express at optimal level. Moreover, the A/T rich region may also contain transcriptional termination sites (AATAAA polyadenylation), mRNA instability motif (ATTTA) and cryptic mRNA splicing sites. It has been observed that the codon usage of a native Bt cry gene is significantly different from that of a plant gene. As a result, the mRNA from this gene may not be efficiently utilized. Codon usage might influence the expression of genes at the level of translation or transcription or mRNA processing. To optimize an insecticidal gene for expression in plants, attempts have been made to alter the gene to resemble, as much as possible, genes naturally contained within the host plant to be transformed.

[0010] However, development of crop plant varieties expressing high/optimum level of Bt cry protein conferring resistance to certain insect pests is still a major problem in agricultural field. Increased expression of insect-control protein genes has been critical to the development of genetically improved plants with agronomically acceptable levels of insect resistance. Various attempts to control or prevent insect infestation of crop plants have been made, yet certain insect pests remain to be a significant problem in agriculture. Therefore, there remains a need for insect resistant transgenic crop plants with desired expression levels of insecticidal proteins in the transgenic plants.

[0011] The present invention provides herein a solution to the existing problem of insect pest infestation by providing plant codon optimized synthetic DNA sequence encoding Bt Cry2Ai protein having insecticidal activity against insect pests.

SUMMARY

[0012] Disclosed herein are codon optimised synthetic nucleotide sequences encoding *B. thuringiensis* protein having insecticidal activity against insect pests. The disclosure is drawn to methods for enhancing expression of heterologous genes in plant cells. A gene or coding region of *cry2Ai* gene is constructed to provide a plant specific preferred codon sequence. In this manner, codon usage for a Cry2Ai protein is altered for expression in a plant. Such plant optimized coding sequences can be operably linked to promoter capable of directing expression of the coding sequences in a plant cell. Transformed host cells and transgenic plants comprising the codon optimised *B. thuringiensis* synthetic nucleotide sequences are also aspects of the present disclosure.

[0013] One of the examples of the present disclosure refers to codon optimized synthetic nucleotide sequences encoding insecticidal protein, wherein the nucleotide sequences have been optimized for expression in plants.

[0014] Another example of the present disclosure refers to codon optimized nucleotide sequences encoding insecticidal Bt protein to maximize the expression of Bt proteins in a plant, preferably in a plant selected from a group consisting of eggplant, cotton, rice, tomato, wheat, corn, sorghum, oat, millet, legume, cabbage, cauliflower, broccoli, Brassica sp., beans, pea, pigeon-pea, potato, pepper, cucurbit, lettuce, sweet potato canola, soybean, alfalfa, peanuts, and sunflower.

[0015] According to the present disclosure, the inventors have synthesized Bt insecticidal *Cry2Ai* crystal protein genes in which the codon usage has been altered in order to increase expression in plant. However, rather than alter the codon usage to resemble plant gene in terms of overall codon distribution, the inventors have optimized the codon usage by using the codons which are most preferred in plants in the synthesis of the nucleotide sequences of the disclosure. The optimized plant preferred codon usage is effective for expression of high level of the Bt insecticidal protein in dicot plants such as cotton, eggplant and tomato; in monocots such as rice and in legumes such as chickpea and pigeon pea.

[0016] The codon optimized synthetic nucleotide sequences of the present disclosure have been derived from the *Bacillus thuringiensis* Cry2Ai protein having amino acid sequence as set forth in SEQ ID NO: 1 (NCBI GenBank: ACV97158.1). The protein having amino acid sequence as set forth in SEQ ID NO: 1 is active against various lepidopteran insects, including *Helicoverpa armigera* -the cotton bollworm and corn earworm, *Cnaphalocrocis medinalis* -the rice leaffolder, and *Scirpophaga incertulas* -the rice yellow stem borer and *Pectinophora gossypiella*.

[0017] While the present disclosure has been exemplified by the synthesis of codon optimized Bt *cry2Ai* nucleotides for expression in plants. It is recognized that the codon optimized Bt *cry2Ai* nucleotides can be utilized to optimize expression of the protein in plants such as cotton, eggplant, tomato, rice, and maize.

[0018] Accordingly one aspect of present disclosure refers to a codon optimized synthetic nucleotide sequence encoding protein having amino acid sequence as set forth in SEQ ID NO: 1, wherein said nucleotide sequence is selected from a group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

[0019] In one example, the present disclosure refers to a nucleic acid molecule comprising a codon optimized sequence for expression in a plant selected from the group consisting of: (a) the nucleotide sequence as set forth in SEQ ID NO: 2; (b) a nucleotide sequence which specifically hybridizes to at least 10 nucleotides of the nucleotide sequence as set forth in SEQ ID NO: 2 from nucleotide position 262 to 402 and/or 1471 to 1631; and (c) a nucleotide sequence complementary to the nucleotide sequence of (a) and b).

