

FASCICULE DE BREVET D'INVENTION

21 Numéro de dépôt : 1201900135
PCT/US2017/055731

22 Date de dépôt : 09/10/2017

30 Priorité(s) :
US n° 62/406,082 du 10/10/2016

24 Délivré le : 30/07/2020

45 Publié le : 23.10.2020

73 Titulaire(s) :

Monsanto Technology LLC,
800 North Lindbergh Blvd.,
ST LOUIS, MO 63167 (US)

72 Inventeur(s) :

HOWE Arlene R. (US)
BOWEN David J. (US)
CHAY Catherine A. (US)
KESANAPALLI Uma (US)

74 Mandataire : Cabinet ÉKÉMÉ LYSAGHT SARL,
B.P. 6370, YAOUNDE (CM).

54 Titre : Novel insect inhibitory proteins.

57 Abrégé :

Pesticidal proteins exhibiting toxic activity against Lepidopteran pest species are disclosed, and include, but are not limited to, TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL. DNA constructs are provided which contain a recombinant nucleic acid sequence encoding one or more of the disclosed pesticidal proteins. Transgenic plants, plant cells, seed, and plant parts resistant to Lepidopteran infestation are provided which contain recombinant nucleic acid sequences encoding the pesticidal proteins of the present invention. Methods for detecting the presence of the recombinant nucleic acid sequences or the proteins of the present invention in a biological sample, and methods of controlling Lepidopteran species pests using any of the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL pesticidal proteins are also provided.

Monsanto Technology LLC

NOVEL INSECT INHIBITORY PROTEINS**REFERENCE TO RELATED APPLICATION**

[01] This application claims the benefit of United States provisional application No. 62/406,082, filed October 10, 2016, which is herein incorporated by reference in its entirety.

5

INCORPORATION OF SEQUENCE LISTING

[02] The file named "MONS426WO_ST25.txt" containing a computer-readable form of the Sequence Listing was created on October 8, 2017. This file is 77,030 bytes (measured in MS-Windows®), filed contemporaneously by electronic submission (using the United States Patent Office EFS-Web filing system), and incorporated herein by reference in its entirety.

10

FIELD OF THE INVENTION

[03] The invention generally relates to the field of insect inhibitory proteins. A novel class of proteins exhibiting insect inhibitory activity against agriculturally-relevant pests of crop plants and seeds are disclosed. In particular, the disclosed class of proteins is insecticidally active against agriculturally-relevant pests of crop plants and seeds, particularly Lepidopteran species of insect pests. Plants, plant parts, and seeds containing a recombinant polynucleotide construct encoding one or more of the disclosed toxin proteins are provided.

15

BACKGROUND OF THE INVENTION

20

[04] Improving crop yield from agriculturally significant plants including, among others, corn, soybean, sugarcane, rice, wheat, vegetables, and cotton, has become increasingly important. In addition to the growing need for agricultural products to feed, clothe and provide energy for a growing human population, climate-related effects and pressure from the growing population to use land other than for agricultural practices are predicted to reduce the amount of arable land available for farming. These factors have led to grim forecasts of food security, particularly in the absence of major improvements in plant biotechnology and agronomic practices. In light of these pressures, environmentally sustainable improvements in technology, agricultural techniques, and pest management are vital tools to expand crop production on the limited amount of arable land available for farming.

25

30

[05] Insects, particularly insects within the order Lepidoptera and Coleoptera, are considered a major cause of damage to field crops, thereby decreasing crop yields over infested areas. Lepidopteran pest species which negatively impact agriculture include, but are not limited to, Beet armyworm (*Spodoptera exigua*), Corn earworm (*Helicoverpa zea*), Cotton leaf worm
 5 (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Old World bollworm (*Helicoverpa armigera*), Oriental leaf worm (*Spodoptera litura*), Pink bollworm (*Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (*Pectinophora gossypiella*), Soybean looper (*Chrysodeixis includens*), Southern armyworm (*Spodoptera eridania*), Southwestern corn borer (*Diatraea grandiosella*), Spotted bollworm
 10 (*Earias vittella*), Sugarcane borer (*Diatraea saccharalis*), Tobacco budworm (*Heliothis virescens*), and Velvet bean caterpillar (*Anticarsia gemmatilis*).

[06] Historically, the intensive application of synthetic chemical insecticides was relied upon as the pest control agent in agriculture. Concerns for the environment and human health, in addition to emerging resistance issues, stimulated the research and development of biological
 15 pesticides. This research effort led to the progressive discovery and use of various entomopathogenic microbial species, including bacteria.

[07] The biological control paradigm shifted when the potential of entomopathogenic bacteria, especially bacteria belonging to the genus *Bacillus*, was discovered and developed as a biological pest control agent. Strains of the bacterium *Bacillus thuringiensis* (*Bt*) have been used as a
 20 source for pesticidal proteins since it was discovered that *Bt* strains show a high toxicity against specific insects. *Bt* strains are known to produce delta-endotoxins that are localized within parasporal crystalline inclusion bodies at the onset of sporulation and during the stationary growth phase (e.g., Cry proteins), and are also known to produce secreted insecticidal protein. Upon ingestion by a susceptible insect, delta-endotoxins as well as secreted toxins exert their
 25 effects at the surface of the midgut epithelium, disrupting the cell membrane, leading to cell disruption and death. Genes encoding insecticidal proteins have also been identified in bacterial species other than *Bt*, including other *Bacillus* and a diversity of additional bacterial species, such as *Brevibacillus laterosporus*, *Lysinibacillus sphaericus* (“*Ls*” formerly known as *Bacillus sphaericus*) and *Paenibacillus popilliae*.

[08] Crystalline and secreted soluble insecticidal toxins are highly specific for their hosts and have gained worldwide acceptance as alternatives to chemical insecticides. For example,

insecticidal toxin proteins have been employed in various agricultural applications to protect agriculturally important plants from insect infestations, decrease the need for chemical pesticide applications, and increase yields. Insecticidal toxin proteins are used to control agriculturally-relevant pests of crop plants by mechanical methods, such as spraying to disperse microbial formulations containing various bacteria strains onto plant surfaces, and by using genetic transformation techniques to produce transgenic plants and seeds expressing insecticidal toxin protein.

[09] The use of transgenic plants expressing insecticidal toxin proteins has been globally adapted. For example, in 2012, 26.1 million hectares were planted with transgenic crops expressing *Bt* toxins (James, C., Global Status of Commercialized Biotech/GM Crops: 2012. ISAAA Brief No. 44). The global use of transgenic insect-protected crops and the limited number of insecticidal toxin proteins used in these crops has created a selection pressure for existing insect alleles that impart resistance to the currently-utilized insecticidal proteins.

[010] The development of resistance in target pests to insecticidal toxin proteins creates the continuing need for discovery and development of new forms of insecticidal toxin proteins that are useful for managing the increase in insect resistance to transgenic crops expressing insecticidal toxin proteins. New protein toxins with improved efficacy and which exhibit control over a broader spectrum of susceptible insect species will reduce the number of surviving insects which can develop resistance alleles. In addition, the use in one plant of two or more transgenic insecticidal toxin proteins toxic to the same insect pest and displaying different modes of action reduces the probability of resistance in any single target insect species.

[011] Thus, the inventors disclose herein a novel protein toxin family from *Bacillus thuringiensis*, along with similar toxin proteins, variant proteins, and exemplary recombinant proteins that exhibit insecticidal activity against target Lepidopteran species, particularly against Beet armyworm (*Spodoptera exigua*), Corn earworm (*Helicoverpa zea*), Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Old World bollworm (*Helicoverpa armigera*), Oriental leaf worm (*Spodoptera litura*), Pink bollworm (*Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (*Pectinophora gossypiella*), Soybean looper (*Chrysodeixis includens*), Southern armyworm (*Spodoptera eridania*), Southwestern corn borer (*Diatraea grandiosella*), Spotted bollworm

(*Earias vittella*), Sugarcane borer (*Diatraea saccharalis*), Tobacco budworm (*Heliothis virescens*), and Velvet bean caterpillar (*Anticarsia gemmatilis*).

SUMMARY OF THE INVENTION

5 [012] Disclosed herein is a novel group of pesticidal proteins with insect inhibitory activity (toxin proteins), referred to herein as TIC4472, TIC1425, and TIC2613 belonging to the TIC4472 protein toxin class, which are shown to exhibit inhibitory activity against one or more pests of crop plants. The TIC4472 protein and proteins in the TIC4472 protein toxin class can be used alone or in combination with other insecticidal proteins and toxic agents in formulations
10 and *in planta*, thus providing alternatives to insecticidal proteins and insecticide chemistries currently in use in agricultural systems.

[013] In one embodiment, disclosed in this application is a recombinant nucleic acid molecule comprising a heterologous promoter fragment operably linked to a polynucleotide segment encoding a pesticidal protein or fragment thereof, wherein (a) said pesticidal protein comprises
15 the amino acid sequence of SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) said pesticidal protein comprises an amino acid sequence having: (i) at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (ii) at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10; or (c) said polynucleotide segment hybridizes to a
20 polynucleotide having the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9; or (d) said polynucleotide segment encoding a pesticidal protein or fragment thereof comprises a polynucleotide sequence having at least 65%, or 70%, or 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99%, or about 100% sequence identity to the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID
25 NO:9; or (e) said recombinant nucleic acid molecule is in operable linkage with a vector, and said vector is selected from the group consisting of a plasmid, phagemid, bacmid, cosmid, and a bacterial or yeast artificial chromosome. The recombinant nucleic acid molecule can comprise a sequence that functions to express the pesticidal protein in a plant; or is expressed in a plant cell to produce a pesticidally effective amount of pesticidal protein.

30 [014] In another embodiment of this application, host cells comprising a recombinant nucleic acid molecule of the application are provided, wherein the host cell is selected from the group

consisting of a bacterial and a plant cell. Contemplated bacterial host cells include *Agrobacterium*, *Rhizobium*, *Bacillus*, *Brevibacillus*, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Pantoec*, and *Erwinia*. In certain embodiments, said *Bacillus* species is *Bacillus cereus* or *Bacillus thuringiensis*, said *Brevibacillus* is *Brevibacillus laterosperous*, or *Escherichia* is *Escherichia coli*. Contemplated plant host cells include a dicotyledonous plant cell and a monocotyledonous plant cell. Contemplated plant cells further include an alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton (*Gossypium* sp.), a cucurbit, cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeonpea, pine, potato, poplar, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat plant cell.

[015] In another embodiment, the pesticidal protein exhibits activity against Lepidopteran insects, including Beet armyworm (*Spodoptera exigua*), Corn earworm (*Helicoverpa zea*), Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Old World bollworm (*Helicoverpa armigera*), Oriental leaf worm (*Spodoptera litura*), Pink bollworm (*Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (*Pectinophora gossypiella*), Soybean looper (*Chrysodeixis includens*), Southern armyworm (*Spodoptera eridania*), Southwestern corn borer (*Diatraea grandiosella*), Spotted bollworm (*Earias vittella*), Sugarcane borer (*Diatraea saccharalis*), Tobacco budworm (*Heliothis virescens*), and Velvet bean caterpillar (*Anticarsia gemmatilis*).

[016] Also contemplated in this application are plants comprising a recombinant nucleic acid molecule comprising a heterologous promoter fragment operably linked to a polynucleotide segment encoding a pesticidal protein or fragment thereof, wherein: (a) said pesticidal protein comprises the amino acid sequence of SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) said pesticidal protein comprises an amino acid sequence having: (i) at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (ii) at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10; or (c) said polynucleotide

segment hybridizes under stringent hybridization conditions to the compliment of the nucleotide sequence of SEQ ID NO:3 or SEQ ID NO:9; or (d) said plant exhibits a detectable amount of said pesticidal protein. In certain embodiments, the pesticidal protein comprises SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. In one embodiment, the plant is either a dicotyledonous plant or a monocotyledonous plant. In another embodiment, the plant is further selected from the group consisting of an alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton, a cucurbit, cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeon pea, pine, potato, poplar, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat.

[017] In further embodiments, seeds comprising the recombinant nucleic acid molecules are disclosed.

[018] In another embodiment, an insect inhibitory composition comprising the recombinant nucleic acid molecules disclosed in this application are contemplated. The insect inhibitory composition can further comprise a nucleotide sequence encoding at least one other pesticidal agent that is different from said pesticidal protein. In certain embodiments, the at least one other pesticidal agent is selected from the group consisting of an insect inhibitory protein, an insect inhibitory dsRNA molecule, and an ancillary protein. It is also contemplated that the at least one other pesticidal agent in the insect inhibitory composition exhibits activity against one or more pest species of the orders Lepidoptera, Coleoptera, or Hemiptera. The at least one other pesticidal agent in the insect inhibitory composition is in one embodiment selected from the group consisting of a Cry1A, Cry1Ab, Cry1Ac, Cry1A.105, Cry1Ae, Cry1B, Cry1C, Cry1C variants, Cry1D, Cry1E, Cry1F, Cry1A/F chimeras, Cry1G, Cry1H, Cry1I, Cry1J, Cry1K, Cry1L, Cry2A, Cry2Ab, Cry2Ae, Cry3, Cry3A variants, Cry3B, Cry4B, Cry6, Cry7, Cry8, Cry9, Cry15, Cry34, Cry35, Cry43A, Cry43B, Cry51Aa1, ET29, ET33, ET34, ET35, ET66, ET70, TIC400, TIC407, TIC417, TIC431, TIC800, TIC807, TIC834, TIC853, TIC900, TIC901, TIC1201, TIC1415, TIC2160, TIC3131, TIC836, TIC860, TIC867, TIC869, TIC1100, VIP3A, VIP3B, VIP3Ab, AXMI-AXMI-, AXMI-88, AXMI-97, AXMI-102, AXMI-112, AXMI-117, AXMI-

100, AXMI-115, AXMI-113, and AXMI-005, AXMI134, AXMI-150, AXMI-171, AXMI-184, AXMI-196, AXMI-204, AXMI-207, AXMI-209, AXMI-205, AXMI-218, AXMI-220, AXMI-221z, AXMI-222z, AXMI-223z, AXMI-224z and AXMI-225z, AXMI-238, AXMI-270, AXMI-279, AXMI-345, AXMI-335, AXMI-R1 and variants thereof, IP3 and variants thereof, DIG-3, DIG-5, DIG-10, DIG-657 and a DIG-11 protein.

[019] Commodity products comprising a detectable amount of the recombinant nucleic acid molecules disclosed in this application are also contemplated. Such commodity products include commodity corn bagged by a grain handler, corn flakes, corn cakes, corn flour, corn meal, corn syrup, corn oil, corn silage, corn starch, corn cereal, and the like, and corresponding soybean, rice, wheat, sorghum, pigeon pea, peanut, fruit, melon, and vegetable commodity products including, where applicable, juices, concentrates, jams, jellies, marmalades, and other edible forms of such commodity products containing a detectable amount of such polynucleotides and or polypeptides of this application, whole or processed cotton seed, cotton oil, lint, seeds and plant parts processed for feed or food, fiber, paper, biomasses, and fuel products such as fuel derived from cotton oil or pellets derived from cotton gin waste, whole or processed soybean seed, soybean oil, soybean protein, soybean meal, soybean flour, soybean flakes, soybean bran, soybean milk, soybean cheese, soybean wine, animal feed comprising soybean, paper comprising soybean, cream comprising soybean, soybean biomass, and fuel products produced using soybean plants and soybean plant parts.

[020] Also contemplated in this application are methods of producing seed comprising the recombinant nucleic acid molecules disclosed in this application. The method comprises planting at least one of the seed comprising the recombinant nucleic acid molecules disclosed in this application; growing plant from the seed; and harvesting seed from the plants, wherein the harvested seed comprises the recombinant nucleic acid molecules in this application.

[021] In another illustrative embodiment, a plant resistant to insect infestation, is provided wherein the cells of said plant comprise: (a) a recombinant nucleic acid molecule encoding an insecticidally effective amount of a pesticidal protein as set forth in SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) an insecticidally effective amount of a protein comprising an amino acid sequence having: (i) at least 93%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (ii) at least 73%, or 75%, or 80%,

or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10.

[022] Also disclosed in this application are methods for controlling a Lepidopteran species pest, and controlling a Lepidopteran species pest infestation of a plant, particularly a crop plant. The method comprises, in one embodiment, (a) contacting the pest with an insecticidally effective amount of a pesticidal proteins as set forth in SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) contacting the pest with an insecticidally effective amount of one or more pesticidal proteins comprising an amino acid sequence having: (i) at least 93%, or 95%, or about 100% amino acid sequence identity to identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (ii) least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10.

[023] Further provided herein is a method of detecting the presence of a recombinant nucleic acid molecule comprising a polynucleotide segment encoding a pesticidal protein or fragment thereof, wherein: (a) said pesticidal protein comprises the amino acid sequence of SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) said pesticidal protein comprises an amino acid sequence having: (i) at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (ii) at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10; or (c) said polynucleotide segment hybridizes to a polynucleotide having the nucleotide sequence of SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10. In one embodiment of the invention, the method comprises contacting a sample of nucleic acids with a nucleic acid probe that hybridizes under stringent hybridization conditions with genomic DNA from a plant comprising a polynucleotide segment encoding a pesticidal protein or fragment thereof provided herein, and does not hybridize under such hybridization conditions with genomic DNA from an otherwise isogenic plant that does not comprise the segment, wherein the probe is homologous or complementary to SEQ ID NO:3 or SEQ ID NO:9, or a sequence that encodes a pesticidal protein comprising an amino acid sequence having: (a) at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10. The method may further

comprise (a) subjecting the sample and probe to stringent hybridization conditions; and (b) detecting hybridization of the probe with DNA of the sample.

[024] Also provided by the invention are methods of detecting the presence of a pesticidal protein or fragment thereof in a sample comprising protein, wherein said pesticidal protein
 5 comprises the amino acid sequence of SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10; or said pesticidal protein comprises an amino acid sequence having: (a) at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or (b) at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10. In one
 10 embodiment, the method comprises: (a) contacting a sample with an immunoreactive antibody; and (b) detecting the presence of the protein. In some embodiments the step of detecting comprises an ELISA, or a Western blot.

BRIEF DESCRIPTION OF THE SEQUENCES

15 [025] SEQ ID NO:1 is a nucleic acid sequence encoding a TIC4472 pesticidal protein obtained from *Bacillus thuringiensis* species EG10742.

[026] SEQ ID NO:2 is the amino acid sequence of the TIC4472 pesticidal protein.

[027] SEQ ID NO:3 is a synthetic coding sequence encoding a TIC4472PL pesticidal protein designed for expression in a plant cell wherein an additional alanine codon is inserted
 20 immediately following the initiating methionine codon.

[028] SEQ ID NO:4 is the amino acid sequence of TIC4472PL encoded by a synthetic coding sequence designed for expression in a plant cell (SEQ ID NO:3), and wherein an additional alanine amino acid is inserted immediately following the initiating methionine.

25 [029] SEQ ID NO:5 is a nucleic acid sequence encoding a TIC1425 pesticidal protein obtained from *Bacillus thuringiensis* species EG10731.

[030] SEQ ID NO:6 is the amino acid sequence of the TIC1425 pesticidal protein.

[031] SEQ ID NO:7 is a nucleic acid sequence encoding a TIC2613 pesticidal protein obtained from *Bacillus thuringiensis* species EG5408.

[032] SEQ ID NO:8 is the amino acid sequence of the TIC2613 pesticidal protein.

[033] SEQ ID NO:9 is a synthetic coding sequence encoding a TIC2613PL pesticidal protein designed for expression in a plant cell wherein an additional alanine codon is inserted immediately following the initiating methionine codon.

[034] SEQ ID NO:10 is the amino acid sequence of TIC2613PL encoded by a synthetic coding sequence designed for expression in a plant cell (SEQ ID NO:9), and wherein an additional alanine amino acid is inserted immediately following the initiating methionine.

DETAILED DESCRIPTION OF THE INVENTION

[035] The problem in the art of agricultural pest control can be characterized as a need for new toxin proteins that are efficacious against target pests, exhibit broad spectrum toxicity against target pest species, are capable of being expressed in plants without causing undesirable agronomic issues, and provide an alternative mode of action compared to current toxins that are used commercially in plants.

[036] Novel pesticidal proteins exemplified by TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL are disclosed herein, and address each of these needs, particularly against a broad spectrum of Lepidopteran insect pests, and more particularly against Beet armyworm (*Spodoptera exigua*), Corn earworm (*Helicoverpa zea*), Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Old World bollworm (*Helicoverpa armigera*), Oriental leaf worm (*Spodoptera litura*), Pink bollworm (*Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (*Pectinophora gossypiella*), Soybean looper (*Chrysodeixis includens*), Southern armyworm (*Spodoptera eridania*), Southwestern corn borer (*Diatraea grandiosella*), Spotted bollworm (*Earias vittella*), Sugarcane borer (*Diatraea saccharalis*), Tobacco budworm (*Heliothis virescens*), and Velvet bean caterpillar (*Anticarsia gemmatilis*).

[037] Reference in this application to TIC4472, "TIC4472 protein", "TIC4472 protein toxin", "TIC4472 toxin protein", "TIC4472 pesticidal protein", "TIC4472-related toxins", "TIC4472-related toxin proteins", TIC4472PL, "TIC4472PL protein", "TIC4472PL protein toxin", "TIC4472PL toxin protein", "TIC4472PL pesticidal protein", "TIC4472PL-related toxins", "TIC4472PL-related toxin proteins", TIC1425, "TIC1425 protein", "TIC1425 protein toxin", "TIC1425 toxin protein", "TIC1425 pesticidal protein", "TIC1425-related toxins", "TIC1425-related toxin proteins", TIC2613, "TIC2613protein", "TIC2613protein toxin", "TIC2613toxin

protein”, “TIC2613pesticidal protein”, “TIC2613-related toxins”, “TIC2613-related toxin proteins”, TIC2613PL, “TIC2613PL protein”, “TIC2613PL protein toxin”, “TIC2613PL toxin protein”, “TIC2613PL pesticidal protein”, “TIC2613PL-related toxins”, “TIC2613PL-related toxin proteins”, and the like, refer to any novel pesticidal protein or insect inhibitory protein, that comprises, that consists of, that is substantially homologous to, that is similar to, or that is derived from any pesticidal protein or insect inhibitory protein sequence of TIC4472 (SEQ ID NO:2), TIC4472PL (SEQ ID NO:4), TIC1425 (SEQ ID NO:6), TIC2613 (SEQ ID NO:8), or TIC2613PL (SEQ ID NO:10) and pesticidal or insect inhibitory segments thereof, or combinations thereof, that confer activity against Lepidopteran pests, including any protein exhibiting pesticidal or insect inhibitory activity if alignment of such protein with TIC4472, TIC4472PL, or TIC1425 results in amino acid sequence identity of any fraction percentage from about 93% to about 100% percent; or if alignment of such protein with TIC2613 or TIC2613PL results in amino acid sequence identity of any fraction percentage from about 73% to about 100% percent. The TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL proteins include both the plastid-targeted and non-plastid targeted form of the proteins.

