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(71) **Applicant** *(for all designated States except US)*: MONSANTO COMPANY [US/US]; 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: BROWN, Sherri, Marie [US/US]; 15504 Twingate Drive, Chesterfield, MO 63017 (US). GREENPLATE, John, Thomas [US/US]; 747 Sherwick Terrace, Manchester, MO 63021 (US). ISAAC, Barbara, Guenther [US/US]; 5165 Rosemount Drive, St. Charles, MO 63304 (US). JENNINGS, Michael, Girard [US/US]; Apartment 307, 14550 Rialto Drive, Chesterfield, MO 63017 (US). LEVINE, Elaine, Beatrice [US/US]; 12392 Eastland Court, St. Louis, MO 63146 (US). PURCELL, John, Patrick [US/US]; 615 Charbray Drive, Ballwin, MO 63011 (US).

(74) **Agent:** BOLDING, James, Clifton; Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).

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(54) **Title:** METHOD OF CONTROLLING INSECTS IN PLANTS

(57) **Abstract**

Patatins control insects, primarily by stunting growth of larvae, thereby preventing maturation and reproduction. Genes encoding for one or more of these proteins may be cloned into vectors for transformation of plant-colonizing microorganisms or plants, thereby providing a method of controlling insect infestation.

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METHOD OF CONTROLLING INSECTS

FIELD OF THE INVENTION

This invention relates to a method of controlling insect infestation of plants by providing a protein which may be applied directly to the plant or produced thereon by microorganisms or by genetically modifying the plant to produce the protein, and to microorganisms and plants useful in that method.

BACKGROUND OF THE INVENTION

The use of natural products, including proteins, is a well known method of controlling many insect pests. For example, endotoxins of *Bacillus thuringiensis (B.t.)* are used to control both lepidopteran and coleopteran insect pests. Genes producing these endotoxins have been introduced into and expressed by various plants, including cotton, tobacco, and tomato. There are, however, several economically important insect pests that are not susceptible to *B.t.* endotoxins. Examples of such important pests are the boll weevil (BWV), *Anthonomus grandis*, and corn rootworm (CRW), *Diabrotica* spp. In addition, having other, different gene products for control of insects which are susceptible to *B.t.* endotoxins is important, if not vital, for resistance management.

Several other known insecticidal proteins are found in plants. These include lectins, amylase inhibitors, and protease inhibitors, which can affect insect growth and development when ingested at high doses [Boulter et al.,1989; Broadway and Duffey, 1986; Czapla and Lang, 1990; Gatehouse et al., 1986; Heusing et al., 1991; Ishimoto and K. Kitamura, 1989; Murdock et al., 1990; Shukle and Murdock, 1983], but do not provide the acute mortality afforded by *B.t.* proteins.

It is an object of the present invention to provide proteins capable of controlling BWV, CRW, or other insect pests, and genes useful in producing such proteins. It is a further object of the present invention to provide genetic constructs for and methods of inserting such genetic material into microorganisms and plant cells. It is another object of the present invention to provide transformed microorganisms and plants containing such genetic material.

SUMMARY OF THE INVENTION

It has been discovered that patatins, the major storage protein of potato tubers, will control various insects, including western corn rootworm (WCRW), *Diabrotica virgifera*, southern corn rootworm (SCRW), *Diabrotica undecimpunctata,* and boll weevil (BWV), *Anthonomus grandis.* Patatins are lethal to some larvae and will stunt the growth of survivors so that maturation is prevented or severely delayed resulting in no reproduction. These proteins, which are known to have esterase (lipid acyl hydrolase) activity, may be applied directly to plants or introduced in other ways such as through the application of plant-colonizing microorganisms, which have been transformed to produce the enzymes, or by the plants themselves after similar transformation.

Patatins are a family of proteins found in potato [Gaillaird, 1971; Racusen, 1984; Andrews et al., 1988] and other plants, particularly in solanaceous plants [Ganal et al., 1991; Vancanneyt et al., 1989]. In potato, the patatins are found predominantly in tubers, but also at much lower levels in other plant organs [Hofgen and Willmitzer, 1990]. The esterase substrate specificities of several patatin isozymes have been examined [Hofgen and Willmitzer, 1990; Racusen, 1986]

Genes that encode patatins have been previously isolated by Mignery et al., 1984, Mignery et al., 1988, Stiekema et al., 1988, and others. Rosahl et al., 1987, transferred it to tobacco plants, and observed expression of patatin. This demonstrates that the patatin genes can be heterologously expressed by plants.

Genes for patatins may be similarly isolated and inserted into appropriate transformation vector cassettes which are then (1) used to transform plant-colonizing microorganisms which when applied to plants express the genes producing a patatin, thereby providing control of insects, or (2) incorporated into the genome of a plant, which then protects itself from attack by insects by expressing the gene and producing a patatin. Additionally, the plant may also be transformed or bred to co-express one or more *B.t.* genes which code for proteins for the control of insects. This would provide plants that are either (1) protected from a wider range of pests and/or (2) have two modes of action against some pests, which is an important tool in resistance management. Examples of plants

transformed to express *B.t.* genes are disclosed in European Patent Publication No. 0 385 962, which corresponds to U.S. Serial Number 476,661, filed February 12, 1990, [Fischhoff et al.], which is incorporated herein by reference. Additionally, the plant may also be transformed or bred to co-express proteinase inhibitor genes, such as those encoding potato papain inhibitor [Rodis and Hoff, 1984] or soybean trypsin inhibitor [for review see Ryan, 1990] as proteinase inhibitors have been shown to potentiate the activity of other insecticidal proteins.

In accomplishing the foregoing, there is provided, in accordance with one aspect of the present invention, a method of controlling insect infestation of plants comprising providing an effective amount of an insecticidal patatin for ingestion by the insect. This method may be effected by providing plant-colonizing microorganisms which have been transformed to express a gene for a patatin and which are introduced to the plant, express such gene, and provide an insecticidally effective amount of a patatin. This method may also be effected by genetically transforming the plant to be protected with a DNA molecule comprising

(i) a promoter which functions in plant cells to cause the production of an RNA sequence;

(ii) a structural coding sequence that encodes for a patatin;

(iii) a 3' non-translated region which functions in said plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA sequence,

wherein said promoter is heterologous with respect to said structural coding sequence and wherein said promoter is operatively linked with said structural coding sequence, which is in turn operably linked with said non-translated region. Preferably the plant will express patatin at a level of about 0.1-0.5% of total protein.

Also provided by the present invention are genetically transformed, insect-resistant corn, cotton, tomato and potato plants.

As used herein, the term "controlling insect infestation" means reducing the number of insects which cause reduced beneficial yield, either through mortality, retardation of larval development (stunting), or reduced reproductive efficiency. As used herein, the term "insecticidal" means capable of reducing the number of insects which cause reduced beneficial

yield, either through mortality, retardation of larval development (stunting), or reduced reproductive efficiency.

As used herein, the term "structural coding sequence" means a DNA sequence which encodes for a polypeptide, which may be made by a cell following transcription of the DNA to mRNA, followed by translation to the desired polypeptide.

As used herein, the term "patatin" means a plant protein having 75% or more homology to the protein encoded by SEQ ID NO:31, shown below, or more preferably at least 80% homology, or even more preferably at least 85% homology. This term also includes proteins produced from synthetic DNA sequences which have been designed for improved expression in monocots.

DETAILED DESCRIPTION OF THE INVENTION

Patatins are a family of esterases found in potato [Gaillaird, 1971; Racusen, 1984; Andrews et al., 1988] and other plants, particularly solanaceous plants [Ganal et al., 1991; Vancanneyt et al., 1989]. In potato, the patatins are found predominantly in tubers, but also at very low levels in other plant organs [Hofgen and Willmitzer, 1990]. The esterase substrate specificities of several patatin isozymes have been examined [Hofgen and Willmitzer, 1990; Racusen, 1986] and found to have broad substrate specificity, showing that these enzymes have limited substrate requirements. The use of all plant-derived patatins and their equivalents, both those disclosed in detail herein and homologous proteins, whether derived from natural DNA sequences or synthetic DNA sequences, for the purpose of controlling insect infestation of plants is within the scope of the present invention.

Crude patatin preparations from potato are available commercially. For example, Sigma Chemical Company, St. Louis, MO offers potato protein preparations denominated by Sigma as acid phosphatase (P-1146 and P-3752) or apyrase (A-9149). Potato tubers may also be acquired and protein extracts can be prepared by methods described in the literature (Racusen and Foote, 1980; Park et al., 1983).

BIOEFFICACY ASSAYS

Artificial Diet Bioassays

Assays for activity against larvae of SCRW, BWV, Colorado potato beetle (CPB), *Leptinotarsa decemlineata*, and European corn borer (ECB), *Ostrinia nubilalis*, are carried out by overlaying the test sample on an agar diet similar to that described for SCRW by Marrone et al., 1985. Test samples were prepared by solubilization of the protein in 4-5 mL 10 mM HEPES, pH 7.5, followed by dialysis in this same buffer using 3500 molecular weight cutoff tubing. Neonate larvae are allowed to feed on the treated diet at 26 °C and mortality and growth stunting are evaluated at 5 or 6 days. The results of the assays of P-3752 (Sigma) are given in Table 1. This crude potato preparation showed broad spectrum insecticidal activity.

Table 1

Rate	% Mortality/Stunting[a]			
	SCRW	BWV	CPB	ECB
0.01X	0	11	0	0
0.03X	0*	20*	0	13
0.10X	19**	20**	6	0*
0.30X	6**-***	46***	13*	6*
1.00X	6***	73***	13**	6**

[a] *=slight stunting (approximately 30-40% size reduction)
**=moderate stunting (approximately 50-80% size reduction)
***=severe stunting (>90% size reduction)

Precise quantitative measurements of the weights of SCRW (Table 2) after 5 days exposure and ECB (Table 3) after 6 days exposure were made and are presented below. SCRW larvae developing on diet containing P-3752 showed a 92% reduction in weight compared to controls and ECB larvae showed a 62% reduction in weight compared to controls.

Table 2

Treatment	Mean Surv.Wgt.(SEM)	%Wt Rdct	%Mortality
Tris	4.00 mg (0.60)[a]	--control level--	
P-3752	0.30 mg (0.03)[a]	92	6

[a]Mean Surv. Wgts. significantly different at 95% (One Factor ANOVA).

Table 3

Treatment	Mean Surv.Wgt.(SEM)	%Wt Rdct	%Mortality
Tris	5.39 mg (0.49)[a]	--control level--	
P-3752	2.05 mg (0.27)[a]	62	7

[a]Mean Surv. Wgts. significantly different at 95% (One Factor ANOVA).

The proteinaceous nature of the insecticidal component of P-3752 which is active against southern corn rootworm (SCRW) and boll weevil (BWV) was determined by heat lability, ammonium sulfate precipitation, molecular size fractionation, and protease susceptibility experiments.

To confirm that the effects of P-3752 are due to direct effects of ingested patatin and not indirect effects due to an antifeedant response, a diet choice study was conducted with ECB and SCRW. Results of this choice study indicated that there was feeding on both P-3752- and Tris-treated diet with no overwhelming preference. There appeared to be no avoidance of P-3752-treated diet in relation to Tris-treated diet.

A long term (25 day) assay of P-3752 against SCRW utilized 2nd instar larvae and several transfers of surviving insects onto freshly-treated diet. At the end of the study, all of the control larvae had pupated. In contrast, 50% of the treatment larvae were dead and the other 50% had increased in body weight by only 16% of their initial weight (2.48 mg vs. 2.14 mg). This demonstrates that the larval development is arrested, not just slowed. This has important ramifications from an insect control standpoint as the larvae will not develop to adulthood. Thus the number of rootworms in future generations will be reduced.

Larvae of western corn rootworm (WCRW), *Diabrotica virgifera*, can only be used in laboratory experiments in the 2nd instar larval stage. To test P-3752 against WCRW, a side-by-side assay with 2nd instar SCRW larvae was designed. P-3752 treatment resulted in only 13% and 11% weight gain, respectively, of SCRW and WCRW 2nd instar larvae. Control SCRW increased in weight by 474% and WCRW grew 200% in 7 days. This suggests that patatin activity against WCRW is roughly equivalent to its activity against SCRW.