[0020] Another example of the present disclosure refers to a codon optimized synthetic nucleotide sequence encoding protein having the amino acid sequence as set forth in SEQ ID NO: 1, wherein said nucleotide sequence is

a. as set forth in SEQ ID NO: 2, or a nucleotide sequence complementary thereto; or

b. a nucleotide sequence which specifically hybridizes to at least 10 nucleotides of the nucleotide sequence as set forth in SEQ ID NO: 2 from nucleotide position 262 to 402 and/or 1471 to 1631 or a nucleotide sequence complementary thereto

[0021] Another aspect of the present disclosure refers to a recombinant DNA comprising the codon optimized synthetic nucleotide sequence as disclosed herein, wherein the nucleotide sequence is operably linked to a heterologous regulatory element.

[0022] Another aspect of the present disclosure refers to a DNA construct for expression of an insecticidal protein of interest comprising a 5' non-translated sequence, a coding sequence encoding an insecticidal Cry2Ai protein comprising the amino acid sequence of SEQ ID NO: 1 or an insecticidal portion thereof, and a 3' non-translated region, wherein said 5' non-translated sequence comprises a promoter functional in a plant cell, said coding sequence is a codon optimized synthetic nucleotide sequence as disclosed herein, and wherein said 3' non-translated sequence comprises a transcription termination sequence and a polyadenylation signal.

[0023] Another aspect of the present disclosure refers to a plasmid vector comprising the recombinant DNA disclosed herein, or the DNA construct as disclosed herein.

[0024] Another aspect of the present disclosure refers to a host cell comprising the codon optimized synthetic nucleotide sequence as disclosed herein.

[0025] Another aspect of the present disclosure refers to a method for conferring an insect resistance in a plant comprising

(a) inserting into a plant cell a codon optimized synthetic nucleotide sequence as disclosed herein, wherein the nucleotide sequence is operably linked to a (i) promoter functional in a plant cell and (ii) a terminator;

(b) obtaining a transformed plant cell from the plant cell of step (a), wherein said transformed plant cell comprises the said codon optimized synthetic nucleotide sequence as disclosed herein; and

(c) generating a transgenic plant from said transformed plant cell of step (b), wherein said transgenic plant comprises the said codon optimized synthetic nucleotide sequence as disclosed herein.

[0026] Another aspect of the present disclosure refers to a transgenic plant obtained by the method as disclosed herein. Yet another aspect of the present disclosure refers to a transgenic plant comprising the codon optimized synthetic nucleotide sequence as disclosed herein. A further aspect of the present disclosure refers to a tissue, seed or a progeny obtained from the transgenic plant as disclosed herein, wherein said seed or progeny comprises the codon optimized synthetic nucleotide sequence as disclosed herein. In another aspect, the present disclosure refers to a biological sample derived from the tissues or seed or progeny as disclosed herein, wherein said sample comprising a detectable amount of said codon optimized synthetic nucleotide sequence as disclosed herein.

[0027] Another aspect of the present disclosure refers to a composition comprising *Bacillus thuringiensis* comprising the codon optimized synthetic nucleotide sequence as disclosed herein encoding Cry2Ai protein having amino acid sequence as set forth in SEQ ID NO: 1.

[0028] Another aspect of the present disclosure refers to a method of controlling insect infestation in a crop plant and providing insect resistance management, wherein said method comprising contacting said crop plant with an insecticidally effective amount of the composition as disclosed herein.

[0029] Yet another aspect of the present disclosure is use of the codon optimized synthetic nucleotide sequence, the DNA construct or the plasmid as disclosed herein for production of insect resistant transgenic plants.

[0030] Yet another aspect of the present disclosure is use of the codon optimized synthetic nucleotide sequence as disclosed herein for production of insecticidal composition, wherein the composition comprises *Bacillus thuringiensis* cells comprising the said nucleotide sequences.

BRIEF DESCRIPTION OF THE ACCOMPANYING FIGURES

[0031] FIGURE 1shows T-DNA construct map of pGreen0029-CaMV35S-201D1.

[0032] FIGURE 2 shows genetic transformation and regeneration of transgenic cotton plants.

BRIEF DESCRIPTION OF THE SEQUENCES

[0033] SEQ ID NO: 1 is amino acid sequence of Cry2Ai protein (NCBI GenBank: ACV97158.1).

[0034] SEQ ID NO: 2 is a codon optimized synthetic *cry2Ai* nucleotide sequence (201D1) encoding the Cry2Ai protein (SEQ ID NO: 1).

[0035] SEQ ID NO: 3 is a codon optimized synthetic *cry2Ai* nucleotide sequence (201D2) encoding the Cry2Ai protein (SEQ ID NO: 1).