[038] The term “segment” or “fragment” is used in this application to describe consecutive amino acid or nucleic acid sequences that are shorter than the complete amino acid or nucleic acid sequence describing a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein. A segment or fragment exhibiting insect inhibitory activity is also disclosed in this application if alignment of such segment or fragment, with the corresponding section of the TIC4472 protein set forth in SEQ ID NO:2, TIC4472PL protein set forth in SEQ ID NO:4, TIC1425 protein set forth in SEQ ID NO:6, results in amino acid sequence identity of any fraction percentage from about 93 to about 100 percent between the segment or fragment and the corresponding section of the TIC4472, TIC4472PL, or TIC1425 protein; or if alignment of such segment or fragment, with the corresponding section of the TIC2613 set forth in SEQ ID NO:8, or TIC2613PL protein set forth in SEQ ID NO:10, results in amino acid sequence identity of any fraction percentage from about 73 to about 100 percent between the segment or fragment and the corresponding section of the TIC2613 or TIC2613PL protein.

[039] In still further specific embodiments, a fragment of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein may be defined as exhibiting pesticidal activity possessed by the starting protein molecule from which it is derived. A fragment of a nucleic acid sequence

encoding a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein may be defined as encoding a protein exhibiting the pesticidal activity possessed by the protein molecule encoded by the starting nucleic acid sequence from which it is derived. A fragment or variant described herein may further comprise a domain identified herein which is responsible for the pesticidal activity of a protein.

[040] In specific embodiments, fragments of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein are provided comprising at least about 50, at least about 75, at least about 95, at least about 100, at least about 125, at least about 150, at least about 175, at least about 200, at least about 225, at least about 250, at least about 275, at least about 300, at least about 500, at least about 600, at least about 700, at least about 750, at least about 800, at least about 900, at least about 1000, at least about 1100, at least about 1150, or at least about 1175 contiguous amino acids, or longer, of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein having pesticidal activity as disclosed herein. In certain embodiments, the invention provides fragments of any one of SEQ ID NOs: 2, 4, 6, 8, or 10, having the activity of the full length sequence. Methods for producing such fragments from a starting molecule are well known in the art.

[041] Reference in this application to the terms “active” or “activity”, “pesticidal activity” or “pesticidal” or “insecticidal activity”, “insect inhibitory” or “insecticidal” refer to efficacy of a toxic agent, such as a protein toxin, in inhibiting (inhibiting growth, feeding, fecundity, or viability), suppressing (suppressing growth, feeding, fecundity, or viability), controlling (controlling the pest infestation, controlling the pest feeding activities on a particular crop containing an effective amount of the TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein) or killing (causing the morbidity, mortality, or reduced fecundity of) a pest. These terms are intended to include the result of providing a pesticidally effective amount of a toxic protein to a pest where the exposure of the pest to the toxic protein results in morbidity, mortality, reduced fecundity, or stunting. These terms also include repulsion of the pest from the plant, a tissue of the plant, a plant part, seed, plant cells, or from the particular geographic location where the plant may be growing, as a result of providing a pesticidally effective amount of the toxic protein in or on the plant. In general, pesticidal activity refers to the ability of a toxic protein to be effective in inhibiting the growth, development, viability, feeding behavior, mating behavior, fecundity, or any measurable decrease in the adverse effects caused by an insect feeding on this protein,

protein fragment, protein segment or polynucleotide of a particular target pest, including but not limited to insects of the order Lepidoptera. The toxic protein can be produced by the plant or can be applied to the plant or to the environment within the location where the plant is located. The terms “bioactivity”, “effective”, “efficacious” or variations thereof are also terms
 5 interchangeably utilized in this application to describe the effects of proteins of the present invention on target insect pests.

[042] A pesticidally effective amount of a toxic agent, when provided in the diet of a target pest, exhibits pesticidal activity when the toxic agent contacts the pest. A toxic agent can be a pesticidal protein or one or more chemical agents known in the art. Pesticidal or insecticidal
 10 chemical agents and pesticidal or insecticidal protein agents can be used alone or in combinations with each other. Chemical agents include but are not limited to dsRNA molecules targeting specific genes for suppression in a target pest, organochlorides, organophosphates, carbamates, pyrethroids, neonicotinoids, and ryanoids. Pesticidal or insecticidal protein agents include the protein toxins set forth in this application, as well as other proteinaceous toxic agents
 15 including those that target Lepidopterans, as well as protein toxins that are used to control other plant pests such as Cry and Cyt proteins available in the art for use in controlling Coleopteran, Hemipteran and Homopteran species.

[043] It is intended that reference to a pest, particularly a pest of a crop plant, means insect pests of crop plants, particularly those Lepidoptera insect pests that are controlled by the
 20 TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein toxin class. However, reference to a pest can also include Coleopteran, Hemipteran and Homopteran insect pests of plants, as well as nematodes and fungi when toxic agents targeting these pests are co-localized or present together with the TIC4472, TIC4472PL, or TIC1425 protein or a protein that is 93 to about 100 percent identical to TIC4472, TIC4472PL, or TIC1425; or the TIC2613, or
 25 TIC2613PL protein or a protein that is 73 to about 100 percent identical to TIC2613, or TIC2613PL.

[044] The TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL proteins are related by a common function and exhibit insecticidal activity towards insect pests from the Lepidoptera insect species, including adults, pupae, larvae, and neonates.

30 [045] The insects of the order Lepidoptera include, but are not limited to, armyworms, cutworms, loopers, and heliothines in the Family Noctuidae, *e.g.*, Fall armyworm (*Spodoptera*

frugiperda), Beet armyworm (*Spodoptera exigua*), Black armyworm (*Spodoptera exempta*), Southern armyworm (*Spodoptera eridania*), bertha armyworm (*Mamestra configurata*), black cutworm (*Agrotis ipsilon*), cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), velvetbean caterpillar (*Anticarsia gemmatilis*), green cloverworm (*Hypena scabra*), tobacco budworm (*Heliothis virescens*), granulate cutworm (*Agrotis subterranea*), armyworm (*Pseudaletia unipuncta*), western cutworm (*Agrotis orthogonia*); borers, casebearers, webworms, coneworms, cabbageworms and skeletonizers from the Family Pyralidae, e.g., European corn borer (*Ostrinia nubilalis*), navel orangeworm (*Amyelois transitella*), corn root webworm (*Crambus caliginosellus*), sod webworm (*Herpetogramma licarsisalis*), sunflower moth (*Homoeosoma electellum*), lesser cornstalk borer (*Elasmopalpus lignosellus*); leafrollers, budworms, seed worms, and fruit worms in the Family Tortricidae, e.g., codling moth (*Cydia pomonella*), grape berry moth (*Endopiza viteana*), oriental fruit moth (*Grapholita molesta*), sunflower bud moth (*Suleima helianthana*); and many other economically important Lepidoptera, e.g., diamondback moth (*Plutella xylostella*), pink bollworm (*Pectinophora gossypiella*), and gypsy moth (*Lymantria dispar*). Other insect pests of order Lepidoptera include, e.g., cotton leaf worm (*Alabama argillacea*), fruit tree leaf roller (*Archips argyrospila*), European leafroller (*Archips rosana*) and other *Archips* species, (*Chilo suppressalis*, Asiatic rice borer, or rice stem borer), rice leaf roller (*Cnaphalocrocis medinalis*), corn root webworm (*Crambus caliginosellus*), bluegrass webworm (*Crambus teterrellus*), southwestern corn borer (*Diatraea grandiosella*), sugarcane borer (*Diatraea saccharalis*), spiny bollworm (*Earias insulana*), spotted bollworm (*Earias vittella*), American bollworm (*Helicoverpa armigera*), corn earworm (*Helicoverpa zea*, also known as soybean podworm and cotton bollworm), tobacco budworm (*Heliothis virescens*), sod webworm (*Herpetogramma licarsisalis*), Western bean cutworm (*Striacosta albicosta*), European grape vine moth (*Lobesia botrana*), citrus leafminer (*Phyllocnistis citrella*), large white butterfly (*Pieris brassicae*), small white butterfly (*Pieris rapae*, also known as imported cabbageworm), beet armyworm (*Spodoptera exigua*), tobacco cutworm (*Spodoptera litura*, also known as cluster caterpillar), and tomato leafminer (*Tuta absoluta*).

[046] Reference in this application to an “isolated DNA molecule”, or an equivalent term or phrase, is intended to mean that the DNA molecule is one that is present alone or in combination with other compositions, but not within its natural environment. For example, nucleic acid elements such as a coding sequence, intron sequence, untranslated leader sequence, promoter

sequence, transcriptional termination sequence, and the like, that are naturally found within the DNA of the genome of an organism are not considered to be “isolated” so long as the element is within the genome of the organism and at the location within the genome in which it is naturally found. However, each of these elements, and subparts of these elements, would be “isolated” within the scope of this disclosure so long as the element is not within the genome of the organism and at the location within the genome in which it is naturally found. Similarly, a nucleotide sequence encoding an insecticidal protein or any naturally occurring insecticidal variant of that protein would be an isolated nucleotide sequence so long as the nucleotide sequence was not within the DNA of the bacterium from which the sequence encoding the protein is naturally found. A synthetic nucleotide sequence encoding the amino acid sequence of the naturally occurring insecticidal protein would be considered to be isolated for the purposes of this disclosure. For the purposes of this disclosure, any transgenic nucleotide sequence, *i.e.*, the nucleotide sequence of the DNA inserted into the genome of the cells of a plant or bacterium, or present in an extrachromosomal vector, would be considered to be an isolated nucleotide sequence whether it is present within the plasmid or similar structure used to transform the cells, within the genome of the plant or bacterium, or present in detectable amounts in tissues, progeny, biological samples or commodity products derived from the plant or bacterium.

[047] As described further in this application, an open reading frame (ORF) encoding TIC4747 (SEQ ID NO:1) was discovered in DNA obtained from *Bacillus thuringiensis* strain EG10742.

The coding sequence was cloned and expressed in microbial host cells to produce recombinant proteins used in bioassays. An open reading frame (ORF) encoding TIC1425 (SEQ ID NO:5) was discovered in DNA obtained from *Bacillus thuringiensis* strain EG10731. An open reading frame (ORF) encoding TIC2613 (SEQ ID NO:7) was discovered in DNA obtained from *Bacillus thuringiensis* strain EG5408. Bioassay using microbial host cell-derived proteins of TIC4472 demonstrated activity against the Lepidopteran species Beet armyworm (*Spodoptera exigua*), Corn earworm (*Helicoverpa zea*), Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Old World bollworm (*Helicoverpa armigera*), Oriental leaf worm (*Spodoptera litura*), Pink bollworm (*Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (*Pectinophora gossypiella*), Soybean looper (*Chrysodeixis includens*), Southern armyworm (*Spodoptera eridania*), Southwestern corn borer (*Diatraea grandiosella*), Spotted bollworm (*Earias vittella*), Sugarcane borer (*Diatraea saccharalis*),

Tobacco budworm (*Heliothis virescens*), and Velvet bean caterpillar (*Anticarsia gemmatilis*). In addition, activity was also observed against Yellow fever mosquito (*Aedes aegypti*). Bioassay using microbial host cell-derived proteins of TIC1425 demonstrated activity against the Lepidopteran species Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Sugarcane borer (*Diatraea saccharalis*), and Southwestern corn borer (*Diatraea grandiosella*). Bioassay using microbial host cell-derived proteins of TIC2613 demonstrated activity against the Lepidopteran species Corn earworm (*Helicoverpa zea*), Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Soybean looper (*Chrysodeixis includens*), Southwestern corn borer (*Diatraea grandiosella*), and Tobacco budworm (*Heliothis virescens*).

[048] For expression in plant cells, the TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL proteins can be expressed to reside in the cytosol or targeted to various organelles of the plant cell. For example, targeting a protein to the chloroplast may result in increased levels of expressed protein in a transgenic plant while preventing off-phenotypes from occurring.

Targeting may also result in an increase in pest resistance efficacy in the transgenic event. A target peptide or transit peptide is a short (3-70 amino acids long) peptide chain that directs the transport of a protein to a specific region in the cell, including the nucleus, mitochondria, endoplasmic reticulum (ER), chloroplast, apoplast, peroxisome and plasma membrane. Some target peptides are cleaved from the protein by signal peptidases after the proteins are transported.

For targeting to the chloroplast, proteins contain transit peptides which are around 40-50 amino acids. For descriptions of the use of chloroplast transit peptides, see U.S. Patent Nos. 5,188,642 and 5,728,925. Many chloroplast-localized proteins are expressed from nuclear genes as precursors and are targeted to the chloroplast by a chloroplast transit peptide (CTP). Examples of such isolated chloroplast proteins include, but are not limited to, those associated with the small subunit (SSU) of ribulose-1,5-bisphosphate carboxylase, ferredoxin, ferredoxin oxidoreductase, the light-harvesting complex protein I and protein II, thioredoxin F, enolpyruvyl shikimate phosphate synthase (EPSPS), and transit peptides described in U.S. Patent No. 7,193,133. It has been demonstrated *in vivo* and *in vitro* that non-chloroplast proteins may be targeted to the chloroplast by use of protein fusions with a heterologous CTP and that the CTP is sufficient to target a protein to the chloroplast. Incorporation of a suitable chloroplast transit peptide such as the *Arabidopsis thaliana* EPSPS CTP (CTP2) (see, Klee *et al.*, *Mol. Gen. Genet.*

210:437-442, 1987) or the *Petunia hybrida* EPSPS CTP (CTP4) (*see, della-Cioppa et al., Proc. Natl. Acad. Sci. USA* 83:6873-6877, 1986) has been shown to target heterologous EPSPS protein sequences to chloroplasts in transgenic plants (*see, U.S. Patent Nos. 5,627,061; 5,633,435; and 5,312,910; and EP 0218571; EP 189707; EP 508909; and EP 924299*). For targeting the

5 TIC6757 or TIC6757PL toxin protein to the chloroplast, a sequence encoding a chloroplast transit peptide is placed 5' in operable linkage and in frame to a synthetic coding sequence encoding the TIC6757 or TIC6757PL toxin protein that has been designed for optimal expression in plant cells.

[049] It is contemplated that additional toxin protein sequences related to TIC4472, TIC1425,

10 or TIC2613 can be created by using the amino acid sequence of TIC4472, TIC1425, or TIC2613 to create novel proteins with novel properties. The TIC4472, TIC1425, or TIC2613 toxin proteins can be aligned to combine differences at the amino acid sequence level into novel amino acid sequence variants and making appropriate changes to the recombinant nucleic acid sequence encoding the variants.

15 [050] This disclosure further contemplates that improved variants of the TIC4472 protein toxin class can be engineered *in planta* by using various gene editing methods known in the art. Such technologies used for genome editing include, but are not limited to, ZFN (zinc-finger nuclease), meganucleases, TALEN (Transcription activator-like effector nucleases), and CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas (CRISPR-associated) systems.

20 These genome editing methods can be used to alter the toxin protein coding sequence transformed within a plant cell to a different toxin coding sequence. Specifically, through these methods, one or more codons within the toxin coding sequence are altered to engineer a new protein amino acid sequence. Alternatively, a fragment within the coding sequence is replaced or deleted, or additional DNA fragments are inserted into the coding sequence, to engineer a new

25 toxin coding sequence. The new coding sequence can encode a toxin protein with new properties such as increased activity or spectrum against insect pests, as well as provide activity against an insect pest species wherein resistance has developed against the original insect toxin protein. The plant cell comprising the gene edited toxin coding sequence can be used by methods known in the art to generate whole plants expressing the new toxin protein.

30 [051] It is also contemplated that fragments of TIC4472, TIC1425, or TIC2613 or protein variants thereof can be truncated forms wherein one or more amino acids are deleted from the N-

terminal end, C-terminal end, the middle of the protein, or combinations thereof wherein the fragments and variants retain insect inhibitory activity. These fragments can be naturally occurring or synthetic variants of TIC4472, TIC1425, or TIC2613 or derived protein variants, but should retain the insect inhibitory activity of at least TIC4472, TIC1425, or TIC2613. A
 5 fragment or variant described herein may further comprise a domain identified herein which is responsible for the pesticidal activity of a protein.

[052] Proteins that resemble the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins can be identified and compared to each other using various computer based algorithms known in the art (*see* Tables 1 and 2). Amino acid sequence identities reported in this
 10 application are a result of a Clustal W alignment using these default parameters: Weight matrix: blosum, Gap opening penalty: 10.0, Gap extension penalty: 0.05, Hydrophilic gaps: On, Hydrophilic residues: GPSNDQERK, Residue-specific gap penalties: On (Thompson, *et al* (1994) Nucleic Acids Research, 22:4673-4680). Percent amino acid identity is further calculated by the product of 100% multiplied by (amino acid identities/length of subject protein). Other alignment
 15 algorithms are also available in the art and provide results similar to those obtained using a Clustal W alignment and are contemplated herein.

[053] It is intended that a protein exhibiting insect inhibitory activity against a Lepidopteran insect species is related to TIC4472, TIC4472PL, or TIC1425 if the protein is used in a query, *e.g.*, in a Clustal W alignment, and the proteins of the present invention as set forth as SEQ ID
 20 NO:2, SEQ ID NO:4, or SEQ ID NO:6 are identified as hits in such alignment in which the query protein exhibits at least 93% to about 100% amino acid identity along the length of the query protein that is about 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or any fraction percentage in this range. It is also intended that a protein exhibiting insect inhibitory activity against a Lepidopteran insect species is related to TIC2613 or TIC2613PL if the protein is used
 25 in a query, *e.g.*, in a Clustal W alignment, and the proteins of the present invention as set forth as SEQ ID NO:8, or SEQ ID NO:10 are identified as hits in such alignment in which the query protein exhibits at least 73% to about 100% amino acid identity along the length of the query protein that is about 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, or any
 30 fraction percentage in this range

[054] Exemplary proteins TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL were aligned with each other using a Clustal W algorithm. A pair-wise matrix of percent amino acid sequence identities for each of the full-length proteins was created, as reported in Table 1.

5 **Table 1. Pair-wise matrix display of exemplary proteins TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL.**

Toxin	TIC4472 (SEQ ID NO:2)	TIC4472PL (SEQ ID NO:4)	TIC1425 (SEQ ID NO:6)	TIC2613 (SEQ ID NO:8)	TIC2613PL (SEQ ID NO:10)
TIC4472 (SEQ ID NO:2)	-	99.9 (1186)	99.9 (1186)	68.1 (808)	68 (807)
TIC4472PL (SEQ ID NO:4)	99.8 (1186)	-	99.7 (1185)	67.9 (807)	68.1 (809)
TIC1425 (SEQ ID NO:6)	99.9 (1186)	99.8 (1185)	-	68.2 (809)	68.1 (808)
TIC2613 (SEQ ID NO:8)	68.6 (808)	68.5 (807)	68.7 (809)	-	99.9 (1177)
TIC2613PL (SEQ ID NO:10)	68.4 (807)	68.6 (809)	68.5 (808)	99.8 (1177)	-

10 Table Description: Clustal W alignment between (X) and (Y) are reported in a pair-wise matrix. The percent amino acid identity between all pairs is calculated and is represented by the first number in each box. The second number (in parentheses) in each box represents the number of identical amino acids between the pair.

15 [055] In addition to percent identity, TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL and related proteins can also be related by primary structure (conserved amino acid motifs), by length (about 1187 amino acids), and by other characteristics. Characteristics of the

TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL protein toxins are reported in Table 2.

Table 2. Selected characteristics of the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins.

<u>Protein</u>	<u>Molecular</u> <u>Weight</u> <u>(in</u> <u>Daltons)</u>	<u>Amino</u> <u>Acid</u> <u>Length</u>	<u>Isoelectric</u> <u>Point</u>	<u>Charge</u> <u>at PH</u> <u>7.0</u>	<u>No. of</u> <u>Strongly</u> <u>Basic (-)</u> <u>Amino</u> <u>Acids</u>	<u>No. of</u> <u>Strongly</u> <u>Acidic</u> <u>Amino</u> <u>Acids</u>	<u>No. of</u> <u>Hydrophobic</u> <u>Amino Acids</u>	<u>No. of</u> <u>Polar</u> <u>Amino</u> <u>Acids</u>
TIC4472	134446.86	1187	4.7545	-36	137	162	611	576
TIC4472PL	134517.94	1188	4.7545	-36	137	162	612	576
TIC1425	134460.88	1187	4.7545	-36	137	162	611	576
TIC2613	134636.07	1178	4.6653	-42	132	162	588	590
TIC2613PL	134707.15	1179	4.6653	-42	132	162	589	590

[056] As described further in the Examples of this application, synthetic nucleic acid molecule sequences encoding a variant of TIC4472, TIC4472PL, and a variant of TIC2613, TIC2613PL, were designed for use in plants. An exemplary recombinant nucleic acid molecule sequence that was designed for use in plants encoding the TIC4472PL protein is presented as SEQ ID NO:3. An exemplary recombinant nucleic acid molecule sequence that was designed for use in plants encoding the TIC2613PL protein is presented as SEQ ID NO:9. The TIC4472PL and TIC2613PL proteins have an additional alanine amino acid immediately following the initiating methionine relative to the TIC4472 and TIC2613 proteins, respectively. The additional alanine residue inserted into the TIC4472 and TIC2613 amino acid sequences are believed to improve expression of the protein *in planta*. Likewise, synthetic nucleic acid molecule sequences encoding variants of TIC1425 and can designed for use in plants.

[057] Leaf disc assay using R₀ cotton leaf tissue expressing TIC4472PL protein demonstrated high activity against Soybean looper (*Chrysodeixis includens*) and Tobacco budworm (*Heliothis virescens*) and low activity against Cotton bollworm (*Helicoverpa zea*) and Fall armyworm

(*Spodoptera frugiperda*). Leaf disc assay using R₀ soybean leaf tissue expressing TIC4472PL protein demonstrated activity against Southern armyworm (*Spodoptera eridania*) and Soybean looper (*Chrysodeixis includens*).

[058] Leaf samples from R₀ soybean plants expressing TIC4472PL and TIC2613PL proteins demonstrated activity against Southern armyworm (*Spodoptera eridania*) and Soybean looper (*Chrysodeixis includens*).

[059] Expression cassettes and vectors containing a recombinant nucleic acid molecule sequence can be constructed and introduced into corn, soybean, cotton or other plant cells in accordance with transformation methods and techniques known in the art. For example, *Agrobacterium*-mediated transformation is described in U.S. Patent Application Publications 2009/0138985A1 (soybean), 2008/0280361A1 (soybean), 2009/0142837A1 (corn), 2008/0282432 (cotton), 2008/0256667 (cotton), 2003/0110531 (wheat), 2001/0042257 A1 (sugar beet), U.S. Patent Nos. 5,750,871 (canola), 7,026,528 (wheat), and 6,365,807 (rice), and in Arencibia *et al.* (1998) Transgenic Res. 7:213-222 (sugarcane) all of which are incorporated herein by reference in their entirety. Transformed cells can be regenerated into transformed plants that express TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins and demonstrate pesticidal activity through bioassays performed in the presence of Lepidopteran pest larvae using plant leaf disks obtained from the transformed plants. Plants can be derived from the plant cells by regeneration, seed, pollen, or meristem transformation techniques. Methods for transforming plants are known in the art.

