop on Plant Growth-promoting

30 33:390-95 (1987), Young et al., "PGPR: Is There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," *Promoting Rhizobacteria: Progress and Prospects*, Second International Workshop on Plant Growth-promoting

35 33:390-95 (1987), Young et al., "PGPR: Is There Relationship Between Plant Growth Regulators and the Stimulation of Plant Growth or Biological Activity?," *Promoting Rhizobacteria: Progress and Prospects*, Second International Workshop on Plant Growth-promoting

152:247-54 (1989); however, the production of plant growth regulators has been proposed as the mechanism mediating these effects. Many bacteria produce various plant growth regulators *in vitro* (Atzorn et al., 5 "Production of Gibberellins and Indole-3-Acetic Acid by *Rhizobium phaseoli* in Relation to Nodulation of *Phaseolus vulgaris* Roots," Planta 175:532-38 (1988) and M. E. Brown, "Plant Growth Substances Produced by Micro-Organism of Solid and Rhizosphere," J. Appl. Bact. 10 35:443-51 (1972)) or antibiotics (Gardner et al., "Growth Promotion and Inhibition by Antibiotic-Producing Fluorescent Pseudomonads on Citrus Roots," Plant Soil 77:103-13 (1984)). Siderophore production is another mechanism proposed for some PGPR strains (Ahl et al., 15 "Iron Bound-Siderophores, Cyanic Acid, and Antibiotics Involved in Suppression of *Thievaliopsis basicola* by a *Pseudomonas fluorescens* Strain," J. Phytopathol. 116:121-34 (1986), Kloepper et al., "Enhanced Plant Growth by Siderophores Produced by Plant Growth-Promoting Rhizobacteria," Nature 286:885-86 (1980), and Kloepper et 20 al., "Pseudomonas siderophores: A Mechanism Explaining Disease-Suppressive Soils," Curr. Microbiol. 4:317-20 (1980)). The colonization of root surfaces and thus the direct competition with pathogenic bacteria on the 25 surfaces is another mechanism of action (Kloepper et al., "Relationship of *in vitro* Antibiosis of Plant Growth-Promoting Rhizobacteria to Plant Growth and the Displacement of Root Microflora," Phytopathology 71:1020-24 (1981), Weller, et al., "Increased Growth of Wheat by 30 Seed Treatments With Fluorescent Pseudomonads, and Implications of *Pythium* Control," Can. J. Microbiol. 8:328-34 (1986), and Suslow et al., "Rhizobacteria of Sugar Beets: Effects of Seed Application and Root Colonization on Yield," Phytopathology 72:199-206 35 (1982)). Canola (rapeseed) studies have indicated PGPR

increased plant growth parameters including yields, seedling emergence and vigor, early-season plant growth (number of leaves and length of main runner), and leaf area (Kloepper et al., "Plant Growth-Promoting Rhizobacteria on Canola (rapeseed)," Plant Disease 72:42-46 (1988)). Studies with potato indicated greater yields when *Pseudomonas* strains were applied to seed potatoes (Burr et al., "Increased Potato Yields by Treatment of Seed Pieces With Specific Strains of *Pseudomonas* Fluorescens and *P. putida*," Phytopathology 68:1377-83 (1978), Kloepper et al., "Effect of Seed Piece Inoculation With Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers," Phytopathology 73:217-19 (1983), Geels et al., "Reduction of Yield Depressions in High Frequency Potato Cropping Soil After Seed Tuber Treatments With Antagonistic Fluorescent *Pseudomonas* spp.," Phytopathol. Z. 108:207-38 (1983), Howie et al., "Rhizobacteria: Influence of Cultivar and Soil Type on Plant Growth and Yield of Potato," Soil Biol. Biochem. 15:127-32 (1983), and Vrany et al., "Growth and Yield of Potato Plants Inoculated With Rhizosphere Bacteria," Folia Microbiol. 29:248-53 (1984)). Yield increase was apparently due to the competitive effects of the PGPR to eliminate pathogenic bacteria on the seed tuber, possibly by antibiosis (Kloepper et al., "Effect of Seed Piece Inoculation With Plant Growth-Promoting Rhizobacteria on Populations of *Erwinia carotovora* on Potato Roots and in Daughter Tubers," Phytopathology 73:217-19 (1983), Kloepper et al., "Effects of Rhizosphere Colonization by Plant Growth-Promoting Rhizobacteria on Potato Plant Development and Yield," Phytopathology 70:1078-82 (1980), Kloepper et al., "Emergence-Promoting Rhizobacteria: Description and Implications for Agriculture," pp. 155-164, Iron, Siderophores, and Plant Disease, T.R.

Swinburne, ed. Plenum, New York (1986), and Kloepfer et al., "Relationship of *in vitro* Antibiosis of Plant Growth-Promoting Rhizobacteria to Plant Growth and the Displacement of Root Microflora," Phytopathology 71:1020-24 (1981)). In several studies, plant emergence was improved using PGPR (Tipping et al., "Development of Emergence-Promoting Rhizobacteria for Supersweet Corn," Phytopathology 76:938-41 (1990) (abstract) and Kloepfer et al., "Emergence-Promoting Rhizobacteria: Description and Implications for Agriculture," pp. 155-164, Iron, Siderophores, and Plant Disease, T.R. Swinburne, ed. Plenum, New York (1986)). Numerous other studies indicated improved plant health upon treatment with rhizobacteria, due to biocontrol of plant pathogens (B. Schippers, "Biological Control of Pathogens With Rhizobacteria," Phil. Trans. R. Soc. Lond. B. 318:283-93 (1988), Schroth et al., "Disease-Suppressive Soil and Root-Colonizing Bacteria," Science 216:1376-81 (1982), Stutz et al., "Naturally Occurring Fluorescent Pseudomonads Involved in Suppression of Black Root Rot of Tobacco," Phytopathology 76:181-85 (1986), and D.M. Weller, "Biological Control of Soilborne Plant Pathogens in the Rhizosphere With Bacteria," Annu. Rev. Phytopathol. 26:379-407 (1988)).

Pathogen-induced immunization of a plant has been found to promote growth. Injection of *Peronospora tabacina* externally to tobacco xylem not only alleviated stunting but also promoted growth and development. Immunized tobacco plants, in both greenhouse and field experiments, were approximately 40% taller, had a 40% increase in dry weight, a 30% increase in fresh weight, and 4-6 more leaves than control plants (Tuzun, S., et al., "The Effect of Stem Injection with *Peronospora tabacina* and Metalaxyl Treatment on Growth of Tobacco and Protection Against Blue Mould in the Field,"

- 7 -

Phytopathology, 74:804 (1984). These plants flowered approximately 2-3 weeks earlier than control plants (Tuzun, S., et al., "Movement of a Factor in Tobacco Infected with *Peronospora tabacina* Adam which Systemically Protects Against Blue Mould," Systemically Protects Against Blue Mould," Physiological Plant Pathology, 26:321-30 (1985).

The present invention is directed to an improvement over prior plant growth enhancement procedures.

The discussion of the background to the invention herein is included to explain the context of the invention. This is not to be taken as admission that 10 any of the material referred to was published, known or part of the common general knowledge in Australia as at the priority date of any of the claims.

SUMMARY OF THE INVENTION

15 The present invention relates to a method of enhancing growth in plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or plant seeds under conditions to impart enhanced growth to the plants or to plants grown from the plant seed.

In one aspect the present invention provides a method of enhancing 20 growth in plants compared to untreated plants comprising:

applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants grown from the plant seed, compared to an untreated plant or plant seed, wherein the hypersensitive response elicitor 25 polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwina*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

In another aspect the present invention provides a method of enhancing growth in plants compared to untreated plants comprising:

30 providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein; and



- 7a -

growing the transgenic plants or transgenic plants grown from the transgenic plant seeds under conditions effective to enhance plant growth, compared to an untreated plant or plant seed, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a 5 pathogen selected from the group consisting of *Erwina*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

As an alternative to applying hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart enhanced growth to the plants or to plants grown from the seed, transgenic plants or plant 10 seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DMA molecule to enhance growth.

In one aspect the present invention provides a transgenic plant 15 transformed with a DNA molecule encoding a hypersensitive response elicitor in a form effective to enhance growth of the plant, wherein the hypersensitive response elicitor is derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

In another aspect the present invention provides a transgenic plant seed 20 transformed with a DNA molecule encoding a hypersensitive response elicitor in a form effective to enhance growth of a plant grown from the plant seed, wherein the hypersensitive response elicitor is derived from a pathogen, selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

25 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 DMA molecule to enhance growth.



The present invention is directed to effecting any form of plant growth enhancement or promotion. 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 5 quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit colouration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short 10 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. It is thus apparent that the present invention constitutes a significant advance in 15 agricultural efficiency.

Throughout the description and claims of this specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a map of plasmid vector pCPP2139 which contains the *Erwinia amylovora* hypersensitive response elicitor gene.

Figure 2 is a map of plasmid vector pCPP50 which does not contain the 25 *Erwinia amylovora* hypersensitive response elicitor gene but is otherwise the same as plasmid vector pCPP2139 shown in Figure 1. See Masui, et al., Bio/Technology 2:81-85 (1984), which is hereby incorporated by reference.

30



DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of enhancing growth in plants. This method involves 5 applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions to impart enhanced growth to the plant or to a plant grown from the plant seed. Alternatively, plants can be treated in this 10 manner to produce seeds, which when planted, impart enhanced growth in progeny plants.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart enhanced growth to the 15 plants or to plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to 20 permit that DNA molecule to enhance growth.

Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in 25 soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to enhance growth.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention 30 can correspond to hypersensitive response elicitor polypeptides or proteins derived from a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor.

- 10 -

Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, 5 *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solancearum*, *Xanthomonas campestris*, and mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide 10 is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora pythium*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The embodiment of the present invention where 15 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 polypeptide or protein; 2) application of bacteria which do not cause 20 disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the 25 hypersensitive response elicitor polypeptide or protein. In addition, seeds in accordance with the present invention can be recovered from plants which have been treated with a hypersensitive response elicitor protein or polypeptide in accordance with the present invention.

30 In one embodiment of the present invention, the hypersensitive response elicitor polypeptides or proteins can be isolated from their corresponding organisms and applied to plants or plant seeds. Such isolation procedures are well known, as described in 35 Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet,

- 11 -

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. 13:543-553 (1994); He, S. Y., H. 5 C. Huang, and A. Collmer, "Pseudomonas syringae pv. *syringae* Harpin_{ps}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, 10 and S. V. Beer, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*, Science 257:85-88 (1992), which are hereby incorporated by reference. See also U.S. Patent Nos. 5,849,868 and 5,708,139, which 15 are hereby incorporated by reference. Preferably, however, the isolated hypersensitive response elicitor polypeptides or proteins of the present invention are produced recombinantly and purified as described below.

In other embodiments of the present invention, 20 the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting 25 or exporting the polypeptide or protein so that the elicitor can contact plant or plant seeds cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria *in planta* or on seeds or just prior to introduction of the 30 bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive 35 response elicitor polypeptide or protein. For example,



- 12 -

E. coli, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than 5 *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a 10 hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or their seeds which are not susceptible to the disease carried by the bacteria. For example, *Erwinia amylovora* 15 causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato plants or seeds to enhance 20 growth without causing disease in that species.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

25

	Met	Gln	Ile	Thr	Ile	Lys	Ala	His	Ile	Gly	Gly	Asp	Leu	Gly	Val	Ser	
	1				5				10					15			
30		Gly	Leu	Gly	Ala	Gln	Gly	Leu	Lys	Gly	Leu	Asn	Ser	Ala	Ala	Ser	Ser
			20						25					30			
		Leu	Gly	Ser	Ser	Val	Asp	Lys	Leu	Ser	Ser	Thr	Ile	Asp	Lys	Leu	Thr
				35					40				45				
35		Ser	Ala	Leu	Thr	Ser	Met	Met	Phe	Gly	Gly	Ala	Leu	Ala	Gln	Gly	Leu
			50				55					60					
		Gly	Ala	Ser	Ser	Lys	Gly	Leu	Gly	Met	Ser	Asn	Gln	Leu	Gly	Gln	Ser
40			65					70				75		80			
		Phe	Gly	Asn	Gly	Ala	Gln	Gly	Ala	Ser	Asn	Leu	Leu	Ser	Val	Pro	Lys
					85					90				95			

- 13 -

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
100 105 110

5 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115 120 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130 135 140

10 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145 150 155 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165 170 175

15 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
180 185 190

20 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
195 200 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210 215 220

25 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225 230 235 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245 250 255

30 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260 265 270

35 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275 280 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290 295 300

40 Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305 310 315 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
325 330 335

45 Asn Ala

50 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 *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

- 14 -

CGATTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTGCA CACCGTTACG	60
GCCTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC	120
5 GATCTGGTAT TTCAGTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG	180
CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCAGCAGAG	240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
10 CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTGAACG GGCAGGAATG	360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC	420
15 CGATCATTAA GATAAAGGCG GCTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
CACCGTCGGC GTCACTCAGT AACAAAGTATC CATCATGATG CCTACATCGG GATCGCGTG	540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
20 AATTACGATC AAAGCGCACA TCGGGCGTGA TTTGGCGTC TCCGGTCTGG GGCTGGGTGC	660
TCAGGGACTG AAAGGACTGA ATTCCCGGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT	720
25 GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTG GCGGCGCGCT	780
GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGCGA	900
30 TCGTTGTCA AAAATGTTG ATAAAGCGCT GGACGATCTG CTGGGTATG ACACCGTGAC	960
CAAGCTGACT AACCAAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
35 CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAAC GCACTGTCGT CCATTCTCGG	1080
CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGG CAGGCGGCTT	1140
40 GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGCGT	1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
45 TCAGTATCCG GAAATATTG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
GACGGACGAC AAATCCTGGG CTAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440