P-3752 was slightly active against tobacco budworm (TBW), *Heliothis virescens*, beet armyworm (BAW), *Spodoptera exigua*, corn

earworm, *Helicoverpa zea*, pink bollworm, *Pectinophora gossypiella*, and tobacco hornworm, *Manduca sexta*, with stunting ratings of 1 to 1.5 at the same concentration at which a stunting rating of 3 is demonstrated for SCRW. P-3752 gave a stunting rating of 2.5 for black cutworm, *Agrotis ipsilon*. (The stunting ratings are defined above in Table 1.) It was inactive against green peach aphid, *Myzus persicae*, at the concentration tested.

Plant Tissue Bioassays

(1) Potato: One g of crude P-3752 was dissolved in 4 mL 25 mM Tris, pH 7.5 buffer, then dialyzed and filtered through a 0.2 μm membrane. Triton® X-100 was added to generate a 0.1% solution. Potato leaves were dipped into the enzyme preparation and placed on moistened filter paper in petri dishes. CPB larvae were added and the plates were incubated at 27 °C for 3 days. P-3752 treatment of potato leaves resulted in stunting and reduced feeding of CPB larvae. At the conclusion of the assay, significantly less leaf tissue remained on control leaves compared to P-3752-treated leaves.

(2) Corn and cotton: Black Mexican sweet corn callus (BMS) or cotton callus was removed from agar plates and transferred into 50 mL centrifuge tubes. Callus was vortexed and centrifuged in an IEC Clinical centrifuge for 5 min. at setting 8. The supernatant was decanted. To a 50 mL tube containing 15 mL of callus pellet was added 30 mL of liquid 2% agar. Following thorough mixing, the diet was pipetted into an assay arena for insect bioassay. Dialyzed P-3752 was added as a diet overlay (at 20% volume) and the assay was carried out as described above.

Excised corn roots and shoots were vacuum-infiltrated (Inflt.) with crude P-3752 or 25 mM Tris, pH 7.5 buffer. The control sample was tissue submersed in Tris buffer. Approximately 10-15 pieces of root or 3 pieces of shoot tissue were placed in wells of a 24-well tissue culture plate and replicated 4 times. Four neonate SCRW larvae were added to each well. The assay was incubated at 26 °C for 4 days, at which time observations were made with respect to mortality and average larval weight. The results of these assays are shown in Table 4.

Table 4

Tissue	Insect	% Mortality	%Wt.Reduction
Inflt corn roots	SCRW	90	44

Inflt corn shoots	SCRW	51	52
Trtd BMS callus	SCRW	24	51
Trtd BMS callus	WCRW	0	23
Trtd BMS callus	ECB	0	33
Trtd cotton callus	BWV	60	no data

Thus, insecticidal activity on all four insects (SCRW, BWV, CPB, and ECB) is retained when P-3752 is co-ingested with plant tissue. These diet studies demonstrate that the patatins are insecticidally active when assayed in diets whose nutrients are comprised solely of plant tissue (roots, shoots, callus or leaves).

MODE OF ACTION STUDIES

The following studies suggest that patatin, the insecticidally active component of P-3752, has a direct effect on the insect itself and that the activity demonstrated in the experiments described above cannot be attributed to the active component's effect(s) on the insect's diet prior to ingestion.

Diet Effect Study

One gram of P-3752 was dissolved in 10 mL of 25 mM Tris, pH 7.6 buffer, then dialyzed in MWCO 12-14,000 tubing against this same buffer. Following 0.2 μm filtration, 50 μL aliquots were added to insect diet wells on two plates four days prior to insect addition. Both plates were incubated at 27 °C for four days. Following incubation, one plate was heated to 80 °C for 1 hour to inactivate the enzyme(s). Fifty μL aliquots were added to a third plate. Thus, incubated, incubated + heat, and unincubated plates were utilized for SCRW bioassay.

SCRW activity in the diet pre-incubation study was as follows:

unincubated P-3752 - 6***

incubated P-3752 - 0**

incubated, heated P-3752 - 0

While some of the activity was lost during the diet incubation, a complete loss of activity resulted from heat treatment. This data is consistent with a direct, post-ingestion mode-of-action, and when considered in conjunction with the plant tissue assays and the variability against different insects

indicates that the activity of the protein on SCRW and other insects is not via a dietary effect.

PROTEIN IDENTIFICATION

The insecticidally active component from P-3752 has been purified, partially sequenced, and characterized. The active agent(s) has been identified as patatin, a family of lipid acyl hydrolases from potato.

Protein Isolation

Four distinct protocols were used to purify the SCRW bioactive component from P-3752.

Purification of SCRW activity by anion exchange chromatography - The SCRW-active component from P-3752 was purified by Q-Sepharose (Pharmacia) anion exchange chromatography followed by MONO-Q (HR 5/5, Pharmacia) anion exchange chromatography. The protein levels in SCRW-active fractions indicated that the observed ** stunting was achieved with a protein concentration of 31 ppm of diet. SDS-PAGE indicated that three major protein bands (M_r 42,000, ~26,000 and ~16,000) were present in the active fractions.

Five step purification of SCRW activity - Five sequential purification steps were used to purify the SCRW-active component from P-3752. These were membrane sizing, ammonium sulfate precipitation, Q-Sepharose IEC, S-Sepharose IEC, and P-200 SEC. SDS-PAGE of the purest SCRW-active fractions showed protein bands at M_r 42,000, ~26,000, and ~16,000.

Purification of bioactivity by isoelectric focusing - The SCRW-bioactive potato proteins were purified by two sequential runs on the RF3 protein fractionator (Rainin) according to the manufacturer's instructions. The SDS-PAGE profile of the SCRW-active fractions was very similar to the profile observed in active fractions from the 5-step purification and the anion exchange purification. The IEF gel showed that the proteins fractionate from pH 4.6 to 5.1, consistent with the reported pI range for patatin (Racusen and Foote, 1980).

Consecutive isoelectric focusings on the RF3 over a narrow pH range (pH 4-5) were used to attempt to resolve the patatin isozymes. As

expected, a peak of bioactivity was seen with proteins of pIs 4.6-5.1. These fractions have distinct isozyme patterns and different levels of bioactivity. Bioactivity in the fractions ranged from 0 mortality with *-** stunting at doses of 80-512 ppm. Some of the bioactive fractions have only 2 major isozymes, demonstrating that a complex pattern of isozymes is not required for bioactivity.

Purification by Native PAGE - P-3752 was electrophoresed under native conditions and an esterase-active (using α-naphthyl acetate as substrate) triplet of bands was isolated. The gel-purified esterase-positive material was active against SCRW yielding 1.5* stunting. SDS-PAGE of this material revealed major bands at M_r 42,000, ~26,000 and ~16,000, a profile previously observed with the other purifications. This is further confirmation that patatin is the insecticidal component from potato.

Amino Acid Sequences

NH_2-terminal amino acid sequence was obtained on all the protein bands (M_r 42,000, ~26,000 and ~16,000) in the SCRW-active chromatography fraction from the anion exchange purification and the five step purification. Overall, sequence data were generated for all bands in the active fractions. Most of the bands showed >85% homology with a 15-amino acid sequence at either the NH_2-terminus (SEQ ID NO:1) or an internal sequence (SEQ ID NO:7) of an isozyme of patatin (Stiekema et al., 1988). One of the 17 kD bands showed 75% homology with the initial eight amino acids of the published NH_2-terminus sequence of patatin. The other 17 kD band showed >85% homology with the initial eight amino acids of the published internal sequence. These bands represent proteolyzed products of patatin. The presence of isozymes is clearly indicated by variability in amino acids at positions 1 and 3 for both NH_2-terminus and internal sequences.

N-terminal Amino Acid Sequence

Pub. Seq.:	K**L** E **EMVTVLSIDGGG** (SEQ ID NO:1)
Band 1 (42 kD):	X**L** G **EMVTVLSIDGGG** (SEQ ID NO:2)
Band 2 (28 kD):	T**L** G **EMVTVLSIDGGG** (SEQ ID NO:3)
Band 3 (26 kD):	T**L** G **EMVTVLSIDGGG** (SEQ ID NO:4)
Band 4 (24 kD):	K**L** X **EMVTVLSIDGGG** (SEQ ID NO:5)

Band 5a (17 kD): XXE**EMVTV** (SEQ ID NO:6)

Internal Sequence (amino acid position 224)

Pub. Seq.: **SLDYKQMLLLSLGTG** (SEQ ID NO:7)

Band 5b (17 kD): **KL**D**YKQML** (SEQ ID NO:8)

Band 6 (16 kD): **SL**X**YKQMLLLSLGTG** (SEQ ID NO:9)

Band 7 (15 kD): **SL**N**YKQMLLLSLGTG** (SEQ ID NO:10)

Esterase Activity

Several experiments were run to examine the esterase activity in the SCRW-active fractions.

α-naphthylacetate substrate: SDS-PAGE (10-20%) was utilized to determine if SCRW-active fractions (from the 5-step purification) exhibited esterase activity [Racusen, 1984]. On two halves of the gel, sets of heated and non-heated SCRW-active fractions were loaded. A single esterase-positive band was observed in the non-heated sample, with an M_r of 55,000. The heated sample revealed the original M_r 42,000 band and a concomitant absence of a 55,000 band. This result is consistent with the literature reports of the electrophoretic mobility of patatin's esterase activity (Racusen, 1984). The M_r 55,000 band was not observed in the heated sample, which indicates that the heat treatment in SDS eliminates the esterase activity. In the absence of the M_r 55,000 band in the heated sample, the originally observed M_r 42,000 band was observed with coomassie staining.

p-Nitrophenyl substrate specificity studies - A series of *p*-nitrophenyl esters (C-2, C-4, C-6, C-8, C-10, C-12, C-14, and C-16 esters) was tested to determine the substrate specificity. *p*-NP C-8 and C-10 esters were consistently the best substrates for the esterase activity of most of the patatins tested, relative to the other esters.

Lipid ester substrates - A SCRW-active purified fraction (from 5-step purification) was tested for the ability to hydrolyze several lipids Each lipid was dissolved and incubated with an aliquot of a SCRW-active purified fraction. Samples were analyzed by TLC utilizing a three solvent development system (Pernes et al., 1980). Four lipids showed marked

modifications by TLC. These included oleoyl lysolecithin, dioleoyl L-α-phosphatidylcholine, 1-monolinolenoyl-rac-glycerol, and diolein (Sigma). A new TLC spot at R_f 0.37 in the organic extract of these lipid/active fraction reaction mixtures was identified as free fatty acid by comparison with linoleic and oleic fatty acid standards. Thus, SCRW-active material shows esterase activity on these four lipid esters.

WCRW midguts were removed from third instar larvae feeding on corn roots. Midgut lipids were extracted, dissolved and incubated at the pH of the midgut (pH 6.55) with the SCRW-active purified fraction. Samples were analyzed by TLC utilizing the above method. The purified SCRW-active fraction demonstrated esterase activity on WCRW midgut phospholipids at the pH of the midgut. This illustrates a possible mode-of-action for the insecticidal activity of patatin.

Alternate Sources of Patatin

Because all initial experiments were carried out with P-3752, a commercially available enzyme preparation (Sigma) from Minnesota Russet var. Kranz potato tubers, it was desirable to demonstrate that insecticidally active patatins could be recovered from fresh potato tuber tissue. Tuber extracts were prepared essentially as described in the literature (Racusen and Foote, 1980; Park et al., 1983). Three commercially available *S. tuberosum* cultivars (Russet, Desiree, and LaChipper) and seven wild type species (*S. kurtzianum*, *S. berthaultii*, *S. tarijense*, *S. acaule*, *S. demissum*, *S. cardiophyllum*, and *S. raphanifolium*, all available from the Inter-Regional Potato Introduction Station, USDA, ARS, Sturgeon Bay, WI) were analyzed. All extracts were positive for patatin by SDS-PAGE and Western blot assays; all were esterase positive by C-10 esterase assay; and all were insecticidally active against SCRW, i.e., had stunting ratings of 2-3. See Table 5. This demonstrates that insecticidally active patatins can be isolated from the tubers of several species and that many members of this entire class of proteins would be expected to have insecticidal properties.

Table 5

Species	[Prot] (mg/mL)	ΔO.D./min•mL	SCRW [a] 1X	SCRW [a] 0.1X
S. acaule	26.6	82,500	2	1
S. berthaultii	23.1	29	3	1
S. cardiophyllum	14.6	89	2.5	1.5
S. demissum	35.3	375,000	3	1.5
S. kurtzianum	25.0	2,700	2.5	1
S. raphanifolium	33.7	3,725	3	2
S. tarijense	27.2	1008	2.5	1

[a] SCRW activity is expressed in terms of larval stunting: 1 = slight stunting (30-40% size reduction), 2 = moderate stunting (50-80% size reduction), 3 = severe stunting (>90% size reduction).