[060] As an alternative to traditional transformation methods, a DNA sequence, such as a transgene, expression cassette(s), etc., may be inserted or integrated into a specific site or locus within the genome of a plant or plant cell via site-directed integration. Recombinant DNA construct(s) and molecule(s) of this disclosure may thus include a donor template sequence comprising at least one transgene, expression cassette, or other DNA sequence for insertion into the genome of the plant or plant cell. Such donor template for site-directed integration may further include one or two homology arms flanking an insertion sequence (*i.e.*, the sequence, transgene, cassette, etc., to be inserted into the plant genome). The recombinant DNA construct(s) of this disclosure may further comprise an expression cassette(s) encoding a site-specific nuclease and/or any associated protein(s) to carry out site-directed integration. These nuclease expressing cassette(s) may be present in the same molecule or vector as the donor

template (in cis) or on a separate molecule or vector (in trans). Several methods for site-directed integration are known in the art involving different proteins (or complexes of proteins and/or guide RNA) that cut the genomic DNA to produce a double strand break (DSB) or nick at a desired genomic site or locus. Briefly as understood in the art, during the process of repairing the DSB or nick introduced by the nuclease enzyme, the donor template DNA may become integrated into the genome at the site of the DSB or nick. The presence of the homology arm(s) in the donor template may promote the adoption and targeting of the insertion sequence into the plant genome during the repair process through homologous recombination, although an insertion event may occur through non-homologous end joining (NHEJ). Examples of site-specific nucleases that may be used include zinc-finger nucleases, engineered or native meganucleases, TALE-endonucleases, and RNA-guided endonucleases (*e.g.*, Cas9 or Cpf1). For methods using RNA-guided site-specific nucleases (*e.g.*, Cas9 or Cpf1), the recombinant DNA construct(s) will also comprise a sequence encoding one or more guide RNAs to direct the nuclease to the desired site within the plant genome.

[061] As used herein, a “recombinant DNA molecule” is a DNA molecule comprising a combination of DNA molecules that would not naturally occur together without human intervention. For instance, a recombinant DNA molecule may be a DNA molecule that is comprised of at least two DNA molecules heterologous with respect to each other, a DNA molecule that comprises a DNA sequence that deviates from DNA sequences that exist in nature, or a DNA molecule that has been incorporated into a host cell’s DNA by genetic transformation or gene editing. Similarly, a “recombinant protein molecule” is a protein molecule comprising a combination of amino acids that would not naturally occur together without human intervention. For example, a recombinant protein molecule may be a protein molecule that is comprised of at least two amino acid molecules heterologous with respect to each other, a protein molecule that comprises an amino acid sequence that deviates from amino acid sequences that exist in nature, or a protein molecule that is expressed in a host cell as a result of genetic transformation of the host cell or by gene editing of the host cell genome.

[062] Recombinant nucleic acid molecule compositions that encode TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL are contemplated. For example, TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins can be expressed with recombinant DNA constructs in which a polynucleotide molecule with an ORF encoding the protein is operably

linked to genetic expression elements such as a promoter and any other regulatory element necessary for expression in the system for which the construct is intended. Non-limiting examples include a plant-functional promoter operably linked to a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequence for expression of the protein in plants or a *Bt*-functional promoter operably linked to a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequence for expression of the protein in a *Bt* bacterium or other *Bacillus* species. Other elements can be operably linked to the TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequence including, but not limited to, enhancers, introns, untranslated leaders, encoded protein immobilization tags (HIS-tag), translocation peptides (*i.e.*, plastid transit peptides, signal peptides), polypeptide sequences for post-translational modifying enzymes, ribosomal binding sites, and RNAi target sites. Exemplary recombinant polynucleotide molecules provided herewith include, but are not limited to, a heterologous promoter operably linked to a polynucleotide such as SEQ ID NO:3, SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:7, and SEQ ID NO:9 that encodes the respective polypeptides or proteins having the amino acid sequence as set forth in SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:10. A heterologous promoter can also be operably linked to synthetic DNA coding sequences encoding a plastid targeted TIC4472PL or TIC2613PL; or an untargeted TIC4472PL or TIC2613PL. The codons of a recombinant nucleic acid molecule encoding for proteins disclosed herein can be substituted by synonymous codons (known in the art as a silent substitution).

[063] A recombinant DNA construct comprising TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequences can further comprise a region of DNA that encodes for one or more insect inhibitory agents which can be configured to concomitantly express or co-express with a DNA sequence encoding a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein, a protein different from a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein, an insect inhibitory dsRNA molecule, or an ancillary protein. Ancillary proteins include, but are not limited to, co-factors, enzymes, binding-partners, or other agents that function to aid in the effectiveness of an insect inhibitory agent, for example, by aiding its expression, influencing its stability in plants, optimizing free energy for oligomerization, augmenting its toxicity, and increasing its spectrum of activity. An ancillary protein may

facilitate the uptake of one or more insect inhibitory agents, for example, or potentiate the toxic effects of the toxic agent.

[064] A recombinant DNA construct can be assembled so that all proteins or dsRNA molecules are expressed from one promoter or each protein or dsRNA molecules is under separate promoter control or some combination thereof. The proteins of this invention can be expressed from a multi-gene expression system in which one or more proteins of TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL are expressed from a common nucleotide segment which also contains other open reading frames and promoters, depending on the type of expression system selected. For example, a bacterial multi-gene expression system can utilize a single promoter to drive expression of multiply-linked/tandem open reading frames from within a single operon (*i.e.*, polycistronic expression). In another example, a plant multi-gene expression system can utilize multiply-unlinked or linked expression cassettes, each cassette expressing a different protein or other agent such as one or more dsRNA molecules.

[065] Recombinant polynucleotides or recombinant DNA constructs comprising a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequence can be delivered to host cells by vectors, *e.g.*, a plasmid, baculovirus, synthetic chromosome, virion, cosmid, phagemid, phage, or viral vector. Such vectors can be used to achieve stable or transient expression of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequence in a host cell, or subsequent expression of the encoded polypeptide. An exogenous recombinant polynucleotide or recombinant DNA construct that comprises a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein encoding sequence and that is introduced into a host cell is referred in this application as a “transgene”.

[066] Transgenic bacteria, transgenic plant cells, transgenic plants, and transgenic plant parts that contain a recombinant polynucleotide that expresses any one or more of TIC4472 or a related family toxin protein encoding sequence are provided herein. The term “bacterial cell” or “bacterium” can include, but is not limited to, an *Agrobacterium*, a *Bacillus*, an *Escherichia*, a *Salmonella*, a *Pseudomonas*, a *Brevibacillus*, a *Klebsiella*, an *Erwinia*, or a *Rhizobium* cell. The term “plant cell” or “plant” can include but is not limited to a dicotyledonous or monocotyledonous plant. The term “plant cell” or “plant” can also include but is not limited to an alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton, a cucurbit,

cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeonpea, pine, potato, poplar, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat plant cell or plant. In certain embodiments, transgenic plants and transgenic plant parts regenerated from a transgenic plant cell are provided. In certain embodiments, the transgenic plants can be obtained from a transgenic seed, by cutting, snapping, grinding or otherwise disassociating the part from the plant. In certain embodiments, the plant part can be a seed, a boll, a leaf, a flower, a stem, a root, or any portion thereof, or a non-regenerable portion of a transgenic plant part. As used in this context, a “non-regenerable” portion of a transgenic plant part is a portion that cannot be induced to form a whole plant or that cannot be induced to form a whole plant that is capable of sexual and/or asexual reproduction. In certain embodiments, a non-regenerable portion of a plant part is a portion of a transgenic seed, boll, leaf, flower, stem, or root.

[067] Methods of making transgenic plants that comprise insect, Lepidoptera-inhibitory amounts of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein are provided. Such plants can be made by introducing a recombinant polynucleotide that encodes any of the proteins provided in this application into a plant cell, and selecting a plant derived from said plant cell that expresses an insect, Lepidoptera-inhibitory amount of the proteins. Plants can be derived from the plant cells by regeneration, seed, pollen, or meristem transformation techniques. Methods for transforming plants are known in the art.

[068] Processed plant products, wherein the processed product comprises a detectable amount of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein, an insect inhibitory segment or fragment thereof, or any distinguishing portion thereof, are also disclosed herein. In certain embodiments, the processed product is selected from the group consisting of plant parts, plant biomass, oil, meal, sugar, animal feed, flour, flakes, bran, lint, hulls, processed seed, and seed. In certain embodiments, the processed product is non-regenerable. The plant product can comprise commodity or other products of commerce derived from a transgenic plant or transgenic plant part, where the commodity or other products can be tracked through commerce

by detecting nucleotide segments or expressed RNA or proteins that encode or comprise distinguishing portions of a TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL protein.

[069] Plants expressing the TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL proteins can be crossed by breeding with transgenic events expressing other toxin proteins and/or
5 expressing other transgenic traits such as herbicide tolerance genes, genes conferring yield or stress tolerance traits, and the like, or such traits can be combined in a single vector so that the traits are all linked.

[070] Reference in this application to an "isolated" DNA molecule or amino acid molecule, or an equivalent term or phrase, is intended to mean that the DNA molecule or amino acid molecule
10 is one that is present alone or in combination with other compositions, but not within its natural environment. For example, a DNA molecule or amino acid molecule would be "isolated" within the scope of this disclosure so long as the element is not within the genome of the organism and at the location within the genome in which it is naturally found. For the purposes of this disclosure, any transgenic nucleotide sequence, i.e., the nucleotide sequence of the DNA inserted
15 into the genome of the cells of a plant or bacterium, or present in an extrachromosomal vector, would be considered to be an isolated nucleotide sequence whether it is present within the plasmid or similar structure used to transform the cells, within the genome of the plant or bacterium, or present in detectable amounts in tissues, progeny, biological samples or commodity products derived from the plant or bacterium.

[071] As further described in the Examples, TIC4472, TIC4472PL, TIC1425, TIC2613, or
20 TIC2613PL protein-encoding sequences and sequences having a substantial percentage identity to TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL can be identified using methods known to those of ordinary skill in the art such as polymerase chain reaction (PCR), thermal amplification and hybridization. For example, the proteins TIC4472, TIC4472PL, TIC1425,
25 TIC2613, or TIC2613PL can be used to produce antibodies that bind specifically to related proteins, and can be used to screen for and to find other protein members that are closely related.

[072] Furthermore, nucleotide sequences encoding the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin proteins can be used as probes and primers for screening to identify other members of the class using thermal-cycle or isothermal amplification and
30 hybridization methods. For example, oligonucleotides derived from the sequence as set forth in SEQ ID NO:3 can be used to determine the presence or absence of a TIC4472PL transgene in a

deoxyribonucleic acid sample derived from a commodity product. Oligonucleotides derived from the sequence as set forth in SEQ ID NO:7 can be used to determine the presence or absence of a TIC2613PL transgene in a deoxyribonucleic acid sample derived from a commodity product. Given the sensitivity of certain nucleic acid detection methods that employ oligonucleotides, it is anticipated that oligonucleotides derived from sequences as set forth in SEQ ID NO:3 or SEQ ID NO:9 can be used to detect a TIC4472PL or TIC2613PL transgene in commodity products derived from pooled sources where only a fraction of the commodity product is derived from a transgenic plant containing any of the transgenes. It is further recognized that such oligonucleotides can be used to introduce nucleotide sequence variation in each of SEQ ID NO:3 and SEQ ID NO:9. Such "mutagenesis" oligonucleotides are useful for identification of TIC4472PL and TIC2613PL amino acid sequence variants exhibiting a range of insect inhibitory activity or varied expression in transgenic plant host cells.

[073] Nucleotide sequence homologs, *e.g.*, insecticidal proteins encoded by nucleotide sequences that hybridize to each or any of the sequences disclosed in this application under stringent hybridization conditions, are also an embodiment of the present invention. The invention also provides a method for detecting a first nucleotide sequence that hybridizes to a second nucleotide sequence, wherein the first nucleotide sequence (or its reverse complement sequence) encodes a pesticidal protein or pesticidal fragment thereof and hybridizes to the second nucleotide sequence. In such case, the second nucleotide sequence can be any of the nucleotide sequences presented as SEQ ID NO:3, SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9 under stringent hybridization conditions. Nucleotide coding sequences hybridize to one another under appropriate hybridization conditions, such as stringent hybridization conditions, and the proteins encoded by these nucleotide sequences cross react with antiserum raised against any one of the other proteins. Stringent hybridization conditions, as defined herein, comprise at least hybridization at 42°C followed by two washes for five minutes each at room temperature with 2X SSC, 0.1% SDS, followed by two washes for thirty minutes each at 65°C in 0.5X SSC, 0.1% SDS. Washes at even higher temperatures constitute even more stringent conditions, *e.g.*, hybridization conditions of 68°C, followed by washing at 68°C, in 2xSSC containing 0.1% SDS.

[074] One skilled in the art will recognize that, due to the redundancy of the genetic code, many other sequences are capable of encoding such related proteins, and those sequences, to the extent

that they function to express pesticidal proteins either in *Bacillus* strains or in plant cells, are embodiments of the present invention, recognizing of course that many such redundant coding sequences will not hybridize under these conditions to the native *Bacillus* sequences encoding TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL. This application contemplates the use of these and other identification methods known to those of ordinary skill in the art, to identify TIC4472, TIC1425, and TIC2613 protein-encoding sequences and sequences having a substantial percentage identity to TIC4472, TIC1425, and TIC2613 protein-encoding sequences.

[075] This disclosure also contemplates the use of molecular methods known in the art to engineer and clone commercially useful proteins comprising chimeras of proteins from pesticidal proteins; *e.g.*, the chimeras may be assembled from segments of the TIC4472, TIC4472PL, TIC1425, TIC2613, or TIC2613PL, proteins to derive additional useful embodiments including assembly of segments of TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins with segments of diverse proteins different from TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL and related proteins. The TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins may be subjected to alignment to each other and to other *Bacillus thuringiensis* or other pesticidal proteins (whether or not these are closely or distantly related phylogenetically), and segments of each such protein may be identified that are useful for substitution between the aligned proteins, resulting in the construction of chimeric proteins. Such chimeric proteins can be subjected to pest bioassay analysis and characterized for the presence or absence of increased bioactivity or expanded target pest spectrum compared to the parent proteins from which each such segment in the chimera was derived. The pesticidal activity of the polypeptides may be further engineered for activity to a particular pest or to a broader spectrum of pests by swapping domains or segments with other proteins or by using directed evolution methods known in the art.

[076] Methods of controlling insects, in particular Lepidoptera infestations of crop plants, with the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL proteins are also disclosed in this application. Such methods can comprise growing a plant comprising an insect- or Lepidoptera-inhibitory amount of a TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein. In certain embodiments, such methods can further comprise any one or more of: (i) applying any composition comprising or encoding a TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein to a plant or a seed that gives rise to a plant; and (ii) transforming a

plant or a plant cell that gives rise to a plant with a polynucleotide encoding a TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein. In general, it is contemplated that a TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein can be provided in a composition, provided in a microorganism, or provided in a transgenic plant to confer insect inhibitory activity against Lepidopteran insects.

[077] In certain embodiments, a recombinant nucleic acid molecule of TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin proteins is the insecticidally active ingredient of an insect inhibitory composition prepared by culturing recombinant *Bacillus* or any other recombinant bacterial cell transformed to express a TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein under conditions suitable to express the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein. Such a composition can be prepared by desiccation, lyophilization, homogenization, extraction, filtration, centrifugation, sedimentation, or concentration of a culture of such recombinant cells expressing/producing said recombinant polypeptide. Such a process can result in a *Bacillus* or other entomopathogenic bacterial cell extract, cell suspension, cell homogenate, cell lysate, cell supernatant, cell filtrate, or cell pellet. By obtaining the recombinant polypeptides so produced, a composition that includes the recombinant polypeptides can include bacterial cells, bacterial spores, and parasporal inclusion bodies and can be formulated for various uses, including as agricultural insect inhibitory spray products or as insect inhibitory formulations in diet bioassays.

[078] In one embodiment, to reduce the likelihood of resistance development, an insect inhibitory composition comprising TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL can further comprise at least one additional polypeptide that exhibits insect inhibitory activity against the same Lepidopteran insect species, but which is different from the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL toxin protein. Possible additional polypeptides for such a composition include an insect inhibitory protein and an insect inhibitory dsRNA molecule. One example for the use of such ribonucleotide sequences to control insect pests is described in Baum, *et al.* (U.S. Patent Publication 2006/0021087 A1). Such additional polypeptide for the control of Lepidopteran pests may be selected from the group consisting of an insect inhibitory protein, such as, but not limited to, Cry1A (U.S. Patent No. 5,880,275), Cry1Ab, Cry1Ac, Cry1A.105, Cry1Ae, Cry1B (U.S. Patent Publication No. 10/525,318), Cry1C (U.S. Patent No. 6,033,874), Cry1D, Cry1Da and variants thereof, Cry1E, Cry1F, and Cry1A/F

chimeras (U.S. Patent Nos. 7,070,982; 6,962,705; and 6,713,063), Cry1G, Cry1H, Cry1I, Cry1J, Cry1K, Cry1L, Cry1-type chimeras such as, but not limited to, TIC836, TIC860, TIC867, TIC869, and TIC1100 (International Application Publication WO2016/061391 (A2)), TIC2160 (International Application Publication WO2016/061392(A2)), Cry2A, Cry2Ab (U.S. Patent No. 7,064,249), Cry2Ae, Cry4B, Cry6, Cry7, Cry8, Cry9, Cry15, Cry43A, Cry43B, Cry51Aa1, ET66, TIC400, , TIC800, TIC834, TIC1415, Vip3A, VIP3Ab, VIP3B, AXMI-001, AXMI-002, AXMI-030, AXMI-035, AND AXMI-045 (U.S. Patent Publication 2013-0117884 A1), AXMI-52, AXMI-58, AXMI-88, AXMI-97, AXMI-102, AXMI-112, AXMI-117, AXMI-100 (U.S. Patent Publication 2013-0310543 A1), AXMI-115, AXMI-113, AXMI-005 (U.S. Patent Publication 2013-0104259 A1), AXMI-134 (U.S. Patent Publication 2013-0167264 A1), AXMI-150 (U.S. Patent Publication 2010-0160231 A1), AXMI-184 (U.S. Patent Publication 2010-0004176 A1), AXMI-196, AXMI-204, AXMI-207, axmi209 (U.S. Patent Publication 2011-0030096 A1), AXMI-218, AXMI-220 (U.S. Patent Publication 2014-0245491 A1), AXMI-221z, AXMI-222z, AXMI-223z, AXMI-224z, AXMI-225z (U.S. Patent Publication 2014-0196175 A1), AXMI-238 (U.S. Patent Publication 2014-0033363 A1), AXMI-270 (U.S. Patent Publication 2014-0223598 A1), AXMI-345 (U.S. Patent Publication 2014-0373195 A1), AXMI-335 (International Application Publication WO2013/134523(A2)), DIG-3 (U.S. Patent Publication 2013-0219570 A1), DIG-5 (U.S. Patent Publication 2010-0317569 A1), DIG-11 (U.S. Patent Publication 2010-0319093 A1), AfIP-1A and derivatives thereof (U.S. Patent Publication 2014-0033361 A1), AfIP-1B and derivatives thereof (U.S. Patent Publication 2014-0033361 A1), PIP-1A/PIP-1B (U.S. Patent Publication 2014-0007292 A1), PSEEN3174 (U.S. Patent Publication 2014-0007292 A1), AECFG-592740 (U.S. Patent Publication 2014-0007292 A1), Pput_1063 (U.S. Patent Publication 2014-0007292 A1), DIG-657 (International Application Publication WO2015/195594(A2)), Pput_1064 (U.S. Patent Publication 2014-0007292 A1), GS-135 and derivatives thereof (U.S. Patent Publication 2012-0233726 A1), GS153 and derivatives thereof (U.S. Patent Publication 2012-0192310 A1), GS154 and derivatives thereof (U.S. Patent Publication 2012-0192310 A1), GS155 and derivatives thereof (U.S. Patent Publication 2012-0192310 A1), SEQ ID NO:2 and derivatives thereof as described in U.S. Patent Publication 2012-0167259 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent Publication 2012-0047606 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent Publication 2011-0154536 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent Publication

2011-0112013 A1, SEQ ID NO:2 and 4 and derivatives thereof as described in U.S. Patent Publication 2010-0192256 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent Publication 2010-0077507 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent Publication 2010-0077508 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent
 5 Publication 2009-0313721 A1, SEQ ID NO:2 or 4 and derivatives thereof as described in U.S. Patent Publication 2010-0269221 A1, SEQ ID NO:2 and derivatives thereof as described in U.S. Patent No. 7,772,465 (B2), CF161_0085 and derivatives thereof as described in WO2014/008054 A2, Lepidopteran toxic proteins and their derivatives as described in US Patent Publications US2008-0172762 A1, US2011-0055968 A1, and US2012-0117690 A1; SEQ ID
 10 NO:2 and derivatives thereof as described in US7510878(B2), SEQ ID NO:2 and derivatives thereof as described in U.S. Patent No. 7812129(B1); and the like.

[079] In other embodiments, such composition/formulation can further comprise at least one additional polypeptide that exhibits insect inhibitory activity to an insect that is not inhibited by an otherwise insect inhibitory protein of the present invention to expand the spectrum of insect
 15 inhibition obtained. For example, for the control of Hemipteran pests, combinations of insect inhibitory proteins of the present invention can be used with Hemipteran-active proteins such as TIC1415 (US Patent Publication 2013-0097735 A1), TIC807 (U.S. Patent No. 8609936), TIC834 (U.S. Patent Publication 2013-0269060 A1), AXMI-036 (U.S. Patent Publication 2010-0137216 A1), and AXMI-171 (U.S. Patent Publication 2013-0055469 A1). Further a polypeptide
 20 for the control of Coleopteran pests may be selected from the group consisting of an insect inhibitory protein, such as, but not limited to, Cry3Bb (U.S. Patent No. 6,501,009), Cry1C variants, Cry3A variants, Cry3, Cry3B, Cry34/35, 5307, AXMI134 (U.S. Patent Publication 2013-0167264 A1) AXMI-184 (U.S. Patent Publication 2010-0004176 A1), AXMI-205 (U.S. Patent Publication 2014-0298538 A1), axmi207 (U.S. Patent Publication 2013-0303440 A1),
 25 AXMI-218, AXMI-220 (U.S. Patent Publication 20140245491A1), AXMI-221z, AXMI-223z (U.S. Patent Publication 2014-0196175 A1), AXMI-279 (U.S. Patent Publication 2014-0223599 A1), AXMI-R1 and variants thereof (U.S. Patent Publication 2010-0197592 A1, TIC407, TIC417, TIC431, TIC807, TIC853, TIC901, TIC1201, TIC3131, DIG-10 (U.S. Patent Publication 2010-0319092 A1), eHIPs (U.S. Patent Application Publication No. 2010/0017914),
 30 IP3 and variants thereof (U.S. Patent Publication 2012-0210462 A1), and ω -Hexatoxin-Hv1a (U.S. Patent Application Publication US2014-0366227 A1).

[080] Additional polypeptides for the control of Coleopteran, Lepidopteran, and Hemipteran insect pests can be found on the *Bacillus thuringiensis* toxin nomenclature website maintained by Neil Crickmore (on the world wide web at btnomenclature.info).