- 15 -

CGCCAGCATG	GACAAATTCC	GTCAGGCGAT	GGGTATGATC	AAAAGCGCGG	TGGCGGGTGA	1500	
TACCGGCAAT	ACCAACCTGA	ACCTGCGTGG	CGCGGGCGGT	GCATCGCTGG	GTATCGATGC	1560	
5	GGCTGTCGTC	GGCGATAAAA	TAGCCAACAT	GTCGCTGGGT	AAGCTGGCCA	ACGCCTGATA	1620
	ATCTGTGCTG	GCCTGATAAA	GCGGAAACGA	AAAAAGAGAC	GGGGAAGCCT	GTCTCTTTTC	1680
10	TTATTATGCG	TTTTATGCGG	TTACCTGGAC	CGGTTAACATCA	TCGTCATCGA	TCTGGTACAA	1740
	ACGCACATTT	TCCC GTTCAT	TCGCGTCGTT	ACGCGCCACA	ATCGCGATGG	CATCTTCCTC	1800
	GTCGCTCAGA	TTGCGCGGCT	GATGGGAAC	GCCGGGTGGA	ATATAGAGAA	ACTCGCCGGC	1860
15	CAGATGGAGA	CACGTCTGCG	ATAAATCTGT	GCCGTAACGT	TTTCTATCC	GCCCCTTTAG	1920
	CAGATAGATT	GCGGTTTCGT	AATCAACATG	GTAATGCGGT	TCCGCCTGTG	CGCCGGCCGG	1980
20	GATCACCACA	ATATTCA TAG	AAAGCTGTCT	TGCACCTACC	GTATCGCGGG	AGATAACCGAC	2040
	AAAATAGGGC	AGTTTTGCG	TGGTATCCGT	GGGGTGTTC	GGCCTGACAA	TCTTGAGTTG	2100
	GTTCGTCATC	ATCTTTCTCC	ATCTGGCGA	CCTGATCGGT	T		2141

25 The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

30	Met	Ser	Leu	Asn	Thr	Ser	Gly	Leu	Gly	Ala	Ser	Thr	Met	Gln	Ile	Ser
	1							5					10		15	
	Ile	Gly	Gly	Ala	Gly	Gly	Asn	Asn	Gly	Leu	Leu	Gly	Thr	Ser	Arg	Gln
35								20					25		30	
	Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Asn	
							35					40		45		
40	Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
							50					55		60		
	Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Leu	Met	Gly	Gly	Leu		
45							65					70		75		80
	Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu
						85					90			95		
50	Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Gly	Asn
							100					105		110		

- 16 -

/

	Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
	115						120							125		
5	Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
	130						135						140			
	Thr	Ser	Gly	Thr	Asp	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln	Gln
	145						150					155		160		
10	Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
							165				170		175			
	Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
							180				185		190			
15	Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
							195				200		205			
20	Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
							210			215		220				
	Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
							225			230		235		240		
25	Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gly
							245			250		255				
	Leu	Gly	Asn	Ala	Val	Gly	Thr	Gly	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gly
							260			265		270				
30	Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
							275			280		285				
35	Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
							290			295		300				
	Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
							305			310		315		320		
40	Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
							325			330		335				
	Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn
							340			345		350				
45	Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn
							355			360		365				
50	Gly	Asn	Leu	Gln	Ala	Arg	Gly	Ala	Gly	Gly	Ser	Ser	Leu	Gly	Ile	Asp
							370			375		380				
	Ala	Met	Met	Ala	Gly	Asp	Ala	Ile	Asn	Asn	Met	Ala	Leu	Gly	Lys	Leu
							385			390		395		400		
55	Gly	Ala	Ala													

60 This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at

- 17 -

least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully 5 described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The 10 DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

15	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTGAA TTATTCATAA	60
	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTCT	120
20	ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAAATCAAAT ATGATACCGT CAATCAGCTG	240
	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
25	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
	GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
30	GGCGGCAACA ATACCACCTTC AACAAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
	TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
	CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
35	CAAGATGGCA CCCAGGGCAG TTCCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
40	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
	GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCGGGTGGA CTACCAGCAG	840
	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAAGGC GCTGAATGAT	900
45	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGCGA TCGGGCGATG	960
	GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTGGCAA GCCGCAGTAC	1020
50	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGCAAA AGCACTGAGC	1080
	AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
	ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
55	GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
	CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

- 18 -

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

5	Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
	1 5 10 15
10	Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
	20 25 30
15	Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
	35 40 45
20	Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
	50 55 60
25	Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
	65 70 75 80
30	Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
	85 90 95
35	Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
	100 105 110
40	Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
	115 120 125
45	Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
	130 135 140
50	Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
	145 150 155 160
55	Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
	165 170 175
60	Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
	180 185 190
65	Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
	195 200 205
70	Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
	210 215 220
75	Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
	225 230 235 240
80	Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
	245 250 255
85	Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Leu Gly Thr Pro Val
	260 265 270
90	Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
	275 280 285

- 19 -

	Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala
	290						295					300				
5	Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala
	305					310				315		320				
	Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg
					325				330				335			
10	Asn	Gln	Ala	Ala	Ala											
					340											

This hypersensitive response elicitor polypeptide or protein 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 hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin_{ps}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

	ATGCAGAGTC	TCAGTCTTAA	CAGCAGCTCG	CTGCAAACCC	CGGCAATGGC	CCTTGTCTG	60
30	GTACGTCCTG	AAGCCGAGAC	GACTGGCAGT	ACGTCGAGCA	AGGCGCTTCA	GGAAGTTGTC	120
	GTGAAGCTGG	CCGAGGAACT	GATGCGCAAT	GGTCAACTCG	ACGACAGCTC	GCCATTGGGA	180
35	AAACTGTTGG	CCAAGTCGAT	GGCCGCAGAT	GGCAAGGCCG	GCGGCCGTAT	TGAGGATGTC	240
	ATCGCTGCGC	TGGACAAGCT	GATCCATGAA	AAGCTCGGTG	ACAACCTCGG	CGCGTCTGCG	300
	GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
40	AAGTCGATGC	TCGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
	GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCGC	ACAGTTTCCC	480
45	AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAACCTCCT	TGATGGCGAC	540
	GAAACGGCTG	CGTTCCGTT	GGCACTCGAC	ATCATTGCC	AGCAACTGGG	TAATCAGCAG	600
	AGTGACGCTG	GCAGTCTGGC	AGGGACGGGT	GGAGGTCTGG	GCACTCCGAG	CAGTTTTCC	660
50	AACAACTCGT	CCGTGATGGG	TGATCCGCTG	ATCGACGCCA	ATACCGGTCC	CGGTGACAGC	720
	GGCAATACCC	GTGGTGAAGC	GGGGCAACTG	ATCGGCGAGC	TTATCGACCG	TGGCCTGCAA	780
	TCGGTATTGG	CCGGTGGTGG	ACTGGGCACA	CCCGTAAACA	CCCCGCAGAC	CGGTACGTCG	840

- 20 -

	GCGAATGGCG	GACAGTCCGC	TCAGGATCTT	GATCAGTTGC	TGGGCGGCTT	GCTGCTCAAG	900
	GGCCTGGAGG	CAACGCTCAA	GGATGCCGGG	CAAACAGGCA	CCGACGTGCA	GTCGAGCGCT	960
5	GCGCAAATCG	CCACCTTGCT	GGTCAGTACG	CTGCTGCAAG	GCACCCGCAA	TCAGGCTGCA	1020
	GCCTGA						1026

10 The hypersensitive response elicitor
polypeptide or protein derived from *Pseudomonas*
solanacearum has an amino acid sequence corresponding to
SEQ. ID. No. 7 as follows:

15 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
 1 5 10 15
 Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
 20 25 30
 20 Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
 35 40 45
 25 Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60
 Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
 65 70 75 80
 30 Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
 85 90 95
 Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
 100 105 110
 35 Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
 115 120 125
 40 Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
 130 135 140
 Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
 145 150 155 160
 45 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
 165 170 175
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
 50 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 55 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 60

- 21 -

	Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn
									245			250				255
5	Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Gly	Asn	Gln
				260				265			270					
	Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly
					275			280				285				
10	Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser
					290			295			300					
	Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val
15					305			310			315				320	
	Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Gly	Ser	Gln
					325			330			335					
	Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met								
20					340											

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 8 as follows:

25	ATGTCAGTCG	GAAACATCCA	GAGCCCGTCG	AACCTCCCGG	GTCTGCAGAA	CCTGAACCTC	60
	AACACCAACA	CCAACAGCCA	GCAATCGGGC	CAGTCCGTGC	AAGACCTGAT	CAAGCAGGTC	120
30	GAGAAGGACA	TCCTCAACAT	CATCCGAGCC	CTCGTGCAGA	AGGCCGCACA	GTCGGCGGGC	180
	GGCAACACCG	GTAACACCGG	CAACCGGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
35	AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
	GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAG	CGCTGATGCA	GCTGCTGGAA	360
	GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCCGGCGG	CAATGACAAG	420
40	GGCAACGGCG	TGGGCGGTGC	CAACGGCGCC	AAGGGTGCCG	GCGGCCAGGG	CGGCCTGGCC	480
	GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	GCGGCCGGCGG	TGCTGGCGCC	540
45	GGCGCGCGGG	GTGGCGGTGT	CGGCGGTGCT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
	GGCGCAGGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
	GGCCCGCAGA	ACGCAGGCCA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAC	720
50	CAGGGCGGCC	TCACCGGGCT	GCTGAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
	ATGATGCAGC	AAGGCGGCC	CGGCGGCC	AACCAAGGCC	AGGGCGGCTC	GAAGGGTGCC	840
55	GGCAACGCCT	CGCCGGCTTC	CGGCGCGAAC	CCGGGCGCGA	ACCAGCCCGG	TTCGGCGGGAT	900
	GATCAATCGT	CCGGCCAGAA	CAATCTGCAA	TCCCAGATCA	TGGATGTGGT	GAAGGAGGTC	960
	GTCCAGATCC	TGCAGCAGAT	GCTGGCGGCC	CAGAACGGCG	GCAGCCAGCA	GTCCACCTCG	1020
60	ACGCAGGCCA	TGTAA					1035

- 22 -

Further information regarding 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 5 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.

The hypersensitive response elicitor 10 polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

15 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

20 This sequence is an amino terminal sequence having 26 residues only from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins 25 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 an amino acid 30 sequence corresponding to SEQ. ID. No. 10 as follows:

35 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
 1 5 10 15
 Leu Leu Ala Met
 20

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

subsp. *carotovora* Strain Ecc71 Overexpress *hrp N_{Ecc}* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response 5 elicitor protein or polypeptide is shown 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 10 *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, 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 Specificity and Induction of Resistance to Bacterial and Fungal 15 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 20 Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al., "A New Elicitor of the Hypersensitive Response in 25 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 30 and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

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

It is also possible to use fragments of the above hypersensitive response elicitor polypeptides or 10 proteins as well as fragments of full length elicitors from other pathogens, in the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene 5 fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or a peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor 10 protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino 15 acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the 20 elicitor 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

- 25 -

increase and expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out 5 using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

10 An example of a useful fragment is the popA1 fragment of the hypersensitive response elicitor polypeptide or protein from *Pseudomonas solanacearum*. See Arlat, M., F. Van Gijsegem, J.C. Huet, J.C. Pemollet, and C.A. Boucher, "PopA1, a Protein Which Induces a 15 Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), which is hereby incorporated by reference. As to *Erwinia amylovora*, a suitable fragment can be, for example, either or both the 20 polypeptide extending between and including amino acids 1 and 98 of SEQ. ID. No. 3 and the polypeptide extending between and including amino acids 137 and 204 of SEQ. ID. No. 3.

25 Variants may also (or alternatively) be modified 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- 30 terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide.

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

The DNA molecule encoding the hypersensitive response elicitor polypeptide or 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 proper 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 prokaryotic organisms and eucaryotic cells grown in tissue culture.

5 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.

10 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 15 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 20 thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard 25 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.

30 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 cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; 35 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 5 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.

Different genetic signals and processing events 10 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 promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby 15 promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, 20 procaryotic promotors 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. Efficient 25 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 30 protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably 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.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors 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 number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, lac promotor, trp promotor, recA promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to lacUV5, ompF, bla, lpp, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid trp-lacUV5 (tac) promotor or other *E. coli* promotors 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 inhibit the action of the promotor 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 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 promotor, may also contain any combination of various "strong" 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 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 hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. 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.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to enhance growth. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: rose, *Saintpaulia*,

- 31 -

petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

The method of the present invention involving 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.

5 Suitable application methods include topical application (e.g., high or low pressure spraying), injection, dusting, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by topical application (low or high pressure spraying), coating, immersion, dusting, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they 10 are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated 15 using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide 20 to enhance growth in the plants. Such propagated plants may, in turn, be useful in producing seeds or propagules (e.g., cuttings) that produce plants capable of enhanced 25 growth.

The hypersensitive response elicitor 30 polypeptide or protein can be applied to plants or plant

seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials 5 being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier.

10 Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 0.5 nM hypersensitive response elicitor polypeptide or protein.

15 Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, herbicide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

20 Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein 25 can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

30 In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced 35 according to procedures well known in the art, such as by

biolistics or *Agrobacterium* mediated transformation. Examples of suitable hypersensitive response elicitor polypeptides or proteins and the nucleic acid sequences for their encoding DNA are disclosed *supra*. Once 5 transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in enhanced growth of the plant. Alternatively, 10 transgenic seeds are recovered from the transgenic plants. These 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 under conditions 15 effective to impart enhanced growth. While not wishing to be bound by theory, such growth enhancement may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used 20 in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including 25 hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, dusting, and immersion. Similarly, after plants have been propagated from the transgenic 30 plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to enhance plant growth. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.). The transgenic plants 35 of the present invention are useful in producing seeds or

- 34 -

propagules (e.g., cuttings) from which plants capable of enhanced growth would be produced.