Extracts of *S. berthaultii, S. kurtzianum,* and *S. tarijense* were bioassayed against two additional target insects, CPB and ECB. Bioassay data is summarized below in Table 6. Very little activity was noted with these extracts against CPB whereas the ECB larvae were moderately to severely stunted at the 1X rate. However, the ECB larvae appear to be slightly less sensitive to these potato extracts than the SCRW larvae, as indicated by a complete absence of activity at 0.1X against ECB.

Table 6

Species	CPB[a] 1X	CPB[a] 0.1X	ECB [a] 1X	ECB [a] 0.1X
S. berthaultii	0	0	3	0
S. kurtzianum	1	0	2.5	0
S. tarijense	0	0	3	0

[a] Larval stunting

Genomic DNA from nine different plants was tested by Southern analysis for proteins homologous to patatin. Southern blots probed with a α-^{32}P-labelled probe of SEQ ID NO:11 indicated that there are homologous sequences in several other plant species. Strong signals were obtained in corn, tomato, sugar beet, rice, and potato. Individual bands were unable to be resolved in this experiment; however, the size of the smears and their intensities were similar in all of these species. Weaker signals were also

seen in zucchini, soybean, and canola, and appeared as a small number of discreet bands in the DNA from each species. Cucumber and *Arabidopsis* did not exhibit detectable hybridization with the patatin probe under the conditions used in this experiment, perhaps due to the smaller amount of DNA loaded as seen in the ethidium bromide stain of the gel.

The DNA sequences for these homologous proteins can be readily obtained by one of ordinary skill in the art and inserted into plants or other organisms by known means. The insecticidal properties of such proteins can be best tested after heterologous expression, for example, from baculovirus or *E. coli*. Thus, other proteins which can be used in the methods of the present invention may be obtained with a normal amount of experimentation using known methods and thereafter used to provide plants with protection from insect infestation.

GENETIC IDENTIFICATION

Genes for patatins have been cloned by several investigators. The sequence disclosed by Mignery et al., 1984, was referred to as GM203. It has an incomplete signal sequence. Mignery et al., 1988, identified a genomic clone, designated PS20, encompassing GM203 and containing a complete signal sequence. SEQ ID NO:11 was constructed with the signal sequence of PS20 and the cDNA coding portion of GM203, hereinafter referred to as PatA+. It also contains an NcoI restriction site and an EcoRI site immediately following the translation termination codon.

Solanum tuberosum cv. Russet Burbank

Twenty cDNAs were isolated from the tubers of potato cultivar Russet Burbank and sequenced. The deduced amino acid sequences show that these cDNAs encode eleven different patatin isozymes. These eleven proteins are from about 82% to 100% identical as compared to PatA+, SEQ ID NO:11, with differences occurring at numerous positions throughout the length of the cDNA. The sequences for eleven different representative cDNAs encoding the eleven different patatin isozymes are denominated as shown in Table 7. The cDNAs were engineered by PCR procedures using primers SEQ ID NO:26 and SEQ ID NO:27, corresponding to the 5' nucleotides encoding the first few codons of the signal sequence and the 3' end of the coding sequence, respectively, for later cloning manipulations. A

"+" symbol indicates that the native signal coding sequence is included. Some cDNAs did not contain the complete native signal coding sequence and only the mature protein coding sequence was obtained from a similar PCR procedure using primers SEQ ID NO:32 and SEQ ID NO:27. These are so designated with the subscript "m."

Table 7

Isozyme	Sequence ID Number
PatA+	SEQ ID NO:11
$PatA_m$	SEQ ID NO:14
PatB+	SEQ ID NO:16
PatC+	SEQ ID NO:17
$PatD_m$	SEQ ID NO:18
PatE+	SEQ ID NO:19
$PatE_m$	SEQ ID NO:20
$PatF_m$	SEQ ID NO:21
PatG+	SEQ ID NO:22
$PatH_m$	SEQ ID NO:15
$PatI_m$	SEQ ID NO:23
PatL+	SEQ ID NO:24
PatM+	SEQ ID NO:25

Solanum berthaultii

Patatin cDNAs from the diploid potato *S. berthaultii* were isolated by reverse transcription of tuber mRNA followed by PCR with primers SEQ ID NO:26 and SEQ ID NO:27, described above. Multiple independent PCR reactions were performed to avoid the isolation of duplicate clones due to the amplification process.

A total of 14 patatin cDNAs were partially sequenced. All fourteen cDNAs (denominated Pat1 through Pat14) appear to have a unique nucleotide sequence, suggesting that at least 14 different patatin mRNAs are expressed in *S. berthaultii* tubers. The sequence for Pat3+ is SEQ ID NO:28. The sequence for Pat10+ is SEQ ID NO:29. The deduced amino acid sequence shows that the 14 cDNAs encode at least 11 different proteins. In general, the cDNA sequences from the *S. berthaultii* tubers

were very similar. Only 12 amino acid positions of the total 367 residues (3%) showed sequence variability. The amino acid residues present in each of those positions is shown in Table 8. At five of these positions, there was only a single variant clone with a unique residue. These changes could reflect actual differences between mRNAs or could have resulted from errors made during the PCR process. At the other seven positions, there was more variability; at least two cDNAs had an alternate amino acid. Each of the nine different amino acid sequence groups had a unique pattern of residues at these seven positions. In some cases, the changes were conservative such as the Thr to Ser change at position 164. In other cases, there were more dramatic differences such as introduction of a proline at position 148.

TABLE 8

cDNA	Position of Amino Acid Difference											
	89		96		106	113	120	123	148	164	187	200
PAT3+	GLN	LEU	GLN		TYR	GLU	VAL	ALA	ALA	THR	ASP	ASP
PAT4+	GLN		SER	ASP	HIS	GLU	VAL	ALA	PRO	SER	ASP	VAL
PAT5+	GLN		SER	ASP	HIS	GLU	VAL	ALA	PRO	THR	ASP	ASP
PAT7+	GLN	LEU	GLN		TYR	GLU	VAL	ALA	ALA	THR	ASN	ASP
PAT8+	LYS		SER	GLY	TYR	LYS	VAL	ALA	PRO	THR	ASP	ASP
PAT9+	LYS		SER	ASP	TYR	LYS	VAL	ALA	PRO	THR	ASP	ASP
PAT10+	GLN		SER	ASP	HIS	GLU	VAL	THR	PRO	THR	ASP	ASP
PAT11+	GLN		SER	ASP	HIS	GLU	ALA	ALA	ALA	THR	ASP	ASP
PAT12+	GLN		SER	GLY	HIS	GLU	VAL	ALA	ALA	THR	ASP	ASP
PATA+	HIS	---	SER	---	TYR	GLU	VAL	ALA	ALA	THR	GLU	ASP

Solanum cardiophyllum

Ten cDNA clones were generated via PCR utilizing mRNA isolated from *Solanum cardiophyllum* tubers as described above. Nucleotide sequence was obtained on at least 75% of the length of each clone. The full length sequence of one clone denominated Pat17+ is SEQ ID NO:30. SEQ ID NO:31 is the engineered mature form, $Pat17_m$. The *S. cardiophyllum* clones were almost identical, with only random nucleotide sequence changes that could be actual differences or PCR errors. However at positions 54

and 519, several clones were observed to have identical changes, suggesting that they are not due to the amplification process. The patterns of nucleotides at these positions indicated that there are at least 4 different mRNAs represented. mRNAs from two of the groups were isolated several times and mRNA from the other two groups were only isolated once in this set of cDNA clones.

The deduced amino acid sequences of the *S. cardiophyllum* clones were also extremely similar. There were 8 unique amino acid sequence groups, each differing from the other sequences by a single residue. cDNA clones encoding an amino acid sequence identical to the Pat17+ sequence were recovered twice and the other seven cDNAs (Pat 18+, 19+, 20+, 21+, 22+, 23+, and 24+) contained a single unique residue.

GENETIC TRANSFORMATION

As discussed above, patatin genes can be isolated from various plant sources. One or more of these genes may then be used to transform bacterial cells or plant cells to enable the production of patatin and carry out the methods of this invention. Examples of how this may be done with various sequences for patatin are given below.

Engineering of the Patatin cDNAs

In order to incorporate a patatin gene into vectors appropriate for expression of patatin in heterologous host cells, it was necessary to introduce appropriate restriction sites near the ends of the gene. The goals of this mutagenesis were to create cassettes that included the protein coding sequence with minimal noncoding flanking sequences and to incorporate useful restriction sites to mobilize these cassettes. Cassettes were designed that would allow mobilization of the intact coding sequence including the signal peptide or just the mature coding sequence. For $PatA_m$, two mutagenesis primers were designed to create these cassettes. Mutagenesis with SEQ ID NO:12 substituted two amino acids (methionine-alanine) for lysine at the N-terminus of the mature protein and introduced an NcoI site, and SEQ ID NO:13 added a second termination codon and an EcoRI site.

The resulting modified sequence was identified as $PatA_m$, SEQ ID NO:14. For all other cDNAs, similar modifications and introduction of

restriction sites were done using PCR and either primers SEQ ID NO:26 and SEQ ID NO:27 or primers SEQ ID NO:32 and SEQ ID NO:27, as described previously.

Expression of Patatins in *E. coli*

The DNA coding sequence for $PatA_m$ (SEQ ID NO:14) was inserted into pMON5766, an *E. coli* expression vector derived from pBR327 (Soberon et al., 1980) with a recA promoter and a G10 leader (Olins et al., 1989). The resulting vector, pMON19714, was mobilized into *E. coli* strain JM101, which subsequently produced $PatA_m$ as confirmed by Western blot analysis and esterase activity using *p*-nitrophenyl C-10 ester.

The DNA coding sequence for $Pat17_m$ as well as that for $PatA_m$ were each inserted into an *E. coli* expression vector derived from pMON6235 with the AraBAD promoter (inducible when cells are grown in arabinose), a G10 leader, and an ampicillin resistance marker gene. The resulting vectors, pMON25213, containing $Pat17_m$, and pMON25216, containing $PatA_m$, were introduced into *E. coli* strain JM101.

Patatin is expressed by the transformed *E. coli*; however, it is compartmentalized in refractile bodies (RBs). Intact cells and solubilized RBs were used in SCRW assays. The results are shown in Table 9.

Table 9

Sample (pMON)	Rep	Intact cells SCRW activity[1]	Solubilized RBs SCRW activity[1]
19714 A_m	1	2.5	nt
	2	1.5	1.0
25216 A_m	1	1.0	0
	2	0	1.0
25213 17_m	1	3.0	3.0[2]
	2	1.5	0.5

[1] SCRW activity is expressed in terms of larval stunting: 1=slight stunting (30-40% size reduction), 2=moderate stunting (50-80% size reduction), 3=severe stunting (>90% size reduction).

[2] Mortality rate with this sample was 81%.

Expression of Patatins in Plant-Colonizing Bacteria

To control insects, it may be desirable to express one or more patatins in a plant-colonizing bacterium, and then apply this bacterium to the plant. As the insect feeds on the plant, it ingests a toxic dose of patatin produced by the plant colonizers. Plant-colonizers can be either those that inhabit the plant surface, such as *Pseudomonas* or *Agrobacterium* species, or endophytes that inhabit the plant vasculature such as *Clavibacter* species. For surface colonizers, the patatin gene may be inserted into a broad host range vector capable of replicating in these Gram-negative hosts. Examples of such vectors are pKT231 of the IncQ incompatibility group [Bagdasarian et al., 1981] or pVK100 of the IncP group [Knauf, 1982]. For endophytes the patatin gene can be inserted into the chromosome by homologous recombination or by incorporation of the gene onto an appropriate transposon capable of chromosomal insertion in these endophytic bacteria.

Expression of Patatins in Baculovirus

Patatin genes were cloned into the baculovirus donor vector pMON14327, described in co-pending U.S. Serial Number 07/941,363, filed September 4, 1992, which is hereby incorporated by reference, as NcoI/EcoRI fragments. Donor vector pMON14327 contains an ampicillin resistance gene, the left and right arms of the Tn7 transposon, and, between these arms, a gentamicin resistance gene, the strong baculovirus polyhedrin promoter and a polylinker. The baculovirus shuttle vector or bacmid is composed of a mini-attTn7 site in frame within the lacZ gene and a kanamycin resistance gene recombined into the AcNPV viral genome. With the help of a tetracycline-resistant helper plasmid, pMON7124, recombinant AcNPV virus were produced by transposition of the patatin or GUS genes and marker genes into the viral genome (Luckow et al., 1993). The following genes were inserted into pMON14327: the genes listed in Table 7 along with Pat3+, Pat10+, and Pat17+.

