FORM 2

THE PATENTS ACT, 1970
(39 of 1970)
&
THE PATENTS RULES, 2003

COMPLETE SPECIFICATION
(See section 10, rule 13)

***1. Title of the invention*:** INSECT RESISTANT TRANSGENIC CAULIFLOWER PLANT COMPRISING EVENT CFE-4 AND METHODS OF DETECTION THEREOF

2. Applicant(s)

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3. Preamble to the description

COMPLETE SPECIFICATION

The following specification particularly describes the invention and the manner in which it is to be performed.

FIELD OF INVENTION

[0001] The present disclosure relates to transgenic cauliflower event CFE-4, comprising *cry1Ac* gene. The transgenic cauliflower event CFE-4 confers resistance against lepidopteran insects. The invention provides polynucleotide molecules and sequences that are unique to this event. The invention also relates to insect resistant transgenic plants and methods for detecting the presence of said cauliflower event in a cauliflower plant, parts thereof by detection of specific polynucleotide molecules that are unique to the transgenic event.

BACKGROUND OF THE INVENTION

[0002] Cauliflower is a popular and widely consumed vegetable that has its origin in the Mediterranean coast. India is one of the major cauliflower producing countries (FAOSTAT 2010, http://faostat.fao.org/site/339/default.aspx). Cauliflower is grown for its tender white head (curd) formed by flower parts. Cauliflower curds are mainly consumed in various forms after cooking. The cauliflower vegetable is a good source of vitamin B, vitamin C, vitamin A, minerals and proteins. (Salunkhe *et al*., *Postharvest Biotechnology of Fruits*, 1984, Vol.2, CRC Press, Boca Raton, FL, USA).

[0003] Cauliflower production suffers from various insect infestations during different growth stages. The predominant insect pest species attacking the crop is *Plutella xylostella* L., commonly known as diamondback moth (DBM). The larvae of the DBM feed on leaves, buds, flowers, green pods and stems causing extensive damage. The management of DBM is generally through insecticide sprays, which over time leads to development of resistance against the insecticide and pollution of the environment. Farmers use large quantities of chemical insecticides routinely. It has been reported that the damage caused by DBM results in an annual loss of $16 million US dollars in India (Mohan *et al*., *Crop* Protection, 2003, *22*, 495-504). In India, losses in yield of cabbage and cauliflower due to DBM can reach up to 90% if no chemicals are used. In contrast this number is reduced to 35% when chemicals are used. The cost of insecticides accounts for one-third of the total input costs and just under one-fourth of total cost of

cultivation (Sandur S. *Consultant Report for the Center for Environmental Stress and Adaptation Research. LaTrobe University, Victoria, Australia*, 2004, 31pp). Farmers use large quantities of chemical insecticides singly or in combination to get blemish free cauliflower head, which fetch premium prices in the market. This practice of indiscriminate use of insecticides leads to the build-up of insecticide residues in the agricultural product, local ecological imbalance, pest resurgence due to resistance and environmental pollution.

[0004] To reduce pest-linked damage in cauliflower crop as well as to protect the environment from the adverse effects of insecticides, deploying the lepidopteran specific *cry1Ac* gene under the control of a suitable promoter for high level of expression in cauliflower would provide an effective built-in mechanism for pest control. This would result in reduction of cauliflower cultivation costs, as the contribution of chemical insecticides in cauliflower cultivation is sizable. Efforts to control this pest solely through conventional insecticides have led to development of resistance in the pests to most of the insecticides available in India (Singh *et al*., *Impact of Vegetable Research in India, Proceedings 13,* March, 2002). Management of DBM by integrated pest management using Bt crystal protein for spraying externally has been tested successfully. These Bt formulations are effective in target pest control, but the formulations get washed out in rain and get degraded when exposed to sunlight. Current control methods for DBM and other brassica insect pests and the prospects for improved management with lepidopteran-resistant Bt vegetable brassicas in Asia and Africa (Grzywacz *et al*., *Crop Protection*, 2010, *29*, 68-79). An alternative to external application is to develop transgenic cauliflower expressing Bt genes for specific insect resistance. A number of groups have carried out successful transformation of cauliflower using *Agrobacterium*-mediated methods for transformation (Chakrabarty *et al*., *J Biosci*., 2002, *27*, 495-502). The source organism for *cry1Ac* gene is *Bacillus thuringiensis* (Bt), which is a gram-positive bacterium that synthesizes insecticidal crystalline (Cry) inclusions during sporulation. The *cry1Ac* gene encodes the 130kDa Cry1Ac protein and is highly specific and toxic to Lepidpoteran larvae. Cry1Ac protein