[081] The possibility for insects to develop resistance to certain insecticides has been documented in the art. One insect resistance management strategy is to employ transgenic crops that express two distinct insect inhibitory agents that operate through different modes of action. Therefore, any insects with resistance to either one of the insect inhibitory agents can be controlled by the other insect inhibitory agent. Another insect resistance management strategy employs the use of plants that are not protected to the targeted Lepidopteran pest species to provide a refuge for such unprotected plants. One particular example is described in U.S. Patent No. 6,551,962, which is incorporated by reference in its entirety.

[082] Other embodiments such as topically applied pesticidal chemistries that are designed for controlling pests that are also controlled by the proteins disclosed herein to be used with proteins in seed treatments, spray on, drip on, or wipe on formulations can be applied directly to the soil (a soil drench), applied to growing plants expressing the proteins disclosed herein, or formulated to be applied to seed containing one or more transgenes encoding one or more of the proteins disclosed. Such formulations for use in seed treatments can be applied with various stickers and tackifiers known in the art. Such formulations can contain pesticides that are synergistic in mode of action with the proteins disclosed, so that the formulation pesticides act through a different mode of action to control the same or similar pests that can be controlled by the proteins disclosed, or that such pesticides act to control pests within a broader host range or plant pest species that are not effectively controlled by the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL pesticidal proteins.

[083] The aforementioned composition/formulation can further comprise an agriculturally-acceptable carrier, such as a bait, a powder, dust, pellet, granule, spray, emulsion, a colloidal suspension, an aqueous solution, a *Bacillus* spore/crystal preparation, a seed treatment, a recombinant plant cell/plant tissue/seed/plant transformed to express one or more of the proteins, or bacterium transformed to express one or more of the proteins. Depending on the level of insect inhibitory or insecticidal inhibition inherent in the recombinant polypeptide and the level of formulation to be applied to a plant or diet assay, the composition/formulation can include

various by weight amounts of the recombinant polypeptide, *e.g.* from 0.0001% to 0.001% to 0.01% to 1% to 99% by weight of the recombinant polypeptide.

[084] In view of the foregoing, those of skill in the art should appreciate that changes can be made in the specific aspects which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention. Thus, specific structural and functional details disclosed herein are not to be interpreted as limiting. It should be understood that the entire disclosure of each reference cited herein is incorporated within the disclosure of this application.

EXAMPLES

Example 1

Discovery, cloning, and expression of TIC4472, TIC1425, and TIC2613

[085] Sequences encoding three novel *Bacillus thuringiensis* pesticidal proteins were identified, cloned, sequence confirmed, and tested in insect bioassay. The pesticidal proteins, TIC4472, TIC1425 and TIC2613, isolated from the *Bacillus thuringiensis* strains EG10742, EG10731, and EG5408, respectively, represent novel Cry1Ca-like proteins.

[086] Polymerase chain reaction (PCR) primers were designed to amplify a full length copy of the coding region for TIC4472, TIC1425 and TIC2613 from total genomic DNA isolated from the *Bacillus thuringiensis* strains EG10742, EG10731, and EG5408, respectively. The PCR amplicons also included the translational initiation and termination codons of each coding sequence.

[087] Each of the amplicons were cloned using methods known in the art into *Bt* (*Bacillus thuringiensis*) expression vectors in operable linkage with a *Bt* expressible promoter.

Example 2

TIC4472, TIC1425, and TIC2613 demonstrates Lepidopteran activity in insect bioassay

[088] Bioactivity of the pesticidal proteins TIC4472, TIC1425 and TIC2613 was evaluated by producing the protein in a *Bt* expression host. A *Bt* strain expressing TIC4472, TIC1425 and TIC2613 was grown for twenty four (24) hours and then either a spore crystal preparation or solubilized protein preparation was added to insect diet. Mortality and stunting were evaluated

by comparing the growth and development of insects on a diet with a culture from the *Bt* strain expressing TIC4472, TIC1425 and TIC2613 to insects on a diet with an untreated control culture.

[089] Preparations of TIC4472 were assayed against the Lepidopteran species Corn earworm (two colonies (CEW and CEWUC), *Helicoverpa zea*, also herein referred to as Cotton bollworm and Soybean pod worm), Cotton leaf worm (CLW, *Alabama argillacea*), European corn borer (ECB, *Ostrinia nubilalis*), Fall armyworm (FAW, *Spodoptera frugiperda*), Soybean looper (SBL, *Chrysodeixis includens*), Southern armyworm (SAW, *Spodoptera eridania*), Southwestern corn borer (SWCB, *Diatraea grandiosella*), Sugarcane borer (SCB, *Diatraea saccharalis*), a Cry2Ab resistant colony of Sugarcane borer (SCB2R), Tobacco budworm (TBW, *Heliothis virescens*), and Velvet bean caterpillar (VBW, *Anticarsia gemmatilis*); the Coleopteran species Colorado potato beetle (CPB, *Leptinotarsa decemlineata*) and Western corn rootworm (WCB, *Diabrotica virgifera virgifera*); the Hemipteran species Tarnished plant bug (TPB, *Lygus lineolaris*), Western tarnished plant bug (WTP, *Lygus hesperus*); and the Dipteran species Yellow fever mosquito (*Aedes aegypti*). Preparations of TIC1425 were assayed against the Lepidopteran species Black cutworm (BCW, *Agrotis ipsilon*), Cotton leaf worm (CLW, *Alabama argillacea*), European corn borer (ECB, *Ostrinia nubilalis*), Fall armyworm (FAW, *Spodoptera frugiperda*), Black cutworm (BCW, *Agrotis ipsilon*), Southwestern corn borer (SWCB, *Diatraea grandiosella*), and Sugarcane borer (SCB, *Diatraea saccharalis*); and the Coleopteran species Western corn rootworm (WCB, *Diabrotica virgifera virgifera*) and Southern corn rootworm (*Diabrotica undecimpunctata howardi*). Preparations of TIC2613 were assayed against the Lepidopteran species Corn earworm (two colonies (CEW and CEWUC), *Helicoverpa zea*, also herein referred to as Cotton bollworm and Soybean pod worm), Cotton leaf worm (CLW, *Alabama argillacea*), European corn borer (ECB, *Ostrinia nubilalis*), Fall armyworm (FAW, *Spodoptera frugiperda*), Soybean looper (SBL, *Chrysodeixis includens*), Southern armyworm (SAW, *Spodoptera eridania*), Black cutworm (BCW, *Agrotis ipsilon*), Southwestern corn borer (SWCB, *Diatraea grandiosella*), Tobacco budworm (TBW, *Heliothis virescens*), and Velvet bean caterpillar (VBW, *Anticarsia gemmatilis*); the Coleopteran species Colorado potato beetle (CPB, *Leptinotarsa decemlineata*); Western corn rootworm (WCB, *Diabrotica virgifera virgifera*) and Southern corn rootworm (*Diabrotica undecimpunctata howardi*); and the Hemipteran species Tarnished plant bug (TPB, *Lygus lineolaris*), Western tarnished plant bug (WTP, *Lygus hesperus*).

[090] The bioassay activity observed for each protein grown in the *Bt* host is presented in Tables 3 and 4 below, wherein “+” indicates activity, “NT” indicates the toxin was not assayed against that specific insect pest, “S” indicates stunting, and “M” indicates mortality. Preparations of TIC4472, TIC1425, and TIC2613 did not demonstrate activity against the Coleopteran or the Hemipteran insect pests assayed for each protein. TIC4472 also demonstrated activity against Yellow fever mosquito (*Aedes aegypti*). All three toxins demonstrated resistance to multiple Lepidopteran insect pests as shown in Tables 3 and 4.

Table 3. Bioassay activity of TIC4472, TIC1425, and TIC2613 against insect pests.

Toxin	CEW		CEWUC		CLW		ECB		FAW		SBL		SAW	
	S	M	S	M	S	M	S	M	S	M	S	M	S	M
TIC4472	+	+	+	+	+	+	+	+	+		+	+	+	+
TIC1425	NT	NT				+	+	+	+		NT	NT		
TIC2613	+				+	+	+	+	+	+	+	+	+	

Table 4. Bioassay activity of TIC4472, TIC1425, and TIC2613 against insect pests.

Toxin	BCW		SCB		SCB2R		SWCB		TBW		VBC		YFM	
	S	M	S	M	S	M	S	M	S	M	S	M	S	M
TIC4472	NT	NT	+	+	+	+	+	+	+	+	+	+		+
TIC1425				+	NT	NT		+	NT	NT	NT	NT	NT	NT
TIC2613			+	+	NT	NT	+	+	+		+		NT	NT

[091] As can be seen in Tables 3 and 4 above, the insect toxin TIC4472 demonstrated activity against all Lepidopteran insect pests assayed (CEW, CEWUC, CLW, ECB, FAW, SBL, SAW, SCB, SCB2R, SWCB, TBW, and VBC); as well as YFM. The insect toxin TIC1425 demonstrated activity against CLW, ECB, FAW, SCB, and SWCB. The insect toxin TIC2613 demonstrated activity against CEW, CLW, ECB, FAW, SBL, SAW, SCB, SWCB, TBW, and VBC. Activity was not observed for TIC1425 and TIC2613 when assayed against BCW.

[092] In a separate series of experiments, protein preparations of TIC4472 were assayed using a diet overlay assay against the Lepidopteran insect pests Beet armyworm (BAW, *Spodoptera exigua*), Pink bollworm (PBW, *Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (PBW_Cry1Ac^r, *Pectinophora gossypiella*), Old world bollworm (OWB, *Helicoverpa armigera*), Oriental leaf worm (OLW, *Spodoptera litura*), and Spotted bollworm (SBW, *Earias vittella*). Table 5 shows the activity observed against each of these Lepidopteran insect pests assayed in the diet overlay bioassay, wherein “+” indicates activity.

Table 5. Bioassay activity of TIC4472 against Lepidopteran insect pests.

Lepidopteran Insect Pest					
BAW	PBW	PBW_Cry1Ac ^r	OWB	OLW	SBW
+	+	+	+	+	+

[093] As can be seen in Table 5 above, TIC4472 demonstrated activity against all of the Lepidopteran insect pests assayed in the diet overlay bioassay, including the Cry1Ac resistant colony of Pink bollworm.

[094] As demonstrated in Tables 3-5, TIC4472, TIC1425, and TIC2613 demonstrate activity across a wide range of Lepidopteran insect pest species.

Example 3

Design of synthetic coding sequences encoding TIC4472PL and TIC2613PL for expression in plant cells

[095] Synthetic coding sequences were constructed for use in expression of the encoded protein in plants, cloned into a binary plant transformation vectors, and used to transform plant cells. The synthetic sequences were synthesized according to methods generally described in U.S. Patent 5,500,365, avoiding certain inimical problem sequences such as ATTTA and A/T rich plant polyadenylation sequences. The synthetic coding sequences encode TIC4472PL and TIC2613PL proteins which comprise an additional alanine residue immediately following the initiating methionine relative to the TIC4472 and TIC2613 proteins, respectively. The additional

alanine residue is incorporated into the synthetic coding sequence to improve expression of the insect toxin protein.

[096] The synthetic coding sequence encoding TIC4472PL (SEQ ID NO:4) is presented herein as SEQ ID NO:3. The synthetic coding sequence encoding TIC2613PL (SEQ ID NO:10) is presented as SEQ ID NO:9. The synthetic coding sequences were used in binary plant transformation vectors to generate transgenic plants expressing the TIC4472PL and TIC2613PL proteins and assayed for activity against Lepidopteran insect pests.

Example 4

Assay of TIC4472PL activity against Lepidopteran pests in stably transformed cotton plants

[097] Binary plant transformation vectors comprising transgene cassettes designed to express untargeted TIC4472PL pesticidal protein were cloned using methods known in the art. The resulting vectors were used to stably transform cotton plants. Tissues were harvested from the transformants and used in insect bioassay against various Lepidopteran insect pests.

[098] Synthetic coding sequences were constructed for use in expression of the encoded protein in plants, cloned into a binary plant transformation vector, and used to transform cotton plant cells. The resulting plant transformation vectors comprised a first transgene cassette for expression of the TIC4472PL pesticidal protein which comprised a constitutive promoter, operably linked 5' to a leader, operably linked 5' to a synthetic coding sequence encoding an untargeted TIC4472PL protein, which was in turn operably linked 5' to a 3' UTR; and a second transgene cassette for the selection of transformed plant cells using spectinomycin selection.

[099] Three binary plant transformation vector constructs were constructed as described above and comprised different constitutive promoters. Two constructs, Construct 1 and Construct 3 also comprised an intron sequence that was operably linked 3' to the leader and 5' to the synthetic coding sequence encoding an untargeted TIC4472PL protein. Construct 2 was intronless. Cotton plants were transformed with the three different binary transformation vectors using an *Agrobacterium*-mediated transformation method. The transformed cells were induced to form plants by methods known in the art. Bioassays using plant leaf disks were performed analogous to those described in U.S. Patent No. 8,344,207. A single freshly hatched neonate larvae less than one day old was placed on each leaf disc sample and allowed to feed for approximately four days. A non-transformed cotton plant was used to obtain tissue to be used as

a negative control. Multiple transformation R_0 single-copy insertion events from each binary vector were assessed against Cotton bollworm (CBW, *Helicoverpa zea*), Fall armyworm (FAW, *Spodoptera frugiperda*), Soybean looper (SBL, *Chrysodeixis includens*), and Tobacco budworm (TBW, *Heliothis virescens*). Leaf Damage Rating (LDR) scores ranging from one (1) to four (4) were applied to event. The LDR scores are based upon the percentage of damage observed for each leaf disc. Table 6 below shows the LDR scores and corresponding range of percent damage associated with each score. For LDR scores of one (1), the penetrance of the trait is also assessed. High penetrance (as indicated by “(H)”) is defined as greater than fifty (50) percent of the assayed events for each construct having less than or equal to ten (10) percent leaf damage. Low penetrance (as indicated by “(L)”) is defined as less than or equal to fifty (50) percent of the assayed events for each construct having less than or equal to ten (10) percent leaf damage. Penetrance is not applied to LDR scores greater than one (1).

Table 6. Cotton Leaf Damage Rating (LDR) scores and corresponding percentage damage and Penetrance.

LDR Score	Leaf Damage Rating (LDR) range	Penetrance
1	LDR $\leq$ 10%	High = > 50% of events
1		Low = < 50% of events
2	10% < LDR $\leq$ 17.5%	n/a
3	17.5% < LDR $\leq$ 35%	n/a
4	LDR > 35%	n/a

[0100] The leaf damage rating scores for transformed R_0 cotton plants expressing TIC4472PL are presented in Table 7 below. The number of events demonstrating the LDR score out of the total number events assayed is shown in parenthesis, followed by the penetrance for those LDR scores of one (1).

Table 7. Leaf Damage Rating (LDR) scores, number of events demonstrating the LDR, and penetrance for transformed R_0 cotton plants expressing TIC4472PL.

Construct	CBW	FAW	SBL	TBW
Construct 1	3 (1/25)	2 (2/25)	1 (25/25) H	1 (24/25) H
Construct 2	3 (3/25)	2 (1/25)	1 (24/25) H	1 (16/25) H
Construct 3	4 (23/23)	1 (1/23) L	1 (21/23) H	1 (6/23) L

[0101] Transformed R₀ cotton plants expressing TIC4472PL were highly efficacious (defined as having less than or equal to ten percent leaf damage) against SBL and TBW as shown in Table 7. Activity against CBW and FAW was also observed in several events.

- 5 [0102] R₁ cotton events were selected from the transformed R₀ cotton plants assayed above and were used in a leaf disc assay against FAW, SBL, and TBW. Table 8 shows the leaf damage rating scores for transformed R₁ cotton plants expressing TIC4472PL.

Table 8. Leaf Damage Rating (LDR) scores, number of events demonstrating the LDR, and penetrance for transformed R₁ cotton plants expressing TIC4472PL.

Construct	FAW	SBL	TBW
Construct 1	1 (3/4) H	1 (4/4) H	1 (4/4) H
Construct 2	1 (1/4) L	1 (4/4) H	1 (1/4) L

[0103] As can be seen in Table 8 above, the selected events showed high efficacy against FAW, SBL, and TBW. Penetrance was high for Construct 1 transformed events for all three insect pest species. With respect to Construct 2 transformed events, penetrance was high for SBL.

- 15 [0104] The forgoing demonstrates that transformed cotton plants expressing TIC4472PL protein provide resistance to Fall armyworm (FAW, *Spodoptera frugiperda*), Soybean looper (SBL, *Chrysodeixis includens*) and Tobacco budworm (TBW, *Heliothis virescens*).

Example 5

Assay of TIC4472PL and TIC2613PL activity against Lepidopteran pests in stably transformed soybean plants

[0105] A binary plant transformation vector comprising a transgene cassette designed to express a plastid targeted TIC4472PL or TIC2613PL untargeted pesticidal protein was cloned using methods known in the art. The resulting vector was used to stably transform soybean plants.

Tissues were harvested from the transformants and used in insect bioassay against various Lepidopteran insect pests.

[0106] The synthetic coding sequences designed for plant expression described in Example 3 were cloned into binary plant transformation vector constructs, and used to transform soybean plant cells. Three binary vector constructs were constructed using methods known in the art to express plastid targeted and untargeted TIC4472PL. Construct 4 comprised a first transgene cassette for expression of the untargeted TIC4472PL which comprised a constitutive promoter, operably linked 5' to a leader, operably linked 5' to a synthetic coding sequence encoding an untargeted TIC4472PL protein, which was in turn operably linked 5' to a 3' UTR and; a second transgene cassette for the selection of transformed plant cells using spectinomycin selection. Construct 5 comprised a first transgene cassette for expression of the targeted TIC4472PL which comprised a constitutive promoter, operably linked 5' to a leader, operably linked 5' to an intron, operably linked 5' to a synthetic coding sequence encoding a plastid targeted TIC4472PL protein, which was in turn operably linked 5' to a 3' UTR and; a second transgene cassette for the selection of transformed plant cells using spectinomycin selection. Construct 6 comprised a first transgene cassette for expression of the untargeted TIC4472PL which comprised a constitutive promoter, operably linked 5' to a leader, operably linked 5' to an intron, operably linked 5' to a synthetic coding sequence encoding an untargeted TIC4472PL protein, which was in turn operably linked 5' to a 3' UTR and; a second transgene cassette for the selection of transformed plant cells using spectinomycin selection.

[0107] Two binary vector constructs were constructed using methods known in the art to express untargeted TIC2613PL. Construct 7 comprised a first transgene cassette for expression of the untargeted TIC2613PL which comprised a constitutive promoter, operably linked 5' to a leader, operably linked 5' to a synthetic coding sequence encoding an untargeted TIC2613PL protein, which was in turn operably linked 5' to a 3' UTR and; a second transgene cassette for the selection of transformed plant cells using spectinomycin selection. Construct 8 comprised a first transgene cassette for expression of the untargeted TIC2613PL which comprised a constitutive promoter, operably linked 5' to a leader, operably linked 5' to an intron, operably linked 5' to a synthetic coding sequence encoding an untargeted TIC2613PL protein, which was in turn operably linked 5' to a 3' UTR and; a second transgene cassette for the selection of transformed plant cells using spectinomycin selection.

[0108] The resulting binary transformation vector constructs were used to transform soybean cells using an *Agrobacterium*-mediated transformation method. The transformed soybean cells were induced to form plants by methods known in the art. Bioassays using plant leaf disks were performed analogous to those described in U.S. Patent No. 8,344,207. A non-transformed soybean plant was used to obtain tissue to be used as a negative control. Multiple R₀ soybean transformation events derived from the binary vectors were assessed against Southern armyworm (SAW, *Spodoptera eridania*), Soybean looper (SBL, *Chrysodeixis includens*), and Soybean podworm (SPW, *Helicoverpa zea*). The Leaf Damage Rating (LDR) scores were similar to those as for cotton but differed in the range of percentage damage used to determine the score. The Leaf Damage Rating scores and their corresponding percent leaf damage rating range are presented in Table 9 below.

Table 9. Soybean Leaf Damage Rating (LDR) scores and corresponding percentage damage range.

LDR Score	Leaf Damage Rating (LDR) range
1	$\text{LDR} \leq 20\%$
2	$20\% < \text{LDR} \leq 35\%$
3	$35\% < \text{LDR} \leq 70\%$
4	$\text{LDR} > 70\%$

[0109] The leaf damage rating scores for transformed R₀ cotton plants expressing TIC4472PL and TIC2613PL are presented in Table 10 below. The number of events demonstrating the LDR score out of the total number events assayed is shown in parenthesis.

Table 10. Leaf Damage Rating (LDR) scores and number of events demonstrating the LDR for transformed R₀ soybean plants expressing TIC4472PL and TIC2613PL.

Toxin	Construct	SAW	SBL	SPW
TIC4472PL	Construct 4	1 (23/29)	1 (29/29)	3 (8/29)
TIC4472PL	Construct 5	1 (20/29)	1 (28/29)	1 (1/29)
TIC4472PL	Construct 6	1 (10/29)	1 (15/15)	3 (3/15)
TIC2613PL	Construct 7	1 (22/30)	1 (24/30)	3 (1/30)
TIC2613PL	Construct 8	1 (14/25)	1 (22/25)	2 (2/25)

5 [0110] As can be seen in Table 10 above, both expression of both TIC4472PL and TIC2613PL demonstrated high efficacy against SAW and SBL. Activity was lower for both proteins against SPW.

[0111] R₁ soybean events were selected from the transformed R₀ soybean plants expressing TIC4472PL and TIC2613PL assayed above and were used in a leaf disc assay against SAW, SBL, and SPW. A R₁ soybean event expressing TIC4472PL was also assessed against Velvet
10 bean caterpillar (VBW, *Anticarsia gemmatilis*). Table 11 shows the leaf damage rating scores for transformed R₁ soybean plants expressing TIC4472PL and TIC2613PL.

Table 11. Leaf Damage Rating (LDR) scores and number of events demonstrating the LDR for transformed R₁ soybean plants expressing TIC4472PL and TIC2613PL.

15

Toxin	Construct	SAW	SBL	SPW	VBC
TIC4472PL	Construct 5	1 (3/8)	1 (5/8)	3 (2/8)	
TIC4472PL	Construct 6	1 (1/9)	1 (6/9)	3 (1/9)	1 (3/9)
TIC2613PL	Construct 8	2 (2/10)	1 (9/10)	4 (4/10)	

[0112] As can be seen in Table 11, a number of R₁ soybean events transformed with TIC4472PL demonstrated high efficacy against SAW and SBL. In addition, several R₁ soybean events
20 transformed using Construct 6 demonstrated high efficacy against VBC. R₁ soybean events transformed with TIC2613PL demonstrated high efficacy against SBL.

[0113] The forgoing demonstrates that transformed soybean plants expressing TIC4472PL or TIC2613PL provide resistance to Lepidopteran insects, in particular Southern armyworm (*Spodoptera eridania*) and Soybean looper (*Chrysodeixis includens*) and Velvet bean caterpillar (*Anticarsia gemmatilis*).

5

Example 6

Assay of TIC4472PL and TIC2613PL activity against Lepidopteran pests in stably transformed corn plants

[0114] Binary plant transformation vectors comprising transgene cassettes designed to express both plastid targeted and untargeted TIC4472PL or TIC2613PL pesticidal protein are cloned using methods known in the art. The resulting vectors are used to stably transform corn plants. Tissues are harvested from the transformants and used in insect bioassay against various Lepidopteran insect pests.