Following the procedures of U.S. Serial Number 07/941,363 and Luckow et al., quantities of patatin from the above genes were produced. The presence of patatin was confirmed by Western blot analysis and esterase activity with *p*-nitrophenyl C-10 ester. With the exception of Pat

A_m, each isozyme was scaled-up at least twice for SCRW bioassay. While Pat E+ and $PatE_m$ fermentations consistently showed little or no patatin expression, all other isozymes appeared to be expressed at acceptable levels for bioassay. However, the nature of the post-translational processing of the patatin proteins in baculovirus compared to potato was not determined. Bioactivity of the isozymes as expressed by baculovirus, against SCRW, was observed with Pat 17+, PatB+, $PatD_m$, $PatI_m$, PatL+, and Pat3+.

The effects of multiple isozymes (produced by baculovirus) on insects' growth and development was determined. Aliquots of the eleven Russet isozymes were combined into one sample for bioassay against SCRW, ECB, black cutworm, and TBW. Ten to fifteen mg of each Q-Sepharose-purified isozyme was combined except for $PatD_m$, of which only 1.7 mg was available. This mixture resulted in 100% mortality in TBW assays and 93% weight reduction against ECB. Thus, each isozyme was assayed separately against TBW and ECB. Compared to the vector control larvae, TBW and ECB larvae feeding on diet treated with isozymes PatC+, PatL+, and $PatI_m$ showed significant stunting (≥75%) and/or mortality. Pat B+ and $PatD_m$ also stunted TBW by 69 and 78%, respectively.

Plant Gene Construction

The expression of a plant gene which exists in double-stranded DNA form involves transcription of messenger RNA (mRNA) from one strand of the DNA by RNA polymerase enzyme, and the subsequent processing of the mRNA primary transcript inside the nucleus. This processing involves a 3' non-translated region which adds polyadenylate nucleotides to the 3' end of the RNA. Transcription of DNA into mRNA is regulated by a region of DNA usually referred to as the "promoter." The promoter region contains a sequence of bases that signals RNA polymerase to associate with the DNA and to initiate the transcription of mRNA using one of the DNA strands as a template to make a corresponding strand of RNA.

A number of promoters which are active in plant cells have been described in the literature. Such promoters may be obtained from plants or plant viruses and include, but are not limited to, the nopaline synthase (NOS) and octopine synthase (OCS) promoters (which are carried on

tumor-inducing plasmids of *Agrobacterium tumefaciens*), the cauliflower mosaic virus (CaMV) 19S and 35S promoters, the light-inducible promoter from the small subunit of ribulose 1,5-bis-phosphate carboxylase (ssRUBISCO, a very abundant plant polypeptide), and the Figwort Mosaic Virus (FMV) 35S promoter. All of these promoters have been used to create various types of DNA constructs which have been expressed in plants (see e.g., PCT publication WO 84/02913). One may also want to limit expression to certain plant parts which are susceptible to insect attack. For example, a root-specific promoter may be used to limit expression to the root or a root-enhanced promoter may be used to increase levels of active protein in the roots. This is preferred for plants susceptible to root-eating insects.

Certain plant promoters are also more effective in monocots. For example, the rice actin promoter described in WO 91/09948 is efficacious for expression in corn. The maize ubiquitin promoter, described in EP 0 342 926, may also be used in monocots.

The promoters used in the DNA constructs (i.e. chimeric plant genes) of the present invention may be modified, if desired, to affect their control characteristics. For example, the CaMV35S promoter may be ligated to the portion of the ssRUBISCO gene that represses the expression of ssRUBISCO in the absence of light, to create a promoter which is active in leaves but not in roots. The resulting chimeric promoter may be used as described herein.

For purposes of this description, the phrase "CaMV35S" promoter thus includes variations of CaMV35S promoter, e.g., promoters derived by means of ligation with operator regions, random or controlled mutagenesis, etc. Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression. Examples of such enhancer sequences have been reported by Kay et al. (1987).

The particular promoter selected should be capable of causing sufficient expression of the enzyme coding sequence to result in the production of an effective amount of patatin. A preferred promoter is the CaMV E35S promoter (enhanced CaMV35S).

The RNA produced by a DNA construct of the present invention also contains a 5' non-translated leader sequence. This sequence can be

derived from the promoter selected to express the gene, and can be specifically modified so as to increase translation of the mRNA. The 5' non-translated regions can also be obtained from viral RNA's, from suitable eukaryotic genes, or from a synthetic gene sequence. The present invention is not limited to constructs wherein the non-translated region is derived from the 5' non-translated sequence that accompanies the promoter sequence.

As noted above, the 3' non-translated region of the chimeric plant genes of the present invention contains a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3' end of the RNA. Examples of preferred 3' regions are (1) the 3' transcribed, non-translated regions containing the polyadenylate signal of *Agrobacterium* tumor-inducing (Ti) plasmid genes, such as the nopaline synthase (NOS) gene and (2) plant genes like the soybean 7s storage protein genes and the pea ssRUBISCO E9 gene. [Fischhoff et al.]

Localization

Vectors containing the patatin cassettes described above express the active protein in the cytoplasm or vacuoles of the plant cell. It may be desirable to direct most or all of the patatin into the plant secretory pathway. To achieve this, it may be advantageous to use a signal sequence derived from a bacterial or plant gene, but a plant gene is expected to be preferred. Examples of such signal sequences are those from the endoproteinase B gene (Koehler and Ho) and the tobacco PR1b gene (Cornelissen et al.). pMON10824, disclosed in EP Publ. 0 385 962, is a plant transformation vector designed for the expression of the lepidopteran active *B.t. kurstaki* protein. In pMON10824, the *B.t.k.* coding sequence is fused to the PR1b signal sequence plus 10 amino acids of the mature PR1b coding sequence. To create a vector in which the PR1b signal is fused to the patatin gene, pMON10824 is cut with BglII and NcoI and the small BglII-NcoI fragment that contains the PR1b signal is isolated. In a ligation reaction, the small BglII-NcoI pMON10824 fragment is mixed with the 1.0 kb NcoI-EcoRI fragment from pMON19714 and BamHI-EcoRI digested pMON19470 (Brown et al.). This reaction constructs a plasmid in which the patatin coding sequence is fused to the secretory signal from the PR1b gene and driven by the CaMV35S promoter and an intron for monocot expression. For dicot gene expression, a similar reaction may be performed.

The NotI-NotI fragment of the dicot expression vector may be inserted into a dicot transformation vector as described below and mobilized into a disarmed *Agrobacterium* host and used to transform dicots. Thus, plants which produce patatin that is secreted into the extracellular space can be made.

The NotI-NotI fragment of this monocot plasmid may be inserted into a corn transformation vector (such as pMON18181 described above) to produce a corn plant which secretes patatin.

It may be advantageous to direct the localization of patatin to another cellular compartment, the chloroplast. Proteins can be directed to the chloroplast by including at their N-termini a chloroplast transit peptide (CTP). One CTP that has worked to localize heterologous proteins to the chloroplast is that derived from the RUBISCO small subunit gene of *Arabidopsis*, denoted ats1A. A variant of this transit peptide that encodes the transit peptide, 23 amino acids of mature RUBISCO sequence, plus a reiteration of the transit peptide cleavage site has been constructed for the successful chloroplast localization of the *B.t.k.* protein. pMON19643, described in Brown et al., contains the *Arabidopsis* ats1A transit peptide fused to the GOX gene and may be used as the base for constructing vectors for the chloroplast localization of the patatin. A complete EcoRI and partial NcoI digestion of pMON19643 is performed and the large (4.0 kb) fragment is isolated. In a ligation reaction, the NcoI-EcoRI fragment from pMON19714 is mixed with the large fragment of pMON19643. This reaction constructs a plasmid in which the patatin coding sequence is fused to the *Arabidopsis* transit peptide with 23 amino acids of mature RUBISCO, and driven by the CaMV E35S promoter. Alternatively, a similar plasmid may be constructed to replace the promoter with the FMV35S promoter. Such plasmids are mobilized into disarmed *Agrobacterium* hosts and used to transform dicots. Alternatively, the NotI-NotI fragment is cloned into a corn transformation vector, as described above. Thus, plants can be generated which produce patatin that is localized to the chloroplast.

Plant Transformation and Expression

A chimeric plant gene containing a structural coding sequence of the present invention can be inserted into the genome of a plant by any

suitable method. Suitable plant transformation vectors include those derived from a Ti plasmid of *Agrobacterium tumefaciens*, as well as those disclosed, e.g., by Herrera-Estrella (1983), Bevan (1983), Klee (1985) and EPO publication 0 120 516 (Schilperoort et al.). In addition to plant transformation vectors derived from the Ti or root-inducing (Ri) plasmids of *Agrobacterium*, alternative methods can be used to insert the DNA constructs of this invention into plant cells. Such methods may involve, for example, the use of liposomes, electroporation, chemicals that increase free DNA uptake, free DNA delivery via microprojectile bombardment, and transformation using viruses or pollen.

Transient Expression of Patatin in Tobacco Cells

A particularly useful plasmid cassette vector for transformation of dicotyledonous plants is pMON11794. The expression cassette pMON 11794 consists of the CaMV E35S promoter, the petunia Hsp70 5' untranslated leader, and the 3' end including polyadenylation signals from the NOS gene. pMON11794 includes NcoI and EcoRI sites for insertion of coding sequences and NotI-NotI sites flanking the plant gene expression cassette.

PatA+ (SEQ ID NO:11), PatB+ (SEQ ID NO:16), PatC+ (SEQ ID NO:17), and PatG+ (SEQ ID NO:22), were each inserted into pMON11794 to produce pMON19745, pMON19742, pMON19743, and pMON19744 respectively. Each of these vectors was electroporated into tobacco protoplasts. Expression of patatin by the transformed tobacco cells was confirmed by Western blot analysis.

Stable Transformation of Dicots

Stable transformation of a dicot with a patatin gene has been reported by Rosahl et al. Tobacco was transformed with a patatin gene under the control of a leaf and stem specific promoter. Patatin was expressed.

The NotI-NotI fragment from pMON19745 was inserted into pMON17227, a Ti plasmid vector disclosed and described by Barry et al. in WO 92/04449, incorporated herein by reference, to produce pMON22566. This vector contains the glyphosate resistance gene described by Barry for selection of transformed plants. Similarly SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:22 were used to make vectors pMON22563, 22564, and 22565, respectively.

These vectors were introduced into disarmed *Agrobacterium* ABI and used to transform tomato explants in tissue culture. After selection for glyphosate resistance and plant regeneration, whole plants expressing the patatin gene were recovered. Expression of the patatin gene was confirmed by Western blot analysis, and preliminary results indicate expression at levels between 0.1 and 0.5% of total protein. Bioassays with insect larvae are underway.

Transient Expression of Patatin in Corn Cells

The 1 kb NcoI-EcoRI fragment of pMON19729, described above, was inserted into pMON19433, which is described in WO 93/19189 and co-pending U.S. patent application Serial Number 07/855,857, filed March 19, 1992 (Brown et al.), which is hereby incorporated by reference. The resulting plasmid, pMON19731, was digested with NotI and the resulting fragment inserted into pMON10081, also described by Brown et al., to give pMON19740. This plasmid was electroporated into corn leaf protoplasts as described by Sheen, 1991. Expression of patatin by the transformed corn protoplasts was confirmed by Western blot analysis.

To obtain cytoplasmic expression of patatin, the NcoI-EcoRI fragment of pMON19714 was inserted into pMON19433 to produce pMON19730. The NotI fragment of pMON19730 was inserted into pMON10081 and the resulting plasmid, pMON19739, was electroporated into corn leaf protoplasts, which produced patatin, as confirmed by Western blot analysis.

Pat17, with and without targeting signals, was also expressed in corn protoplasts. pMON19761 was constructed by inserting the 1.1 Kb NcoI-EcoRI fragment (SEQ ID NO:30) encoding the protein Pat17+ (the mature Pat17 protein and its own signal sequence for vacuolar targeting) into pMON19648 . Thus, pMON19761 contains the CaMV E35S promoter, the Hsp 70 intron, the Pat17+ gene, and the NOS terminator for expression in corn cells.

