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(54) Title: NOVEL BACILLUS THURINGIENSIS CRYSTAL POLYPEPTIDES, POLYNUCLEOTIDES, AND COMPOSITIONS THEREOF

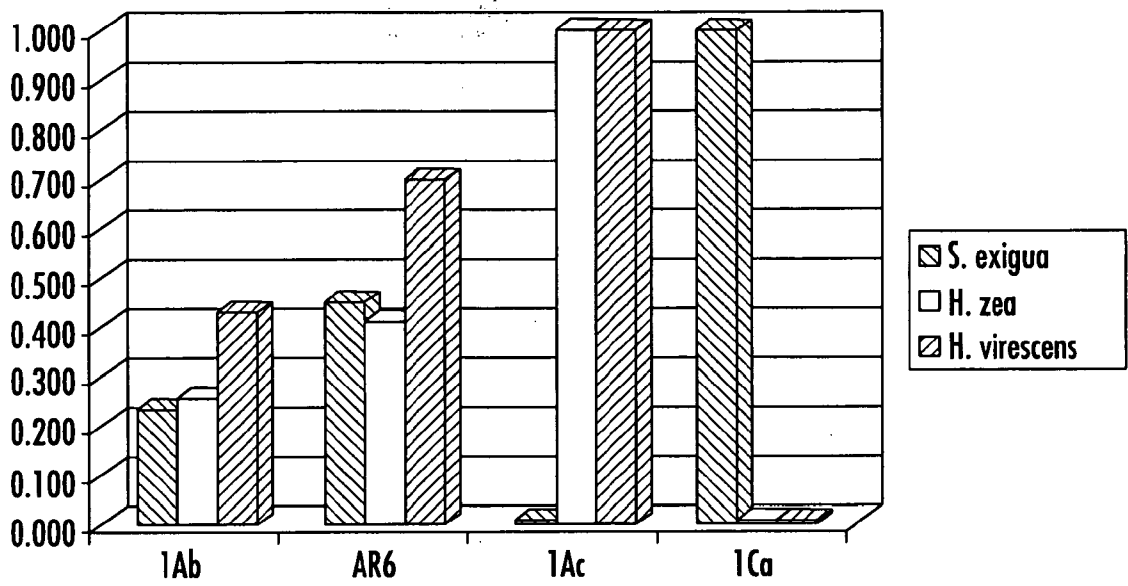


FIG. 2

(57) Abstract: The present invention provides insecticidal polypeptides related to shuffled Bacillus thuringiensis Cry1 polypeptides. Nucleic acids encoding the polypeptides of the invention are also provided. Methods for using the polypeptides and nucleic acids of the invention to enhance resistance of plants to insect predation are encompassed.

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1. An isolated nucleic acid molecule comprising a nucleotide sequence that is at least 99% homologous to the full nucleotide sequence of any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or a complement thereof, wherein said nucleic acid molecule encodes a first polypeptide, said first polypeptide having insecticidal activity.
5
2. The nucleic acid molecule of claim 1 which comprises the nucleotide sequence of any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or a complement thereof.
10
3. (cancelled)
4. The nucleic acid molecule of claim 1 further comprising additional nucleotides, said additional nucleotides encoding additional amino acids, said additional amino acids expressed in conjunction with said first polypeptide to create a protoxin.
15
5. The nucleic acid molecule of claim 4 wherein said additional amino acids are separated from said first polypeptide in an insect.
20
6. A vector comprising at least one nucleic acid molecule of claim 1.
7. An expression cassette comprising at least one nucleotide of claim 1 operably linked to a promoter, wherein the polynucleotide is in a sense orientation.
25
8. A host cell into which is successfully introduced at least one expression cassette of claim 7.

9. The host cell of claim 8 that is a plant cell.
10. A transgenic plant comprising at least one expression cassette of claim 7.
- 5 11. The transgenic plant of claim 10, wherein the plant is rice, wheat, sugarcane, sorghum, maize, cotton, soybean, alfalfa, spinach, tobacco, tomato, potato, sunflower, canola, barley or millet.
- 10 12. A seed from the transgenic plant of claim 10, wherein the seed includes at least one nucleotide of claim 1.
13. The seed of claim 12, wherein the seed is rice, wheat, sugarcane, sorghum, corn, cotton, soybean, alfalfa, spinach, tobacco, tomato, potato, sunflower, canola, barley or millet.
- 15 14. (cancelled)
15. A nucleic acid molecule comprising a nucleotide sequence that has undergone shuffling and is at least 90% homologous to the full nucleotide sequence of any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or a complement thereof, wherein said nucleic acid molecule encodes a first polypeptide, said first polypeptide having insecticidal activity.
- 20 25 16. The nucleic acid molecule of claim 15 that comprises the nucleotide sequence of any of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or a complement thereof.

17. (cancelled)
18. The nucleic acid molecule of claim 15 further comprising additional nucleotides, said additional nucleotides encoding additional amino acids, said additional amino acids expressed in conjunction with said first polypeptide to create a protoxin.
19. The nucleic acid molecule of claim 18 wherein said additional amino acids are separated from said first polypeptide in an insect.
20. A vector comprising a nucleic acid molecule of claim 15.
21. A polypeptide comprising a first polypeptide sequence that is at least 99% homologous to the full polypeptide sequence of any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28.
22. (cancelled)
23. The polypeptide of claim 21 further comprising additional amino acids, said additional amino acids expressed in conjunction with said first polypeptide to create a protoxin.
24. The polypeptide of claim 23 wherein said additional amino acids are separated from said first polypeptide in an insect.
25. A polypeptide comprising a first polypeptide sequence that is at least 90% homologous to the polypeptide sequence of any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28, wherein said

polypeptide has insecticidal activity.