[0115] The synthetic coding sequence encoding TIC4472PL (SEQ ID NO:3) or TIC2613 (SEQ ID NO:9) protein are cloned into binary transformation vectors. For plastid targeted protein, the synthetic TIC4472PL or TIC2613PL pesticidal protein coding sequence is operably linked in frame with a chloroplast targeting signal peptide encoding sequence. The resulting plant transformation vectors comprise a first transgene cassette for expression of the TIC4472PL or TIC2613PL pesticidal protein which comprise a constitutive promoter, operably linked 5' to a leader, operably linked 5' to an intron, operably linked 5' to a synthetic coding sequence encoding a plastid targeted or untargeted TIC4472PL or TIC2613PL protein, which is in turn operably linked 5' to a 3' UTR; and a second transgene cassette for the selection of transformed plant cells using glyphosate selection.

[0116] Corn plants are transformed with the binary transformation vectors described above using an *Agrobacterium*-mediated transformation method. The transformed cells are induced to form plants by methods known in the art. Bioassays using plant leaf disks are performed analogous to those described in U.S. Patent No. 8,344,207. A non-transformed corn plant is used to obtain tissue to be used as a negative control. Multiple transformation events from each binary vector are assessed against Beet armyworm (BAW, *Spodoptera exigua*), Black cutworm (BCW, *Agrotis ipsilon*), Corn earworm (CEW, *Helicoverpa zea*), Cotton leaf worm (CLW, *Alabama argillacea*), European corn borer (ECB, *Ostrinia nubilalis*), Fall armyworm (FAW, *Spodoptera frugiperda*),

Old world bollworm (OWB, *Helicoverpa armigera*), Oriental leaf worm (OLW, *Spodoptera litura*), Pink bollworm (PBW, *Pectinophora gossypiella*), Soybean looper (SBL, *Chrysodeixis includens*), Spotted bollworm (SBW, *Earias vittella*), Southwestern corn borer (SWCB, *Diatraea grandiosella*), Sugarcane borer (SCB, *Diatraea saccharalis*), Tobacco budworm (TBW, *Heliothis virescens*), and Velvet bean caterpillar (VBW, *Anticarsia gemmatilis*), as well as other Lepidopteran insect pests.

[0117] The insect pests are observed for mortality and stunting caused by ingestion of the presented leaf discs expressing TIC4472PL or TIC2613PL and compared to leaf discs derived from non-transformed corn plants.

10 [0118] All of the compositions disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions of this invention have been described in terms of the foregoing illustrative embodiments, it will be apparent to those of skill in the art that variations, changes, modifications, and alterations may be applied to the composition described herein, without departing from the true concept, spirit, and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of the invention as defined by the appended claims.

20 [0119] All publications and published patent documents cited in the specification are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

What is claimed is:

- 5 1. A recombinant nucleic acid molecule comprising a heterologous promoter operably linked to a polynucleotide segment encoding a pesticidal protein or pesticidal fragment thereof, wherein:
 - a. said pesticidal protein comprises the amino acid sequence of SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10; or
 - 10 b. said pesticidal protein comprises an amino acid sequence having:
 - i. at least 93%, or 95%, or 98% or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or
 - ii. at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10; or
 - 15 c. said polynucleotide segment hybridizes under stringent hybridization conditions to a polynucleotide having the nucleotide sequence of SEQ ID NO:3, SEQ ID NO:1, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9.
2. The recombinant nucleic acid molecule of claim 1, wherein:
 - 20 a. the recombinant nucleic acid molecule comprises a sequence that functions to express the pesticidal protein in a plant; or
 - b. the recombinant nucleic acid molecule is expressed in a plant cell to produce a pesticidally effective amount of the pesticidal protein; or
 - c. the recombinant nucleic acid molecule is in operable linkage with a vector, and said vector is selected from the group consisting of a plasmid, phagemid, 25 bacmid, cosmid, and a bacterial or yeast artificial chromosome.
3. The recombinant nucleic acid molecule of claim 1, defined as present within a host cell, wherein said host cell is selected from the group consisting of a bacterial and a plant cell.

4. The recombinant nucleic acid molecule of claim 3, wherein the bacterial host cell is from a genus of bacteria selected from the group consisting of: *Agrobacterium*, *Rhizobium*, *Bacillus*, *Brevibacillus*, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Pantoea*, and *Erwinia*.
- 5 5. The recombinant nucleic acid molecule of claim 1 wherein the *Bacillus* species is *Bacillus cereus* or *Bacillus thuringiensis*, said *Brevibacillus* is *Brevibacillus laterosperous*, and said *Escherichia* is *Escherichia coli*.
6. The recombinant nucleic acid of claim 3, wherein said plant cell is a dicotyledonous or a monocotyledonous plant cell.
- 10 7. The recombinant nucleic acid of claim 6, wherein said plant host cell is selected from the group consisting of an alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton, a cucurbit, cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive,

15 onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeonpea, pine, potato, poplar, pumpkin, Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat plant cell.
- 20 8. The recombinant nucleic acid molecule of claim 1, wherein said protein exhibits activity against a Lepidopteran insect.
9. The recombinant nucleic acid molecule of claim 8, wherein said insect is selected from the group consisting of: Beet armyworm (*Spodoptera exigua*), Corn earworm (*Helicoverpa zea*), Cotton leaf worm (*Alabama argillacea*), European corn borer (*Ostrinia nubilalis*), Fall armyworm (*Spodoptera frugiperda*), Old World bollworm (*Helicoverpa armigera*), Oriental leaf worm (*Spodoptera litura*), Pink bollworm (*Pectinophora gossypiella*), Cry1Ac resistant Pink bollworm (*Pectinophora gossypiella*), Soybean looper (*Chrysodeixis includens*), Southern armyworm (*Spodoptera eridania*), Southwestern corn borer (*Diatraea grandiosella*), Spotted

25 bollworm (*Earias vittella*), Sugarcane borer (*Diatraea saccharalis*), Tobacco budworm (*Heliothis virescens*), and Velvet bean caterpillar (*Anticarsia gemmatilis*).

30

10. A plant or part thereof comprising the recombinant nucleic acid molecule of claim 1.
11. The plant or part thereof of claim 10, wherein said plant is a monocot plant or a dicot plant.
12. The plant of claim 10, wherein the plant is selected from the group consisting of an
5 alfalfa, banana, barley, bean, broccoli, cabbage, brassica, carrot, cassava, castor, cauliflower, celery, chickpea, Chinese cabbage, citrus, coconut, coffee, corn, clover, cotton, a cucurbit, cucumber, Douglas fir, eggplant, eucalyptus, flax, garlic, grape, hops, leek, lettuce, Loblolly pine, millets, melons, nut, oat, olive, onion, ornamental, palm, pasture grass, pea, peanut, pepper, pigeon pea, pine, potato, poplar, pumpkin,
10 Radiata pine, radish, rapeseed, rice, rootstocks, rye, safflower, shrub, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugar beet, sugarcane, sunflower, sweet corn, sweet gum, sweet potato, switchgrass, tea, tobacco, tomato, triticale, turf grass, watermelon, and wheat.
13. A seed of the plant of claim 10, wherein said seed comprises said recombinant
15 nucleic acid molecule.
14. An insect inhibitory composition comprising the recombinant nucleic acid molecule of claim 1.
15. The insect inhibitory composition of claim 14, further comprising a nucleotide
20 sequence encoding at least one other pesticidal agent that is different from said pesticidal protein.
16. The insect inhibitory composition of claim 15, wherein said at least one other
pesticidal agent is selected from the group consisting of an insect inhibitory protein, an insect inhibitory dsRNA molecule, and an ancillary protein.
17. The insect inhibitory composition of claim 16, wherein said at least one other
25 pesticidal agent exhibits activity against one or more pest species of the orders Lepidoptera, Coleoptera, or Hemiptera.
18. The insect inhibitory composition of claim 17, wherein said at least one other
30 pesticidal protein is selected from the group consisting of a Cry1A, Cry1Ab, Cry1Ac, Cry1A.105, Cry1Ae, Cry1B, Cry1C, Cry1C variants, Cry1D, Cry1E, Cry1F, Cry1A/F chimeras, Cry1G, Cry1H, Cry1I, Cry1J, Cry1K, Cry1L, Cry2A, Cry2Ab, Cry2Ae, Cry3, Cry3A variants, Cry3B, Cry4B, Cry6, Cry7, Cry8, Cry9, Cry15, Cry34, Cry35,

Cry43A, Cry43B, Cry51Aa1, ET29, ET33, ET34, ET35, ET66, ET70, TIC400, TIC407, TIC417, TIC431, TIC800, TIC807, TIC834, TIC853, TIC900, TIC901, TIC1201, TIC1415, TIC2160, TIC3131, TIC836, TIC860, TIC867, TIC869, TIC1100, VIP3A, VIP3B, VIP3Ab, AXMI-AXMI-, AXMI-88, AXMI-97, AXMI-102, AXMI-112, AXMI-117, AXMI-100, AXMI-115, AXMI-113, and AXMI-005, AXMI134, AXMI-150, AXMI-171, AXMI-184, AXMI-196, AXMI-204, AXMI-207, AXMI-209, AXMI-205, AXMI-218, AXMI-220, AXMI-221z, AXMI-222z, AXMI-223z, AXMI-224z and AXMI-225z, AXMI-238, AXMI-270, AXMI-279, AXMI-345, AXMI-335, AXMI-R1 and variants thereof, IP3 and variants thereof, DIG-3, DIG-5, DIG-10, DIG-657 and a DIG-11 protein.

19. The insect inhibitory composition of claim 14, defined as comprising a plant cell that expresses said recombinant nucleic acid molecule.

20. A commodity product produced from the plant or part thereof of claim 10, wherein the commodity product comprises a detectable amount of said recombinant nucleic acid molecule or a pesticidal protein.

21. The commodity product of claim 20, selected from the group consisting of commodity corn bagged by a grain handler, corn flakes, corn cakes, corn flour, corn meal, corn syrup, corn oil, corn silage, corn starch, corn cereal, and the like, and corresponding soybean, rice, wheat, sorghum, pigeon pea, peanut, fruit, melon, and vegetable commodity products including, where applicable, juices, concentrates, jams, jellies, marmalades, and other edible forms of such commodity products containing a detectable amount of such polynucleotides and or polypeptides of this application, whole or processed cotton seed, cotton oil, lint, seeds and plant parts processed for feed or food, fiber, paper, biomasses, and fuel products such as fuel derived from cotton oil or pellets derived from cotton gin waste, whole or processed soybean seed, soybean oil, soybean protein, soybean meal, soybean flour, soybean flakes, soybean bran, soybean milk, soybean cheese, soybean wine, animal feed comprising soybean, paper comprising soybean, cream comprising soybean, soybean biomass, and fuel products produced using soybean plants and soybean plant parts.

22. A method of producing seed comprising:

a. planting a first seed according to claim 13;

- b. growing a plant from the seed; and
- c. harvesting seed from the plants, wherein said harvested seed comprises said recombinant nucleic acid molecule.

23. A plant resistant to insect infestation, wherein the cells of said plant comprise the recombinant nucleic acid molecule of claim 1.

24. A method for controlling a Lepidopteran species pest or pest infestation, said method comprising:

- a. contacting the pest with an insecticidally effective amount of a pesticidal protein as set forth in SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10; or

- b. contacting the pest with an insecticidally effective amount of one or more pesticidal proteins comprising an amino acid sequence having:

- i. at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6;

or

- ii. at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10.

25. A method of detecting the presence of the recombinant nucleic acid molecule of claim 1 in a sample comprising plant genomic DNA, comprising:

- a. contacting the sample with a nucleic acid probe that hybridizes under stringent hybridization conditions with genomic DNA from a plant comprising the DNA molecule of claim 1, and does not hybridize under such hybridization conditions with genomic DNA from an otherwise isogenic plant that does not comprise the recombinant nucleic acid molecule of claim 1, wherein the probe is homologous or complementary to SEQ ID NO:3 or SEQ ID NO:9, or a sequence that encodes a pesticidal protein comprising an amino acid sequence having:

- i. at least 93%, or 95%, or 98%, or 99%, or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or
- ii. at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10;

- b. subjecting the sample and probe to stringent hybridization conditions; and
- c. detecting hybridization of the probe with DNA of the sample.

26. A method of detecting the presence of a pesticidal protein or fragment thereof in a sample comprising protein, wherein said pesticidal protein comprises the amino acid sequence of SEQ ID NO:4, SEQ ID NO:2, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:10; or said pesticidal protein comprises an amino acid sequence having:

- a. at least 93%, or 95%, or 98%, or 99% or about 100% amino acid sequence identity to SEQ ID NO:4, SEQ ID NO:2, or SEQ ID NO:6; or
- b. at least 73%, or 75%, or 80%, or 85%, or 90%, or 95%, or about 100% amino acid sequence identity to SEQ ID NO:8 or SEQ ID NO:10; comprising:
- c. contacting the sample with an immunoreactive antibody; and
- d. detecting the presence of the protein.

27. The method of claim 26, wherein the step of detecting comprises an ELISA, or a Western blot.

ABSTRACT
NOVEL INSECT INHIBITORY PROTEINS

5 Pesticidal proteins exhibiting toxic activity against Lepidopteran pest species are disclosed, and include, but are not limited to, TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL. DNA constructs are provided which contain a recombinant nucleic acid sequence encoding one or more of the disclosed pesticidal proteins. Transgenic plants, plant cells, seed, and plant parts resistant to Lepidopteran infestation are provided which contain recombinant
10 nucleic acid sequences encoding the pesticidal proteins of the present invention. Methods for detecting the presence of the recombinant nucleic acid sequences or the proteins of the present invention in a biological sample, and methods of controlling Lepidopteran species pests using any of the TIC4472, TIC4472PL, TIC1425, TIC2613, and TIC2613PL pesticidal proteins are also provided.

pct-us2017-055731 sequence listing
SEQUENCE LISTING

<110> Monsanto Technology LLC
Bowen, David J
Chay, Catherine A
Howe, Arlene R
Kesanapalli, Uma

<120> NOVEL INSECT INHIBITORY PROTEINS

<130> MONS:426WO

<150> US 62/406082
<151> 2016-10-10

<160> 10

<170> PatentIn version 3.5

<210> 1
<211> 3564
<212> DNA
<213> Bacillus thuringiensis

<220>
<221> misc_feature
<222> (1)..(3564)
<223> Nucleic acid sequence encoding a TIC4472 pesticidal protein
obtained from Bacillus thuringiensis species EG10742.

<400> 1
atgaataata atattgaaaa ccaatgcgta ccttacaatt gtttaagtaa tcctgaagaa 60
gtaatTTTg atggagaacg gatatcaact ggtaattcat caattgatat ttctctgtca 120
cttgTTcaac ttctggtatc taactTTgta ccaggcgag gattTTtagt agggTTaata 180
gattTTgtat ggggaatagt aggccTTtct ccatgggatg catttctagt gcaaattgaa 240
caattaattc agcaaagaat agaagcatat gctagggctg cagcaatttc taatttagaa 300
ggaataggaa acaatttcaa tatatatgtg gaagcatttc aagaatggga agaagatcct 360
aataatccag caaccaggaa tagagtagtt gatcgctttc gtatacttga tgggctactt 420
gaaagggaca ttccttcggt tcgaatttct ggatttgaag tccccctttt atccgtttat 480
gctcaagcgg ccaatctgca tctagctata ttaagagatt ctgtaatttt tggagaaaga 540
tggggattga caacaacaaa tgtcaatgaa aactataata gacaaatcag gcatattgat 600
gaatatgctg atcactgtgc aaatacgtat aatcggggat taaataattt accgaaatct 660
acgtatcaag attggataac atataatcga ttacggagag aattaacatt gactgtatta 720
gatatcgctg ctttctttcc aaactatgac aataggcggg atccaattca gccagttggt 780
caactaacia gggaagttaa tacggacca ttaattactt ttaatcccca gttacagtct 840
gtagctcaat tacctacttt taacgttatg gaaagcaacg caattagaaa tcctcatttg 900
tttgatatat tgaataatct tacaattttt acggattggt ttagtgttgg acgcaacttt 960
tattggggag gacatcgagt aacttctaac tatataggag gaggcaacat aacatctcct 1020
atatatggaa gagaggcgaa ccaggagcct ccaagatctt ttacttttaa tggacctgtt 1080

pct-us2017-055731 Sequence listing

tttaggactt tatcaaattcc tacttttacga ttattacagc aaccttggcc agcaccacca	1140
tttaattttac gtggtgttga aggagtagaa ttttctacac ctacaaatag ctttacgtat	1200
cgaggaagag gtacagttga ttctttaacc gaattaccgc ctgaggataa tagtgtgcta	1260
cctcgcgagg gatatagtca tcgtttatgt catgcaactt ttgttcaaag atctggaaca	1320
ccatttttaa caacgggtgt agtattttct tggacgcac gcagtgcac tcttacaat	1380
acaattgatc cagacaaaat tactcagata cttttagtga aaggatttag agtttgagt	1440
ggcgcctctg tcgttacagg accaggtttt acaggagggg atacccttcg aagaactaac	1500
tttggggatt ttgtatctat gcaagttaat attaattcac caataacaca aagataccgt	1560
ttaagatttc gttatgcttc cagtagagat gcacgactta cagtagcgac aggagcagca	1620
aacacaggag ttggagggca aattagtgtg gacatggctc ttcagaaaac tatggaaatt	1680
ggagagagct taacatctag aacattttaga tataccgatt ttagtaatcc tttttcattt	1740
agagctaadc cagatataat tcgtataaat gaacaaccac tattcgggtgc aggctctatt	1800
agtagtggtg aactttatat agataaaatt gaaattattc tagcagatgc aacatttgaa	1860
gcagaatatg atttggaaag agcacagaag gcggtgaatg cgctgtttac ttctacaaac	1920
caaagaggat taaaaacaga tgtgacggat tatcatattg atcaagtatc caatttagtt	1980
gagtgtttat cggatgaatt ttgtctggat gaaaaacgag aattgtccga gaaagtcaaa	2040
catgcgaagc gactcagtga tgagcgaat ttactccagg atcgaaattt cacatccatt	2100
aatgggcaac tagaccgtgg ctggagagga agtacggata ttaccatcca aggaggagat	2160
gacgtattca aagagaatta cgtcacacta ctgggtacct ttgatgagtg ctatccaacg	2220
tatttatatc aaaaaataga tgagtcgaaa ttaaaagcct ataccgcta tgaattaaga	2280
gggtatatcg aggatagtca agacttagaa atctatttaa ttcgctacaa tgcaaaacac	2340
gaaacagtaa atgtgccagg tacaggttcc ttatggccgc tttcagtcga aagtccaatc	2400
ggaaagtgtg gagaaccgaa tcgatgcaca ccacacctg aatggaatcc taatctagat	2460
tgttcctgca gagacgggaa aacatgtgca catcattctc atcatttctc cttggacatt	2520
gatgttggat gtacagactt aaatgaagat ctaggtgtat gggatgatatt caagattaag	2580
acgcaagatg gtcacgaag actaggaaat ctagagtttc tcgaagagaa accattagta	2640
ggagaagcgt tagctcgtgt gaaaagagcg gagaaaaaat ggagagacaa acgcgaaaaa	2700
ttgcaattag aaacaaatat cgtttacaaa gaggcaaaag aagctgtgga tgctttattt	2760
gtaaactctc aatatgatag attacaagtg gatacgaaca ttgccatgat tcatgcggca	2820
gataaacgcg ttcatagaat ccgagaagcg tatcttcag agttatctgt gattccgggt	2880
gtcaatgcgg ctattttcga agaattagaa ggggtgtgtt tctactgcatt ctccctatat	2940
gatgcgagaa atgtcattaa aaatggcgat ttaataatg gcttatcatg ctggaacgtg	3000
aaagggcatg tagaagaaca aaacgaccat cgttcgggtc ttgttgtccc ggaatgggaa	3060
gcagaagtgt cacaagaggt tcgtgtctgt ccagggtcgtg gctatatacct tcgtgttaca	3120

pct-us2017-055731 Sequence listing

gcatacaaag aggggatatgg agaaggctgt gtaaccattc atgggatcga gaacaataca	3180
gacgaactga agtttagcaa ctgcgtagaa gaggaagtct atccaaacaa cacggtaacg	3240
tgtaatgatt atcctgcaaa tcaagaagaa tacgggggtg cgtacacttc tcgtaatcgt	3300
ggatatgacg aaacttatgg aagcaattct tccgtatcag ctgattatgc gtcagtttat	3360
gaagaaaaag cgtatacaga tggacgaaga gacaatccat gtgaatttaa cagagggtat	3420
ggggattata cgccactacc agctggctat gtaacaaaag aattagaata cttcccagaa	3480
accgataagg tatggattga gattggagaa acggaaggaa cattcatcgt ggacagtgtg	3540
gaattactcc ttatggagga atag	3564

<210> 2
 <211> 1187
 <212> PRT
 <213> Bacillus thuringiensis

<220>
 <221> MISC_FEATURE
 <222> (1)..(1187)
 <223> The amino acid sequence of the TIC4472 pesticidal protein.

