combinations with various promoters functional in plants. However, only low levels of protein have been obtained in transgenic plants.

[009] One of the reasons for low level expression of the Bt cry gene in plant is high A/T content in Bt DNA sequence than plant genes in which G/C ratio is higher than A/T. The overall value of A/T for bacterial genes is 60-70% and plant genes with 40-50%. As a consequence, GC ratio in cry genes codon usage is significantly insufficient to 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 such as rice leaf folder, rice yellow stem is still a major problem in agricultural field. 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 including, but not limited to, insect pests belonging to the order Lepidoptera.

SUMMARY

[0012] Disclosed herein are codon optimised synthetic nucleotide sequences encoding *B. thuringiensis* Cry2Ai 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 to increase 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 objects of the present disclosure is to provide codon optimized synthetic nucleotide sequences encoding insecticidal protein, wherein the nucleotide sequences have been optimized for expression in plants.

[0014] It is another object of the present disclosure to provide 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. It is one feature of the present disclosure that codon optimized synthetic Bt *cry2Ai* genes are constructed using the most preferred eggplant, rice and legumes codons.

[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 plants, particularly in eggplant, rice, and legumes. However, rather than alter the codon usage to resemble a eggplant, rice or legumes gene in terms of overall codon distribution, the inventors have optimized the codon usage by using the codons which are most preferred in plants such as rice, eggplant, tomato, maize and legumes 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 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 eggplant, rice and legumes optimized Bt *cry2Ai* nucleotides for expression in rice, eggplant, and chickpea respectively, it is recognized that the eggplant optimized Bt *cry2Ai* nucleotides can be utilized to optimize expression of the protein in other plants such as tomato, maize and cotton as well. Further, it is recognized that the rice optimized Bt *cry2Ai* nucleotides can be utilized to optimize expression of the protein in other plants as well. Similarly it is recognized that the legume optimized Bt *cry2Ai* nucleotides can be utilized to optimize expression of the protein in other plants as well.

[0018] Accordingly, one of the aspects of the present disclosure is to provide 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 under high stringency conditions to at least 10 nucleotides of the nucleotide sequence as set forth in SEQ ID NO: 2 from nucleotide position 251 to 402 and/or 1456 to, selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5. SEQ ID NO: 6, and SEQ ID NO: 7.

[0019] Another aspect of the present disclosure is to provide 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.

I/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

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

    b. a nucleotide sequence which specifically hybridizes under high stringency conditions to at least 10 nucleotides of the nucleotide sequence as set forth in SEQ ID NO: 2 from nucleotide position 251 to 402 and/or 1456 to 1628, selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5. SEQ ID NO: 6, and SEQ ID NO: 7.

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 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 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 recombinant DNA as claimed in claim 3, or the DNA construct as claimed in claim 5.

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 comprising the codon optimized synthetic nucleotide sequence as claimed in claim 1.

12. The transgenic plant as claimed in claim 11, 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.

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

14. 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.

15. The composition as claimed in claim 14, 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.

16. The composition as claimed in claim 15, 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.

17. A method of controlling insect infestation in a crop plant and providing insect resistance management, wherein said method comprises contacting said crop plant with a insecticidally effective amount of the composition as claimed in claim 14.

18. Use of the codon optimized synthetic nucleotide sequence as claimed in claim 1, the DNA construct as claimed in claim 5 or the plasmid as claimed in claim 6 for production of insect resistant transgenic plants.

19. 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.