EXAMPLES

5

Example 1 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Germination Percentage

10 Seeds of the Marglobe Tomato Variety were submerged in 40ml of *Erwinia amylovora* hypersensitive response elicitor solution ("harpin"). Harpin was prepared by growing *E. coli* strain DH5 containing the plasmid pCPP2139 (see Figure 1), lysing the cells by 15 sonication, heat treating by holding in boiling water for 5 minutes before centrifuging to remove cellular debris, and precipitating proteins and other heat-labile components. The resulting preparation ("CFEP") was diluted serially. These dilutions (1:40, 1:80, 1:160, 20 1:320 and 1:640) contained 20, 10, 5, 2.5, and 1.25 μ gm/ml, respectively, of harpin based on Western Blot assay. Seeds were soaked in harpin or buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After 25 soaking, the seeds were sown in germination pots with artificial soil on day 1. This procedure was carried out on 100 seeds per treatment.

Treatments:

30 1. Seeds in harpin (1:40) (20 μ gm/ml).
2. Seeds in harpin (1:80) (10 μ gm/ml).
3. Seeds in harpin (1:160) (5 μ gm/ml).
4. Seeds in harpin (1:320) (2.5 μ gm/ml).
5. Seeds in harpin (1:640) (1.25 μ gm/ml).
6. Seeds in buffer (5mM KPO₄, pH 6.8).

35

- 35 -

Table 1 - Number of Seedlings After Seed Treatment

	Treatment	Day 0	Number of seeds germinated			
			Day 1	Day 5	Day 7	Day 9
5	Harpin seed soak (20 μ gm/ml)	sowing	43	57	59	
	Harpin seed soak (10 μ gm/ml)	sowing	43	52	52	
	Harpin seed soak (5 μ gm/ml)	sowing	40	47	51	
	Harpin seed soak (2.5 μ gm/ml)	sowing	43	56	58	
10	Harpin seed soak (1.25 μ gm/ml)	sowing	38	53	57	
	Buffer seed soak	sowing	27	37	40	

As shown in Table 1, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor reduced the time needed for germination and greatly increased the percentage of germination.

Example 2 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

Seeds of the Marglobe Tomato Variety were submerged in *Erwinia amylovora* harpin (1:15, 1:30, 1:60, and 1:120) or buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with artificial soil on day 1.

Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

Treatments:

1. Harpin (1:15) (52 μ gm/ml).
2. Harpin (1:30) (26 μ gm/ml).
3. Harpin (1:60) (13 μ gm/ml).
4. Harpin (1:120) (6.5 μ gm/ml).
5. Buffer (5mM KPO₄, pH 6.8).

Table 2 - Seedling Height (cm) 15 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
52 μ gm/ml	10	5.6	5.8	5.8	5.6	6.0	6.0	5.8	5.4	5.8	5.6	5.7
26 μ gm/ml	10	6.8	7.2	6.6	7.0	6.8	6.8	7.0	7.4	7.2	7.0	7.0
13 μ gm/ml	10	5.8	5.6	6.0	5.6	5.8	5.8	5.6	5.8	6.0	5.6	5.9
6.5 μ gm/ml	10	5.4	5.2	5.6	5.4	5.2	5.4	5.6	5.6	5.4	5.2	5.4
Buffer	10	5.6	5.4	5.2	5.2	5.4	5.2	5.0	5.2	5.4	5.6	5.3

-36-

Table 3 - Seedling Height (cm) 21 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
52 μ gm/ml	10	7.6	7.8	7.6	7.6	7.8	7.8	7.8	7.4	7.6	7.6	7.7
26 μ gm/ml	10	8.2	8.2	8.0	9.0	8.4	8.6	8.6	9.0	9.2	9.0	8.6
13 μ gm/ml	10	6.8	6.6	6.8	6.8	6.8	6.8	6.6	7.2	7.0	7.2	6.9
6.5 μ gm/ml	10	6.8	6.6	6.6	6.4	6.8	6.6	6.8	6.6	6.6	6.8	6.7
Buffer	10	6.6	6.4	6.2	6.6	6.4	6.6	6.8	6.4	6.4	6.6	6.5

Table 4 - Seedling Height (cm) 27 Days After Seed Treatment.

Treat	1	2	3	4	5	6	7	8	9	10	Mean
52 μ gm/ml	10.2	10.6	10.4	10.6	10.4	10.6	10.8	10.4	10.8	10.6	10.5
26 μ gm/ml	11.6	11.4	11.6	11.8	11.8	11.8	11.6	11.4	11.6	11.4	11.6
13 μ gm/ml	9.8	9.6	9.8	9.6	9.8	9.8	9.6	9.4	9.6	9.8	9.7
6.5 μ gm/ml	9.4	9.4	9.6	9.4	9.6	9.4	9.6	9.6	9.4	9.2	9.5
Buffer	9.6	10.2	10.0	9.8	10.0	10.2	10.0	10.2	10.4	9.6	10.0

Table 5 - Summary--Mean Height of Tomato Plants after Treatment.

5	Treatment	Mean height of tomato plants (cm)			
		Day 0	Day 1	Day 15	Day 21
	Harpin seed soak (1:15)	sowing	5.7	7.7	10.5
	Harpin seed soak (1:30)	sowing	7.0	8.6	11.6
	Harpin seed soak (1:60)	sowing	5.9	6.9	9.7
10	Harpin seed soak (1:120)	sowing	5.4	6.7	9.5
	Buffer seed soak	sowing	5.3	6.5	10.0

As shown in Tables 2-5, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor increased plant growth. A 1:30 dilution had the greatest effect -- a 16% increase in 5 seedling height.

Example 3 - Effect of Treating Tomato Plants with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

When Marglobe tomato plants were 4 weeks old, they were sprayed with 6 ml/plant of *Erwinia amylovora* harpin solution containing 13 μ gm/ml (1:60) or 8.7 μ gm/ml (1:90) of harpin or buffer (5mM KPO₄) in a growth chamber at 28°C. The heights of tomato plants were measured 2 weeks after spraying harpin (6-week-old tomato plants) and 2 weeks plus 5 days after spraying. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the 20 surface of soil to the top of plant.

Treatments:

1. Harpin (1:60) (13 μ gm/ml).
2. Harpin (1:90) (8.7 μ gm/ml).
3. Buffer (5mM KPO₄, pH 6.8).

Table 6 - Mean Height of Tomato Plants after Treatment With Harpin.

5	Operation and Treatment			Mean height (cm) of tomato plants	
	Day 0 sowing	Day 14 transplant	Day 28 harpin 1:60 (13 μ gm/ml)	Day 42	Day 47
10				35.5	36.0
	sowing	transplant	harpin 1:90 (8.7 μ gm/ml)	35.7	36.5
15	sowing	transplant	buffer	32.5	33.0

As shown in Table 6, spraying tomato seedlings with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. Similar increases in growth were noted for the two doses of the hypersensitive response elicitor tested compared with the buffer-treated control.

Example 4 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Tomato Plant Height

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor solution ("harpin") (1:40, 1:80, 1:160, 1:320, and 1:640) or buffer in beakers on day 0 for 24 hours at 28°C in the growth chamber. After soaking seeds in harpin or buffer, they were sown in germination pots with artificial soil on day 1. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

- 40 -

Treatments:

1. Harpin (1:40) (20 μ gm/ml).
2. Harpin (1:80) (10 μ gm/ml).
3. Harpin (1:160) (5 μ gm/ml).
4. Harpin (1:320) (2.5 μ gm/ml).
5. Harpin (1:640) (1.25 μ gm/ml).
6. Buffer (5mM KPO₄, pH 6.8).

Table 7 - Seedling Height (cm) 12 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
20 μ gm/ml	10	6.5	6.8	6.8	6.5	6.4	6.4	6.8	6.4	6.8	6.6	6.6
10 μ gm/ml	10	6.8	6.2	6.6	6.4	6.8	6.8	6.6	6.4	6.8	6.4	6.6
5 μ gm/ml	10	6.2	6.6	6.0	6.6	6.4	6.2	6.6	6.2	6.0	6.6	6.3
2.5 μ gm/ml	10	6.4	6.2	6.6	6.0	6.2	6.4	6.0	6.0	6.2	6.2	6.2
1.25 μ gm/ml	10	6.2	6.2	6.0	6.4	6.0	6.0	6.4	6.2	6.4	6.2	6.2
Buffer	10	5.8	6.0	6.2	6.2	5.8	5.8	6.0	6.2	6.0	6.0	6.0

Table 8 - Seedling Height (cm) 14 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
20 μ gm/ml	10	7.8	7.8	8.2	8.0	8.2	8.4	7.8	8.4	7.6	7.8	8.0
10 μ gm/ml	10	8.6	8.8	8.4	9.2	8.4	8.6	7.8	7.8	8.4	8.4	8.4
5 μ gm/ml	10	9.8	9.2	9.8	9.6	9.2	9.4	8.6	9.2	9.0	8.6	9.2
2.5 μ gm/ml	10	8.8	8.6	8.6	8.4	7.8	8.6	8.4	9.0	8.0	7.8	8.4
1.25 μ gm/ml	10	8.4	7.8	8.4	8.0	8.6	8.4	8.0	8.2	8.4	8.2	8.2
Buffer	10	7.2	8.2	7.4	7.6	7.8	7.6	7.8	7.4	7.8	7.6	7.6

Table 9 - Seedling Height (cm) 17 Days After Seed Treatment.

Treat	Plants	1	2	3	4	5	6	7	8	9	10	Mean
20 $\mu\text{gm}/\text{ml}$	10	11.2	11.6	11.4	11.6	11.4	11.2	11.8	11.4	11.8	11.6	11.5
10 $\mu\text{gm}/\text{ml}$	10	13.4	13.4	13.8	13.2	13.4	12.6	12.4	13.4	13.2	13.4	13.2
5 $\mu\text{gm}/\text{ml}$	10	13.6	12.8	13.6	13.2	14.2	13.8	12.6	13.4	13.8	13.6	13.5
2.5 $\mu\text{gm}/\text{ml}$	10	11.6	12.4	12.4	11.8	11.6	12.2	12.6	11.8	12.0	11.6	12.0
1.25 $\mu\text{gm}/\text{ml}$	10	12.8	12.6	12.0	12.4	11.6	11.8	12.2	11.4	11.2	11.4	11.9
Buffer	10	10.0	10.4	10.6	10.6	10.4	10.4	10.8	10.2	10.4	10.0	10.4

Table 10 -Summary - Mean Height of Tomato Plants After Treatment

Operation and Treatment	Mean height of tomato plants(cm)				
	Day 0	Day 1	Day 12	Day 14	Day 17
Harpin seed soak (20 $\mu\text{gm}/\text{ml}$)	sowing	6.6	8.0	11.5	
Harpin seed soak (10 $\mu\text{gm}/\text{ml}$)	sowing	6.6	8.4	13.2	
Harpin seed soak (5 $\mu\text{gm}/\text{ml}$)	sowing	6.3	9.2	13.5	
Harpin seed soak (2.5 $\mu\text{gm}/\text{ml}$)	sowing	6.2	8.4	12.0	
Harpin seed soak (1.25 $\mu\text{gm}/\text{ml}$)	sowing	6.2	8.2	11.9	
Buffer seed soak	sowing	6.0	7.6	10.4	

As shown in Tables 7-10, the treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. A 1:160 dilution (5 μ g/ml harpin) had the greatest effect -- seedling height was increased more than 20% over the buffer treated plants.

10 **Example 5 - Effect of Treating Tomato Seeds with *Erwinia amylovora* Hypersensitive Response Elicitor on Seed Germination Percentage**

15 Marglobe tomato seeds were submerged in 40ml of *Erwinia amylovora* hypersensitive response elicitor ("harpin") solution (dilutions of CFEP from *E. coli* DH5 (pCPP2139) of 1:50 or 1:100 which contained, respectively, 8 μ gm/ml and 4 μ gm/ml of hypersensitive response elicitor) and buffer in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking, the seeds were sown in germination pots with artificial soil 20 on day 1. This treatment was carried out on 20 seeds per pot and 4 pots per treatment.

Treatments:

25 1. Harpin (8 μ gm/ml).
2. Harpin (8 μ gm/ml).
3. Harpin (8 μ gm/ml).
4. Harpin (8 μ gm/ml).
5. Harpin (4 μ gm/ml).
6. Harpin (4 μ gm/ml).
30 7. Harpin (4 μ gm/ml).
8. Harpin (4 μ gm/ml).
9. Buffer (5mM KPO₄, pH 6.8).
10. Buffer (5mM KPO₄, pH 6.8).
11. Buffer (5mM KPO₄, pH 6.8).
35 12. Buffer (5mM KPO₄, pH 6.8).

- 44 -

Table 11 - Number of Seedlings After Seed Treatment With Harpin

5	Operation and Treatment	Number of seeds germinated (out of a total of 20)					
		Day 0	Day 1	Day 5	Day 42	Day 47	
				Mean		Mean	Mean
10	Harpin (8 μ gm/ml)	sowing		11	15		19
	Harpin (8 μ gm/ml)	sowing		13	17		20
	Harpin (8 μ gm/ml)	sowing		10	13		16
	Harpin (8 μ gm/ml)	sowing		9 10.8	15 15.0	16	17.8
15	Harpin (4 μ gm/ml)	sowing		11	17		17
	Harpin (4 μ gm/ml)	sowing		15	17		18
	Harpin (4 μ gm/ml)	sowing		9	12		14
	Harpin (4 μ gm/ml)	sowing		9 11.0	14 15.0	16	16.3
20	Buffer	sowing		11	11		14
	Buffer	sowing		9	14		15
	Buffer	sowing		10	14		14
	Buffer	sowing		10 10.0	12 12.8	14	14.3

25

As shown in Table 11, treatment of tomato seeds with *Erwinia amylovora* hypersensitive response elicitor can increase germination rate and level of tomato seeds. The higher dose used appeared to be more effective than buffer at the end of the experiment.