<400> 2

Met Asn Asn Asn Ile Glu Asn Gln Cys Val Pro Tyr Asn Cys Leu Ser
 1 5 10 15

Asn Pro Glu Glu Val Ile Leu Asp Gly Glu Arg Ile Ser Thr Gly Asn
 20 25 30

Ser Ser Ile Asp Ile Ser Leu Ser Leu Val Gln Leu Leu Val Ser Asn
 35 40 45

Phe Val Pro Gly Gly Gly Phe Leu Val Gly Leu Ile Asp Phe Val Trp
 50 55 60

Gly Ile Val Gly Pro Ser Pro Trp Asp Ala Phe Leu Val Gln Ile Glu
 65 70 75 80

Gln Leu Ile Gln Gln Arg Ile Glu Ala Tyr Ala Arg Ala Ala Ile
 85 90 95

Ser Asn Leu Glu Gly Ile Gly Asn Asn Phe Asn Ile Tyr Val Glu Ala
 100 105 110

Phe Gln Glu Trp Glu Glu Asp Pro Asn Asn Pro Ala Thr Arg Asn Arg
 115 120 125

Val Val Asp Arg Phe Arg Ile Leu Asp Gly Leu Leu Glu Arg Asp Ile
 130 135 140

Pro Ser Phe Arg Ile Ser Gly Phe Glu Val Pro Leu Leu Ser Val Tyr
 145 150 155 160

pct-us2017-055731 Sequence listing

Ala Gln Ala Ala Asn₁₆₅ Leu His Leu Ala Ile₁₇₀ Leu Arg Asp Ser Val₁₇₅ Ile
 Phe Gly Glu Arg₁₈₀ Trp Gly Leu Thr Thr₁₈₅ Thr Asn Val Asn₁₉₀ Glu Asn Tyr
 Asn Arg Gln₁₉₅ Ile Arg His Ile Asp₂₀₀ Glu Tyr Ala Asp His₂₀₅ Cys Ala Asn
 Thr Tyr₂₁₀ Asn Arg Gly Leu Asn₂₁₅ Asn Leu Pro Lys Ser₂₂₀ Thr Tyr Gln Asp
 Trp Ile Thr Tyr Asn Arg₂₃₀ Leu Arg Arg Glu Leu₂₃₅ Thr Leu Thr Val Leu₂₄₀
 Asp Ile Ala Ala Phe₂₄₅ Phe Pro Asn Tyr Asp₂₅₀ Asn Arg Arg Tyr Pro₂₅₅ Ile
 Gln Pro Val Gly₂₆₀ Gln Leu Thr Arg Glu₂₆₅ Val Tyr Thr Asp Pro₂₇₀ Leu Ile
 Thr Phe Asn₂₇₅ Pro Gln Leu Gln Ser₂₈₀ Val Ala Gln Leu Pro₂₈₅ Thr Phe Asn
 Val Met₂₉₀ Glu Ser Asn Ala Ile₂₉₅ Arg Asn Pro His Leu₃₀₀ Phe Asp Ile Leu
 Asn Asn Leu Thr Ile Phe₃₁₀ Thr Asp Trp Phe Ser₃₁₅ Val Gly Arg Asn Phe₃₂₀
 Tyr Trp Gly Gly His₃₂₅ Arg Val Thr Ser Asn₃₃₀ Tyr Ile Gly Gly Gly₃₃₅ Asn
 Ile Thr Ser Pro₃₄₀ Ile Tyr Gly Arg Glu₃₄₅ Ala Asn Gln Glu Pro₃₅₀ Pro Arg
 Ser Phe Thr₃₅₅ Phe Asn Gly Pro Val₃₆₀ Phe Arg Thr Leu Ser₃₆₅ Asn Pro Thr
 Leu Arg₃₇₀ Leu Leu Gln Gln Pro₃₇₅ Trp Pro Ala Pro Pro₃₈₀ Phe Asn Leu Arg
 Gly Val Glu Gly Val Glu₃₉₀ Phe Ser Thr Pro Thr₃₉₅ Asn Ser Phe Thr Tyr₄₀₀
 Arg Gly Arg Gly Thr₄₀₅ Val Asp Ser Leu Thr₄₁₀ Glu Leu Pro Pro Glu₄₁₅ Asp
 Asn Ser Val₄₂₀ Leu Pro Arg Glu Gly Tyr₄₂₅ Ser His Arg Leu Cys₄₃₀ His Ala

pct-us2017-055731 Sequence listing

Thr Phe Val Gln Arg Ser Gly Thr Pro Phe Leu Thr Thr Gly Val Val
 435 440 445
 Phe Ser Trp Thr His Arg Ser Ala Thr Leu Thr Asn Thr Ile Asp Pro
 450 455 460
 Asp Lys Ile Thr Gln Ile Pro Leu Val Lys Gly Phe Arg Val Trp Ser
 465 470 475 480
 Gly Ala Ser Val Val Thr Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu
 485 490 495
 Arg Arg Thr Asn Phe Gly Asp Phe Val Ser Met Gln Val Asn Ile Asn
 500 505 510
 Ser Pro Ile Thr Gln Arg Tyr Arg Leu Arg Phe Arg Tyr Ala Ser Ser
 515 520 525
 Arg Asp Ala Arg Leu Thr Val Ala Thr Gly Ala Ala Asn Thr Gly Val
 530 535 540
 Gly Gly Gln Ile Ser Val Asp Met Ala Leu Gln Lys Thr Met Glu Ile
 545 550 555 560
 Gly Glu Ser Leu Thr Ser Arg Thr Phe Arg Tyr Thr Asp Phe Ser Asn
 565 570 575
 Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Arg Ile Asn Glu Gln
 580 585 590
 Pro Leu Phe Gly Ala Gly Ser Ile Ser Ser Gly Glu Leu Tyr Ile Asp
 595 600 605
 Lys Ile Glu Ile Ile Leu Ala Asp Ala Thr Phe Glu Ala Glu Tyr Asp
 610 615 620
 Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Thr Asn
 625 630 635 640
 Gln Arg Gly Leu Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln Val
 645 650 655
 Ser Asn Leu Val Glu Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu Lys
 660 665 670
 Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu
 675 680 685
 Arg Asn Leu Leu Gln Asp Arg Asn Phe Thr Ser Ile Asn Gly Gln Leu
 690 695 700

pct-us2017-055731 Sequence listing

Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp
 705 710 715 720
 Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Leu Gly Thr Phe Asp Glu
 725 730 735
 Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys
 740 745 750
 Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp
 755 760 765
 Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn
 770 775 780
 Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Glu Ser Pro Ile
 785 790 795 800
 Gly Lys Cys Gly Glu Pro Asn Arg Cys Thr Pro His Leu Glu Trp Asn
 805 810 815
 Pro Asn Leu Asp Cys Ser Cys Arg Asp Gly Lys Thr Cys Ala His His
 820 825 830
 Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn
 835 840 845
 Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly
 850 855 860
 His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val
 865 870 875 880
 Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp
 885 890 895
 Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala
 900 905 910
 Lys Glu Ala Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu
 915 920 925
 Gln Val Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val
 930 935 940
 His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly
 945 950 955 960
 Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Cys Val Phe Thr Ala
 965 970 975

pct-us2017-055731 Sequence listing

Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn
 980 985 990

Asn Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Glu Glu Gln Asn
 995 1000 1005

Asp His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val
 1010 1015 1020

Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg
 1025 1030 1035

Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile
 1040 1045 1050

His Gly Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys
 1055 1060 1065

Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp
 1070 1075 1080

Tyr Pro Ala Asn Gln Glu Glu Tyr Gly Gly Ala Tyr Thr Ser Arg
 1085 1090 1095

Asn Arg Gly Tyr Asp Glu Thr Tyr Gly Ser Asn Ser Ser Val Ser
 1100 1105 1110

Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ala Tyr Thr Asp Gly
 1115 1120 1125

Arg Arg Asp Asn Pro Cys Glu Phe Asn Arg Gly Tyr Gly Asp Tyr
 1130 1135 1140

Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe
 1145 1150 1155

Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly
 1160 1165 1170

Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
 1175 1180 1185

<210> 3
 <211> 3567
 <212> DNA
 <213> Artificial

<220>
 <223> A synthetic coding sequence encoding a TIC4472PL pesticidal protein designed for expression in a plant cell wherein an additional alanine codon is inserted immediately following the initiating methionine codon.

pct-us2017-055731 Sequence listing

<400> 3
atggctaata acaacatcga gaaccagtgc gtgccctaca actgcctttc gaacccggag 60
gaagtgatcc tggacggcga aaggatctcg accgggaata gcagcatcga catctcgctt 120
tcgctcgtgc agcttctagt cagtaacttc gttccgggcg gagggtttct cgtgggcctt 180
attgacttcg tttggggcat cgtggggcca tctccttggg acgcattcct cgtgcagatc 240
gagcaactga tccagcagcg tatcgaggcg tacgctaggg ctgccgctat ctccaacctg 300
gagggcatcg gcaacaactt caacatctac gttgaagcct tccaagaatg ggaggaagat 360
cctaataacc cagctacgcg gaacagagtg gtggatcgct ttagaatcct cgacggcctc 420
ctggaaaggg acatcccagag cttccgtatt tccggcttcg aggtgccgct gctgagcgtg 480
tacgcgcaag cggccaatct gcacctggcg attctccggg actctgtgat cttcggcgag 540
cgggtggggcc tcaccaccac taacgtgaac gagaactaca accgccagat ccgccacatc 600
gacgagtacg cggaccactg cgccaacaca tacaatcgcg ggctgaacaa cctccctaag 660
agcacttacc aagattggat cacctacaac aggctccgcc ggggaactcac tctcacagtc 720
ctcgacatcg ctgccttctt cccgaactac gacaaccgcc gctacccgat tcagccagtc 780
ggccagctca cccgtgaggt gtacaccgat cactgataa ctttcaatcc gcagctccag 840
tctgtcgcac agttgccac cttcaacgtc atggaaagca acgccatcag gaaccacac 900
ttgttcgaca tccttaacaa cctgactatc ttcaccgact gggttcagcgt cggacggaac 960
ttctactggg gcggacaccg cgtcacctca aactacatcg gcggcggaac cattacttcg 1020
cccatctacg gccgggaggc gaatcaggag ccgccacgca gctttacatt caacggctct 1080
gtgtttcgca cgttatcgaa cccgacactc aggctgctcc agcagccctg gcctgcgccg 1140
ccgtttaatt tgcgcggcgt cgaaggcgtc gagttcagta cgccgaccaa cagcttcacc 1200
tatcgcggac gcgggactgt tgactccctg acagagctgc cgccggagga caactcgggt 1260
ctgccgcgtg agggctacag ccataggctt tgtcacgcga cttttgtgca gcgatccggg 1320
acaccgttcc ttacaaccgg cgtggtgttc tcctggacac accgcagtgc aactctgacg 1380
aacacgattg acccagacaa gatcacgcag atcccgttag tgaagggtt ccgggtttgg 1440
tctggtgcct ctgtagtcac tgggcctggc ttacgggtg gcgacatcct ccgtcgcacg 1500
aactttggcg acttcgtgtc catgcaagtg aacattaaca gccctattac gcaacgctac 1560
cggctgaggt tcagatacgc ttcctcgcgg gacgccgctc ttacggtggc gacgggcgca 1620
gcgaacactg gagttggcgg ccaaactctc gtggacatgg ctttgagaa gactatggag 1680
atcggtgagt ctctcacatc tcgcacgttc cgctacacgg atttctccaa ccctttctcc 1740
ttccgcgcca atccggacat cattcggatc aacgaacagc cgctcttcgg cgcgggctcc 1800
atctcatccg gtgagcttta cattgataag attgagataa ttctggccga cgcgaccttt 1860
gaggcagagt atgatctgga gcgcgcacag aaggccgtga acgcgctgtt tacgtccacg 1920
aaccagcgcg ggctcaagac agacgtcaca gactaccaca tcgaccaagt ctccaacctt 1980

pct-us2017-055731 Sequence listing

gtcgagtgtc tctccgacga gttctgcctg gacgagaagc gggagcttag tgagaaggtg	2040
aagcacgcaa agcgccctgtc tgacgagcgg aaccttctac aagaccgtaa cttcacctcc	2100
attaacgggc agctagaccg tggctggcgc ggggtccaccg acatcactat ccaaggtggc	2160
gacgacgtct tcaaggagaa ctacgtgacg ctgctcggca cttttgacga gtgctacccg	2220
acatacctct atcagaagat tgacgagtct aagctcaagg cttacacccg ttacgagctg	2280
cgtggctaca tcgaggactc ccaggatctg gaaatctatc tcatcagata caacgcgaag	2340
cacgagacag tcaacgtacc tgggacaggc tctctctggc ctctgtctgt ggagagtccc	2400
atcggcaaat gtggcgagcc gaacagatgt actccgcacc tggagtggaa tcccaacttg	2460
gactgtagtt gccgcgacgg caagacctgc gcgcaccact cccaccactt ctccctggac	2520
attgacgtcg gctgcacgga tctcaacgag gatctgggcg tttgggtcat cttcaagata	2580
aagaccaggg acggacacgc cagactggga aacctagagt tccttgagga gaagccgctg	2640
gtcggcgaag cactggcgcg ggtcaagagg gccgagaaga agtggcggga caaacgggag	2700
aaacttcaac tcgaaacgaa catcgtttac aaggaggcaa aggaggccgt ggacgactg	2760
ttcgtgaact cgcagtacga ccgcctccaa gtggacacca acatcgccat gatccatgca	2820
gcggacaagc gcgtgcaccg aatcagggaa gcgtacttgc ccgagttgtc cgttatccct	2880
ggcgtgaacg ctgccatctt cgaggaactg gagggctgcg tgttcaccgc attctccctg	2940
tacgacgcac gaaacgtcat caagaatggc gacttcaaca acggcctgag ctgctggaac	3000
gtgaagggcc acgtggagga gcagaacgac caccgctccg tgttagtggc cccggaatgg	3060
gaagccgaag tgagccagga ggtcaggggtg tgtcccggtc gcgggttacat cctccgcgtg	3120
accgcctaca aggagggcta tggcgagggc tgcgtgacga tacacggtat cgagaacaac	3180
accgatgagc ttaagttctc gaactgcgtg gagggaggagg tgtacccgaa taacacagtg	3240
acgtgcaatg actacccggc caaccaggag gagtacggcg gtgcctacac gagccgaaac	3300
cgtggctatg acgaaactta cggctcgaac agcagcgtgt ctgcggatta tgccagtgtg	3360
tacgaggaga aggcgtacac ggacgggagg cgcgacaacc cttgcgagtt caatagaggc	3420
tatggcgact acacgccgct gcccgccggt tatgtgacga aggagttgga atacttccca	3480
gagacggaca aggtgtggat cgagattggc gagaccgagg gcacgttcat tgtggacagc	3540
gttgagctgc tactgatgga ggagtga	3567

<210> 4
 <211> 1188
 <212> PRT
 <213> Artificial

<220>
 <223> The amino acid sequence of TIC4472PL encoded by a synthetic coding sequence designed for expression in a plant cell (SEQ ID NO:3), and wherein an additional alanine amino acid is inserted immediately following the initiating methionine.

<400> 4

pct-us2017-055731 Sequence listing

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

pct-us2017-055731 Sequence listing

Ile Thr Phe Asn Pro Gln Leu Gln Ser Val Ala Gln Leu Pro Thr Phe
 275 280 285

Asn Val Met Glu Ser Asn Ala Ile Arg Asn Pro His Leu Phe Asp Ile
 290 295 300

Leu Asn Asn Leu Thr Ile Phe Thr Asp Trp Phe Ser Val Gly Arg Asn
 305 310 315 320

Phe Tyr Trp Gly Gly His Arg Val Thr Ser Asn Tyr Ile Gly Gly Gly
 325 330 335

Asn Ile Thr Ser Pro Ile Tyr Gly Arg Glu Ala Asn Gln Glu Pro Pro
 340 345 350

Arg Ser Phe Thr Phe Asn Gly Pro Val Phe Arg Thr Leu Ser Asn Pro
 355 360 365

Thr Leu Arg Leu Leu Gln Gln Pro Trp Pro Ala Pro Pro Phe Asn Leu
 370 375 380

Arg Gly Val Glu Gly Val Glu Phe Ser Thr Pro Thr Asn Ser Phe Thr
 385 390 395 400

Tyr Arg Gly Arg Gly Thr Val Asp Ser Leu Thr Glu Leu Pro Pro Glu
 405 410 415

Asp Asn Ser Val Leu Pro Arg Glu Gly Tyr Ser His Arg Leu Cys His
 420 425 430

Ala Thr Phe Val Gln Arg Ser Gly Thr Pro Phe Leu Thr Thr Gly Val
 435 440 445

Val Phe Ser Trp Thr His Arg Ser Ala Thr Leu Thr Asn Thr Ile Asp
 450 455 460

Pro Asp Lys Ile Thr Gln Ile Pro Leu Val Lys Gly Phe Arg Val Trp
 465 470 475 480

Ser Gly Ala Ser Val Val Thr Gly Pro Gly Phe Thr Gly Gly Asp Ile
 485 490 495

Leu Arg Arg Thr Asn Phe Gly Asp Phe Val Ser Met Gln Val Asn Ile
 500 505 510

Asn Ser Pro Ile Thr Gln Arg Tyr Arg Leu Arg Phe Arg Tyr Ala Ser
 515 520 525

Ser Arg Asp Ala Arg Leu Thr Val Ala Thr Gly Ala Ala Asn Thr Gly
 530 535 540

pct-us2017-055731 Sequence listing

Val Gly Gly Gln Ile Ser Val Asp Met Ala Leu Gln Lys Thr Met Glu
 545 550 555 560
 Ile Gly Glu Ser Leu Thr Ser Arg Thr Phe Arg Tyr Thr Asp Phe Ser
 565 570 575
 Asn Pro Phe Ser Phe Arg Ala Asn Pro Asp Ile Ile Arg Ile Asn Glu
 580 585 590
 Gln Pro Leu Phe Gly Ala Gly Ser Ile Ser Ser Gly Glu Leu Tyr Ile
 595 600 605
 Asp Lys Ile Glu Ile Ile Leu Ala Asp Ala Thr Phe Glu Ala Glu Tyr
 610 615 620
 Asp Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu Phe Thr Ser Thr
 625 630 635 640
 Asn Gln Arg Gly Leu Lys Thr Asp Val Thr Asp Tyr His Ile Asp Gln
 645 650 655
 Val Ser Asn Leu Val Glu Cys Leu Ser Asp Glu Phe Cys Leu Asp Glu
 660 665 670
 Lys Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp
 675 680 685
 Glu Arg Asn Leu Leu Gln Asp Arg Asn Phe Thr Ser Ile Asn Gly Gln
 690 695 700
 Leu Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly
 705 710 715 720
 Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Leu Gly Thr Phe Asp
 725 730 735
 Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu
 740 745 750
 Lys Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln
 755 760 765
 Asp Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val
 770 775 780
 Asn Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Glu Ser Pro
 785 790 795 800
 Ile Gly Lys Cys Gly Glu Pro Asn Arg Cys Thr Pro His Leu Glu Trp
 805 810 815

pct-us2017-055731 Sequence listing

Asn Pro Asn Leu Asp Cys Ser Cys Arg Asp Gly Lys Thr Cys Ala His
 820 825 830
 His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu
 835 840 845
 Asn Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp
 850 855 860
 Gly His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu
 865 870 875 880
 Val Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg
 885 890 895
 Asp Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val Tyr Lys Glu
 900 905 910
 Ala Lys Glu Ala Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg
 915 920 925
 Leu Gln Val Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg
 930 935 940
 Val His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro
 945 950 955 960
 Gly Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Cys Val Phe Thr
 965 970 975
 Ala Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe
 980 985 990
 Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Glu Glu Gln
 995 1000 1005
 Asn Asp His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu
 1010 1015 1020
 Val Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu
 1025 1030 1035
 Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr
 1040 1045 1050
 Ile His Gly Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn
 1055 1060 1065
 Cys Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn
 1070 1075 1080

pct-us2017-055731 Sequence listing

Asp Tyr Pro Ala Asn Gln Glu Glu Tyr Gly Gly Ala Tyr Thr Ser
 1085 1090 1095

Arg Asn Arg Gly Tyr Asp Glu Thr Tyr Gly Ser Asn Ser Ser Val
 1100 1105 1110

Ser Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ala Tyr Thr Asp
 1115 1120 1125

Gly Arg Arg Asp Asn Pro Cys Glu Phe Asn Arg Gly Tyr Gly Asp
 1130 1135 1140

Tyr Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr
 1145 1150 1155

Phe Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu
 1160 1165 1170

Gly Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
 1175 1180 1185

<210> 5
 <211> 3564
 <212> DNA
 <213> Bacillus thuringiensis

<220>
 <221> misc_feature
 <222> (1)..(3564)
 <223> Nucleic acid sequence encoding a TIC1425 pesticidal protein
 obtained from Bacillus thuringiensis species EG10731.

<400> 5
 atgaataata atattgaaaa ccaatgcgta ccttacaatt gtttaagtaa tcctgaagaa 60
 gtaatttttg atggagaacg gatatcaact ggtaattcat caattgatat ttctctgtca 120
 cttgttcaac ttctggtatc taactttgta ccaggcggag gatttttagt agggttaata 180
 gattttgtat ggggaatagt aggcccttct ccatgggatg cttttctagt gcaaattgaa 240
 caattaattc agcaaagaat agaagcatat gctagggctg cagcaatttc taatttagaa 300
 ggaataggaa acaatttcaa tatatatgtg gaagcatttc aagaatggga agaagatcct 360
 aataatccag caaccaggaa tagagtagtt gatcgctttc gtatacttga tgggctactt 420
 gaaagggaca ttccttcggt tcgaatttct ggatttgaag tccccctttt atccgtttat 480
 gctcaagcgg ccaatctgca tctagctata ttaagagatt ctgtaatttt tggagaaaga 540
 tggggattga caacaacaaa tgtcaatgaa aactataata gacaaatcag gcatattgat 600
 gaatatgctg atcactgtgc aaatacgtat aatcggggat taaataattt accgaaatct 660
 acgtatcaag attggataac atataatcga ttacggagag aattaacatt gactgtatta 720
 gatatcgctg ctttctttcc aaactatgac aataggcggg atccaattca gccagttggt 780
 caactaaca ggggaagtta tacggacca ttaattactt ttaatcccca gttacagtct 840

pct-us2017-055731 Sequence listing

gtagctcaat tacctacttt taacgttatg gaaagcaacg caattagaaa tcctcatttg	900
tttgatatat tgaataatct tacaatTTTT acggattggg ttagtggttg acgcaacttt	960
tattggggag gacatcgagt aacttctaac tatataggag gaggcaacat aacatctcct	1020
atatatggaa gagaggcgaa ccaggagcct ccaagatctt ttacttttaa tggacctgtt	1080
tttaggactt tatcaaatcc tacttttacga ttattacagc aaccttggcc agcaccacca	1140
tttaatttac gtggtgttga aggagtagaa ttttctacac ctacaaatag ctttacgtat	1200
cgaggaagag gtacagttga ttctttaacc gaattaccgc ctgaggataa tagtggtgcta	1260
cctcgcgagg gatatatgtca tcgtttatgt catgcaactt ttgttcaaag atctggaaca	1320
ccatttttaa caacgggtgt agtattttct tggacgcac gcagtgaac tcttacaat	1380
acaattgatc cagacaaaat tactcagata ctttagtgga aaggatttag agtttggagt	1440
ggcgcctctg tcgttacagg accaggTTTT acaggagggg atatccttcg aagaactaac	1500
tttggggatt ttgtatctat gcaagttaat attaattcac caataacaca aagataccgt	1560
ttaagatttc gttatgcttc cagtagagat gcacgactta cagtagcgac aggagcagca	1620
aacacaggag ttggagggca aattagtgtg gacatggctc ttcagaaaac tatggaaatt	1680
ggagagagct taacatctag aacatttaga tataccgatt ttagtaatcc tttttcattt	1740
agagctaadc cagatataat tcgtataaat gaacaaccac tattcggtgc aggtctctatt	1800
agtagtggtg aactttatat agataaaatt gaaattattc tagcagatgc aacatttgaa	1860
gcagaatatg atttggaaag agcacagaag gcggtgaatg cgctgtttac ttctacaaac	1920
caaagaggat taaaaacaga tgtgacggat tatcatattg atcaagtatc caatttagtt	1980
gagtgtttat cggatgaatt ttgtctggat gaaaaacgag aattgtccga gaaagtcaaa	2040
catgcgaagc gactcagtga tgagcgaaat ttactccagg atcgaaattt cacatccatt	2100
aatgggcaac tagaccgtgg ctggagagga agtacggata ttaccatcca aggaggagat	2160
gacgtattca aagagaatta cgtcacacta ctgggtacct ttgatgagtg ctatccaacg	2220
tatttatatc aaaaaataga tgagtcgaaa ttaaaagcct ataccgcta tgaattaaga	2280
gggtatatcg aggatagtca agacttagaa atctatttaa ttcgctacaa tgcaaaacac	2340
gaaacagtaa atgtgccagg tacaggttcc ttatggccgc tttcagtcga aagtccaatc	2400
ggaaagtgtg gagaaccgaa tcgatgcaca ccacacctg aatggaatcc taatctagat	2460
tgttcctgca gagacgggaa aacatgtgca catcattctc atcatttctc cttggacatt	2520
gatgttggat gtacagactt aaatgaagat ctaggtgtat gggatgatatt caagattaag	2580
acgcaagatg gtcatgcaag actaggaaat ctagagtttc tcgaagagaa accattagta	2640
ggagaagcgt tagctcgtgt gaaaagagcg gagaaaaaat ggagagacaa acgcgaaaaa	2700
ttgcaattag aaacaaatat cgtttacaaa gaggcaaaag aagctgtgga tgctttattt	2760
gtaaactctc aatatgatag attacaagtg gatacgaaca ttgcatgat tcatgcggca	2820
gataaacgcg ttcatagaat ccgagaagcg tatcttcag agttatctgt gattccgggt	2880

pct-us2017-055731 Sequence listing

```

gtcaatgcgg ctattttcga agaattagaa ggggtgtattt tctactgcatt ctccctatat      2940
gatgcgagaa atgtcattaa aaatggcgat tttaataatg gcttatcatg ctggaacgtg      3000
aaagggcatg tagaagaaca aaacgaccat cgttcgggtcc ttgttggtccc ggaatgggaa      3060
gcagaagtgt cacaagaggt tcgtgtctgt ccaggtcgtg gctatatcct tcgtgttaca      3120
gcatacaaag agggatatgg agaaggctgt gtaaccattc atgggatcga gaacaatata      3180
gacgaactga agtttagcaa ctgcgtagaa gaggaagtct atccaaacaa cacggtaacg      3240
tgtaatgatt atcctgcaaa tcaagaagaa tacgggggtg cgtacacttc tcgtaatcgt      3300
ggatatgacg aaacttatgg aagcaattct tccgtatcag ctgattatgc gtcagtttat      3360
gaagaaaaag cgtatacaga tggacgaaga gacaatccat gtgaatttaa cagaggggat      3420
ggggattata cgccactacc agctggctat gtaacaaaag aattagaata cttcccagaa      3480
accgataagg tatggattga gattggagaa acggaaggaa cattcatcgt ggacagtgtg      3540
gaattactcc ttatggagga ataa                                             3564