To obtain a vector for cytoplasmic expression, the Pat17+ sequence in pMON19761 was replaced by a NcoI-EcoRI fragment encoding the $Pat17_m$ protein (SEQ ID NO:31) from pMON25213 to form the construct pMON25223.

pMON25224 was made by inserting two fragments, 0.3 Kb XbaI-NcoI fragment containing the chloroplast transit peptide (CTP) from the *Arabidopsis thaliana* SSU 1a gene (Timko et al.) from pMON19643 (Brown, et al.) and the 1Kb NcoI-EcoRI fragment for $Pat17_m$ from pMON25213, inserted into pMON19761 (XbaI-EcoRI). Thus, pMON25224 contains the CaMV E35S promoter, the Hsp 70 intron, CTP/$Pat17_m$ coding sequence, and the NOS terminator.

For extracellular targeting, the 5' end of the endoproteinase B cDNA (Koehler and Ho) encoding the extracellular signal peptide of the secreted protein was joined to the gene for $Pat17_m$ from pMON25213. A BglII-EcoRI fragment containing the chimeric gene was made by a splicing overlap extension technique (Horton et al.) and inserted into pMON19761 (BamHI-EcoRI) to make pMON25225.

All of these constructs were electroporated into corn leaf protoplasts and the expression of $Pat17_m$ was confirmed by Western blot analysis.

Stable Transformation of Corn with an Patatin Gene

The corn transformation vector, pMON18181 was constructed from pMON19653 and pMON19643 (Brown et al.). This construct contains a cassette of the CaMV E35S promoter, the Hsp70 intron, the CP4 glyphosate selection marker, and the NOS terminator; a cassette of the CaMV E35S promoter, the Hsp70 intron, the GOX glyphosate selection marker, and the NOS terminator; and a single NotI site for insertion of a gene expression cassette containing a patatin gene. SEQ ID NO:11 and SEQ ID NO:30 were each inserted as NotI-NotI fragments into pMON18181 to produce pMON19746 and pMON19764, respectively.

These vectors have been inserted by bombardment of embryogenic tissue culture cells using a biolistic particle gun as described by Brown et al. Transformed cells were selected for glyphosate resistance and whole plants are being regenerated. Insect-resistant plants will be confirmed to be expressing the gene at 0.1-0.5% of total protein by Western blot analysis, esterase activity assay, and/or insect resistance assay.

Synthetic Genes for Improved Monocot Expression

Modification of coding sequences has been demonstrated to improve expression of other insecticidal protein genes such as the delta endotoxin

sequences from Bacillus thuringiensis (Fischhoff and Perlak; WO 93/07278, Ciba-Geigy). A modified coding sequence was thus designed to improve patatin expression in plants, especially corn. The modified Pat17+ sequence is shown in SEQ ID NO:33. A DNA fragment containing SEQ ID NO:33 will be synthesized and inserted into a corn expression cassette vector such as pMON19470 (Brown et al.). The corn expression cassette is then inserted into pMON18181 or other corn plant transformation vector containing a selectable marker gene for corn transformation and whole corn plants expressing Pat17+ will be obtained.

From the foregoing, it will be seen that this invention is one well adapted to attain all the ends and objects hereinabove set forth together with advantages which are obvious and which are inherent to the invention. It will be understood that certain features and subcombinations are of utility and may be employed without reference to other features and subcombinations. This is contemplated by and is within the scope of the claims. Since many possible embodiments may be made of the invention without departing from the scope thereof, it is to be understood that all matter herein set forth is to be interpreted as illustrative and not in a limiting sense.

All publications and patents mentioned in this specification are herein incorporated by reference as if each individual publication or patent was specifically and individually stated to be incorporated by reference.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Monsanto Company
(B) STREET: 800 North Lindbergh Boulevard
(C) CITY: St. Louis
(D) STATE: Missouri
(E) COUNTRY: United States of America
(F) POSTAL CODE (ZIP): 63167
(G) TELEPHONE: (314)694-3131
(H) TELEFAX: (314)694-5435

(ii) TITLE OF INVENTION: Method of Controlling Insects

(iii) NUMBER OF SEQUENCES: 33

(iv) 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 #1.25 (EPO)

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/031146
(B) FILING DATE: 12-MAR-1993

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Lys Leu Glu Glu Met Val Thr Val Leu Ser Ile Asp Gly Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Xaa Leu Gly Glu Met Val Thr Val Leu Ser Ile Asp Gly Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Thr Leu Gly Glu Met Val Thr Val Leu Ser Ile Asp Gly Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Thr Leu Gly Glu Met Val Thr Val Leu Ser Ile Asp Gly Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Lys Leu Xaa Glu Met Val Thr Val Leu Ser Ile Asp Gly Gly Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Xaa Xaa Glu Glu Met Val Thr Val
1 5

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Ser Leu Asp Tyr Lys Gln Met Leu Leu Leu Ser Leu Gly Thr Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Lys Leu Asp Tyr Lys Gln Met Leu
1 5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Ser Leu Xaa Tyr Lys Gln Met Leu Leu Leu Ser Leu Gly Thr Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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

Ser Leu Asn Tyr Lys Gln Met Leu Leu Leu Ser Leu Gly Thr Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1171 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCATGGCAAC TACTAAATCT TTTTTAATTT TATTTTTTAT GATATTAGCA ACTACTAGTT 60

CAACATGTGC TAAGTTGGAA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCAGCTATC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATAAAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA AGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCCA 300

AAGATATTGT ACCCTTTTAC TTCGAACATG GCCCTCATAT TTTTAATTAT AGTGGTTCAA 360

TTATTGGCCC AATGTATGAT GGAAAATATC TTCTGCAAGT TCTTCAAGAA AAACTTGGAG 420

AAACTCGTGT GCATCAAGCT TTGACAGAAG TTGCCATCTC AAGCTTTGAC ATCAAAACAA 480

ATAAGCCAGT AATATTCACT AAGTCAAATT TAGCAAAGTC TCCAGAATTG GATGCTAAGA 540

TGTATGACAT ATGCTATTCC ACAGCAGCAG CTCCAATATA TTTTCCTCCA CATTACTTTA 600

TTACTCATAC TAGTAATGGT GATATATATG AGTTCAATCT TGTTGATGGT GGTGTTGCTA 660

CTGTTGGTGA TCCGGCGTTA TTATCCCTTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTTC TTCAATTAAG TCATTGGATT ACAAGCAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAA TTCAGAGTTT GATAAAACAT ATACAGCACA AGAGGCAGCT AAATGGGGTC 840

CTCTACGATG GATGTTAGCT ATACAGCAAA TGACTAATGC AGCAAGTTCT TACATGACTG 900

ATTATTACAT TTCTACTGTT TTTCAAGCTC GTCATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAACAGGC ACAACTACTG AAATGGATGA TGCGTCTGAG GCTAATATGG 1020

AATTATTAGT ACAAGTTGGT GAAACATTAT TGAAGAAACC AGTTTCCAAA GACAGTCCTG 1080

AAACCTATGA GGAAGCTCTA AAGAGGTTTG CAAAATTGCT CTCTGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TGATAGAATT C 1171

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CATGTGCTCT AGAAGATCTC CACCATGGCG TTGGAAG 37

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTTCTTATT GATAGAATTC AAGGTC 26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1105 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCATGGCGTT GGAAGAAATG GTGACTGTTC TTAGTATTGA TGGAGGTGGA ATTAAGGGAA 60

TCATTCCAGC TATCATTCTC GAATTTCTTG AAGGACAACT TCAGGAAGTG GACAATAATA 120

AAGATGCAAG ACTTGCAGAT TACTTTGATG TAATTGGAGG AACAAGTACA GGAGGTTTAT 180

TGACTGCTAT GATAACTACT CCAAATGAAA ACAATCGACC CTTTGCTGCT GCCAAAGATA 240

TTGTACCCTT TTACTTCGAA CATGGCCCTC ATATTTTTAA TTATAGTGGT TCAATTATTG 300

GCCCAATGTA TGATGGAAAA TATCTTCTGC AAGTTCTTCA AGAAAAACTT GGAGAAACTC 360

GTGTGCATCA AGCTTTGACA GAAGTTGCCA TCTCAAGCTT TGACATCAAA ACAAATAAGC 420

CAGTAATATT CACTAAGTCA AATTTAGCAA AGTCTCCAGA ATTGGATGCT AAGATGTATG 480

ACATATGCTA TTCCACAGCA GCAGCTCCAA TATATTTTCC TCCACATTAC TTTATTACTC 540

ATACTAGTAA TGGTGATATA TATGAGTTCA ATCTTGTTGA TGGTGGTGTT GCTACTGTTG 600

GTGATCCGGC GTTATTATCC CTTAGCGTTG CAACGAGACT TGCACAAGAG GATCCAGCAT 660

TTTCTTCAAT TAAGTCATTG GATTACAAGC AAATGTTGTT GCTCTCATTA GGCACTGGCA 720

CTAATTCAGA GTTTGATAAA ACATATACAG CACAAGAGGC AGCTAAATGG GGTCCTCTAC 780

GATGGATGTT AGCTATACAG CAAATGACTA ATGCAGCAAG TTCTTACATG ACTGATTATT 840

ACATTTCTAC TGTTTTTCAA GCTCGTCATT CACAAAACAA TTACCTCAGG GTTCAAGAAA 900

ATGCATTAAC AGGCACAACT ACTGAAATGG ATGATGCGTC TGAGGCTAAT ATGGAATTAT 960

TAGTACAAGT TGGTGAAACA TTATTGAAGA AACCAGTTTC CAAAGACAGT CCTGAAACCT 1020

ATGAGGAAGC TCTAAAGAGG TTTGCAAAAT TGCTCTCTGA TAGGAAGAAA CTCCGAGCAA 1080

ACAAAGCTTC TTATTGATAG AATTC 1105

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1106 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCATGGCGTT GGAAGAAATG GTGACTGTTC TTAGTATTGA TGGAGGTGGA ATTAAGGGAA 60

TCATTCCAGC TACCATTCTC GAATTTCTTG AAGGACAACT TCAGGAAGTG GACAATAATA 120

AAGATGCAAG ACTTGCAGAT TACTTTGATG TAATTGGAGG AACAAGTACA GGAGGTTTAT 180

TGACTGCTAT GATAACTACT CCAAATGAAA ACAATCGACC CTTTGCTGCT GCCAAAGATA 240

TTGTACCCTT TTACTTCGAA CATGGCCCTC ATATTTTTAA TTATAGTGGT TCAATTATTG 300

GCCCAATGTA TGATGGAAAA TATCTTCTGC AAGTTCTTCA AGAAAAACTT GGAGAAACTC 360

GTGTGCATCA AGCTTTGACA GAAGTTGCCA TCTCAAGCTT TGACATCAAA ACAAATAAGC 420

CAGTAATATT CACTAAGTCA AATTTAGCAA AGTCTCCAGA ATTGGATGCT AAGATGTATG 480

ACATATGCTA TTCCACAGCA GCAGCTCCAA TATATTTTCC TCCACATTAC TTTATTACTC 540

ATACTAGTAA TGGTGATATA TATGAGTTCA ATCTTGTTGA TGGTGGTGTT GCTACTGTTG 600

GTGATCCGGC GTTATTATCC CTTAGCGTTG CAACGAGACT TGCACAAGAG GATCCAGCAT 660

TTTCTTCAAT TAAGTCATTG GATTACAAGC AAATGTTGTT GCTCTCATTA GGCACTGGCA 720

CTAATTCAGA GTTTGATAAA ACATATACAG CACAAGAGGC AGCTAAATGG GGTCCTCATC 780

GATGGATGTT AGCTATACAG CAAATGACTA ATGCAGCAAG TTCTTACATG ACTGATTATT 840

ACATTTCTAC TGTTTTTCAA GCTGGTCATT CACAAAACAA TTACCTCAGG GTTCAAGAAA 900

ATGCATTAAC AGGCACAACT ACTGAAATGG ATGATGCGTC TGAGGCTAAT ATGGAATTAT 960

TAGTACAAGT TGGTGAAAAA TTATTGAAGG AACCAGTTTC CAAAGACAGT CCTGAAACCT 1020

CTGAGGAAGC TCTAAAGAGG TTTGCAAAAT TGCTCTCTGA TAGAAAGAAA CTCCGAGCAA 1080

ACAAAGCTTC TTATTAATGA GAATTC 1106

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CCATGGCAAC TACTAAATCT GTTTTAGTTT TATTTTTTAT GATATTAGCA ACTACTAGTT 60