containing leaves when ingested by pests causes paralysis of the insect gut and subsequent death due to starvation. Bt protein does not affect non target organisms (Birds, mammals etc) as they lack receptors or have a acidic pH where Bt proteins do not work.

5 **[0005]** The expression of a foreign gene in plants is known to be influenced by the location of the transgene in the genome of the plant. Variations in transgene expression occur due to insertion of the transgene into different chromatin regions, which may be more (euchromatin) or less (heterochromatin) transcriptionally active. Examples of these are methylated regions in which, gene expression is suppressed, or in the 10 proximity transcriptional regulation elements like enhancers and suppressors, which increase or decrease gene expression respectively. Therefore, it is necessary to screen a large number of independent transformation events for the expression of the transgene and to identify the event showing desired expression characteristics of the heterologous inserted gene (Chakrabarty *et al*., *J Biosci*., 2002, 27, 495-502).

15 **[0006]** US 20100003269 discloses a method of generation of transgenic *Brassica oleracea* plants. The invention also provides a method for the production of heterologous proteins using a cruciferae-based plant system, for example pharmaceutical and/or recombinant proteins. In addition, the invention also relates to a method for the production of transgenic collard and cauliflower, and to the large scale 20 production of pharmaceutical and/or therapeutic production, such as production of cruciferae-based vaccine production however, the invention does not disclose event development and its identification.

SUMMARY OF INVENTION

25 **[0007]** An aspect of the present disclosure relates to a method of detecting cauliflower event CFE-4 event in a biological sample, said method comprising of (a) obtaining a biological sample comprising of cauliflower DNA; (b) contacting said biological sample with a first DNA primer and a second DNA primer, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule; and (d) detecting the

presence of said DNA amplicon molecule, wherein the detection of presence of said DNA amplicon molecule is diagnostic for said CFE-4 event.

[0008] An aspect of the present disclosure relates to a method of determining the zygosity of a transgenic cauliflower plant comprising of event CFE-4, said method comprising: (a) obtaining a biological sample comprising of cauliflower DNA; (b) contacting said biological sample with a first DNA primer, a second DNA primer, and a third DNA primer, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule; and (d) detecting the presence of said DNA amplicon molecule, wherein detection of presence of more than one DNA amplicon having different nucleotide sequences is indicative of heterozygosity of the transgenic cauliflower event CFE-4, while detection of presence of one or more DNA amplicon with identical nucleotide sequence is indicative of homozygosity of the transgenic event CFE-4.

[0009] Another aspect of the present disclosure relates to synthetic DNA molecules comprising of any of the DNA amplicons obtained from a method of detecting cauliflower event CFE-4 event in a biological sample, said method comprising of (a) obtaining a biological sample comprising of cauliflower DNA; (b) contacting said biological sample with a first DNA primer and a second DNA primer, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule; and (d) detecting the presence of said DNA amplicon molecule, wherein the detection of presence of said DNA amplicon molecule is diagnostic for said CFE-4 event.

[00010] Yet another aspect of the present disclosure relates to a kit for detection of transgenic cauliflower event CFE-4 comprising of forward and reverse primers having at least 10 contiguous nucleotides selected from the group of sequences consisting of SEQ ID NO 14, SEQ ID NO: 22, SEQ ID NO: 15, and SEQ ID NO: 27.

BRIEF DESCRIPTION OF ACCOMPANYING DRAWINGS

[00011] The following drawings form part of the present specification and are included to further illustrate embodiments of the present invention. The invention may be better

understood by reference to the drawings in combination with the detailed description of the specific embodiments presented herein.