```

```

<210> 6
<211> 1187
<212> PRT
<213> Bacillus thuringiensis

```

```

<220>
<221> MISC_FEATURE
<222> (1)..(1187)
<223> The amino acid sequence of the TIC1425 pesticidal protein.
<400> 6

```

```

Met Asn Asn Asn Ile Glu Asn Gln Cys Val Pro Tyr Asn Cys Leu Ser
1           5           10           15

```

```

Asn Pro Glu Glu Val Ile Leu Asp Gly Glu Arg Ile Ser Thr Gly Asn
          20           25           30

```

```

Ser Ser Ile Asp Ile Ser Leu Ser Leu Val Gln Leu Leu Val Ser Asn
          35           40           45

```

```

Phe Val Pro Gly Gly Gly Phe Leu Val Gly Leu Ile Asp Phe Val Trp
          50           55           60

```

```

Gly Ile Val Gly Pro Ser Pro Trp Asp Ala Phe Leu Val Gln Ile Glu
65           70           75           80

```

```

Gln Leu Ile Gln Gln Arg Ile Glu Ala Tyr Ala Arg Ala Ala Ile
          85           90           95

```

```

Ser Asn Leu Glu Gly Ile Gly Asn Asn Phe Asn Ile Tyr Val Glu Ala
          100          105          110

```

```

Phe Gln Glu Trp Glu Glu Asp Pro Asn Asn Pro Ala Thr Arg Asn Arg

```

pct-us2017-055731 Sequence listing

115 Val Val Asp Arg Phe Arg Ile Leu Asp Gly Leu Leu Glu Arg Asp Ile
 130 135 140
 Pro Ser Phe Arg Ile Ser Gly Phe Glu Val Pro Leu Leu Ser Val Tyr
 145 150 155 160
 Ala Gln Ala Ala Asn Leu His Leu Ala Ile Leu Arg Asp Ser Val Ile
 165 170 175
 Phe Gly Glu Arg Trp Gly Leu Thr Thr Thr Asn Val Asn Glu Asn Tyr
 180 185 190
 Asn Arg Gln Ile Arg His Ile Asp Glu Tyr Ala Asp His Cys Ala Asn
 195 200 205
 Thr Tyr Asn Arg Gly Leu Asn Asn Leu Pro Lys Ser Thr Tyr Gln Asp
 210 215 220
 Trp Ile Thr Tyr Asn Arg Leu Arg Arg Glu Leu Thr Leu Thr Val Leu
 225 230 235 240
 Asp Ile Ala Ala Phe Phe Pro Asn Tyr Asp Asn Arg Arg Tyr Pro Ile
 245 250 255
 Gln Pro Val Gly Gln Leu Thr Arg Glu Val Tyr Thr Asp Pro Leu Ile
 260 265 270
 Thr Phe Asn Pro Gln Leu Gln Ser Val Ala Gln Leu Pro Thr Phe Asn
 275 280 285
 Val Met Glu Ser Asn Ala Ile Arg Asn Pro His Leu Phe Asp Ile Leu
 290 295 300
 Asn Asn Leu Thr Ile Phe Thr Asp Trp Phe Ser Val Gly Arg Asn Phe
 305 310 315 320
 Tyr Trp Gly Gly His Arg Val Thr Ser Asn Tyr Ile Gly Gly Gly Asn
 325 330 335
 Ile Thr Ser Pro Ile Tyr Gly Arg Glu Ala Asn Gln Glu Pro Pro Arg
 340 345 350
 Ser Phe Thr Phe Asn Gly Pro Val Phe Arg Thr Leu Ser Asn Pro Thr
 355 360 365
 Leu Arg Leu Leu Gln Gln Pro Trp Pro Ala Pro Pro Phe Asn Leu Arg
 370 375 380
 Gly Val Glu Gly Val Glu Phe Ser Thr Pro Thr Asn Ser Phe Thr Tyr

pct-us2017-055731 Sequence listing

385						390												400
Arg	Gly	Arg	Gly	Thr	Val	Asp	Ser	Leu	Thr	Glu	Leu	Pro	Pro	Glu	Asp			
				405					410					415				
Asn	Ser	Val	Leu	Pro	Arg	Glu	Gly	Tyr	Ser	His	Arg	Leu	Cys	His	Ala			
			420					425					430					
Thr	Phe	Val	Gln	Arg	Ser	Gly	Thr	Pro	Phe	Leu	Thr	Thr	Gly	Val	Val			
		435					440					445						
Phe	Ser	Trp	Thr	His	Arg	Ser	Ala	Thr	Leu	Thr	Asn	Thr	Ile	Asp	Pro			
	450					455					460							
Asp	Lys	Ile	Thr	Gln	Ile	Pro	Leu	Val	Lys	Gly	Phe	Arg	Val	Trp	Ser			
465					470					475					480			
Gly	Ala	Ser	Val	Val	Thr	Gly	Pro	Gly	Phe	Thr	Gly	Gly	Asp	Ile	Leu			
				485					490					495				
Arg	Arg	Thr	Asn	Phe	Gly	Asp	Phe	Val	Ser	Met	Gln	Val	Asn	Ile	Asn			
			500					505					510					
Ser	Pro	Ile	Thr	Gln	Arg	Tyr	Arg	Leu	Arg	Phe	Arg	Tyr	Ala	Ser	Ser			
		515					520					525						
Arg	Asp	Ala	Arg	Leu	Thr	Val	Ala	Thr	Gly	Ala	Ala	Asn	Thr	Gly	Val			
	530					535					540							
Gly	Gly	Gln	Ile	Ser	Val	Asp	Met	Ala	Leu	Gln	Lys	Thr	Met	Glu	Ile			
545					550					555					560			
Gly	Glu	Ser	Leu	Thr	Ser	Arg	Thr	Phe	Arg	Tyr	Thr	Asp	Phe	Ser	Asn			
				565					570					575				
Pro	Phe	Ser	Phe	Arg	Ala	Asn	Pro	Asp	Ile	Ile	Arg	Ile	Asn	Glu	Gln			
			580					585					590					
Pro	Leu	Phe	Gly	Ala	Gly	Ser	Ile	Ser	Ser	Gly	Glu	Leu	Tyr	Ile	Asp			
		595					600					605						
Lys	Ile	Glu	Ile	Ile	Leu	Ala	Asp	Ala	Thr	Phe	Glu	Ala	Glu	Tyr	Asp			
	610					615					620							
Leu	Glu	Arg	Ala	Gln	Lys	Ala	Val	Asn	Ala	Leu	Phe	Thr	Ser	Thr	Asn			
625					630					635					640			
Gln	Arg	Gly	Leu	Lys	Thr	Asp	Val	Thr	Asp	Tyr	His	Ile	Asp	Gln	Val			
				645					650					655				
Ser	Asn	Leu	Val	Glu	Cys	Leu	Ser	Asp	Glu	Phe	Cys	Leu	Asp	Glu	Lys			

pct-us2017-055731 Sequence listing

```

660      Arg Glu Leu Ser Glu Lys Val Lys His Ala Lys Arg Leu Ser Asp Glu
        675                        680                        685

690      Arg Asn Leu Leu Gln Asp Arg Asn Phe Thr Ser Ile Asn Gly Gln Leu
        695                        700

705      Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile Gln Gly Gly Asp
        710                        715                        720

730      Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Leu Gly Thr Phe Asp Glu
        725                        730                        735

740      Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu Ser Lys Leu Lys
        740                        745                        750

755      Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu Asp Ser Gln Asp
        755                        760                        765

770      Leu Glu Ile Tyr Leu Ile Arg Tyr Asn Ala Lys His Glu Thr Val Asn
        770                        775                        780

785      Val Pro Gly Thr Gly Ser Leu Trp Pro Leu Ser Val Glu Ser Pro Ile
        785                        790                        795                        800

805      Gly Lys Cys Gly Glu Pro Asn Arg Cys Thr Pro His Leu Glu Trp Asn
        805                        810                        815

820      Pro Asn Leu Asp Cys Ser Cys Arg Asp Gly Lys Thr Cys Ala His His
        820                        825                        830

835      Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys Thr Asp Leu Asn
        835                        840                        845

850      Glu Asp Leu Gly Val Trp Val Ile Phe Lys Ile Lys Thr Gln Asp Gly
        850                        855                        860

865      His Ala Arg Leu Gly Asn Leu Glu Phe Leu Glu Glu Lys Pro Leu Val
        865                        870                        875                        880

885      Gly Glu Ala Leu Ala Arg Val Lys Arg Ala Glu Lys Lys Trp Arg Asp
        885                        890                        895

900      Lys Arg Glu Lys Leu Gln Leu Glu Thr Asn Ile Val Tyr Lys Glu Ala
        900                        905                        910

915      Lys Glu Ala Val Asp Ala Leu Phe Val Asn Ser Gln Tyr Asp Arg Leu
        915                        920                        925

930      Gln Val Asp Thr Asn Ile Ala Met Ile His Ala Ala Asp Lys Arg Val

```

pct-us2017-055731 Sequence listing

930
 935
 940
 His Arg Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser Val Ile Pro Gly
 945 950 955 960
 Val Asn Ala Ala Ile Phe Glu Glu Leu Glu Gly Cys Ile Phe Thr Ala
 965 970 975
 Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn Gly Asp Phe Asn
 980 985 990
 Asn Gly Leu Ser Cys Trp Asn Val Lys Gly His Val Glu Glu Gln Asn
 995 1000 1005
 Asp His Arg Ser Val Leu Val Val Pro Glu Trp Glu Ala Glu Val
 1010 1015 1020
 Ser Gln Glu Val Arg Val Cys Pro Gly Arg Gly Tyr Ile Leu Arg
 1025 1030 1035
 Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly Cys Val Thr Ile
 1040 1045 1050
 His Gly Ile Glu Asn Asn Thr Asp Glu Leu Lys Phe Ser Asn Cys
 1055 1060 1065
 Val Glu Glu Glu Val Tyr Pro Asn Asn Thr Val Thr Cys Asn Asp
 1070 1075 1080
 Tyr Pro Ala Asn Gln Glu Glu Tyr Gly Gly Ala Tyr Thr Ser Arg
 1085 1090 1095
 Asn Arg Gly Tyr Asp Glu Thr Tyr Gly Ser Asn Ser Ser Val Ser
 1100 1105 1110
 Ala Asp Tyr Ala Ser Val Tyr Glu Glu Lys Ala Tyr Thr Asp Gly
 1115 1120 1125
 Arg Arg Asp Asn Pro Cys Glu Phe Asn Arg Gly Tyr Gly Asp Tyr
 1130 1135 1140
 Thr Pro Leu Pro Ala Gly Tyr Val Thr Lys Glu Leu Glu Tyr Phe
 1145 1150 1155
 Pro Glu Thr Asp Lys Val Trp Ile Glu Ile Gly Glu Thr Glu Gly
 1160 1165 1170
 Thr Phe Ile Val Asp Ser Val Glu Leu Leu Leu Met Glu Glu
 1175 1180 1185

<210> 7

pct-us2017-055731 Sequence listing

<211> 3537
 <212> DNA
 <213> *Bacillus thuringiensis*

<220>
 <221> misc_feature
 <222> (1)..(3537)
 <223> Nucleic acid sequence encoding a TIC2613 pesticidal protein obtained from *Bacillus thuringiensis* species EG5408.

<400> 7
 atggataaca atatcaagaa ccaatgcatt ccttacaatt gtttaaataga tcctgaggta 60
 gaaatattag gtgaagaggg gataactact agtaatgaaa atctcgaatt tttcttatcg 120
 ctaacgaaat ttgtcttgaa taggtttgtc cctggtggag catatgtagc tggcctatatt 180
 gatgtattct ggggatgggt aaaaccttct gattgggtctg caatccttga acaaattgaa 240
 gaattaatta accaaaaaat tgagacgttt gctagaaatc aagcaattag tagattggaa 300
 gggttaagca acctttatga aatttacgca gatactttta aagaatggga aaaagatccg 360
 actaatccag cattaagaga agaaatgcgt acacaattta atgacatgaa cagctctttt 420
 gtaacagcta tgcctctttt ttcagttcaa aattttgaag ttcctctttt agcagtatac 480
 gctcaagctg caaatttaca tctatcagtt ttaagggatg tctcagtttt tgggtcaaaat 540
 tggggatttg attcagccac tgtcaatagt cgttataatg atttagtaag aaatattcgt 600
 acctatacaa attatgtcgt acgttgggtat aacacaggat tagcaagggt acgagggtact 660
 acgtaccaag attgggttaa ttatcatcgc tttagaagag aattaacaat aactgcattg 720
 gatatcatta ccatattccc aactacgat aataaaatgt atccaattca accccatttt 780
 caattaacaa gagagattta tacggatcca ctaattaatt tcaatccggc gttacagtct 840
 gtagcacaat tacctctatt taatgagatg gaaaatagta caattagaag ccctcattta 900
 gttgattttt taaataggct tacaatttat acagattgggt atagtctcgg aagacactat 960
 tattggggag gacatcaaat agtctctaga caaacaggat caacttccac tattacattc 1020
 cctatatatg gaagagaggc gaatcaagag gcccctagaa catataattt tagtcaacct 1080
 gtcttttagaa cactgtcaaa tcctacttta acacgtttta tgcaaccttg gccagcccca 1140
 gcatttcagt tgcgtcgtct tgagggagtt gaatttcaaa caactacagg taattttacg 1200
 tatcgaggaa gaggtacggt agattccttt gatgaattac caccagatga tacaagcgta 1260
 ccagcgcgtg aaggatatag tcatcgttta tgtcatgcaa ctttatcag aaaatctggg 1320
 acgccgtatt taacaacggg tgtaacacta tcttgacac acaatagcaa tacacctacg 1380
 aatataattt atcctgataa aatcactcaa gtaccattgg tgaaagcatc taaccttcac 1440
 tctagtgttt tcgtttttaa aggaccagga tttacaggag gggacatact tggaagaact 1500
 agtgtgggca acatagcaga tatccaaatg aatattactg caccgttatc acaaagatat 1560
 cgcgtacgaa ttcggtatgc ctctactaca aacttacaat ttcatacgac aattaacggc 1620
 agggccgtaa atcaggctaa tttcccagca actatgaata gagtagaaga cttagaatat 1680

pct-us2017-055731 Sequence listing

aattccttta gaacgataag ttctcggtact ctttttaact ttttagatgc tcaaagtacc	1740
ttcagggttag gtgtatggag cttttcttca ggtaccgttt taatagatag aattgaagtt	1800
gtaccaatgg aagtaacatt tgaagcagaa tctgatttag aaagagcaca aaaggcggtg	1860
aacgctctgt ttacttctat aaatcaaaaa ggactaaaaa cagatgtaac agattatcac	1920
attgatcaag tatccaattt ggtcgaatgt ctatccgatg aattttgtct agatgaaaag	1980
agagaactat ttgagaaagt caaatatgcg aaacgactca gtgacgaacg gaatttactt	2040
gcagatccaa atttcacatc tattaatggg caactagatc gtggatggag aggaagtacg	2100
gatattacca ttcaaggggg cgatgacgta ttcaaagaaa actacgtcac actatcaggt	2160
acccttgatg agtggttatcc aacctattta tatcaaaaaa tagacgaatc gaaattaa	2220
gcgtataccc gttacgagtt acgaggatat atcgaagata gtcaagattt agaagtatac	2280
ttgattcggt acaataccaa acatgaaaca ttgaatgtac caggtagagg gggcctatgg	2340
ccgcttgtag tagaaagttc aatcggaggg tgtggcgaac caaacgatg cgcaccacaa	2400
atggaatggg atccaaatct agaatgttct tgtagcgacg aggagaaatg tgcgcatcat	2460
tcccatcatt tctctctcga tattgatgtt ggatgtactg atttaaataa aaatctaggt	2520
atatgggtta tatttaaaat taaaacgcag aacggttatg caaaattagg aaatttagag	2580
tttctcgaag agaaaccatt aataggggaa gcgttagctc gtgttaagcg agtggagaaa	2640
aaatggaaaag acaaacgtga aaaattagaa ttgaaacga atatagtcta caacgaggca	2700
aaagaagctg tggatgcact attcgtaa atcacaatatg atagattgca agctgataca	2760
aatatcgcaa tgattcatgc ggcggataac aaagttcata aaattcgcg ggcgtacctc	2820
ccagagttat ctgtgatacc aggtgtaaat gcgaccgttt ttgaagaatt agaagagcgt	2880
atttttacag cattctccct ttacgatgca agaaatgtga taaaaaatgg ggatttcaat	2940
aatggattat cttgttggaa tgtgaagggc caagtagatg tagacaaaaa tgaccatcgt	3000
tctgtccttg ttattccagg atgggaatcg gaagtatcac aagaagttca tgtatgtcca	3060
gatcgtggat acattcttcg tgttacggcg tacaaagaag gatattggaga aggctgcgta	3120
acaatccatg agattgataa tcatacagac gaactgaaat ttaaaaactg ctttgaagag	3180
gaagtatctc taaataatgc ggtgacatgt gatgagtata ctacaaatca agaagtagga	3240
gggtatgcgg atgtacgtca atccaataat cgtggatcta atgaggccta tgtaaatacct	3300
acttccacat caactgatta tgcacgctc tacgaggaag agtcgtatac gaatgaacag	3360
acataataatt cttgtgaatc taacagaggg tatggtaatc aaatgccatt accgtctggc	3420
tatgtgacaa aagaattaga atattttcca gagacagata aagtatggat tgagattgga	3480
gaaacagaag gaacattcat cgtagacagt gtggaattac tccttatgga ggaataa	3537

<210> 8
 <211> 1178
 <212> PRT
 <213> Bacillus thuringiensis

pct-us2017-055731 Sequence listing

<220>

<221> MISC_FEATURE

<222> (1)..(1178)

<223> The amino acid sequence of the TIC2613 pesticidal protein.