CAACATGTGC TACGTTGGGA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCGGCTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATAAAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA AGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCCA 300

AAGATATTGT ACCTTTTTAC TTCGAACATG GCCCTCATAT TTTTAATTCT AGTGGTTCAA 360

TTTTTGGCCC AATGTATGAT GGAAAATATT TTCTGCAAGT TCTTCAAGAA AAACTTGGAG 420

AAACTCGTGT GCATCAAGCT TTGACAGAAG TTGCCATCTC AAGCTTTGAC ATCAAAACAA 480

ATAAGCCAGT AATATTCACT AAGTCAAATT TAGCAAAGTC TCCAGAATTG GATGCTAAGA 540

TGAATGACAT ATGCTATTCC ACAGCAGCAG CTCCAACATA TTTTCCTCCA CATTACTTTG 600

TTACTCATAC TAGTAATGGA GATAAATATG AGTTCAATCT TGTTGATGGT GCTGTTGCTA 660

CTGTTGGTGA TCCGGCGTTA TTATCCCTTA GCGTTCGAAC GAAACTTGCA CAAGTGGATC 720

CAAAATTTGC TTCAATTAAG TCATTGAATT ACAACGAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAA TTCAGAGTTT GATAAAACAT ATACAGCAGA AGAGGCAGCT AAATGGGGTC 840

CTCTACGATG GATATTAGCT ATACAGCAAA TGACTAATGC AGCAAGTTCT TACATGACTG 900

ATTATTACCT TTCTACTGTT TTTCAAGCTC GTCATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAACAGGC ACAACTACTG AAATGGATGA TGCGTCTGAG GCTAATATGG 1020

AATTATTAGT ACAAGTTGGT GAAAAATTAT TGAAGAAACC AGTTTCCAAA GACAGTCCTG 1080

AAACCTATGA GGAAGCTCTA AAGAGGTTTG CAAAATTGCT CTCTGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CCATGGCAAC TACTAAATCT TTTTTAATTT TAATTGTTAT GATATTAGCA ACTACTAGTT 60

CAACATTTGC TTCGTTGGAA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCGGGTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG AAAATGGACA 180

ATAATGCAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA AGTACAGGAG 240

GTTTATTGAC TTCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCCA 300

ATGAAATTGT ACCTTTTTAC TTCGAACATG GCCCTCATAT TTTTAATTCT AGGTACTGGC 360

CAATTTTTTG GCCAAAATAT GATGGAAAAT ATCTTATGCA AGTTCTTCAA GAAAACCTTG 420

GAGAAACTCG TGTGCATCAA GCTTTGACTG AAGTTGCCAT CTCAAGCTTT GACATCAAAA 480

CAAATAAGCC AGTAATATTC ACCAAGTCAA ATTTAGCAAA GTCTCCAGAA TTGGATGCTA 540

AGATGTATGA CATATGTTAT TCCACAGCAG CAGCTCCAAC ATATTTTCCT CCACATTACT 600

TTACTACTAA TACTATTAAT GGAGATAAAT ATGAGTTCAA TCTTGTTGAT GGTGCTGTTG 660

CTACTGTTGC TGATCCGGCG TTATTATCCA TTAGCGTTGC AACGAGACTT GCAGAAAAGG 720

ATCCAGCATT TGCTTCAATT AGGTCATTGA ATTACAAAAA AATGTTGTTG CTCTCATTAG 780

GCACTGGCAC TACTTCAGAG TTTGATAAAA CATATACAGC AGAAGAGACA GCTAAATGGG 840

GTGCTATACA ATGGATGTTG GTTATACAGC GAATGACTGA TGCAGCAAGT TCTTACATGA 900

CTGATTATTA CCTTTCTACT GTTTTTCAAG CTCAAAATTC ACAAAAGAAT TACCTCAGGG 960

TTCAAGAAAA TGCGTTAACA GGCACAACTA CTGAAATGGA TGATGCTTCT GAGGCTAATA 1020

TGGAATCATT AGTACAAGTT GGTGAAAATT TATTGAAGAA ACCAGTTCCC AAAGACAATC 1080

CTGAAACCTA TGAGGAAGCT CTAAAGAGGT TTGCAAAATT GCTTTCTGAT AGGAAGAAAC 1140

TTCGAGCAAA CAAAGCTTCT TATTAATGAG AATTC 1175

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1106 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCATGGCGTT GGAAGAAATG GTGACTGTTC TTAGTATTGA TGGAGGTGGA ATTAAGGGAA 60

TCATTCCGGC TACCATTCTC GAATTTCTTG AAGGACAACT TCAGGAAGTG GACAATAATA 120

AAGATGCAAG ACTTGCAGAT TACTTTGATG TAATTGGAGG AACAAGTACA GGAGGTTTAT 180

TGACTGCTAT GATAACTACT CCAAATGAAA ACAATCGACC CTTTGCTGCT GCCAAAGATA 240

TTGTACCTTT TTACTTCGAA CATGGCCCTC ATATTTTTAA TTCTAGTGGT TCAATTTTTG 300

GCCCAATGTA TGATGGAAAA TATTTTCTGC AAGTTCTTCA AGAAAAACTT GGAGAAACTC 360

GTGTGCATCA AGCTTTGACA GAAGTTGCCA TCTCAAGCTT TGACATCAAA ACAAATAAGC 420

CAGTAATATT CACTAAGTCA AATTTAGCAA AGTCTCCAGA ATTGGATGCT AAGATGTATG 480

ACATATGTTA TTCCACAGCA GCAGCTCCAA CATATTTTCC TCCACATTAC TTTGTTACTC 540

ATACTAGTAA TGGAGATAAA TATGAGTTCA ATCTTGTTGA TGGTGCTGTT GCTACTGTTG 600

GTGATCCGGC GTTATTATCC CTTAGCGTTG CAACGAAACT TGCACAAGTG GATCCAAAAT 660

TTGCTTCAAT TAAGTCATTG AATTACAAGC AAATGTTGTT GCTCTCATTA GGCACTGGCA 720

CTAATTCAGA GTTTGATAAA ACATATACAG CAGAAGAGGC AGCTAAATGG GGTCCTCTAC 780

GATGGATATT AGCTATACAG CAAATGACTA ATGCAGCAAG TTCTTACATG ACTGATTATT 840

ACCTTTCTAC TGTTTTTCAA GCTCGTCATT CACAAAACAA TTACCTCAGG GTTCAAGAAA 900

ATGCATTAAC AGGCATAACT ACTGAAATGG ATGATGCGTC TGAGGCTAAT ATGGAATTAT 960

TAGTACAAGT TGGTGAAAAA TTATTGAAGA AACCAGTTTC CAAAGACAGT CCTGAAACCT 1020

ATGAGGAAGC TCTAAAGAGG TTTGCAAAAT TGCTCTCTGA TAGGAAGAAA CTCCGAGCAA 1080

ACAAAGCTTC TTATTAATGA GAATTC 1106

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CCATGGCAAC TACTAAATCT TTTACAATTT TAATTTTTAT GATGTTAGCA ACTACTAGTT 60

CAACATTTGC TACATTGGGA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCGGCTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATGCAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA GGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCTTTT GCTGCTGCTA 300

AAGATATTAT ACCTTTTTAC TTCGAACACG GCCCTCATAT TTTTAATTAT AGTGGTTCAA 360

TTTTAGGCCC AATGTATGAT GGAAAATATC TTCTGCAAGT TCTTCAAGAA AAACTTGGAG 420

AAACTCGTGT GCATCAAGCT TTGACAGAAG TTGCCATCTC AAGCTTTGAC ATCAAAACAA 480

ATAAGCCAGT AATATTCACT AAGTCAAATT TAGCAAAGTC TCCAGAATTG GATGCTAAGA 540

TGTATGACAT ATGCTATTCC ACAGCAGCAG CTCCAATATA TTTTCCTCCA CATCACTTTG 600

TTACTCATAC TAGTAATGGT GCTAGATATG AGTTCAATCT TGTTGATGGT GCTGTTGCTA 660

CTGTTGGTGA TCCGGCGTTA TTATCCCTTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTTC TTCAATTAAG TCATTGGATT ACAAGCAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAA TTCAGAGTTT GATAAAACAT ATACAGCAGA AGAGGCAGCT AAATGGGGTC 840

CTCTACGATG GATGTTAGCT ATACAGCAAA TGACTAATGC AGCAAGTTCT TACATGACTG 900

ATTATTACAT TTCTACTGTT TTTCAAGCTC GTCATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAAATGGC ACAACTACTG AAATGGATGA TGCGTCTGAG GCTAATATGG 1020

AATTATTAGT ACAAGTTGGT GAAACATTAT TGAAGAAACC AGTCTCCAAA GACAGTCCTG 1080

AAACCTATGA GGAAGCTCTA AAGAGATTTG CAAAATTGCT CTCTGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1106 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCATGGCGTT GGAAGAAATG GTGACTGTTC TTAGTATTGA TGGAGGTGGA ATTAAGGGAA 60

TCATTCCGGC TACCATTCTC GAATTTCTTG AAGGACAACT TCAGGAAGTG GACAATAATG 120

CAGATGCAAG ACTTGCAGAT TACTTTGATG TAATTGGAGG AACAGGTACA GGAGGTTTAT 180

TGACTGCTAT GATAACTACT CCAAATGAAA ACAATCGACC TTTTGCTGCT GCTAAAGATA 240

TTATACCTTT TTACTTCGAA CACGGCCCTC ATATTTTTAA TTATAGTGGT TCAATTTTAG 300

GCCCAATGTA TGATGGAAAA TATCTTCTGC AAGTTCTTCA AGAAAAACTT GGAGAAACTC 360

GTGTGCATCA AGCTTTGACA GAAGTTGCCA TCTCAAGCTT TGACATCAAA ACAAATAAGC 420

CAGTAATATT CACTAAGTCA AATTTAGCAA AGTCTCCAGA ATTGGATGCT AAGATGTATG 480

ACATATGCTA TTCCACAGCA GCAGCTCCAA TATATTTTCC TCCACATCAC TTTGTTACTC 540

ATACTAGTAA TGGTGCTAGA TATGAGTTCA ATCTTGTTGA TGGTGCTGTT GCTACTGTTG 600

GTGATCCGGC GTTATTATCC CTTAGCGTTG CAACGAGACT TGCACAAGAG GATCCAGCAT 660

TTTCTTCAAT TAAGTCATTG GATTACAAGC AAATGTTGTT GCTCTCATTA GGCACTGGCA 720

CTAATTCAGA GTTTGATAAA ACATATACAG CAGAAGAGGC AGCTAAATGG GGTCCTCTAC 780

GATGGATGTT AGCTATACAG CAAATGACTA ATGCAGCAAG TTCTTACATG ACTGATTATT 840

ACATTTCTAC TGTTTTTCAA GCTCGTCATT CACAAAACAA TTACCTCAGG GTTCAAGAAA 900

ATGCATTAAA TGGCACAACT ACTGAAATGG ATGATGCGTC TGAGGCTAAT ATGGAATTAT 960

TAGTACAAGT TGGTGAAACA TTATTGAAGA AACCAGTCTC CAAAGACAGT CCTGAAACCT 1020

ATGAGGAAGC TCTAAAGAGA TTTGCAAAAT TGCTCTCTGA TAGGAAGAAA CTCCGAGCAA 1080

ACAAAGCTTC TTATTAATGA GAATTC 1106

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1109 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCATGGCGTT GGAAGAAATG GTGGCTGTTC TTAGTATTGA TGGAGGTGGA ATTAAGGGAA 60