[00012] Figure 1 shows T-DNA elements of the construct used for transformation (pC2300ve10518c), in accordance with an embodiment of the present disclosure. Directional arrows represent the direction of transcription.

[00013] Figure 2 shows the location and orientation of primers used to detect the presence of event CFE-4, and zygosity, in accordance with an embodiment of the present disclosure. Numbers denote the sequence identification number (SEQ ID NO:). Lines represent the approximate span of the nucleotide sequence encoded by the corresponding sequence. Uni-directional arrows represent the location and direction of DNA primers. Adapters used for identification and analysis of transformants are represented as boxes at each end.

[00014] Figure 3 shows the agarose gel image of the CFE-4 event using CFE-4 event specific primers, in accordance with an embodiment of the present disclosure.

DETAILED DISCRIPTION OF THE INVENTION

[00015] Those skilled in the art will be aware that the invention described herein is subject to variations and modifications other than those specifically described. It is to be understood that the invention described herein includes all such variations and modifications. The invention also includes all such steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any or more of such steps or features.

Definitions

[00016] For convenience, before further description of the present invention, certain terms employed in the specification, example and appended claims are collected here. These definitions should be read in the light of the remainder of the disclosure and understood as by a person of skill in the art. The terms used herein have the meanings recognized and known to those of skill in the art, however, for convenience and completeness, particular terms and their meanings are set forth below.

[00017] The articles “a”, “an” and “the” are used to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article.

[00018] The term “plurality” means more than one.

[00019] The terms “at least two”, “more than one” and “plurality” are used
5 interchangeably.

[00020] The terms “comprise” and “comprising” are used in the inclusive, open sense, meaning that additional elements may be included. It is not intended to be construed as “consists of only.

[00021] Throughout this specification, unless the context requires otherwise the word
10 “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated element or step or group of element or steps but not the exclusion of any other element or step or group of element or steps. The term “including” is used to mean “including but not limited to”. “Including” and “including but not limited to” are used interchangeably.

15 **[00022]** The term “heterologous Gene/DNA” refers to DNA sequence of foreign origin inserted into the plant genome.

[00023] A "junction" is where one end of the transgenic DNA has inserted into the host genomic DNA. A junction spans a portion of the inserted transgenic DNA and the adjacent flanking cauliflower genomic DNA and as such comprises the connection
20 point of these two as one contiguous sequence. One junction is at the 5' end of the inserted transgenic DNA and the other one at the 3' end of the inserted transgenic DNA. A "junction sequence" or "junction region" refers to the DNA sequence and/or corresponding DNA molecule of the junction.

[00024] A transgenic "event" as described in the present disclosure is produced by
25 transformation of plant cells with heterologous DNA (a nucleic acid construct that includes a transgene of interest), regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and selection of a particular plant characterized by insertion into a particular genome location. The term "event" refers to the original transformant and progeny of the transformant that include the

heterologous DNA. The term "event" also refers to progeny having the heterologous DNA produced by a sexual cross between the transformant and another variety. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed parent is present in the progeny of the cross at the same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA and flanking genomic sequence immediately adjacent to the inserted DNA that would be expected to be transferred to a progeny that receives the inserted as a result of a sexual cross of one parental line that includes the inserted DNA (e.g., the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA. The present invention relates to the event CFE-4 DNA, plant cells, tissues, seeds and commodities derived from event CFE-4.

[00025] "Primers" are synthesized nucleic acids that anneal to a complementary target DNA strand by hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by polymerase activity, e.g., a DNA polymerase. Primer pairs described in the present invention refer to their use for amplification of a target nucleic acid sequence, e.g., by polymerase chain reaction or other conventional nucleic-acid amplification methods.

[00026] Methods for preparing and using probes and primers are described, for example, in Molecular Cloning: A Laboratory Manual, 2nd ed., vol. 1-3, ed. Sambrook et al., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 (hereinafter, "Sambrook et al., 1989"); Current Protocols in Molecular Biology, ed. Ausubel et al., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel et al., 1992"); and Innis et al., PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. PCR-primers can be derived from a known DNA sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

[00027] Primers based on the flanking DNA and insert sequences disclosed herein can be used to confirm the disclosed sequences by conventional methods, e.g., by re-cloning and sequencing such sequences.