10

Example 6 - Effect on Plant Growth of Treating Tomato Seeds with Proteins Prepared from *E. coli* Containing a Hypersensitive Response Elicitor Encoding Construct, pCPP2139, or Plasmid Vector pCPP50

15

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor ("harpin") (from *E. coli* DH5 α (pCPP2139) (Figure 1) or vector preparation (from DH5 α (pCPP50) (Figure 2) with added BSA protein as control. The control vector preparation contained, per ml, 33.6 μ l of BSA (10 mg/ml) to provide about the same amount of protein as contained in the

- 45 -

pCPP2139 preparation due to harpin. Dilutions of 1:50 (8.0 μ g/ml), 1:100 (4.0 μ g/ml), and 1:200 (2.0 μ g/ml) were prepared in beakers on day 1, and seed was submerged for 24 hours at 28°C in a controlled environment chamber.

5 After soaking, seeds were sown in germination pots with artificial soil on day 2. Ten uniform appearing plants per treatment were chosen randomly and measured at three times after transplanting. The seedlings were measured by ruler from the surface of soil to the top of plant.

10

Treatments:

15

1.	Harpin	1:50	(8.0 μ g/ml)
2.	Harpin	1:100	(4.0 μ g/ml)
3.	Harpin	1:200	(2.0 μ g/ml)
4.	Vector + BSA	1:50	(0 harpin)
5.	Vector + BSA	1:100	(0 harpin)
6.	Vector + BSA	1:200	(0 harpin)

Table 12 - Seedling Height (cm) 18 Days After Seed Treatment

Treat	Harpin	1	2	3	4	5	6	7	8	9	10	Mean
H1:50	8.0	3.6	5.0	4.8	5.0	4.2	5.2	5.8	4.6	4.0	4.8	4.7
H1:100	4.0	4.6	5.8	6.2	6.0	5.6	6.8	6.0	4.8	5.6	6.2	5.8
H1:200	2.0	4.0	5.8	5.8	4.6	5.4	5.0	5.8	4.6	4.6	5.8	5.1
V1:50	0	3.8	5.0	4.6	5.4	5.6	4.6	5.0	5.2	4.6	4.8	4.9
V1:100	0	4.4	5.2	4.6	4.4	5.4	4.8	5.0	4.6	4.4	5.2	4.8
V1:200	0	4.2	4.8	5.4	4.6	5.0	4.8	4.8	5.4	4.6	5.0	4.9

Table 13 - Seedling Height (cm) 22 Days After Seed Treatment.

Treat	Harpin	1	2	3	4	5	6	7	8	9	10	Mean
H1:50	8.0	4.2	5.6	5.2	6.0	4.8	5.4	5.0	5.2	5.4	5.0	5.2
H1:100	4.0	7.6	6.8	7.0	7.2	6.8	7.4	7.6	7.0	6.8	7.4	7.2
H1:200	2.0	7.0	6.6	6.8	7.2	7.4	6.8	7.0	7.2	6.8	7.2	7.0
V1:50	0	5.6	5.8	6.2	6.4	5.6	5.2	5.6	5.8	6.0	5.8	5.8
V1:100	0	5.4	6.0	5.8	6.2	5.8	5.6	5.4	5.2	6.0	5.6	5.7
V1:200	0	5.2	6.2	5.8	5.4	6.2	6.0	5.6	6.4	5.8	6.0	5.9

Table 14 - Seedling Height (cm) 26 Days After Seed Treatment.

Treat.	Harpin	1	2	3	4	5	6	7	8	9	10	Mean
H1:50	8.0	7.6	8.4	8.8	6.8	9.6	8.2	7.4	9.8	9.2	9.0	8.5
H1:100	4.0	12.0	11.4	11.2	11.0	10.8	12.0	11.2	11.6	10.4	10.2	11.2
H1:200	2.0	10.6	11.2	11.6	10.2	11.0	10.8	10.0	11.8	10.2	10.6	10.8
V1:50	0	9.0	9.4	8.8	8.4	9.6	9.2	9.2	8.6	8.0	9.4	9.2
V1:100	0	9.2	10.0	9.8	9.6	8.4	9.4	9.6	9.8	8.0	9.6	9.3
V1:200	0	8.8	9.6	8.2	9.2	8.4	8.0	9.8	9.0	9.4	9.2	9.0

Table 15 - Mean Height of Tomato Plants After Treatment

-47-

Operation and Treatment Day 1	Day 2	Mean height of tomato plants (cm)		
		Day 18	Day 22	Day 26
Harpin (1:50) (8.0 μ gm/ml)	sowing	4.7	5.2	8.5
Harpin (1:100) (4.0 μ gm/ml)	sowing	5.8	7.2	11.2
Harpin (1:200) (2.0 μ gm/ml)	sowing	5.1	7.0	10.8
Vector + BSA (1:50) (0)	sowing	4.9	5.8	9.2
Vector + BSA (1:100) (0)	sowing	4.8	5.7	9.3
Vector + BSA (1:200) (0)	sowing	4.9	5.9	9.0

- 48 -

As shown in Tables 12-15, treatment with *E. coli* containing the gene encoding the *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. The 1:100 dilution (4.0 µg/ml) had the 5 greatest effect, while higher and lower concentrations had less effect. Mean seedling height for treatment with 4.0 µg/ml of harpin was increased about 20% relative to vector control preparation, which contained a similar amount of non-harpin protein. Components of the lysed 10 cell preparation from the strain *E. coli* DH5α(pCPP50), which harbors the vector of the *hrpN* gene in *E. coli* strain DH5α(pCPP2139), do not have the same growth-promoting effect as the harpin-containing preparation, even given that it is supplemented with BSA protein to 15 the same extent as the DH5α(pCPP2139) preparation, which contains large amounts of harpin protein.

20 **Example 7 - Effect on Tomato Plant Growth of Treating Tomato Seeds with Proteins Prepared from *E. coli* Containing a Hypersensitive Response Elicitor Encoding Construct, pCPP2139, or its Plasmid Vector pCPP50**

Marglobe tomato seeds were submerged in *Erwinia amylovora* hypersensitive response elicitor solution ("harpin") (from the harpin encoding plasmid pCPP2139 vector) and from pCPP50 vector-containing solution at dilutions of 1:25, 1:50, and 1:100 in beakers on day 1 for 24 hours at 28°C in a growth chamber. After soaking 30 seeds, they were sown in germination pots with artificial soil on day 2. Ten uniform appearing plants per treatment were chosen randomly and measured. The seedlings were measured by ruler from the surface of soil to the top of plant.

- 49 -

Treatments:

1. Harpin 16 μ gm/ml
2. Harpin 8 μ gm/ml
3. Harpin 4 μ gm/ml
4. Vector 16 μ gm/ml
5. Vector 8 μ gm/ml
6. Vector 4 μ gm/ml

Table 16 - Seedling Height (cm) 11 Days After Seed Treatment

Treat.	Harpin	Plants	1	2	3	4	5	6	7	8	9	10	Mean
H1:25	16 μ gm/ml	10	5.0	5.2	4.8	4.6	4.4	4.6	3.8	4.2	3.8	4.2	4.5
H1:50	8 μ gm/ml	10	5.6	5.4	6.0	5.8	4.8	6.8	5.8	5.0	5.2	4.8	5.5
H1:100	4 μ gm/ml	10	5.2	5.6	5.0	5.0	5.0	4.8	5.0	5.6	4.8	5.2	5.1
V1:25	0	10	4.4	4.4	4.8	4.6	4.8	4.6	4.0	4.8	4.4	4.6	4.5
V1:50	0	10	4.8	4.4	4.6	4.0	4.4	4.2	4.6	4.0	4.4	4.2	4.4
V1:100	0	10	4.6	4.2	4.8	4.4	4.4	4.0	4.2	4.0	4.4	4.0	4.3

-50-

Table 17 - Seedling Height (cm) 14 Days After Seed Treatment

Treat.	Harpin	Plants	1	2	3	4	5	6	7	8	9	10	Mean
H1:25	16 μ gm/ml	10	7.6	7.6	7.2	7.4	7.8	7.8	7.6	7.0	7.4	7.0	7.4
H1:50	8 μ gm/ml	10	8.5	8.2	8.4	7.6	7.8	8.4	8.6	9.0	7.6	8.2	8.2
H1:100	4 μ gm/ml	10	7.2	8.4	8.2	7.4	8.0	7.6	7.6	8.0	8.6	7.6	7.9
V1:25	0	10	6.8	6.4	7.8	6.6	6.6	6.8	7.4	6.0	6.4	6.4	6.7
V1:50	0	10	6.6	5.8	6.4	7.6	7.4	7.2	6.8	6.6	6.4	5.8	6.7
V1:100	0	10	6.2	6.0	6.8	6.6	6.4	5.8	6.6	7.0	5.8	6.4	6.4

- 51 -

Table 18 - Mean Height of Tomato Plants After Treatment.

5	Operation and Treatment	Mean height of tomato plants (cm)			
		Day 1	Day 2	Day 11	Day 14
10	Harpin seed soak (16 µgm/ml)	sowing	4.5	7.4	
	Harpin seed soak (8 µgm/ml)	sowing	5.5	8.2	
	Harpin seed soak (4 µgm/ml)	sowing	5.1	7.9	
	Vector seed soak (16 µgm/ml)	sowing	4.5	6.7	
	Vector seed soak (8 µgm/ml)	sowing	4.4	6.7	
	Vector seed soak (4 µgm/ml)	sowing	4.3	6.4	
15	-----	-----	-----	-----	-----

As shown in Tables 16-18, treatment with *Erwinia amylovora* hypersensitive response elicitor can increase growth of tomato plants. A 1:50 dilution (8 µg/ml hypersensitive response elicitor) had the greatest effect with seedling height being increased by about 20% over the control.

10 Example 8 - Effect of Cell-Free *Erwinia amylovora* Hypersensitive Response Elicitor on Growth of Potato

Three-week-old potato plants, variety Norchip, were grown from tuber pieces in individual containers. 15 The foliage of each plant was sprayed with a solution containing *Erwinia amylovora* hypersensitive response elicitor ("harpin"), or a control solution containing proteins of *E. coli* and those of the vector pCPP50 ("vector"), diluted 1:50, 1:100, and 1:200. On day 20, 20 12 uniform appearing plants were chosen randomly for each of the following treatments. One plant from each treatment was maintained at 16°C, in a growth chamber, while two plants from each treatment were maintained on a greenhouse bench at 18-25°C. Twenty-five days after 25 treatment, the shoots (stems) on all plants were measured individually.

- 52 -

Treatments:

1. Harpin 1:50	16 μ gm/ml
2. Harpin 1:100	8 μ gm/ml
3. Harpin 1:200	4 μ gm/ml
4. Vector 1:50	0 harpin
5. Vector 1:100	0 harpin
6. Vector 1:200	0 harpin

Table 19 - Length of Potato Stems of Plants at 16°C

Treatment on day 20	Length of potato stems (cm) stem on day 45						Plant Mean
	stem 1	stem 2	stem 3	stem 4	stem 5	stem 6	
Harpin 1:50	43.0	39.5	42.5	34.0	38.0	39.5	39.4
Harpin 1:100	42.0	38.5	(2 branch)				40.3
Harpin 1:200	35.5	30.5	31.5	(3 branch)			32.5
Vector 1:50	34.0	32.0	31.5	28.0	27.5	(5 branch)	30.6
Vector 1:100	30.0	33.5	33.0	30.0	28.0	33.0	31.3
Vector 1:200	33.5	31.5	32.5	(3 branch)			32.5

Table 20 - Length of Potato Stems of Plants on a Greenhouse Bench

Treatment on day 20	Length of potato stems (cm) on day 45						Treat. Mean
	stem 1	stem 2	stem 3	stem 4	stem 5	stem 6	
Harpin 1:50	65.5	58.5	57.5	62.5	68.5	(5 branch)	62.5
Harpin 1:50	62.5	67.0	65.0	69.0	(4 branch)		65.9
Harpin 1:100	70.5	73.5	74.0	80.5	(4 branch)		74.6
Harpin 1:100	83.0	80.5	76.5	76.0	81.5	(5 branch)	79.5
Harpin 1:200	56.5	59.5	50.5	53.0	55.5	48.0	53.9
Harpin 1:200	57.0	59.5	69.5	(3 branch)			62.0
Vector 1:50	53.0	62.0	59.5	62.5	(4 branch)		59.3
Vector 1:50	52.0	46.0	61.5	56.5	61.5	57.0	55.8
Vector 1:100	62.0	51.5	66.0	67.5	62.0	63.0	62.0
Vector 1:100	61.5	62.5	59.0	65.5	63.0	63.5	62.5
Vector 1:200	62.0	66.0	(2 branch)				64.0
Vector 1:200	61.0	60.0	63.5	(3 branch)			61.5
							62.8

As shown in Tables 19 and 20, treatment of potato plants with *Erwinia amylovora* hypersensitive response elicitor enhanced shoot (stem) growth. Thus, overall growth, as judged by both the number and mean 5 lengths of stems, were greater in the harpin-treated plants in both the greenhouse and growth chamber-grown plants. The potato plants treated with the medium dose of harpin (8 μ gm/ml) seemed enhanced in their stem growth more than those treated with either higher or lower 10 doses. Treatment with the medium dose of harpin resulted in greater growth under both growing conditions.