TCATTCCGGG TACCATTCTC GAATTTCTTG AAGGACAACT TCAGAAAATG GACAATAATG 120

CAGATGCAAG ACTTGCAGAT TACTTTGATG TAATTGGAGG AACAAGTACA GGAGGTTTAT 180

TGACTGCTAT GATAACTACT CCAAATGAAA ACAATCGACC CTTTGCTGCT GCCAATGAAA 240

TTGTACCTTT TTACTTCGAA CATGGCCCTC ATATTTTTAA TTCTAGGTAC TGGCCAATTT 300

TTTGGCCAAA ATATGATGGA AAATATCTTA TGCAAGTTCT TCAAGAAAAA CTTGGAGAAA 360

CTCGTGTGCA TCAAGCTTTG ACAGAAGTTG CCATCTCAAG CTTTGACATC AAAACAAATA 420

AGCCAGTAAT ATTCACTAAG TCAAATTTGG CAAAGTCTCC AGAATTGGAT GCTAAGACGT 480

ATGACATATG TTATTCGACA GCAGCAGCTC CAACATATTT TCCTCCACAT TACTTTGCTA 540

CTAATACTAT TAATGGAGAT AAATATGAGT TCAATCTTGT TGATGGTGCT GTTGCTACTG 600

TTGCTGATCC GGCGTTATTA TCCGTTAGCG TTGCAACGAG ACGTGCACAA GAGGATCCAG 660

CATTTGCTTC AATTAGGTCA TTGAATTACA AAAAAATGTT GTTGCTCTCA TTAGGCACTG 720

GCACTACTTC AGAGTTTGAT AAAACACATA CAGCAGAAGA GACAGCTAAA TGGGGTGCTC 780

TACAATGGAT GTTGGTTATA CAGCAAATGA CTGAGGCAGC AAGTTCTTAC ATGACTGATT 840

ATTACCTTTC TACTGTTTTT CAAGATCTTC ATTCACAAAA CAATTACCTC AGGGTTCAAG 900

AAAATGCATT AACAGGCACA ACTACTAAAG CGGATGATGC TTCTGAGGCT AATATGGAAT 960

TATTAGCACA AGTTGGTGAA AATTTATTGA AGAAACCAGT TTCCAAAGAC AATCCTGAAA 1020

CCTATGAGGA AGCTCTAAAG AGGTTTGCAA AATTGCTTTC TGATAGGAAG AAACTTCGAG 1080

CAAACAAAGC TTCTTATTAA TGAGAATTC 1109

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCATGGCAAC TACTAAATCT TTTTTAATTT TATTTTTTAT GATATTAGCA ACTACTAGTT 60

CAACATGTGC TAAGTTGGAA GAAATGGTTA CTGTTCTAAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCAGCTATC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATAAAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA AGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCCA 300

AAGATATTGT ACCCTTTTAC TTCGAACATG GCCCTCATAT TTTTAATTAT AGTGGTTCAA 360

TTTTAGGCCC AATGTATGAT GGAAAATATC TTCTGCAAGT TCTTCAAGAA AAACTTGGAG 420

AAACTCGTGT GCATCAAGCT TTGACAGAAG TTGCCATCTC AAGCTTTGAC ATCAAAACAA 480

ATAAGCCAGT AATATTCACT AAGTCAAATT TAGCAAAGTC TCCAGAATTG GATGCTAAGA 540

TGTATGACAT ATGCTATTCC ACAGCAGCAG CTCCAATATA TTTTCCTCCA CATCACTTTG 600

TTACTCATAC TAGTAATGGT GCTAGATATG AGTTCAATCT TGTTGATGGT GCTGTTGCTA 660

CTGTTGGTGA TCCGGCGTTA TTATCCCTTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTTC TTCAATTAAG TCATTGGATT ACAAGCAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAA TTCAGAGTTT GATAAAACAT ATACAGCAGA AGAGGCAGCT AAATGGGGTC 840

CTCTACGATG GATGTTAGCT ATACAGCAAA TGACTAATGC AGCAAGTTCT TACATGACTG 900

ATTATTACAT TTCTACTGTT TTTCAAGCTC GTCATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAAATGGC ACAACTACTG AAATGGATGA TGCGTCTGAG GCTAATATGG 1020

AATTATTAGT ACAAGTTGGT GAAACATTAT TGAAGAAACC AGTTTCCAAA GACAGTCCTG 1080

AAACCTATGA GGAAGCTCTA AAGAGATTTG CAAAATTGCT CTCTGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CCATGGTTGG AAGAAATGGT GACTGTTCTA AGTATTGATG GAGGTGGAAT TAAGGGAATC 60

ATTCCAGCTA TCATTCTCGA ATTTCTTGAA GGACAACTTC AGGAAGTGGA CAATAATAAA 120

GATGCAAGAC TTGCAGATTA CTTTGATGTA ATTGGAGGAA CAAGTACAGG AGGTTTATTG 180

ACTGCTATGA TAACTACTCC AAATGAAAAC AATCGACCCT TTGCTGCTGC CAAAGATATT 240

GTACCCTTTT ACTTCGAACA TGGCCCTCAT ATTTTTAATT ATAGTGGTTC AATTTTAGGC 300

CCAATGTATG ATGGAAAATA TCTTCTGCAA GTTCTTCAAG AAAAACTTGG AGAAACTCGT 360

GTGCATCAAG CTTTGACGGA AGTTGCCATC TCAAGCTTTG ACATCAAAAC AAATAAGCCA 420

GTAATATTCA CTAAGTCAAA TTTAGCAAAG TCTCCAGAAT TGGATGCTAA GATGTATGAC 480

ATATGCTATT CCACAGCAGC AGCTCCAATA TATTTTCCTC CACATCACTT TGTTACTCAT 540

ACTAGTAATG GTGCTAGATA TGAGTTCAAT CTTGTTGATG GTGCTGTTGC TACTGTTGGT 600

GATCCGGCGT TATTATCCCT TAGCGTTGCA ACGAGACTTG CACAAGAGGA TCCAGCATTT 660

TCTTCAATTA AGTCATTGGA TTACAAGCAA ATGTTGTTGC TCTCATTAGG CACTGGCACT 720

AATTCAGAGT TTGATAAAAC ATATACAGCA GAAGAGGCAG CTAAATGGGG TCCTCTACGA 780

TGGATGTTAG CTATACAGCA AATGACTAAT GCAGCAAGTT TTTACATGAC TGATTATTAC 840

ATTTCTACTG TTTTTCAAGC TCGTCATTCA CAAAACAATT ACCTCAGGGT TCAAGAAAAT 900

GCATTAAATG GCACAACTAC TGAAATGGAT GATGCGTCTG AGGCTAATAT GGAATTATTA 960

GTACAAGTTG GTGAAACATT ATTGAAGAAA CCAGTTTCCA GAGACAGTCC TGAAACCTAT 1020

GAGGAAGCTC TAAAGAGATT TGCAAAATTG CTCTCTGATA GGAAGAAACT CCGAGCAAAC 1080

AAAGCTTCTT ATTAATGAGA ATTC 1104

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CCATGGCAAC TACTAAATCT TTTTTAATTT TATTTTTTAT GATATTAGCA ACTACTAGTT 60

CAACATGTGC TAAGTTGGAA GAAATGGTTA CTGTTCTAAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCAGCTATC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATAAAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA AGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCCA 300

AAGATATTGT ACCCTTTTAC TTCGAACATG GCCCTCATAT TTTTAATTAT AGTGGTTCAA 360

TTTTAGGCCC AATGTATGAT GGAAAATATC TTCTGCAAGT TCTTCAAGAA AAACTTGGAG 420

AAACTCGTGT GCATCAAGCT TTGACAGAAG TTGCCATCTC AAGCTTTGAC ATCAAAACAA 480

ATAAGCCAGT AATATTCACT AAGTCAAATT TAGCAAAGTC TCCAGAATTG GATGCTAAGA 540

TGTATGACAT ATGCTATTCC ACAGCAGCAG CTCCAATATA TTTTCCTCCA CATCACTTTG 600

TTACTCATAC TAGTAATGGT GCTAGATATG AGTTCAATCT TGTTGATGGT GCTGTTGCTA 660

CTGTTGGTGA TCCGGCGTTA TTATCCCTTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTTC TTCAATTAAG TCATTGGATT ACAAGCAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAA TTCAGAGTTT GATAAAACAT ATACAGCAGA AGAGGCAGCT AAATGGGGTC 840

CTCTACGATG GATGTTAGCT ATACAGCAAA TGACTAATGC AGCAAGTTCT TACATGACTG 900

ATTATTACAT TTCTACTGTT TTTCAAGCTC GTCATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAAATGGC ACAACTACTG AAATGGATGA TGCGTCTGAG GCTAATATGG 1020

AATTATTAGT ACAAGTTGGT GCAACATTAT TGAAGAAACC AGTCTCCAAA GACAGTCCTG 1080

AAACCTATGA GGAAGCTCTA AAGAGATTTG CAAAATTGCT CTCTGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1175 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCATGGCAAC TACTAAATCT TTTACAATTT TAATTTTTAT GATGTTAGCA ACTACTAGTT 60

CAACATTTGC TACATTGGGA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCGGCTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATGCAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA GGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCTTTT GCTGCTGCTA 300

AAGATATTAT ACCTTTTTAC TTCGATCATG GCCCTAAGAT TTTTGAACCT AGTGGTTTTC 360

ACCTTTTTGA GCCAAAATAT GATGGAAAAT ATCTTATGCA AGTTCTTCAA GAAAAACTTG 420

GAGAAACTCG TGTGCATCAA GCTTTGACAG GAGTTGCCAT CTCAAGCTTT GACATCAAAA 480

CAAATAAGCC AGTAATATTC ACTAAGTCAA GTTTAGCAAA AACTCCAGAA TTGGATGCTA 540

AGATGTATGA CATATGTTAT TCCACAGCAG CAGCTCCAAC ATATTTTCCT CCACATTACT 600

TTGCTACTAA TACTAGTAAT GGAGATCAAT ATGACCTCAA TCTTGTTGAT GGCGATGTTG 660

CTGCTGGTGA TCCGTCGTTA TTATCCATTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTGC TTCAATTAAG TCATTGAATT ACAAACAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAA TTCAGAGTTT GCTAAAAACT ATACAGCAGA AGAGGCAGCT AAATGGGGTA 840

TTCTACAATG GGTATTCTCA CCTTTATGGG AAATGAGAAG TGCAGCAAGT TCTTACATGA 900

ATGATTATTA CCTTTCTACT GTTTTTCAAG CTCTTGATTC ACAAAACAAT TACCTCAGGG 960

TTCAAGAAAA TGCATTAACA GGCACAGCTA CTACATTTGA TGATGCTTCT CTGGCTAATA 1020

TGATATTATT AGTACAAGTT GGTGAAAACT TATTGAAGAA ATCAGTTTCC GAAGACAATC 1080

ATGAAACCTA TGAGGTAGCT CTAAAGAGGT TTGCAAAATT GCTCTCTGAT AGGAAGAAAC 1140

TCCGAGCAAA CAAAGCTTCT TATTAATGAG AATTC 1175

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTTAGATCTC ACCATGGCAA CTACTAAATC TTT 33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CCAGAATTCT CATTAATAAG AAGCTTTGTT TGC 33

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CCATGGCAAC TACTAAATCT TTTACAATTT TAATTTTTAT GATGTTAGCA ACTACTAGTT 60

CAACATTTGC TACATTGGGA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCGGCTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATACAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA GGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCTA 300

AAGATATTAT ACCTTTTTAC TTCGATCATG GCCCTCAGAT TTTTGAACCT AGTGGTCTTC 360

AAATTTTTGG CCCAAAATAT GATGGAAAAT ATCTTATGCA AGTTCTTCAA GAAAAACTTG 420

GAGAAACTCG TGTGCATCAA GCTTTGACAG AAGTTGCCAT CTCAAGCTTT GACATCAAAA 480

CAAATAAGCC AGTAATATTC ACTAAGTCAA ATTTAGCAAA AACTCCAGAA TTGGATGCTA 540

AGATGTATGA CATATGTTAT TCCACAGCAG CAGCTCCAAC ATATTTTCCT CCACATTACT 600

TTGCTACTAA TACTAGTAAT GGAGATCAAT ATGACTTCAA TCTTGTTGAT GGTGATGTTG 660

CTGCTGGTGA TCCGTCGTTA TTATCCATTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTGC TTCAATTAGG TCGTTGAATT ACAAACAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAC TTCAGAGTTT TATAAAAACT ATACAGCAGA AGAGGCAGCT AAATGGGGTA 840

TTCTACAATG GCTGTTACCT TTACAGGAAA TGAGAAGTGC AGCAAGTTCT TACATGAATG 900

ATTATTACCT TTCTACTGTT TTTCAAGCTC TTGATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAACAGGC ACAGCTACTA AATTTGATGA TGCTTCTGTG GCTAATATGA 1020

TATTATTAGT ACAAGTTGGT GAAAACTTAT TGAAGAAATC AGTTTCTGAA GACAATCATG 1080

AAACCTATGA GGTAGCTCTA AAGAGGTTTG CAAAATTGCT CTCCGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCATGGCAAC TACTAAATCT TTTACAATTT TAATTTTTAT GATGTTAGCA ACTACTAGTT 60