[00028] "Amplified DNA" or "amplicon" refers to the product of nucleic acid amplification of a target nucleic acid sequence that is part of a nucleic acid template. For example, to determine whether the cauliflower plant progeny resulting from a sexual cross contains the CFE-4 transgenic event, genomic DNA is extracted from a cauliflower plant tissue sample and may be subjected to nucleic acid amplification method using a primer pair that includes a primer derived from flanking sequence in the genome of the cauliflower plant and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the CFE-4 event. The amplicon is of a length and sequence that is diagnostic for the event.

[00029] **SEQ ID NO: 1**. Adapter sequence.

CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAGGT

[00030] **Second adapter sequence**: P-ACCTGCCC-H_2N

[00031] **SEQ ID NO: 2**. SP6 primer used for sequencing.

TATTTAGGTGACACTATAG

[00032] **SEQ ID NO: 3**. Reverse primer sequence complementary to the inserted transgene sequence adjacent to the left border of the transgene useful for PCR detection of said transgenic event.

CAGCGCATCGCCTTCTATC

[00033] **SEQ ID NO: 4**. Forward primer sequence designed from the adapter used for PCR of adapter ligated genomic DNA of CFE-4 event**.**

GGATCCTAATACGACTCACTATAGGGC

[00034] **SEQ ID NO: 5**. Reverse primer sequence complementary to the inserted transgene region adjacent to the left border of the transgene useful for PCR detection of said transgenic event.

GGTTTCGCTCATGTGTTGAGC

[00035] **SEQ ID NO: 6**. Forward primer sequence designed from the adapter used for PCR of adapter ligated genomic DNA CFE-4 event.

TATAGGGCTCGAGCGGC

[00036] **SEQ ID NO: 7**. Amplified DNA fragment sequence using primers with SEQ ID NO: 5 and SEQ ID NO: 6. This SEQ ID NO: 7 consists of a part of the adapter sequence followed by cauliflower genomic DNA sequence flanking the left border of the inserted transgene in event CFE-4, further followed by part of the inserted transgene.

TATAGGGCTCGAGCGGCCGCCCGGGCAGGTAAAACGAAAAGATACAGAAAATAAA
TAAAAAAGGTTGGAGTGATGATGATAAGTCAAAAGTAGCTGGTTTATGATCACATC
TTTCCCGTAGGAAATCAATGGGAATCCGAATGATTTCTTTCATCATCCTTACCAACA
TTTAGCTCCCCTCAATACTTCAAAAACATTCATTCTAATAAACGACTAAGAGAGTA
AAATCACTAACGATATGTGCCTTCTAATGTATCAAAATAAGAATTCCTAAGAGAGA
AATTCAGACAAGAAAATCAATGAAAGACGCGAGATAAGAGCGTTGATGCAAGAAA
ACGTTGTTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATAGTATATATATA
CAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAAT
TAACGCCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGGAA
TTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCT
TATATGCTCAACACATGAGCGAAACC

[00037] **SEQ ID NO: 8**. Forward primer sequence designed from cauliflower genomic DNA adjacent to the left flank border of the inserted transgene in event CFE-4**.**

GTAGGAAATCAATGGGAATCCGA

[00038] **SEQ ID NO: 9**. DNA fragment sequence consisting of a part of the adapter sequence followed by cauliflower genomic sequence flanking the left border of the inserted transgene in event CFE-4, further followed by part of the inserted transgene.