Example 9 - Effect of Spraying Tomatoes With a Cell-Free Elicitor Preparation Containing the 15 *Erwinia amylovora* Harpin

Marglobe tomato plants were sprayed with harpin preparation (from *E. coli* DH5 α (pCPP2139)) or vector preparation (from *E. coli* DH5 α (pCPP50)) with added BSA 20 protein as control 8 days after transplanting. The control vector preparation contained, per ml, 33.6 μ l of BSA (10 mg/ml) to provide about the same amount of protein as contained in the pCPP2139 preparation due to harpin. Dilutions of 1:50 (8.0 μ g/ml), 1:100 25 (4.0 μ g/ml), and 1:200 (2.0 μ g/ml) were prepared and sprayed on the plants to runoff with an electricity-powered atomizer. Fifteen uniform appearing plants per treatment were chosen randomly and assigned to treatment. The plants were maintained at 28°C in a controlled 30 environment chamber before and after treatment.

Overall heights were measured several times after treatment from the surface of soil to the top of the plant. The tops of the tomato plants were weighed immediately after cutting the stems near the surface of 35 the soil.

- 55 -

Treatments: (Dilutions and harpin content)

1. Harpin 1:50 (8.0 µg/ml)
2. Harpin 1:100 (4.0 µg/ml)
3. Harpin 1:200 (2.0 µg/ml)
4. Vector + BSA 1:50 (0 harpin)
5. Vector + BSA 1:100 (0 harpin)
6. Vector + BSA 1:200 (0 harpin)

Table 21 -Tomato plant height (cm) 1 day after spray treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	5.4	5.0	5.6	5.0	5.2	4.8	5.0	5.2	5.4	5.0	5.6	4.8	4.6	5.0	5.8	5.16
H 100	5.0	5.2	5.0	5.4	5.4	5.0	5.2	4.8	5.6	5.2	5.4	5.0	4.8	5.0	5.2	5.15
H 200	5.0	4.6	5.4	4.6	5.0	5.2	5.4	4.8	5.0	5.2	5.4	5.2	5.0	5.2	5.0	5.13
V 50	5.2	4.6	4.8	5.0	5.6	4.8	5.0	5.2	5.6	5.4	5.2	5.8	5.0	4.8	5.2	5.15
V 100	5.2	4.8	5.2	5.0	5.6	4.8	5.4	5.2	5.0	4.8	5.0	4.8	5.6	5.2	5.4	5.13
V 200	5.2	5.4	5.0	5.4	5.2	5.4	5.0	5.2	5.4	5.2	4.6	4.8	5.2	5.0	5.4	5.16

Table 22 -Tomato plant height (cm) 15 days after spray treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	22.0	21.0	22.0	21.5	23.0	22.0	23.5	25.0	22.0	20.5	21.0	23.5	22.0	22.5	21.0	22.2
H 100	26.0	26.5	27.0	29.0	27.5	26.0	28.0	29.0	28.5	26.0	27.5	28.0	28.0	29.0	26.0	27.5
H 200	24.5	26.0	25.0	26.0	26.5	27.5	28.5	28.0	26.0	24.0	26.5	24.5	26.0	24.0	27.5	26.0
V 50	23.5	21.5	20.5	22.5	20.5	21.0	22.0	23.5	22.0	20.5	22.0	21.0	20.5	22.5	21.5	21.7
V 100	22.5	21.0	20.5	23.0	22.0	20.0	20.5	20.0	21.0	22.0	23.0	20.0	22.0	21.0	22.5	21.4
V 200	21.5	20.5	23.5	20.5	22.0	22.0	22.5	20.0	22.0	23.5	23.5	22.0	20.0	23.0	21.0	21.8

Table 23 - Tomato plant height (cm) 21 days after spray treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	28.5	28.0	27.5	26.0	27.0	28.5	28.5	29.0	30.0	28.5	29.0	27.0	28.5	28.0	27.0	28.1
H 100	37.0	38.0	37.5	39.0	37.0	38.5	36.0	38.0	37.0	38.5	37.0	36.0	37.0	37.0	38.5	37.5
H 200	34.5	34.0	36.0	33.5	32.0	34.5	32.5	34.0	32.0	36.5	30.5	32.0	30.0	32.5	34.0	33.2
V 50	30.0	28.0	28.0	28.5	30.0	27.0	26.5	28.0	29.5	28.5	26.5	28.5	27.0	29.5	28.5	28.3
V 100	28.0	27.5	30.0	29.5	28.5	29.0	30.0	26.5	27.5	28.0	30.0	29.0	28.5	28.0	29.5	28.6
V 200	28.5	30.5	27.0	29.0	28.5	27.5	29.0	30.0	28.0	28.5	29.0	30.5	27.5	28.5	28.0	28.7

WO 98/3284

-57-

Table 24 - Mean Height of Tomato Plants After Spraying

Treatment (Dil. & harpin)	Mean height of tomato plants (cm)	Days After Treatment		
		Day 1	Day 11	Day 14
Harpin 1:50	(8.0 μ g/ml)	5.16	22.2	28.1
Harpin 1:100	(4.0 μ g/ml)	5.15	27.5	37.5
Harpin 1:200	(2.0 μ g/ml)	5.13	26.0	33.2
Vector + BSA 1:50	(0)	5.15	21.7	28.5
Vector + BSA 1:100	(0)	5.13	21.4	28.6
Vector + BSA 1:200	(0)	5.16	21.8	28.7

PCT/US98/01507

Table 25 - Fresh Weight of Tomato Plants (g/plant)
21 Days After Spray Treatment

Treat	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Mean
H 50	65.4	60.3	58.9	73.2	63.8	70.1	58.4	60.1	62.7	55.6	58.3	68.9	58.2	64.2	56.4	62.3
H 100	84.3	68.8	74.6	66.7	78.5	58.9	76.4	78.6	84.8	78.4	86.4	66.5	76.5	82.4	80.5	76.2
H 200	80.1	76.5	68.4	79.5	64.8	79.6	76.4	80.2	66.8	72.5	78.8	72.3	62.8	76.4	73.2	73.9
V 50	64.0	56.8	69.4	72.3	56.7	66.8	71.2	62.3	61.0	62.5	63.4	58.3	72.1	67.8	67.0	64.7
V 100	62.8	58.4	70.2	64.2	58.1	72.7	68.4	53.6	67.5	66.3	59.3	68.2	71.2	65.2	59.2	64.4
V 200	64.2	59.6	70.2	66.6	64.3	60.4	60.8	56.7	71.8	60.6	63.6	58.9	68.3	57.2	60.0	62.9

A single spray of tomato seedlings with harpin, in general, resulted in greater subsequent growth than spray treatment with the control (vector) preparation, which had been supplemented with BSA protein. Enhanced 5 growth in the harpin-treated plants was seen in both plant height and fresh weight measurements. Of the three concentrations tested, the two lower ones resulted in more plant growth (based on either measure) than the higher dose (8.0 μ g/ml). There was little difference in 10 the growth of plants treated with the two lower (2 and 4 μ g/ml) concentrations. Components of the lysed cell preparation from the strain *E. coli* DH5 α (pCPP50), which harbors the vector of the *hrpN* gene in *E. coli* strain DH5 α (pCPP2139), do not have the same growth-promoting 15 effect as the harpin-containing preparation, even though it is supplemented with BSA protein to the same extent as the DH5 α (pCPP2139) preparation, which contains large amounts of harpin protein. Thus, this experiment demonstrates that harpin is responsible for enhanced 20 plant growth.

Example 10 - Early Coloration and Early Ripening of Small Fruits

25 A field trial was conducted to evaluate the effect of hypersensitive response elicitor ("harpin") treatment on yield and ripening parameters of raspberry cv. Canby. Established plants were treated with harpin at 2.5 mg/100 square feet in plots 40 feet long x 3 feet 30 wide (1 plant wide), untreated ("Check"), or treated with the industry standard chemical Ronilan at recommended rates ("Ronilan"). Treatments were replicated four times and arranged by rep in an experimental field site. Treatments were made beginning at 5-10% bloom followed by 35 two applications at 7-10 day intervals. The first two harvests were used to evaluate disease control and fruit

- 60 -

yield data was collected from the last two harvests. Observations indicated harpin-treated fruits were larger and exhibited more redness than untreated fruits, indicating ripening was accelerated by 1-2 weeks. The 5 number of ripe fruits per cluster bearing a minimum of ten fruits was determined at this time and is summarized in Table 26. Harpin treated plots had more ripe fruits per 10-berry cluster than either the check or Ronilan treatments. Combined yields from the last two harvests 10 indicated increased yield in harpin and Ronilan treated plots over the untreated control (Table 27).

Table 26 - Number of Ripe Raspberry Fruits Per Clusters With Ten Berries or More on June 20, 1996.

<u>Treatment</u>	<u>Ripe fruit/10 berry clusters</u>	<u>% of Control</u>
Check	2.75	100.0
Ronilan	2.75	100.0
Harpin	7.25	263.6

Table 27 - Mean Raspberry Fruit Yield by Weight (lbs.) Combined in Last Two Harvest.

<u>Treatment</u>	<u>Total Yield</u>	<u>% of Control</u>
Check	32.5	100.0
Ronilan	37.5	115.4
Harpin	39.5	121.5

Example 11 - Growth Enhancement For Snap Beans

Snap beans of the variety Bush Blue Lake were treated by various methods, planted in 25-cm-d plastic 5 pots filled with commercial potting mix, and placed in an open greenhouse for the evaluation of growth parameters. Treatments included untreated bean seeds ("Check"), seeds treated with a slurry of 1.5% methyl cellulose prepared with water as diluent ("M/C"), seeds treated with 1.5% 10 methyl cellulose followed by a foliar application of hypersensitive response elicitor ("harpin") at 0.125

- 61 -

mg/ml ("M/C+H"), and seeds treated with 1.5% methyl cellulose plus harpin spray dried at 5.0 μ g harpin per 50 seeds followed by a foliar application of harpin at 0.125 mg/ml ("M/C-SD+H"). Seeds were sown on day 0, planted 3 per pot, and thinned to 1 plant per pot upon germination. Treatments were replicated 10 times and randomized by rep in an open greenhouse. Bean pods were harvested after 64 days, and fresh weights of bean pods of marketable size (>10 cm x 5 cm in size) were collected as yield. Data were analyzed by analysis of variance with Fisher's LSD used to separate treatment means.

15 Table 28 - Effect of *Erwinia amylovora* Harpin Treatment by Various Methods on Yield of Market Sized Snap Bean Pods

	<u>Treatment</u>	<u>Marketable Yield, g¹</u>	<u>% of Untreated (Check)</u>
20	M/C-SD+H	70.6 a	452
	M/C-H	58.5 ab	375
	M/C	46.3 bc	297
	M/C+H	42.3 bc	271
25	M/C-SD	40.0 cd	256
	Check	15.6 e	100

30 ¹ Marketable yield included all bean pods 10 cm x 0.5 cm or larger. Means followed by the same letter are not significantly different at P=0.05 according to Fisher's LSD.

As shown in Table 28, the application of *Erwinia amylovora* harpin by various methods of application resulted in an increase in the yield of marketable size snap bean pods. Treatment with methyl cellulose alone also results in an increase in bean yield but was substantially increased when combined with harpin as seed (spray dried) and foliar treatments.

40

Example 12 - Yield Increase in Cucumbers from Foliar Application of HP-1000™ to Cucumbers.

Cucumber seedlings and transplants were treated 45 with foliar sprays of HP-1000™ (EDEN Bioscience, Bothell,

Washington) (*Erwinia amylovora* hypersensitive response elicitor formulation) at rates of 15, 30, or 60 $\mu\text{g}/\text{ml}$ active ingredient (a.i.). The first spray was applied when the first true leaves were fully expanded. The 5 second application was made 10 days after the first spray. All sprays were applied using a back-pack sprayer, and an untreated control (UTC) was also included in the trial. Three days after the second application of HP-1000TM, ten plants from each treatment were 10 transplanted into randomized field plots replicated three times. This yielded a total of thirty plants per treatment. Seven days after transplanting, a third foliar spray of HP-1000TM was applied. Although severe drought followed resulting in significant water stress, a total 15 of six harvests were made following a standard commercial harvesting pattern. The total weight of fruit harvested from each treatment is presented in Table 29. Results indicate that plants treated with HP-1000TM at rates of 15 and 30 $\mu\text{g}/\text{ml}$ yielded significantly more fruit than the 20 UTC. Plants treated with HP-1000TM yielded a moderate yield increase. These results indicated that HP-1000TM treated plants were significantly more tolerant to drought stress conditions than untreated plants.

25 Table 29 - Increase yield of cucumbers after treatment with HP-1000TM

30	Treatment	Rate ¹	Yield, ² lbs./10 plants	% above UTC
	UTC	---	9.7 a	---
	HP-1000 TM	15 $\mu\text{g}/\text{ml}$	25.4 b	161.4
	HP-1000 TM	30 $\mu\text{g}/\text{ml}$	32.6 c	236.4
	HP-1000 TM	60 $\mu\text{g}/\text{ml}$	11.2 a	15.9

35 ¹Active ingredient (a.i.). ²Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 63 -

Example 13 - Yield Increase in Cotton from Treatment with HP-1000™

Cotton was planted in four, 12 x 20 foot replicate field plots in a randomized complete block (RCB) field trial. Plants were treated with HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation), HP-1000™+Pix® (Pix® (BASF Corp., Mount Olive, N.J.) is a growth regulator applied to keep cotton plants compact in height) or Early Harvest® (Griffen Corp., Valdosta, Ga.) (a competitive growth enhancing agent). An untreated control (UTC) was also included in the trial. Using a back-pack sprayer, foliar applications were made of all treatments at three crop growth stages; first true leaves, pre-bloom, and early bloom. All fertilizers and weed control products were applied according to conventional farming practices for all treatments. The number of cotton bolls per plant ten weeks before harvest was significantly higher for the HP-1000™ treated plants compared to other treatments. By harvest, HP-1000™ treatment was shown to have a significantly increased lint yield (43%) compared to UTC (Table 30). When HP-1000™ was combined with Pix®, lint yield was increased 20% over UTC. Since Pix® is commonly applied to large acreages of cotton, this result indicates that HP-1000™ may be successfully tank-mixed with Pix®. Application of the competitive growth enhancing agent, Early Harvest® only produced a 9% increase in lint yield vs. UTC.