<400> 8

Met Asp Asn Asn Ile Lys Asn Gln Cys Ile Pro Tyr Asn Cys Leu Asn
 1 5 10 15

Asp Pro Glu Val Glu Ile Leu Gly Glu Glu Gly Ile Thr Thr Ser Asn
 20 25 30

Glu Asn Leu Glu Phe Phe Leu Ser Leu Thr Lys Phe Val Leu Asn Arg
 35 40 45

Phe Val Pro Gly Gly Ala Tyr Val Ala Gly Leu Phe Asp Val Phe Trp
 50 55 60

Gly Trp Leu Lys Pro Ser Asp Trp Ser Ala Ile Leu Glu Gln Ile Glu
 65 70 75 80

Glu Leu Ile Asn Gln Lys Ile Glu Thr Phe Ala Arg Asn Gln Ala Ile
 85 90 95

Ser Arg Leu Glu Gly Leu Ser Asn Leu Tyr Glu Ile Tyr Ala Asp Thr
 100 105 110

Phe Lys Glu Trp Glu Lys Asp Pro Thr Asn Pro Ala Leu Arg Glu Glu
 115 120 125

Met Arg Thr Gln Phe Asn Asp Met Asn Ser Ser Phe Val Thr Ala Met
 130 135 140

Pro Leu Phe Ser Val Gln Asn Phe Glu Val Pro Leu Leu Ala Val Tyr
 145 150 155 160

Ala Gln Ala Ala Asn Leu His Leu Ser Val Leu Arg Asp Val Ser Val
 165 170 175

Phe Gly Gln Asn Trp Gly Phe Asp Ser Ala Thr Val Asn Ser Arg Tyr
 180 185 190

Asn Asp Leu Val Arg Asn Ile Arg Thr Tyr Thr Asn Tyr Val Val Arg
 195 200 205

Trp Tyr Asn Thr Gly Leu Ala Arg Leu Arg Gly Thr Thr Tyr Gln Asp
 210 215 220

Trp Leu Asn Tyr His Arg Phe Arg Arg Glu Leu Thr Ile Thr Ala Leu
 225 230 235 240

pct-us2017-055731 sequence listing

Asp Ile Ile Thr Ile Phe Pro His Tyr Asp Asn Lys Met Tyr Pro Ile
 245 250 255
 Gln Pro His Phe Gln Leu Thr Arg Glu Ile Tyr Thr Asp Pro Leu Ile
 260 265 270
 Asn Phe Asn Pro Ala Leu Gln Ser Val Ala Gln Leu Pro Leu Phe Asn
 275 280 285
 Glu Met Glu Asn Ser Thr Ile Arg Ser Pro His Leu Val Asp Phe Leu
 290 295 300
 Asn Arg Leu Thr Ile Tyr Thr Asp Trp Tyr Ser Leu Gly Arg His Tyr
 305 310 315 320
 Tyr Trp Gly Gly His Gln Ile Val Ser Arg Gln Thr Gly Ser Thr Ser
 325 330 335
 Thr Ile Thr Phe Pro Ile Tyr Gly Arg Glu Ala Asn Gln Glu Ala Pro
 340 345 350
 Arg Thr Tyr Asn Phe Ser Gln Pro Val Phe Arg Thr Leu Ser Asn Pro
 355 360 365
 Thr Leu Thr Arg Leu Met Gln Pro Trp Pro Ala Pro Ala Phe Gln Leu
 370 375 380
 Arg Arg Leu Glu Gly Val Glu Phe Gln Thr Thr Thr Gly Asn Phe Thr
 385 390 395 400
 Tyr Arg Gly Arg Gly Thr Val Asp Ser Phe Asp Glu Leu Pro Pro Asp
 405 410 415
 Asp Thr Ser Val Pro Ala Arg Glu Gly Tyr Ser His Arg Leu Cys His
 420 425 430
 Ala Thr Phe Ile Arg Lys Ser Gly Thr Pro Tyr Leu Thr Thr Gly Val
 435 440 445
 Thr Leu Ser Trp Thr His Asn Ser Asn Thr Pro Thr Asn Ile Ile Tyr
 450 455 460
 Pro Asp Lys Ile Thr Gln Val Pro Leu Val Lys Ala Ser Asn Leu His
 465 470 475 480
 Ser Ser Ala Phe Val Leu Lys Gly Pro Gly Phe Thr Gly Gly Asp Ile
 485 490 495
 Leu Gly Arg Thr Ser Val Gly Asn Ile Ala Asp Ile Gln Met Asn Ile
 500 505 510

pct-us2017-055731 Sequence listing

Thr Ala Pro Leu Ser Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser
 515 520 525
 Thr Thr Asn Leu Gln Phe His Thr Thr Ile Asn Gly Arg Ala Val Asn
 530 535 540
 Gln Ala Asn Phe Pro Ala Thr Met Asn Arg Val Glu Asp Leu Glu Tyr
 545 550 555 560
 Asn Ser Phe Arg Thr Ile Ser Phe Gly Thr Pro Phe Asn Phe Leu Asp
 565 570 575
 Ala Gln Ser Thr Phe Arg Leu Gly Val Trp Ser Phe Ser Ser Gly Thr
 580 585 590
 Val Leu Ile Asp Arg Ile Glu Val Val Pro Met Glu Val Thr Phe Glu
 595 600 605
 Ala Glu Ser Asp Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu Phe
 610 615 620
 Thr Ser Ile Asn Gln Lys Gly Leu Lys Thr Asp Val Thr Asp Tyr His
 625 630 635 640
 Ile Asp Gln Val Ser Asn Leu Val Glu Cys Leu Ser Asp Glu Phe Cys
 645 650 655
 Leu Asp Glu Lys Arg Glu Leu Phe Glu Lys Val Lys Tyr Ala Lys Arg
 660 665 670
 Leu Ser Asp Glu Arg Asn Leu Leu Ala Asp Pro Asn Phe Thr Ser Ile
 675 680 685
 Asn Gly Gln Leu Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr Ile
 690 695 700
 Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Ser Gly
 705 710 715 720
 Thr Leu Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp Glu
 725 730 735
 Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile Glu
 740 745 750
 Asp Ser Gln Asp Leu Glu Val Tyr Leu Ile Arg Tyr Asn Thr Lys His
 755 760 765
 Glu Thr Leu Asn Val Pro Gly Thr Gly Gly Leu Trp Pro Leu Ala Val
 770 775 780

pct-us2017-055731 Sequence listing

Glu Ser Ser Ile Gly Gly Cys Gly Glu Pro Asn Arg Cys Ala Pro Gln
 785 790 795 800
 Met Glu Trp Asp Pro Asn Leu Glu Cys Ser Cys Ser Asp Glu Glu Lys
 805 810 815
 Cys Ala His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly Cys
 820 825 830
 Thr Asp Leu Asn Glu Asn Leu Gly Ile Trp Val Ile Phe Lys Ile Lys
 835 840 845
 Thr Gln Asn Gly Tyr Ala Lys Leu Gly Asn Leu Glu Phe Leu Glu Glu
 850 855 860
 Lys Pro Leu Ile Gly Glu Ala Leu Ala Arg Val Lys Arg Val Glu Lys
 865 870 875 880
 Lys Trp Lys Asp Lys Arg Glu Lys Leu Glu Phe Glu Thr Asn Ile Val
 885 890 895
 Tyr Asn Glu Ala Lys Glu Ala Val Asp Ala Leu Phe Val Asn Ser Gln
 900 905 910
 Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala Ala
 915 920 925
 Asp Asn Lys Val His Lys Ile Arg Glu Ala Tyr Leu Pro Glu Leu Ser
 930 935 940
 Val Ile Pro Gly Val Asn Ala Thr Val Phe Glu Glu Leu Glu Glu Arg
 945 950 955 960
 Ile Phe Thr Ala Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys Asn
 965 970 975
 Gly Asp Phe Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly Gln Val
 980 985 990
 Asp Val Asp Gln Asn Asp His Arg Ser Val Leu Val Ile Pro Gly Trp
 995 1000 1005
 Glu Ser Glu Val Ser Gln Glu Val His Val Cys Pro Asp Arg Gly
 1010 1015 1020
 Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu Gly
 1025 1030 1035
 Cys Val Thr Ile His Glu Ile Asp Asn His Thr Asp Glu Leu Lys
 1040 1045 1050

pct-us2017-055731 Sequence listing

Phe Lys Asn Cys Phe Glu Glu Glu Val Ser Leu Asn Asn Ala Val
 1055 1060 1065

Thr Cys Asp Glu Tyr Thr Thr Asn Gln Glu Val Gly Gly Tyr Ala
 1070 1075 1080

Asp Val Arg Gln Ser Asn Asn Arg Gly Ser Asn Glu Ala Tyr Val
 1085 1090 1095

Asn Pro Thr Ser Thr Ser Thr Asp Tyr Ala Ser Leu Tyr Glu Glu
 1100 1105 1110

Glu Ser Tyr Thr Asn Glu Gln Thr Tyr Asn Ser Cys Glu Ser Asn
 1115 1120 1125

Arg Gly Tyr Gly Asn Gln Met Pro Leu Pro Ser Gly Tyr Val Thr
 1130 1135 1140

Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile Glu
 1145 1150 1155

Ile Gly Glu Thr Glu Gly Thr Phe Ile Val Asp Ser Val Glu Leu
 1160 1165 1170

Leu Leu Met Glu Glu
 1175

<210> 9

<211> 3540

<212> DNA

<213> Artificial

<220>

<223> A synthetic coding sequence encoding a TIC2613PL pesticidal protein designed for expression in a plant cell wherein an additional alanine codon is inserted immediately following the initiating methionine codon.

<400> 9

atggctgaca acaacatcaa gaaccagtgc atcccgtaca actgcctcaa cgacccggag	60
gtcgagatcc tcggcgagga gggcataacg acgagcaacg agaaccttga gttcttcctc	120
agcctcacga agttcgtcct gaaccgcttc gtgccgggag gagcctacgt ggctggcctg	180
ttcgatgtgt tctggggatg gctcaagcca agcgactggt ccgcgattct ggagcagatc	240
gaggaactca tcaaccagaa gatcgagaca ttcgcccgcg accaggccat cagccgcctg	300
gagggcctct cgaacctcta cgaaatctac gccgatacgt tcaaggagtg ggagaaggat	360
ccgacgaacc cggccttgcg cgaggagatg aggacgcaat tcaacgacat gaactccagc	420
ttcgtcaccg ccatgccgct gttctccgtc cagaacttcg aggtgccctt gctcgccgtg	480
tacgcgcaag ctgcgaactt acatcttagc gtctccgcg acgtcagcgt cttcggccag	540
aactggggat tcgattccgc gacggtgaac tcacggtaca atgatctcgt gcggaacatc	600

pct-us2017-055731 Sequence listing

cggacctaca ccaattacgt cgtgcgctgg tacaacacgg gattggcgcg tctgcgcggc	660
actacctacc aggactggct caactaccac cggttccgcc gcgaactcac aatcacagcg	720
ctggacatca ttaccatctt cccgcactac gacaacaaga tgtaccaat ccagcctcac	780
ttccagctta cccgtgagat ctacacggac ccgctcatca acttcaatcc cgcactgcaa	840
tcagtagccc aattgccact cttcaacgag atggagaact cgacaatccg aagccctcac	900
ctcgtggact tcctcaaccg cctgaccatc tacacggatt ggtactctct tggtcggcac	960
tactattggg gcgggcacca aatcgtgtcc aggcagaccg gctctacctc taccataacc	1020
ttcccgatct atggccggga ggccaaccag gaggtccga ggacttaca cttcagtcag	1080
ccagtgttcc gcacactctc caaccgcact ctactcgtt tgatgcagcc ctggcccgcct	1140
cccgcgtttc agctcagaag attggagggc gtggagttcc aaacaacgac gggcaacttc	1200
acctaccgtg gccgtgggac ggtggacagt ttcgacgagt tgccctccga cgacaccagc	1260
gtgcctgcaa gggaaggcta ctgcacagg ctgtgccacg cgacgttcat ccgcaagtct	1320
gggacaccct acctgacaac cggcgctact ctctcctgga cccacaacag caacacaccc	1380
accaacataa tctaccctga caagataaca caagtgccgc tgggtgaaggc ttcgaacctc	1440
cattcctccg ccttcgtcct caagggtcg ggcttcaccg gcggcgacat cctgggtcgc	1500
acgtcggctg gcaacatcgc ggacattcag atgaacatta ccgcacctct gtcccagcgc	1560
tacagagtgc gtatccgcta cgcgagtacg accaacctcc aattccacac tacgatcaat	1620
gggagggcgg ttaatcaggc caacttcccg gccacgatga accgggtcga agacctggag	1680
tacaactcgt ttcggaccat ctctttcggc acgccgttca acttcctaga cgcccagtc	1740
acctttcggc tgggagtttg gagcttcagc agcggcacag tcctcatcga ccgaatagag	1800
gtggttccga tggaggtcac gttcagggcg gagtcggacc tggagcgagc gcagaaggct	1860
gtaaattgctg tgttcacgag cattaaccag aagggcctca agaccgatgt cacagactac	1920
cacatcgacc aagtgtcgaa cctggtggag tgtctgtcgg atgagttctg tcttgacgag	1980
aagcgggagc tgttcgagaa ggtgaagtat gctaagcggc tgagcgacga gcggaacttg	2040
ttggctgacc cgaacttcac cagcatcaac ggacagctcg accgtgggtg gcgaggttcc	2100
accgacatca cgatacaggc cggagacgat gtgttcaagg agaactatgt gaccctctca	2160
ggaacactgg atgagtgcta cccgacctat ctctaccaga agatcgacga gagcaagctc	2220
aaggcttaca cgcgctacga actccgtggc tacatcgaag actcccagga tcttgaggtg	2280
tacctcatac gctacaacac aaagcacgag acgctcaacg ttcctggcac cgggtggtctt	2340
tggcccttgg ccgtggagag tagcatcggc ggggtgcggtg agccaaaccg atgcgcgcc	2400
cagatggaat gggatccgaa cctagagtgc tcctgctcag acgaggagaa gtgcgcccac	2460
cactcccacc acttctcgtc cgacattgac gttggctgca cggatctcaa cgagaaccta	2520
ggaatctggg tgatcttcaa gattaagacc cagaacggct acgccaagct cgggaatctg	2580
gagtttcttg aggagaagcc gctgatcggc gagggccctcg cgcgcgtgaa gcgagtcgag	2640

pct-us2017-055731 Sequence listing

aagaagtgga aagacaagcg ggagaagcta gagtttgaaa cgaacattgt ttacaacgag 2700
gcaaaggaag ccgtggacgc tctgttcgta aacagtcagt acgaccgtct ccaggccgac 2760
acgaacatcg caatgataca cgcggcggat aacaaggtgc acaagattcg ggaggcttac 2820
ctgcccgagc tgtcggtcat cccaggcgta aacgctaccg tgttcgagga gctggaggaa 2880
cggatcttca ccgcgttctc cctctatgac gcaaggaacg tcatcaagaa cggcgacttc 2940
aacaacggcc tgagctgctg gaacgtgaag ggccaagtgg acgtcgatca gaacgatcac 3000
cgctccgttc tggtcattcc aggggtgggag tccgaggtga gccaagaggt ccatgtgtgc 3060
ccggaccgtg gctacatcct tcgggtgacc gcgtacaagg agggctacgg cgaaggctgc 3120
gtgaccatac acgagatcga caaccacacc gacgagctta agttcaagaa ctgcttcgag 3180
gaggaggtgt cactgaacaa cgccgtgacc tgcgacgagt acacgaccaa tcaggaggtc 3240
ggcggctacg ccgacgtccg ccagtcgaac aatcgaggca gcaacgaggc gtacgtgaac 3300
ccaacctcca cctcgacgga ctacgccagc ctctacgagg aggagtccta cacaacgag 3360
cagacctaca actcgtgcga gagcaaccga ggttacggga accagatgcc gctaccgtcc 3420
gggtacgtga cgaaggagct ggagtatttc ccagagaccg acaagggtgtg gatcgagatc 3480
ggcgagacag agggcacgtt catcgtggac agcgtcgagc tgctgttgat ggaggagtga 3540

<210> 10
<211> 1179
<212> PRT
<213> Artificial

<220>
<223> The amino acid sequence of TIC2613PL encoded by a synthetic coding sequence designed for expression in a plant cell (SEQ ID NO:9), and wherein an additional alanine amino acid is inserted immediately following the initiating methionine.

<400> 10

Met Ala Asp Asn Asn Ile Lys Asn Gln Cys Ile Pro Tyr Asn Cys Leu
1 5 10 15

Asn Asp Pro Glu Val Glu Ile Leu Gly Glu Glu Gly Ile Thr Thr Ser
20 25 30

Asn Glu Asn Leu Glu Phe Phe Leu Ser Leu Thr Lys Phe Val Leu Asn
35 40 45

Arg Phe Val Pro Gly Gly Ala Tyr Val Ala Gly Leu Phe Asp Val Phe
50 55 60

Trp Gly Trp Leu Lys Pro Ser Asp Trp Ser Ala Ile Leu Glu Gln Ile
65 70 75 80

Glu Glu Leu Ile Asn Gln Lys Ile Glu Thr Phe Ala Arg Asn Gln Ala
85 90 95

pct-us2017-055731 Sequence listing

Ile Ser Arg Leu Glu Gly Leu Ser Asn Leu Tyr Glu Ile Tyr Ala Asp
 100 105 110
 Thr Phe Lys Glu Trp Glu Lys Asp Pro Thr Asn Pro Ala Leu Arg Glu
 115 120 125
 Glu Met Arg Thr Gln Phe Asn Asp Met Asn Ser Ser Phe Val Thr Ala
 130 135 140
 Met Pro Leu Phe Ser Val Gln Asn Phe Glu Val Pro Leu Leu Ala Val
 145 150 155 160
 Tyr Ala Gln Ala Ala Asn Leu His Leu Ser Val Leu Arg Asp Val Ser
 165 170 175
 Val Phe Gly Gln Asn Trp Gly Phe Asp Ser Ala Thr Val Asn Ser Arg
 180 185 190
 Tyr Asn Asp Leu Val Arg Asn Ile Arg Thr Tyr Thr Asn Tyr Val Val
 195 200 205
 Arg Trp Tyr Asn Thr Gly Leu Ala Arg Leu Arg Gly Thr Thr Tyr Gln
 210 215 220
 Asp Trp Leu Asn Tyr His Arg Phe Arg Arg Glu Leu Thr Ile Thr Ala
 225 230 235 240
 Leu Asp Ile Ile Thr Ile Phe Pro His Tyr Asp Asn Lys Met Tyr Pro
 245 250 255
 Ile Gln Pro His Phe Gln Leu Thr Arg Glu Ile Tyr Thr Asp Pro Leu
 260 265 270
 Ile Asn Phe Asn Pro Ala Leu Gln Ser Val Ala Gln Leu Pro Leu Phe
 275 280 285
 Asn Glu Met Glu Asn Ser Thr Ile Arg Ser Pro His Leu Val Asp Phe
 290 295 300
 Leu Asn Arg Leu Thr Ile Tyr Thr Asp Trp Tyr Ser Leu Gly Arg His
 305 310 315 320
 Tyr Tyr Trp Gly Gly His Gln Ile Val Ser Arg Gln Thr Gly Ser Thr
 325 330 335
 Ser Thr Ile Thr Phe Pro Ile Tyr Gly Arg Glu Ala Asn Gln Glu Ala
 340 345 350
 Pro Arg Thr Tyr Asn Phe Ser Gln Pro Val Phe Arg Thr Leu Ser Asn
 355 360 365

pct-us2017-055731 Sequence listing

Pro Thr Leu Thr Arg Leu Met Gln Pro Trp Pro Ala Pro Ala Phe Gln
 370 375 380
 Leu Arg Arg Leu Glu Gly Val Glu Phe Gln Thr Thr Thr Gly Asn Phe
 385 390 395 400
 Thr Tyr Arg Gly Arg Gly Thr Val Asp Ser Phe Asp Glu Leu Pro Pro
 405 410 415
 Asp Asp Thr Ser Val Pro Ala Arg Glu Gly Tyr Ser His Arg Leu Cys
 420 425 430
 His Ala Thr Phe Ile Arg Lys Ser Gly Thr Pro Tyr Leu Thr Thr Gly
 435 440 445
 Val Thr Leu Ser Trp Thr His Asn Ser Asn Thr Pro Thr Asn Ile Ile
 450 455 460
 Tyr Pro Asp Lys Ile Thr Gln Val Pro Leu Val Lys Ala Ser Asn Leu
 465 470 475 480
 His Ser Ser Ala Phe Val Leu Lys Gly Pro Gly Phe Thr Gly Gly Asp
 485 490 495
 Ile Leu Gly Arg Thr Ser Val Gly Asn Ile Ala Asp Ile Gln Met Asn
 500 505 510
 Ile Thr Ala Pro Leu Ser Gln Arg Tyr Arg Val Arg Ile Arg Tyr Ala
 515 520 525
 Ser Thr Thr Asn Leu Gln Phe His Thr Thr Ile Asn Gly Arg Ala Val
 530 535 540
 Asn Gln Ala Asn Phe Pro Ala Thr Met Asn Arg Val Glu Asp Leu Glu
 545 550 555 560
 Tyr Asn Ser Phe Arg Thr Ile Ser Phe Gly Thr Pro Phe Asn Phe Leu
 565 570 575
 Asp Ala Gln Ser Thr Phe Arg Leu Gly Val Trp Ser Phe Ser Ser Gly
 580 585 590
 Thr Val Leu Ile Asp Arg Ile Glu Val Val Pro Met Glu Val Thr Phe
 595 600 605
 Glu Ala Glu Ser Asp Leu Glu Arg Ala Gln Lys Ala Val Asn Ala Leu
 610 615 620
 Phe Thr Ser Ile Asn Gln Lys Gly Leu Lys Thr Asp Val Thr Asp Tyr
 625 630 635 640

pct-us2017-055731 Sequence listing

His Ile Asp Gln Val Ser Asn Leu Val Glu Cys Leu Ser Asp Glu Phe
 645 650 655
 Cys Leu Asp Glu Lys Arg Glu Leu Phe Glu Lys Val Lys Tyr Ala Lys
 660 665 670
 Arg Leu Ser Asp Glu Arg Asn Leu Leu Ala Asp Pro Asn Phe Thr Ser
 675 680 685
 Ile Asn Gly Gln Leu Asp Arg Gly Trp Arg Gly Ser Thr Asp Ile Thr
 690 695 700
 Ile Gln Gly Gly Asp Asp Val Phe Lys Glu Asn Tyr Val Thr Leu Ser
 705 710 715 720
 Gly Thr Leu Asp Glu Cys Tyr Pro Thr Tyr Leu Tyr Gln Lys Ile Asp
 725 730 735
 Glu Ser Lys Leu Lys Ala Tyr Thr Arg Tyr Glu Leu Arg Gly Tyr Ile
 740 745 750
 Glu Asp Ser Gln Asp Leu Glu Val Tyr Leu Ile Arg Tyr Asn Thr Lys
 755 760 765
 His Glu Thr Leu Asn Val Pro Gly Thr Gly Gly Leu Trp Pro Leu Ala
 770 775 780
 Val Glu Ser Ser Ile Gly Gly Cys Gly Glu Pro Asn Arg Cys Ala Pro
 785 790 795 800
 Gln Met Glu Trp Asp Pro Asn Leu Glu Cys Ser Cys Ser Asp Glu Glu
 805 810 815
 Lys Cys Ala His His Ser His His Phe Ser Leu Asp Ile Asp Val Gly
 820 825 830
 Cys Thr Asp Leu Asn Glu Asn Leu Gly Ile Trp Val Ile Phe Lys Ile
 835 840 845
 Lys Thr Gln Asn Gly Tyr Ala Lys Leu Gly Asn Leu Glu Phe Leu Glu
 850 855 860
 Glu Lys Pro Leu Ile Gly Glu Ala Leu Ala Arg Val Lys Arg Val Glu
 865 870 875 880
 Lys Lys Trp Lys Asp Lys Arg Glu Lys Leu Glu Phe Glu Thr Asn Ile
 885 890 895
 Val Tyr Asn Glu Ala Lys Glu Ala Val Asp Ala Leu Phe Val Asn Ser
 900 905 910

pct-us2017-055731 Sequence listing

Gln Tyr Asp Arg Leu Gln Ala Asp Thr Asn Ile Ala Met Ile His Ala
 915 920 925
 Ala Asp Asn Lys Val His Lys Ile Arg Glu Ala Tyr Leu Pro Glu Leu
 930 935 940
 Ser Val Ile Pro Gly Val Asn Ala Thr Val Phe Glu Glu Leu Glu Glu
 945 950 955 960
 Arg Ile Phe Thr Ala Phe Ser Leu Tyr Asp Ala Arg Asn Val Ile Lys
 965 970 975
 Asn Gly Asp Phe Asn Asn Gly Leu Ser Cys Trp Asn Val Lys Gly Gln
 980 985 990
 Val Asp Val Asp Gln Asn Asp His Arg Ser Val Leu Val Ile Pro Gly
 995 1000 1005
 Trp Glu Ser Glu Val Ser Gln Glu Val His Val Cys Pro Asp Arg
 1010 1015 1020
 Gly Tyr Ile Leu Arg Val Thr Ala Tyr Lys Glu Gly Tyr Gly Glu
 1025 1030 1035
 Gly Cys Val Thr Ile His Glu Ile Asp Asn His Thr Asp Glu Leu
 1040 1045 1050
 Lys Phe Lys Asn Cys Phe Glu Glu Glu Val Ser Leu Asn Asn Ala
 1055 1060 1065
 Val Thr Cys Asp Glu Tyr Thr Thr Asn Gln Glu Val Gly Gly Tyr
 1070 1075 1080
 Ala Asp Val Arg Gln Ser Asn Asn Arg Gly Ser Asn Glu Ala Tyr
 1085 1090 1095
 Val Asn Pro Thr Ser Thr Ser Thr Asp Tyr Ala Ser Leu Tyr Glu
 1100 1105 1110
 Glu Glu Ser Tyr Thr Asn Glu Gln Thr Tyr Asn Ser Cys Glu Ser
 1115 1120 1125
 Asn Arg Gly Tyr Gly Asn Gln Met Pro Leu Pro Ser Gly Tyr Val
 1130 1135 1140
 Thr Lys Glu Leu Glu Tyr Phe Pro Glu Thr Asp Lys Val Trp Ile
 1145 1150 1155
 Glu Ile Gly Glu Thr Glu Gly Thr Phe Ile Val Asp Ser Val Glu
 1160 1165 1170