CAACATTTGC TACATTGGGA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

AGGGAATCAT TCCGGCTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAGTGGACA 180

ATAATACAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA GGTACAGGAG 240

GTTTATTGAC TGCTATGATA ACTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCTA 300

AAGATATTAT ACCTTTTTAC TTCGATCATG GCCCTCAGAT TTTTGAACCT AGTGGTTCAA 360

TTTTTGATGG CCCAAAATAT GATGGAAAAC ATCTTATGCA AGTTCTTCAA GAAAAACTAG 420

GAGAAACTCG TGTGCATCAA ACTTTGACAG AAGTTGCCAT CTCAAGCTTT GACATCAAAA 480

CAAATAAGCC AGTAATATTC ACTAAGTCAA ATTTACCAAA AACTCCAGAA TTGGATGCTA 540

AGATGTATGA CATATGTTAT TCCACAGCAG CAGCTCCAAC ATATTTTCCT CCACATTACT 600

TTGCTACTAA TACTAGTAAT GGAGATCAAT ATGACTTCAA TCTTGTTGAT GGTGATGTTG 660

CTGCTGGTGA TCCGTCGTTA TTATCCATTA GCGTTGCAAC GAGACTTGCA CAAGAGGATC 720

CAGCATTTGC TTCAATTAGG TCGTTGAATT ACAAACAAAT GTTGTTGCTC TCATTAGGCA 780

CTGGCACTAC TTCAGAGTTT TATAAAAACT ATACAGCAGA AGAGGCAGCT AAATGGGGTA 840

TTCTACAATG GCTGTTACCT TTACAGGAAA TGAGAAGTGC AGCAAGTTCT TACATGAATG 900

ATTATTACCT TTCTACTGTT TTTCAAGCTC TTGATTCACA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAACAGGC ACAGCTACTA AATTTGATGA TGCTTCTGTG GCTAATATGA 1020

TATTATTAGT ACAAGTTGGT GAAAACTTAT TGAAGAAATC AGTTTCTGAA GACAATCATG 1080

AAACCTATGA GGTAGCTCTA AAGAGGTTTG CAAAATTGCT CTCCGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CCATGGCAAC TACTAAATCT TTTTTAATTT TAATATTTAT GATATTAGCA ACTACTAGTT 60

CAACATTTGC TCAGTTGGGA GAAATGGTGA CTGTTCTTAG TATTGATGGA GGTGGAATTA 120

GAGGGATCAT TCCGGCTACC ATTCTCGAAT TTCTTGAAGG ACAACTTCAG GAAATGGACA 180

ATAATGCAGA TGCAAGACTT GCAGATTACT TTGATGTAAT TGGAGGAACA AGTACAGGAG 240

GTTTATTGAC TGCTATGATA AGTACTCCAA ATGAAAACAA TCGACCCTTT GCTGCTGCCA 300

AAGAAATTGT ACCTTTTTAC TTCGAACATG GCCCTCAGAT TTTTAATCCT AGTGGTCAAA 360

TTTTAGGCCC AAAATATGAT GGAAAATATC TTATGCAAGT TCTTCAAGAA AAACTTGGAG 420

AAACTCGTGT GCATCAAGCT TTGACAGAAG TTGTCATCTC AAGCTTTGAC ATCAAAACAA 480

ATAAGCCAGT AATATTCACT AAGTCAAATT TAGCAAACTC TCCAGAATTG GATGCTAAGA 540

TGTATGACAT AAGTTATTCC ACAGCAGCAG CTCCAACATA TTTTCCTCCG CATTACTTTG 600

TTACTAATAC TAGTAATGGA GATGAATATG AGTTCAATCT TGTTGATGGT GCTGTTGCTA 660

CTGTTGCTGA TCCGGCGTTA TTATCCATTA GCGTTGCAAC GAGACTTGCA CAAAAGGATC 720

CAGCATTTGC TTCAATTAGG TCATTGAATT ACAAAAAAAT GCTGTTGCTC TCATTAGGCA 780

CTGGCACTAC TTCAGAGTTT GATAAAACAT ATACAGCAAA AGAGGCAGCT ACCTGGACTG 840

CTGTACATTG GATGTTAGTT ATACAGAAAA TGACTGATGC AGCAAGTTCT TACATGACTG 900

ATTATTACCT TTCTACTGCT TTTCAAGCTC TTGATTCAAA AAACAATTAC CTCAGGGTTC 960

AAGAAAATGC ATTAACAGGC ACAACTACTG AAATGGATGA TGCTTCTGAG GCTAATATGG 1020

AATTATTAGT ACAAGTTGGT GAAAACTTAT TGAAGAAACC AGTTTCCGAA GACAATCCTG 1080

AAACCTATGA GGAAGCTCTA AAGAGGTTTG CAAAATTGCT CTCTGATAGG AAGAAACTCC 1140

GAGCAAACAA AGCTTCTTAT TAATGAGAAT TC 1172

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1106 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CCATGGCGTT GGAAGAAATG GTGACTGTTC TTAGTATTGA TGGAGGTGGA ATTAGAGGGA 60

TCATTCCGGC TACCATTCTC GAATTTCTTG AAGGACAACT TCAGGAAATG GACAATAATG 120

CAGATGCAAG ACTTGCAGAT TACTTTGATG TAATTGGAGG AACAAGTACA GGAGGTTTAT 180

TGACTGCTAT GATAAGTACT CCAAATGAAA ACAATCGACC CTTTGCTGCT GCCAAAGAAA 240

TTGTACCTTT TTACTTCGAA CATGGCCCTC AGATTTTTAA TCCTAGTGGT CAAATTTTAG 300

GCCCAAAATA TGATGGAAAA TATCTTATGC AAGTTCTTCA AGAAAAACTT GGAGAAACTC 360

GTGTGCATCA GGCTTTGACA GAAGTTGTCA TCTCAAGCTT TGACATCAAA ACAAATAAGC 420

CAGTAATATT CACTAAGTCA AATTTAGCAA ACTCTCCAGA ATTGGATGCT AAGATGTATG 480

ACATAAGTTA TTCCACAGCA GCAGCTCCAA CATATTTTCC TCCGCATTAC TTTGTTACTA 540

ATACTAGTAA TGGAGATGAA TATGAGTTCA ATCTTGTTGA TGGTGCTGTT GCTACTGTTG 600

CTGATCCGGC GTTATTATCC ATTAGCGTTG CAACGAGACT TGCACAAAAG GATCCAGCAT 660

TTGCTTCAAT TAGGTCATTG AATTACAAAA AAATGCTGTT GCTCTCATTA GGCACTGGCA 720

CTACTTCAGA GTTTGATAAA ACATATACAG CAAAAGAGGC AGCTACCTGG ACTGCTGTAC 780

ATTGGATGTT AGTTATACAG AAAATGACTG ATGCAGCAAG TTCTTACATG ACTGATTATT 840

ACCTTTCTAC TGCTTTTCAA GCTCTTGATT CAAAAAACAA TTACCTCAGG GTTCAAGAAA 900

ATGCATTAAC AGGCACAACT ACTGAAATGG ATGATGCTTC TGAGGCTAAT ATGGAATTAT 960

TAGTACAAGT TGGTGAAAAC TTATTGAAGA AACCAGTTTC CGAAGACAAT CCTGAAACCT 1020

ATGAGGAAGC TCTAAAGAGG TTTGCAAAAT TGCTCTCTGA TAGGAAGAAA CTCCGAGCAA 1080

ACAAAGCTTC TTATTAATGA GAATTC 1106

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCATCTAGAA GATCTCCACC ATGGCGTTGG GAGAAATGGT GACTG 45

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1164 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ATGGCCACCA CCAAGAGCTT CCTCATCCTG ATCTTCATGA TCCTGGCCAC CACCAGCAGC 60

ACCTTCGCCC AGCTCGGCGA GATGGTGACC GTGCTCTCCA TCGACGGCGG TGGCATCAGG 120

GGCATCATCC CGGCCACCAT CCTGGAGTTC CTGGAGGGCC AACTCCAGGA GATGGACAAC 180

AACGCCGACG CCCGCCTGGC CGACTACTTC GACGTGATCG GTGGCACCAG CACCGGCGGT 240

CTCCTGACCG CCATGATCTC CACTCCGAAC GAGAACAACC GCCCCTTCGC CGCTGCGAAG 300

GAGATCGTCC CGTTCTACTT CGAACACGGC CCTCAGATTT TCAACCCCTC GGGTCAAATC 360

CTGGGCCCCA AGTACGACGG CAAGTACCTT ATGCAAGTGC TTCAGGAGAA GCTGGGCGAG 420

ACTAGGGTGC ACCAGGCGCT GACCGAGGTC GTCATCTCCA GCTTCGACAT CAAGACCAAC 480

AAGCCAGTCA TCTTCACCAA GTCCAACCTG GCCAACAGCC CGGAGCTGGA CGCTAAGATG 540

TACGACATCT CCTACTCCAC TGCTGCCGCT CCCACGTACT TCCCTCCGCA CTACTTCGTC 600

ACCAACACCA GCAACGGCGA CGAGTACGAG TTCAACCTTG TTGACGGTGC GGTGGCTACG 660

GTGGCGGACC CGGCGCTCCT GTCCATCAGC GTCGCCACGC GCCTGGCCCA GAAGGATCCA 720

GCCTTCGCTA GCATTAGGAG CCTCAACTAC AAGAAGATGC TGCTGCTCAG CCTGGGCACT 780

GGCACGACCT CCGAGTTCGA CAAGACCTAC ACTGCCAAGG AGGCCGCTAC CTGGACCGCC 840

GTCCATTGGA TGCTGGTCAT CCAGAAGATG ACGGACGCCG CTTCCAGCTA CATGACCGAC 900

TACTACCTCT CCACTGCGTT CCAGGCGCTT GACTCCAAGA ACAACTACCT CCGTGTTCAG 960

GAGAATGCCC TCACTGGCAC CACGACCGAG ATGGACGATG CCTCCGAGGC CAACATGGAG 1020

CTGCTCGTCC AGGTGGGTGA GAACCTCCTG AAGAAGCCCG TCTCCGAAGA CAATCCCGAG 1080

ACCTATGAGG AAGCGCTCAA GCGCTTTGCC AAGCTGCTCT CTGATAGGAA GAAACTCCGC 1140

GCTAACAAGG CCAGCTACTA ATGA 1164

WHAT IS CLAIMED IS:

1. A method of controlling plant-eating insect infestation of a plant comprising providing an effective amount of an insecticidal patatin for ingestion by the insect.

2. The method of Claim 1 wherein said patatin is provided by plant-colonizing microorganisms which produce said patatin after application to the plant.

3. The method of Claim 1 wherein said patatin is provided by expression of a gene for a patatin incorporated in said plant by previous genetic transformation of a parent cell of the plant.

4. The method of Claim 3 wherein said plant is cotton, corn, tomato, or potato.

5. A method of producing genetically transformed, insect-resistant plants which express an insecticidally effective amount of a patatin, comprising the steps of:

a) inserting into the genome of a plant cell a recombinant, double-stranded DNA molecule comprising

(i) a promoter which functions in plant cells to cause the production of an RNA sequence;

(ii) a structural coding sequence that encodes for a patatin;

(iii) a 3' non-translated region which functions in said plant cells to cause the addition of polyadenylate nucleotides to the 3' end of the RNA sequence,

wherein said promoter is heterologous with respect to said structural coding sequence and wherein said promoter is operatively linked with said structural coding sequence, which is in turn operably linked with said non-translated region;

b) obtaining transformed plant cells; and

c) regenerating from the transformed plant cells genetically transformed plants with express an insecticidally effective amount of patatin;

wherein said promoter is heterologous with respect to the structural coding sequence and wherein said plants are selected from cotton, corn, tomato, and potato.

6. The method of Claim 5 wherein said structural coding sequence comprises SEQ ID NO: 30 or SEQ ID NO:31.

7. The method of Claim 5 wherein said plant is corn and said structural coding sequence is synthesized for enhanced expression in monocots.

8. The method of Claim 7 wherein said structural coding sequence comprises SEQ ID NO:32.

9. A plant produced by the method of Claim 5.

10. The plant of Claim 9 wherein one or more genes expressing *B.t.* endotoxins are included in the genome.

11. A seed or seed piece produced by a plant of Claim 9.