- 64 -

Table 30 - Increased lint yield from cotton after treatment with HP-1000™, HP-1000™+Pix®, or Early Harvest®.

	Treatment	Rate ¹	Lint Yield (lbs./ac)	% above UTC
	UTC	---	942.1	---
10	Early Harvest®	2 oz./ac.	1,077.4*	14.3
	HP-1000™+Pix®	40 µg/ml+8 oz./ac.	1,133.1*	20.4
	HP-1000™	40 µg/ml	1,350.0*	43.3
	(*significant at P= 0.05)		lsd = 122.4	

15 -----

¹Rates for HP-1000™ are for active ingredient (a.i.); rates for Early Harvest® and Pix® are formulated product.

20 -----

Example 14 - Yield Increase of Chinese Egg Plant from Treatment with HP-1000™

Nursery grown Chinese egg plant seedlings were 25 sprayed once with HP-1000™ at (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) 15, 30, or 60 µg/ml (a.i.), then transplanted into field plots replicated three times for each treatment. Two weeks after transplanting, a second application of 30 HP-1000™ was made. A third and final application of HP-1000™ was applied approximately two weeks after the second spray. All sprays were applied using a back-pack sprayer; an untreated control (UTC) was also included in the trial. As the season progressed, a total of eight 35 harvests from each treatment were made. Data from these harvests indicate that treatment with HP-1000™ resulted in greater yield of fruit per plant.

- 65 -

Table 31 - Increased yield for Chinese egg plant after treatment with HP-1000™.

	Treatment	Rate (a.i.)	Yield(lbs./plant)	% above UTC
5	UTC	--	1.45	---
	HP-1000™	15 μ g/ml	2.03	40.0
10	HP-1000™	30 μ g/ml	1.90	31.0
	HP-1000™	60 μ g/ml	1.95	34.5

15 Example 15 - Yield Increase of Rice From Treatment with HP-1000™

Rice seedlings were transplanted into field plots replicated three times, then treated with foliar sprays of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) at three different rates using a back-pack sprayer. An untreated control (UTC) was also included in the trial. The first application of HP-1000™ was made one week after transplanting, the second three weeks after the first. A third and final spray was made just before rice grains began to fill the heads. Results at harvest demonstrated that foliar applications of HP-1000™ at both 30 and 60 μ g/ml significantly increased yield by 47 and 56%, respectively (Table 32).

- 66 -

Table 32 - Increase yield of rice after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	Yield ¹ (lbs./ac.)	% above UTC
5	UTC	---	3,853 a	---
	HP-1000™	15 µg/ml	5,265 ab	35.9
	HP-1000™	30 µg/ml	5,710 b	47.3
10	HP-1000™	60 µg/ml	6,043 b	56.1

15 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 16 - Yield Increase of Soybeans From Treatment with HP-1000™

20 Soybeans were planted into randomized field plots replicated three times for each treatment. A back-pack sprayer was used to apply foliar sprays of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) and an untreated control (UTC) was also included in the trial. Three rates of HP-1000™ were applied beginning at four true leaves when plants were approximately eight inches tall. A second spray of HP-1000™ was applied ten days after the first spray and a third spray ten days after the second. Plant height measured ten days after the first spray treatment indicated that application of HP-1000™ resulted in significant growth enhancement (Table 33). In addition, plants treated with HP-1000™ at 30 the rate of 60 µg/ml began to flower five days earlier than the other treatments. Approximately ten days after application of the third spray, the number of soybean pods per plant was counted from ten randomly selected plants per replication. These results indicated that the 35 growth enhancement from treatment with HP-1000™ resulted in significantly greater yield (Table 34).

40

- 67 -

Table 33 - Increased plant height of soybeans after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	Plant Ht. ¹ (in.)	% above UTC
5	UTC	---	12.2 a	---
	HP-1000™	15 µg/ml	13.2 b	8.3
	HP-1000™	30 µg/ml	14.1 c	16.2
10	HP-1000™	60 µg/ml	14.3 c	17.3

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

15

Table 34 - Increased pod set of soybeans after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	No. Pods/plant ¹	% above UTC
20	UTC	---	41.1 a	---
	HP-1000™	15 µg/ml	45.4 ab	10.4
25	HP-1000™	30 µg/ml	47.4 b	15.4
	HP-1000™	60 µg/ml	48.4 b	17.7

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 17 - Yield Increase of Strawberries From Treatment with HP-1000™

35 Two field trials with HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) were conducted on two strawberry varieties, *Camarosa* and *Selva*. For each variety, a 40 randomized complete block (RCB) design was established having four replicate plots (5.33 x 10 feet) per treatment in a commercially producing strawberry field. Within each plot, strawberry plants were planted in a double row layout. An untreated control (UTC) was also 45 included in the trial. Before applications began, all plants were picked clean of any flowers and berries.

Sprays of HP-1000™ at the rate of 40 µg/ml were applied as six weekly using a back-pack sprayer. Just prior to application of each spray, all ripe fruit from each treatment was harvested, weighed, and graded according to 5 commercial standards. Within three weeks of the first application of HP-1000™ to *Selva* strawberry plants, growth enhancement was discernible as visibly greater above-ground biomass and a more vigorous, greener and healthier appearance. After six harvests (i.e. the 10 scheduled life-span for these plants), all yield data were summed and analyzed. For the *Camarosa* variety, yield of marketable fruit from HP-1000™ treated plants was significantly increased (27%) over the UTC when averaged over the last four pickings (Table 35).

15 Significant differences between treatments were not apparent for this variety for the first two pickings. The *Selva* variety was more responsive to the growth enhancing effects from treatment with HP-1000™; *Selva* strawberry plants yielded a statistically significant 64% more 20 marketable fruit vs. the UTC when averaged over six pickings (Table 35).

25 Table 35 - Increased yield of strawberries after foliar treatment with HP-1000™.

	Treatment	Rate (a.i.)	Yield ¹ (lbs./rep)	% above
30	UTC	---		
		Variety: <i>Camarosa</i>		
	UTC	---	1.71 a	---
	HP-1000™	40 µg/ml	2.17 b	27
		Variety: <i>Selva</i>		
35	UTC	---	0.88 a	---
	HP-1000™	40 µg/ml	1.44 b	64

40 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Example 18 - Earlier Maturity and Increased Yield of Tomatoes from Treatment with HP-1000™

Fresh market tomatoes (var. Solar Set) were 5 grown in plots (2 x 30 feet) replicated 5 times in a randomized complete block (RCB) field trial within a commercial tomato production field. Treatments included HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation), an 10 experimental competitive product (Actigard™ (Novartis, Greensboro, N.C.)) and a chemical standard (Kocide® (Griffen Corp., Valdosta, GA)) + Maneb® (DuPont Agricultural Products, Wilmington, D.E.)) for disease control. The initial application of HP-1000™ was made as 15 a 50 ml drench (of 30 µg/ml a.i.) poured directly over the seedling immediately after transplanting. Thereafter, eleven weekly foliar sprays were applied using a back-pack sprayer. The first harvest from all treatments was made approximately six weeks after 20 transplanting and only fully red, ripe tomatoes were harvested from each treatment. Results indicated that HP-1000™ treated plants had a significantly greater amount of tomatoes ready for the first harvest (Table 36). The tomatoes harvested from the HP-1000™ 25 treated plants were estimated to be 10-14 days ahead other treatments.

- 70 -

Table 36 - Increased yield of tomatoes at first harvest after foliar treatment with of HP-1000™.

5	Treatment	Rate (a.i.) ¹	Yield ² (lbs./rep)	% above
UTC	UTC	---	0.61 a	---
10	HP-1000™	30 µg/ml	2.87 b	375
Actigard™	14 g/ac	0.45 a	-25.1	
Kocide®+	2 lbs./ac.	0.31 a	-49.1	
Maneb®	1 lb./ac			

15 ¹Rates for Kocide® and Maneb® are for formulated product. ²Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 Example 19 - Earlier Flowering and Growth Enhancement of Strawberries From Treatment with HP-1000™ When Planted in Non-fumigated Soil.

25 Strawberry plants ("plugs" and "bare-root"), cv. Commander were transplanted into plots (2 x 30 feet) replicated 5 times in a randomized complete block field trial. Approximately sixty individual plants were 30 transplanted into each replicate. Treatments applied in this field trial are listed below:

35	<u>Treatment</u>	<u>Application method</u>
HP-1000™ (plug plants)	50-ml drench solution of HP-1000™ (EDEN Bioscience) (<i>Erwinia amylovora</i> hypersensitive response elicitor formulation) at 40 µg/ml (a.i.) poured directly over the individual plants immediately after transplanting into non-fumigated soil ¹ , followed by foliar applications of HP-1000™ at 40 µg/ml every 14 days.	
40	HP-1000™ (bare-root plants)	root soak in solution of HP-1000™ at µg/ml (a.i.) for 1 hour, immediately before transplanting into non-fumigated

- 71 -

soil,¹ followed by foliar applications of HP-1000™ at 40 µg/ml every 14 days.

5	methyl bromide/ chloropicrin 75/25	soil fumigation at 300 lbs./ac via injection prior to transplanting, no HP-1000™ treatments applied.
10	Telone/chloropicrin 70/30	soil fumigation at 45 gal./ac via injection prior to transplanting, no HP-1000™ treatments applied.
15	untreated control (UTC)	no fumigation, no HP-1000™ treatments

¹Non-fumigated soil had been cropped to vetch for the two previous years.

20 Transplanting was done in late fall when cool weather tended to slow plant growth. Two weeks after transplanting, the first foliar application of HP-1000™ was made at 40 µg/ml (a.i.) with a back-pack sprayer.

25 Three weeks after transplanting, preliminary results were gathered comparing HP-1000™ treatment against methyl bromide and UTC by counting the number of flowers on all strawberry "plug" plants in each replication. Since flowering had not yet occurred in the "bare-root" plants, each plant in replicates for this treatment was assessed

30 for early leaf growth by measuring the distance from leaf tip to stem on the middle leaf of 3-leaf cluster.

35 Results (Tables 37 and 38) indicated that treatment with HP-1000™ provided early enhanced flower growth and leaf size for "plug" and "bare-root" strawberry plants, respectively.

- 72 -

Table 37 - Earlier flowering of "plug" strawberry transplants after foliar treatment with HP-1000™.

5	Treatment	Rate (a.i.)	No. flowers/rep ¹	% above
10	UTC	---	2.0a	---
	HP-1000™	40 µg/ml	7.5 b	275
15	Methyl bromide/ chloropicrin	300 lbs./ac	5.3 b	163

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 Table 38 - Increased leaf growth in "bare-root" strawberry transplants after foliar treatment with HP-1000™.

25	Treatment	Rate (a.i.)	Leaf length ¹ (in.)	% above
30	UTC	---	1.26 a	---
	HP-1000™	40 µg/ml	1.81 b	44

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

40 Example 20 - Early Growth Enhancement of Jalapeño Peppers from Application of HP-1000™

45 Jalapeño pepper (cv. *Mittlya*) transplants were treated with a root drench of HP-1000 (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) (30 µg/ml a.i.) for 1 hour, then transplanted into randomized field plots replicated four times. An untreated control (UTC) was also included. Beginning 14 days after transplanting, treated plants received three foliar sprays of HP-1000™ at 14 day

- 73 -

intervals using a back-pack sprayer. One week after the third application of HP-1000™ (54 days after transplanting), plant height was measured from four randomly selected plants per replication. Results from 5 these measurements indicated that the HP-1000™ treated plants were approximately 26% taller than the UTC plants (Table 39). In addition, the number of buds, flowers or fruit on each plant was counted. These results indicated that the HP-1000™ treated plants had over 61% more 10 flowers, fruit or buds compared to UTC plants (Table 40).

Table 39 - Increased plant height in Jalapeño peppers after treatment with HP-1000™.

	Treatment	Rate (a.i.)	Plant Ht. (in.) ¹	% above UTC
15	UTC	---	a7.0	---
20	HP-1000™	30 µg/ml	8.6 b	23.6

25 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

Table 40 - Increased number of flowers, fruit or buds in Jalapeño peppers after treatment with HP-1000™.

	Treatment	Rate (a.i.)	No. flowers, fruit or buds/plant ¹	% above
30	UTC	---		---
35	UTC	---	20.6 a	---
40	HP-1000™	30 µg/ml	12.8 b	61.3

45 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 74 -

Example 21 - Growth Enhancement of Tobacco from Application of HP-1000™

Tobacco seedlings were transplanted into 5 randomized field plots replicated three times. A foliar spray of HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) was applied after transplanting at one of three rates: 15, 30, or 60 µg/ml a.i. Sixty days later, a second foliar application 10 of HP-1000 was made. Two days after the second application, plant height, number of leaves per plant, and the leaf size (area) were measured from ten, randomly selected plants per treatment. Results from these 15 measurements indicated treatment with HP-1000™ enhanced tobacco plant growth significantly (Tables 41, 42, and 43). Plant height was increased by 6-13%, while plants treated with HP-1000™ at 30 and 60 µg/ml averaged just 20 over 1 more leaf per plant than UTC. Most significantly, however, treatment with HP-1000™ at 15, 30, and 60 µg/ml resulted in corresponding increases in leaf area. Tobacco plants with an extra leaf per plant and an increase in average leaf size (area) represent a commercially significant response.

25

Table 41 - Increased plant height in tobacco after treatment with HP-1000™.

30

	Treatment	Rate (a.i.)	Plant Ht. (cm)	% above UTC
	UTC	---	72.0	---
	HP-1000™	15 µg/ml	76.4	5.3
	HP-1000™	30 µg/ml	79.2	9.0
35	HP-1000™	60 µg/ml	81.3	6.9

40

- 75 -

Table 42 - Increased number of tobacco leaves per plant after treatment with HP-1000™.