26. (cancelled)
- 5 27. The polypeptide of claim 25 further comprising additional amino acids, said additional amino acids expressed in conjunction with said first polypeptide to create a protoxin.
28. The polypeptide of claim 27 wherein said additional amino acids are
10 separated from said first polypeptide in an insect.
29. A transgenic plant comprising a transgene that expresses at least one of
- a. a nucleic acid molecule of any one of SEQ ID NOS: 1, 3, 5,
7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27 or a complement thereof, or
- 15 b. a polypeptide of any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14,
16, 18, 20, 22, 24, 26, or 28, wherein the transgenic plant has increased
resistance to a Lepidopteran insect pest as compared to a plant that is
not transgenic.
- 20 30. The transgenic plant of claim 29, wherein the plant is selected from the
group consisting of maize, soybean, rice, canola, potato, cotton, and
sunflower.
31. (cancelled)
- 25 32. A method for producing a plant with increased insect resistance, the
method comprising:
- a. introducing into plant cells a construct comprising a
polynucleotide encoding an insecticidal polypeptide operably linked to

a promoter functional in plant cells to yield transformed plant cells, and wherein the polynucleotide encoding the insecticidal polypeptide is selected from the group consisting of:

- 5 i. a polynucleotide that encodes the polypeptide of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28;
 - ii. a polynucleotide comprising SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
 - iii. an isolated polynucleotide degenerate from any of (i) to (ii) as a result of the genetic code;
 - 10 iv. a polynucleotide complimentary to a polynucleotide of any one of (i) to (iii);
 - b. regenerating a transgenic plant from said transformed plant cells, wherein said an insect resistance polypeptide when expressed at levels sufficient to increase insect resistance in said transgenic plant as
15 compared to a control plant.
33. The method of claim 32, wherein insecticidal activity is increased as compared to a control plant, wherein the control plant does not contain the polynucleotide encoding the insect resistance polypeptide.
- 20 34. The method of claim 32, wherein said polynucleotide encoding the polypeptide is constitutively expressed.
35. The method of claim 32, wherein the plant is a dicotyledonous plant.
- 25 36. The method of claim 32, wherein the plant is a monocotyledonous plant.

37. An isolated polynucleotide comprising a member selected from the group consisting of:
- a. a polynucleotide that encodes any of the polypeptides set forth in SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28;
 - b. a polynucleotide comprising any of the sequences of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
 - c. a polynucleotide comprising at least 30 nucleotides in length which hybridizes under stringent conditions to a polynucleotide of (a) or (b), wherein the conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C and a wash in 0.5 X to 1 X SSC at 55 to 60°C;
 - d. a shuffled polynucleotide having at least 90% sequence identity to any of the sequences of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27, wherein the % sequence identity is based on the entire encoding region and is determined by BLAST 2.0 under default parameters;
 - e. a polynucleotide having at least 99% sequence identity to any of the sequences of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27, wherein the % sequence identity is based on the entire encoding region and is determined by BLAST 2.0 under default parameters;
 - f. an isolated polynucleotide degenerate from any of (a) to (e) as a result of the genetic code; and
 - g. a polynucleotide complimentary to a polynucleotide of any one of (a) to (c).
38. A recombinant expression cassette comprising a polynucleotide operably linked to a promoter, wherein the polynucleotide encodes the

polypeptide of claim 37.

39. An isolated polypeptide selected from the group consisting of:
- a. an isolated polypeptide comprising any one of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28, said polypeptide having insect resistance activity;
 - b. a polypeptide that is at least 99% identical to the amino acid sequence of any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28, said polypeptide having insect resistance activity;
 - c. a shuffled polypeptide that is at least 90% identical to the amino acid sequence of any of SEQ ID NOS: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, or 28, said shuffled polypeptide having insect resistance activity;
 - d. a polypeptide that is encoded by a nucleic acid molecule comprising a nucleotide sequence that is at least 99% identical to any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27, or a complement thereof, said polypeptide having insect resistance activity;
 - e. a shuffled polypeptide that is encoded by a nucleic acid molecule comprising a shuffled nucleotide sequence that is at least 90% identical to any one of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27, or a complement thereof, said shuffled polypeptide having insect resistance activity;
40. A transformed host cell comprising the isolated polypeptide of claim 39.
41. The host cell of claim 40, wherein the host cell is a transformed plant cell.

42. The plant cell of claim 41, wherein the plant cell is a rice, wheat, sugarcane, sorghum, maize, cotton, soybean, alfalfa, spinach, tobacco, tomato, potato, sunflower, canola, barley or millet cell.
- 5 43. A transformed plant regenerated from the plant cell of claim 41.
44. The plant of claim 43, wherein the plant is rice, wheat, sugarcane, sorghum, corn, cotton, soybean, alfalfa, spinach, tobacco, tomato, potato, sunflower, canola, barley or millet plant.
- 10 45. A transformed seed of the plant of claim 43, wherein the seed includes at least one nucleotide of claim 39.