[0036] SEQ ID NO: 4 is a codon optimized synthetic *cry2Ai* nucleotide sequence (201D3) encoding the Cry2Ai protein (SEQ ID NO: 1).

We Claim:

1. A codon optimized synthetic nucleotide sequence encoding protein having amino acid sequence as set forth in SEQ ID NO: 1, wherein said nucleotide sequence is selected from a group consisting of SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6.

2. A recombinant DNA comprising the codon optimized synthetic nucleotide sequence as claimed in claim 1, wherein the nucleotide sequence is operably linked to a heterologous regulatory element.

3. The recombinant DNA as claimed in claim 2, wherein said codon optimized synthetic nucleotide sequence optionally comprises a selectable marker gene, a reporter gene or a combination thereof.

4. The recombinant DNA as claimed in claim 2, wherein said codon optimized synthetic nucleotide sequence optionally comprises a DNA sequence encoding a targeting or transit peptide for targeting to the vacuole, mitochondrium, chloroplast, plastid, or for secretion.

5. A DNA construct for expression of an insecticidal protein in plant comprising a 5' non-translated sequence, a coding sequence encoding an insecticidal Cry2Ai protein comprising the amino acid sequence of SEQ ID NO: 1 or an insecticidal portion thereof, and a 3' non-translated region, wherein said 5' non-translated sequence comprises a promoter functional in a plant cell, said coding sequence is a codon optimized synthetic nucleotide sequence as claimed in claim 1, and wherein said 3' non-translated sequence comprises a transcription termination sequence and a polyadenylation signal.

6. A plasmid vector comprising the codon optimized synthetic nucleotide sequence as claimed in claim 1.

7. A host cell comprising the codon optimized synthetic nucleotide sequence as claimed in claim 1.

8. The host cell as claimed in claim 7, wherein said host cell is a plant, bacterial, virus, fungi or a yeast cell.

9. The host cell as claimed in claim 8, wherein said bacterial cell is *Agrobacterium or E. coli.*

10. A method for conferring an insect resistance in a plant comprising

    (a) inserting into a plant cell a codon optimized synthetic nucleotide sequence as claimed in claim 1, wherein the nucleotide sequence is operably linked to a (i) promoter functional in a plant cell and (ii) a terminator;

    (b) obtaining a transformed plant cell from the plant cell of step (a), wherein said transformed plant cell comprises the said codon optimized synthetic nucleotide sequence of claim 1; and

    (c) generating a transgenic plant from said transformed plant cell of step (b), wherein said transgenic plant comprises the said codon optimized synthetic nucleotide sequence of claim 1.

11. A transgenic plant obtained by the method as claimed in claim 10.

12. A transgenic plant comprising the codon optimized synthetic nucleotide sequence as claimed in claim 1.

13. The transgenic plant as claimed in claim 11 or 12,wherein said plant is selected from a group consisting of rice, wheat, corn, sorghum, oat, millet, legume, cotton, tomato, eggplant, cabbage, cauliflower, broccoli, Brassica sp., beans, pea, pigeonpea, potato, pepper, cucurbit, lettuce, sweet potato canola, soybean, alfalfa, peanuts, sunflower, safflower, tobacco, sugarcane , cassava , coffee, pineapple, citrus, cocoa, tea , banana and melon.

14. A tissue, seed or a progeny obtained from the transgenic plant as claimed in claim 11 or 12, wherein said seed or progeny comprises the codon optimized synthetic nucleotide sequence as claimed in claim 1.

15. A biological sample derived from the tissues or seed or progeny as claimed in claim 14, wherein said sample comprising a detectable amount of said codon optimized synthetic nucleotide sequence as claimed in claim 1.

16. A composition comprising *Bacillus thuringiensis* comprising the codon optimized synthetic nucleotide sequence as claimed in claim 1 encoding Cry2Ai protein having amino acid sequence as set forth in SEQ ID NO: 1.

17. The composition as claimed in claim 16, wherein said composition optionally comprises an additional insecticidal agent toxic to same insect pest but exhibiting a different mode of its insecticidal activity from said insecticidal protein.

18. The composition as claimed in claim 17, wherein said insecticidal agent is selected from the group consisting of a *Bacillus* toxin, a *Xenorhabdus* toxin, a *Photorhabdus* toxin, and a dsRNA specific for suppression of one or more essential genes in said insect pest.

19. A method of controlling insect infestation in a crop plant and providing insect resistance management, wherein said method comprising contacting said crop plant with an insecticidally effective amount of the composition as claimed in claim 16.

20. Use of the codon optimized synthetic nucleotide sequence as claimed in claim 1 for production of insect resistant transgenic plants.

21. Use of the codon optimized synthetic nucleotide sequence as claimed in claim 1 for production of insecticidal composition, wherein the composition comprises *Bacillus thuringiensis* cells comprising the said nucleotide sequences.