5	Treatment	Rate (a.i.)	Leaves/plant ¹	% above UTC
	UTC	---	16.8	---
	HP-1000™	15 µg/ml	17.4	3.6
10	HP-1000™	30 µg/ml	18.1	7.7
	HP-1000™	60 µg/ml	17.9	6.5

15 Table 43 - Increased leaf area in tobacco after treatment with HP-1000™.

20	Treatment	Rate (a.i.)	Leaf area (cm ²)	% above UTC
	UTC	---	1,246	---
	HP-1000™	15 µg/ml	1,441	16
25	HP-1000™	30 µg/ml	1,543	24
	HP-1000™	60 µg/ml	1,649	32

30 Example 22 - Growth Enhancement of Winter Wheat from Application of HP-1000™

Winter wheat seed was "dusted" with dry HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) powder at the rate of 3 ounces of formulated product (3% a.i.) per 100 lbs. seed, then planted using conventional seeding equipment into randomized test plots 11.7 feet by 100 feet long. Additional treatments included a seed "dusting" with HP-1000™ powder (3% a.i.) at 1 oz. formulated product per 100 lbs. seed, a seed-soak in a solution of HP-1000™ at a concentration of 20 µg/ml, a.i., for four hours, then air-dried before planting, a standard chemical (Dividend®) fungicide "dusting", and an untreated control (UTC). Eight days after planting,

HP-1000™ treated seeds began to emerge, whereas the UTC and chemical standard-treated seed did not emerge until approximately 14 days after planting, the normal time expected. At 41 days after planting, seedlings were 5 removed from the ground and evaluated. Root mass for wheat treated with HP-1000™ as a "dusting" at 3 oz./100 lb. was visually inspected and judged to be approximately twice as great as any of the other treatments.

Following the field trial, a greenhouse 10 experiment was designed to gain confirmation of these results. Treatments included wheat seed dusted with dry HP-1000™(10% a.i.) at a rate of 3 ounces per 100 lbs. of seed, seed soaking of HP-1000™ in solution concentration of 20 mg/ml for four hours before planting, and an 15 untreated control (UTC). Wheat seeds from each treatment were planted at the rate of 25 seeds per pot, with five pots serving as replicates for each treatment. Fifteen days after planting, ten randomly selected seedlings from each treatment pot were removed, carefully cleaned, and 20 measured for root length. Since the above-ground portion of individual seedlings did not exhibit any treatment effect, increased root growth from treatment with HP-1000™ did not influence the selection of samples. The increase in root growth from either HP-1000™ treatment 25 was significantly greater than UTC (Table 49); however, the seed dusting treatment appeared to give slightly better results.

- 77 -

Table 44 - Increased root growth in wheat seedlings after treatment with HP-1000™.

5	Treatment	Rate	Root length. (cm) ¹	% above UTC
10	UTC	---	35.6 a	---
15	HP-1000™ (dusting)	3 oz./100 lbs.	41.0 b	17.4
20	HP-1000™ (soaking)	20 µg/ml	40.8 b	14.6

15 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20 Example 23 - Growth Enhancement of Cucumbers from Application of HP-1000™

A field trial of commercially produced cucumbers consisted of four treatments, HP-1000™ (EDEN Bioscience) (*Erwinia amylovora* hypersensitive response elicitor formulation) at two rates (20 or 40 µg/ml), a chemical standard for disease control (Bravo® (Zeneca Ag Products, Wilmington, Del.) +Maneb®) and an untreated control (UTC). Each treatment was replicated four times in 3 x 75 foot plots with a plant spacing of approximately 2 feet for each treatment. Foliar sprays of HP-1000™ were applied beginning at first true leaf and repeated at 14 day intervals until the last harvest for a total of six applications. The standard fungicide mix was applied every seven days or sooner if conditions warranted. Commercial harvesting began approximately two months after first application of HP-1000™ (after five sprays of HP-1000™ had been applied), and a final harvest was made approximately 14 days after the first harvest.

40 Results from the first harvest indicated that treatment with HP-1000™ enhanced the average cucumber yield by increasing the total number of cucumbers

- 78 -

harvested and not the average weight of individual cucumbers (Tables 45-47). The same trend was noted at the final harvest (Tables 48-49). It was commercially important that the yield increase resulting from treatment with HP-1000™ was not achieved by significantly increasing average cucumber size.

10 Table 45 - Increased cucumber yield after treatment with HP-1000™, first harvest.

Treatment	Rate (a.i.)	Yield/trt ¹ (kg.)	% above UTC
UTC	---	10.0 a	---
Bravo+Maneb	label	10.8 a	8.4
HP-1000™	20 µg/ml	12.3 ab	22.8
HP-1000™	40 µg/ml	13.8 b	38.0

20 -----
 15 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

25 Table 46 - Increased number of fruit in cucumbers after treatment with HP-1000™, first harvest.

Treatment	Rate (a.i.)	No. fruit/trt ¹	% above UTC
UTC	---	24.5 a	---
Bravo+Maneb	label	27.6 ab	12.8
HP-1000™	20 µg/ml	31.2 b	27.0
HP-1000™	40 µg/ml	34.3 b	39.8

30 -----
 35 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 79 -

Table 47 - Average weight of cucumbers after treatment with HP-1000™, first harvest.

5	Treatment	Rate (a.i.)	Weight/fruit(g)	% change vs.
	UTC			
	UTC	---	406	---
10	Bravo+Maneb	label	390	-4
	HP-1000™	20 µg/ml	395	-3
	HP-1000™	40 µg/ml	403	-1

15 Table 48 - Increased cucumber yield after treatment with HP-1000™, third harvest.

20	Treatment	Rate (a.i.)	Yield/trt ¹ (kg.)	% above UTC
	UTC	---	17.5 a	---
25	Bravo+Maneb	label	14.0 b	-20.1
	HP-1000™	20 µg/ml	20.1 a	15.3
	HP-1000™	40 µg/ml	20.2 a	15.6

30 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.35 Table 49 - Increased number of fruit in cucumbers after treatment with HP-1000™, third harvest.

40	Treatment	Rate (a.i.)	No. fruit/trt ¹	% change vs.
	UTC			
	UTC	---	68.8 ab	---
45	Bravo+Maneb	label	60.0 a	-12.7
	HP-1000™	20 µg/ml	82.3 b	19.6
	HP-1000™	40 µg/ml	85.3 b	24.0

50 ¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 80 -

Table 50 - Average weight of cucumbers after treatment with HP-1000™, third harvest.

	Treatment	Rate (a.i.)	Weight/fruit (g)	% change vs.
	UTC			
	UTC	---	255	---
	Bravo+Maneb	label	232	-9
10	HP-1000™	20 µg/ml	247	-3
	HP-1000™	40 µg/ml	237	-7

15 **Example 24 - Harpin_{pss} from *Pseudomonas syringae* pv *syringae* Induces Growth Enhancement in Tomato**

20 To test if harpin_{pss} (i.e. the hypersensitive response elicitor from *Pseudomonas syringae* pv *syringae*) (He, S. Y., et al., "Pseudomonas syringae pv *syringae* Harpin_{pss}. A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-66 (1993), which is hereby incorporated by reference) also stimulates plant growth, tomato seeds (Marglobe variety) were sowed in 8 inches pots with artificial soil. 10 days after sowing, the seedlings were transplanted into individual pots. Throughout the 25 experiment, fertilizer, irrigation of water, temperature, and soil moisture were maintained uniformly among plants. 16 days after transplanting, the initial plant height was measured and the first application of harpin_{pss} was made, this is referred to as day 0. A second application was 30 made on day 15. Additional growth data was collected on day 10 and day 30. The final data collection on day 30 included both plant height and fresh weight.

35 The harpin_{pss} used for application during the experiment was produced by fermenting *E. coli* DH5 containing the plasmid with the gene encoding harpin_{pss} (i.e. *hrpZ*). The cells were harvested, resuspended in 5 mM potassium phosphate buffer, and disrupted by

sonication. The sonicated material was boiled for 5 minutes and then centrifugated for 10 min. at 10,000 rpm. The supernantant was considered as Cell-Free Elicitor Preparation (CFEP). 20 and 50 μ g/ml harpin_{pss} solution was 5 made with the same buffer used to make cell suspension. CFEP prepared from the same strain containing the same plasmid but without *hrpZ* gene was used as the material for control treatment.

10 The wetting agent, Pinene II (Drexel Chemical Co., Memphis, Tenn.) was added to the harpin_{pss} solution at the concentration of 0.1%, then harpin_{pss} was sprayed onto tomato plant until there was run off.

15 Table 51 shows that there was a significant difference between the harpin_{pss} treatment groups and the control group. Harpin_{pss} treated tomato increased more than 10% in height. The data supports the claim that harpin_{pss} does act similar to the hypersensitive response elicitor from *Erwinia amylovora*, in that when applied to tomato and many other species of plants, there is a growth 20 enhancement effect. In addition to a significant increase of tomato height harpin_{pss}-treated tomato had more biomass, big leaves, early flower setting, and over all healthier appearance.

25

Table 51 - Harpin_{pss} enhances the growth of tomato plant

30	Treatment	Plant Height (cm ¹)		
		Day 0	Day 10	Day 30
	CFEP Control	8.5 ² (0.87) a ³	23.9 (1.90) a	68.2 (8.60) a
35	Harpin _{pss} 20 μ g/ml	8.8 (0.98) a	27.3 (1.75) b	74.2 (6.38) b
	Harpin _{pss} 50 μ g/ml	8.8 (1.13) a	26.8 (2.31) b	75.4 (6.30) b

40 ¹Plant height was measured to the nearest 0.5 cm. Day 0 refers to the day the initial plant heights were recorded and the first application was made.

- 82 -

²Means are given with SD in parenthesis (n=20 for all treatment groups).

5 ³Different letters (a and b) indicates significant differences (P 0.05) among means. Differences were evaluated by ANOVA followed by Fisher LSD.

10 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.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: ENHANCEMENT OF GROWTH IN PLANTS
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
 - (B) STREET: Clinton Square, P.O. Box 1051
 - (C) CITY: Rochester
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/036,048
 - (B) FILING DATE: 27-JAN-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Goldman, Michael L.
 - (B) REGISTRATION NUMBER: 30,727
 - (C) REFERENCE/DOCKET NUMBER: 19603/1502
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (716) 263-1304
 - (B) TELEFAX: (716) 263-1600

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 338 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- 84 -

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1 5 10 15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
20 25 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
35 40 45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
50 55 60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65 70 75 80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
85 90 95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
100 105 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
115 120 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
130 135 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145 150 155 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
165 170 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
180 185 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
195 200 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
210 215 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225 230 235 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
245 250 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
260 265 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
275 280 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
290 295 300

- 85 -

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
 305 310 315 320
 Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
 325 330 335
 Asn Ala

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2141 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGATTTCACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTGCA CACCGTTACG	60
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC	120
GATCTGGTAT TTCAGTTGG GGACACCGGG CGTGAACCTCA TGATGCAGAT TCAGCCGGGG	180
CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCAGCAGAG	240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTGAACG GGCAGGAATG	360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC	420
CGATCATTAA GATAAAGGCG GCTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
CACCGTCGGC GTCACTCAGT AACAAAGTATC CATCATGATG CCTACATCGG GATCGCGTG	540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGCGTC TCCGGTCTGG GGCTGGGTGC	660
TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT	720
GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTG GCGCGCGCT	780
GGCGCAGGGG CTGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGCCAGTC	840
TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGCGGGCGA	900
TGCGTTGTCA AAAATGTTG ATAAAGCGCT GGACGATCTG CTGGGTCTG ACACCGTGAC	960
CAAGCTGACT AACCACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAAC GCACTGTCGT CCATTCTCGG	1080
CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140

- 86 -

GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGCGT	1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCAAA GAGATCGGCC AGTTTATGGA	1320
TCAGTATCCG GAAATATTGAG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCAGGCGGT GCATCGCTGG GTATCGATGC	1560
GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC	1680
TTATTATGCCG GTTTATGCCG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
ACGCACATTT TCCCCTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC	1800
GTCGCTCAGA TTGCGCGGCT GATGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
CAGATGGAGA CACGCTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG	1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG	1980
GATCACCACA ATATTCAAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCGTGACAA TCTTGAGTTG	2100
GTTCGTCATC ATCTTCTCC ATCTGGCGA CCTGATCGGT T	2141

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 403 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser			
1	5	10	15
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln			
20	25	30	
Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Asn			
35	40	45	
Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met			
50	55	60	

- 87 -

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
 65 70 75 80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
 85 90 95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
 100 105 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
 115 120 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
 130 135 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
 145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
 165 170 175

Gln Asp Gly Thr Gln Gly Ser Ser Gly Gly Lys Gln Pro Thr Glu
 180 185 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
 195 200 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
 210 215 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
 225 230 235 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
 245 250 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
 260 265 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
 275 280 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
 290 295 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
 305 310 315 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
 325 330 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
 340 345 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
 355 360 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
 370 375 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400

Gly Ala Ala

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1288 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTGAA TTATTCATAA	60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT	120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATAACCGT CAATCAGCTG	240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
GGCGGCAACA ATACCACTTC AACAAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
CTCCTTGGCA ACGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAAGC GCTGAATGAT	900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTCGTCA ATAAAGGCGA TCGGGCGATG	960
GCAGAAGGAAA TCGGTCAAGTT CATGGACCAAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC	1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAAG CACTGAGC	1080
AAGCCAGATG ACGACGGAAT GACACCAAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
ATGATCAAAA GGCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

- 89 -

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 341 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1 5 10 15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
20 25 30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
35 40 45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
50 55 60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val
65 70 75 80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
85 90 95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
100 105 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
115 120 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
130 135 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145 150 155 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
165 170 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
180 185 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
195 200 205

Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
210 215 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225 230 235 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
245 250 255

- 90 -

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
 260 265 270
 Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
 275 280 285
 Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala
 290 295 300
 Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
 305 310 315 320
 Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
 325 330 335
 Asn Gln Ala Ala Ala
 340

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1026 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCAGAGTC	TCAGTCTTAA	CAGCAGCTCG	CTGCAAACCC	CGGCAATGGC	CCTTGTCTTG	60
GTACGTCTG	AAGCCGAGAC	GACTGGCAGT	ACGTCGAGCA	AGGCGCTTCA	GGAAGTTGTC	120
GTGAAGCTGG	CCGAGGAACG	GATGCGCAAT	GGTCAACTCG	ACGACAGCTC	GCCATTGGGA	180
AAACTGTTGG	CCAAGTCGAT	GGCCGCAGAT	GGCAAGGCGG	GCGGCGGTAT	TGAGGGATGTC	240
ATCGCTGCGC	TGGACAAGCT	GATCCATGAA	AAGCTCGGTG	ACAACTTCGG	CGCGTCTGCG	300
GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
AAGTCGATGC	TCGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCAC	ACAGTTTCCC	480
AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAACTTCCT	TGATGGCGAC	540
GAAACGGCTG	CGTTCCGTTG	GGCACTCGAC	ATCATTGGCC	AGCAACTGGG	TAATCAGCAG	600
AGTGACGCTG	GCAGTCTGGC	AGGGACGGGT	GGAGGTCTGG	GCACTCCGAG	CAGTTTTTCC	660
AACAACTCGT	CCGTGATGGG	TGATCCGCTG	ATCGACGCCA	ATACCGGTCC	CGGTGACAGC	720
GGCAATAACCC	GTGGTGAAGC	GGGGCAACTG	ATCGGCGAGC	TTATCGACCG	TGGCCTGCAA	780
TCGGTATTGG	CCGGTGGTGG	ACTGGGCACA	CCCGTAAACA	CCCCGCAGAC	CGGTACGTCG	840

- 91 -

GCAGATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG	900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT	960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA	1020
GCCTGA	1026

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 344 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln			
1	5	10	15
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser			
20	25	30	
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile			
35	40	45	
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly			
50	55	60	
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala			
65	70	75	80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser			
85	90	95	
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met			
100	105	110	
Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala			
115	120	125	
Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val			
130	135	140	
Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala			
145	150	155	160
Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly			
165	170	175	
Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly			
180	185	190	

- 92 -

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln
 260 265 270
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335
 Gln Ser Thr Ser Thr Gln Pro Met
 340

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1035 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC	60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180
GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC	240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC	300
GGCAACGTCTG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA	360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCAGG CAATGACAAG	420
GGCAACGGCG TGGCGGTGC CAACGGCGCC AAGGGTGCCG GCAGGCCAGGG CGGCCTGGCC	480

- 93 -

GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	CGGGCGGCCG	TGCTGGCGCC	540
GGCGGCGCGG	GTGGCGGTGT	CGGCGGTGCT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
GGCGCAGGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
GGCCCGCAGA	ACGCAGGCGA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAGAC	720
CAGGGCGGCC	TCACCGGCGT	GCTGCAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
ATGATGCAGC	AAGGCGGCCT	CGGCGGCCGC	AACCAGGCGC	AGGGCGGCTC	GAAGGGTGCC	840
GGCAACGCCT	CGCCGGCTTC	CGGCGCGAAC	CCGGGCGCGA	ACCAGCCCGG	TTCGGCGGAT	900
GATCAATCGT	CCGGCCAGAA	CAATCTGCAA	TCCCAGATCA	TGGATGTGGT	GAAGGAGGTC	960
GTCCAGATCC	TGCAGCAGAT	GCTGGCGCG	CAGAACGGCG	GCAGCCAGCA	GTCCACCTCG	1020
ACGCAGCCGA	TGTAA					1035

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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								

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

- 94 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15

Leu Leu Ala Met
20

- 95 -

The claims defining the invention are as follows:

1. A method of enhancing growth in plants compared to untreated plants comprising:
 - 5 applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to enhance growth of the plant or plants grown from the plant seed, compared to an untreated plant or plant seed, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected
 - 10 from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora* and mixtures thereof.
2. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.
- 15 3. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.
- 20 4. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.
- 25 5. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.
- 30 6. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.



- 96 -

7. A method according to claim 1, wherein the plant is selected from the group consisting of dicots and monocots.
8. A method according to claim 7, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum and sugarcane.
- 10 9. A method according to claim 7, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium poinsettia, chrysanthemum, carnation and zinnia.
- 15 10. A method according to claim 1, wherein plants are treated during said applying which is carried out by spraying, injection, or leaf abrasion at a time proximate to when said applying takes place.
- 20 11. A method according to claim 1, wherein plant seeds are treated during said applying which is carried out by spraying, injection, coating, dusting, or immersion.
- 25 12. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied to plants or plant seeds as a composition further comprising a carrier.
13. A method according to claim 12, wherein the carrier is selected from the group consisting of water, aqueous solutions, slurries, and powders.
- 30 14. A method according to claim 12, wherein the composition contains greater than 0.5 nM of the hypersensitive response elicitor polypeptide or protein.



- 97 -

15. A method according to claim 12, wherein the composition further contains additives selected from the group consisting of fertilizer, insecticide, fungicide, nematacide, and mixtures thereof.
- 5 16. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is in isolated form.
- 10 17. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria which do not cause disease and are transformed with a gene encoding the hypersensitive response elicitor polypeptide or protein.
- 15 18. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein is applied as bacteria which cause disease in some plant species, but not in those subjected to said applying, and contain a gene encoding the hypersensitive response elicitor polypeptide or protein.
- 20 19. A method according to claim 1, wherein said applying causes infiltration of the polypeptide or protein into the plant.
- 25 20. A method according to claim 16, wherein said applying effects increased plant height, compared to an untreated plant or plant seed.
21. A method according to claim 20, wherein plants are treated during said applying.
22. A method according to claim 20, wherein plant seeds are treated during said applying, said method further comprising:
planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and propagating the plants from the seeds planted in the soil.



- 98 -

23. A method according to claim 1, wherein plant seeds are treated during said applying to increase plant seed quantities which germinate, said method further comprising:

5 planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
 propagating plants from the seeds planted in the soil.

24. A method according to claim 16, wherein said applying effects greater yield, compared to an untreated plant or plant seed.

10

25. A method according to claim 24, wherein plants are treated during said applying.

15

26. A method according to claim 24, wherein plant seeds are treated during said applying, said method further comprising:

 planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
 propagating plants from the seeds planted in the soil.

20

27. A method according to claim 16, wherein said applying effects earlier germination, compared to an untreated plant or plant seed.

28. A method according to claim 27, wherein plant seeds are treated during said applying, said method further comprising:

25

 planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
 propagating plants from the seeds planted in the soil.

30

29. A method according to claim 16, wherein said applying effects earlier maturation, compared to an untreated plant or plant seed.



- 99 -

30. A method according to claim 29, wherein plants are treated during said applying.

31. A method according to claim 29, wherein plant seeds are treated during
5 said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
propagating plants from the seeds planted in the soil.

10 32. A method according to claim 16, wherein plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
propagating plants from the seeds planted in the soil.

15

33. A method according to claim 32 further comprising:

applying the hypersensitive response elicitor protein or polypeptide in a non-infectious form to the propagated plants to enhance growth further.

20

34. A method according to claim 16, wherein said applying effects earlier fruit and plant coloration, compared to an untreated plant or plant seed.

35. A method according to claim 34, wherein plant seeds are treated during said applying, said method further comprising:

25 planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and
propagating plants from the seeds planted in the soil.

30 36. A method of enhancing growth in plants compared to untreated plants comprising:



- 100 -

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein; and

growing the transgenic plants or transgenic plants grown from the

5 transgenic plant seeds under conditions effective to enhance plant growth, compared to an untreated plant or plant seed, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

10 37. A method according to claim 36, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

15 38. A method according to claim 36, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

20 39. A method according to claim 36, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

25 40. A method according to claim 36, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

41. A method according to claim 36, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

30



- 101 -

42. A method according to claim 36, wherein the hypersensitive response eliciting polypeptide or protein corresponds to that derived from a *Phytophthora* species.

5 43. A method according to claim 36, wherein the plant is selected from the group consisting of dicots and monocots.

44. A method according to claim 43, wherein the plant is selected from the group consisting of rice, wheat, barley, rye, cotton, sunflower, peanut, corn, 10 potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum and sugarcane.

15 45. A method according to claim 43, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation and zinnia.

46. A method according to claim 36, wherein a transgenic plant is provided. 20

47. A method according to claim 36, wherein a transgenic plant seed is provided.

48. A method according to claim 36 further comprising: 25 applying the hypersensitive response elicitor polypeptide or protein to the propagated plants to enhance growth of the plant.

49. A transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor in a form effective to enhance growth of the 30 plant, wherein the hypersensitive response elicitor is derived from a plant pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.



- 102 -

50. A transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor in a form effective to enhance growth of a plant grown from the plant seed, wherein the hypersensitive response elicitor is derived from a pathogen selected from the group consisting of *Erwinia*,
5 *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures thereof.

51. A method according to claim 1 substantially as hereinbefore described with reference to any of the Examples.

10

DATED: 3 April 2002
PHILLIPS ORMONDE & FITZPATRICK
Attorneys for:
CORNELL RESEARCH FOUNDATION, INC.

15



1/2

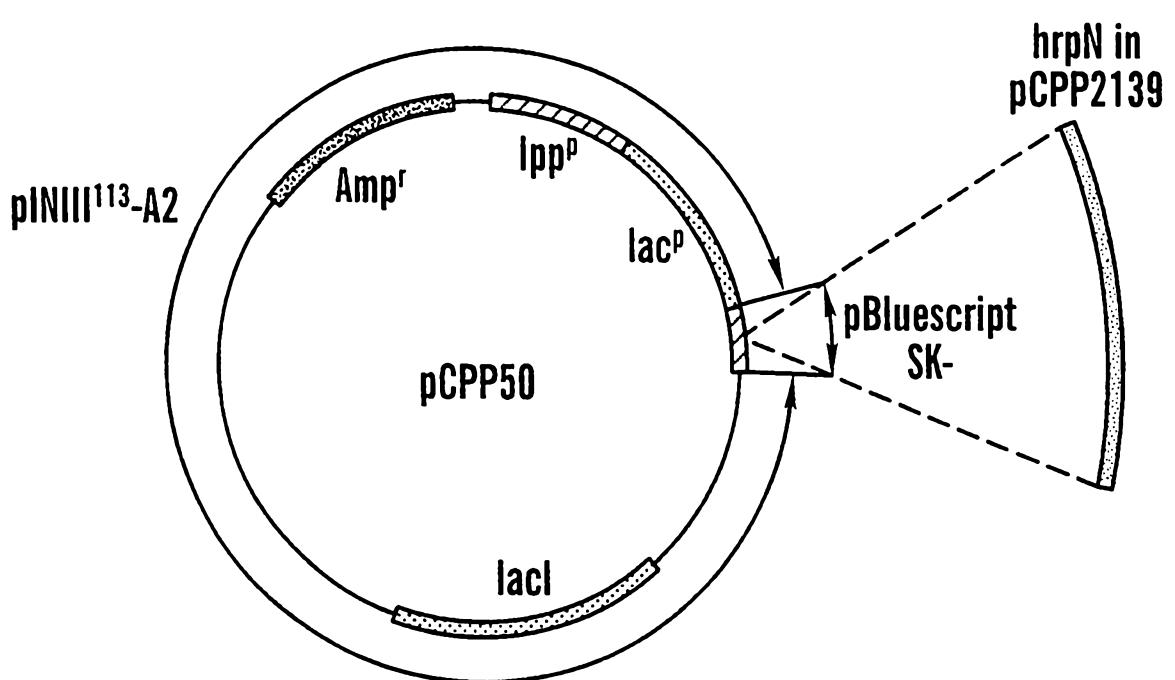


FIG. 1

2/2

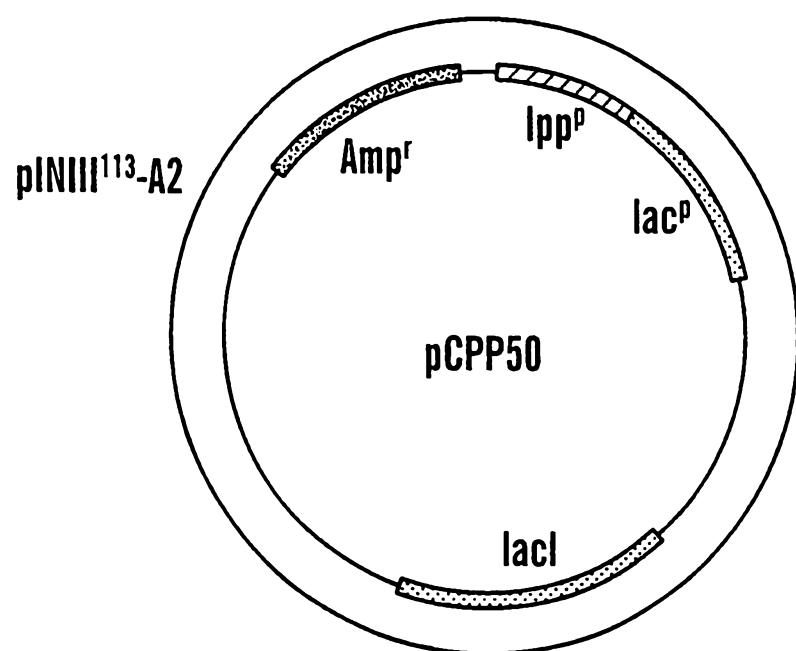


FIG. 2