TATAGGGCTCGAGCGGCCGCCCGGGCAGGTAAAACGAAAAGATACAGAAAATAAA
TAAAAAAGGTTGGAGTGATGATGATAAGTCAAAAGTAGCTGGTTTATGATCACATC
TTTCCCGTAGGAAATCAATGGGAATCCGAATGATTTCTTTCATCATCCTTACCAACA
TTTAGCTCCCCTCAATACTTCAAAAACATTCATTCTAATAAACGACTAAGAGAGTA
AAATCACTAACGATATGTGCCTTCTAATGTATCAAAATAAGAATTCCTAAGAGAGA
AATTCAGACAAGAAAATCAATGAAAGACGCGAGATAAGAGCGTTGATGCAAGAAA

ACGTTGTTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATAGTATATATATA
CAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAAT
TAACGCCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGGAA
TTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCT
5 TATATGCTCAACACATGAGCGAAACCCTATAGGAACCCTAATTCCCTTATCTGGGA
ACTACTCACACATTATTATGGAGAAACTCGAGCTTGTCGATCGACTCTAGCTAGAG
GATCGATCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGA
AGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTC
AGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCT
10 GATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCC
ATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGTGTCACGACGAGATCCT
CGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCC
TGATGCTCTTCG

[00039] SEQ ID NO: 10. Reverse primer sequence complementary to the inserted
15 transgene region adjacent to the left border of the transgene useful for PCR detection of
said transgenic event.

CAGGACATAGCGTTGGCTACC

[00040] SEQ ID NO: 11. Reverse primer sequence complementary to the inserted
transgene region adjacent to the left border of the transgene useful for PCR detection of
20 said transgenic event.

CGAAGAGCATCAGGGGCTC

[00041] SEQ ID NO: 12. Reverse primer sequence complementary to the cauliflower
plant genomic DNA sequence flanking the right border of the inserted transgene and
used for determining zygosity of the CFE-4 event.

25 GCCCATGAGCCCTCATTAG

[00042] SEQ ID NO: 13. DNA sequence spanning the adapter region followed by
cauliflower genomic DNA region flanking the left border of the inserted transgene,
further followed by inserted transgene sequence ending with start of the *cry1Ac* gene
sequence.

30 TATAGGGCTCGAGCGGCCGCCCGGGCAGGTAAAACGAAAAGATACAGAAAATAAA
TAAAAAAGGTTGGAGTGATGATGATAAGTCAAAAGTAGCTGGTTTATGATCACATC

TTTCCCGTAGGAAATCAATGGGAATCCGAATGATTTCTTTCATCATCCTTACCAACA
TTTAGCTCCCCTCAATACTTCAAAAACATTCATTCTAATAAACGACTAAGAGAGTA
AAATCACTAACGATATGTGCCTTCTAATGTATCAAAATAAGAATTCCTAAGAGAGA
AATTCAGACAAGAAAATCAATGAAAGACGCGAGATAAGAGCGTTGATGCAAGAAA
5 ACGTTGTTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATAGTATATATATA
CAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAAT
TAACGCCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGGAA
TTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCT
TATATGCTCAACACATGAGCGAAACCCTATAGGAACCCTAATTCCCTTATCTGGGA
10 ACTACTCACACATTATTATGGAGAAACTCGAGCTTGTCGATCGACTCTAGCTAGAG
GATCGATCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGA
AGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTC
AGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCT
GATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCC
15 ATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGTGTCACGACGAGATCCT
CGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCC
TGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACGT
GCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAAG
CGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAA
20 GGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCTT
CCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCAG
CCACGATAGCCGCGCTGCCTCGTCCTGGAGTTCATTCAGGGCACCGGACAGGTCGG
TCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCATC
AGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAAG
25 CGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCCCCATGGTCGATCGACAG
ATCTGCGAAAGCTCGAGAGAGATAGATTTGTAGAGAGAGACTGGTGATTTCAGCGT
GTCCTCTCCAAATGAAATGAACTTCCTTATATAGAGGAAGGTCTTGCGAAGGATAG
TGGGATTGTGCGTCATCCCTTACGTCAGTGGAGATATCACATCAATCCACTTGCTTT
GAAGACGTGGTTGGAACGTCTTCTTTTTCCACGATGCTCCTCGTGGGTGGGGGTCCA
30 TCTTTGGGACCACTGTCGGCAGAGGCATCTTGAACGATAGCCTTTCCTTTATCGCAA
TGATGGCATTTGTAGGTGCCACCTTCCTTTTCTACTGTCCTTTTGATGAAGTGACAG
ATAGCTGGGCAATGGAATCCGAGGAGGTTTCCCGATATTACCCTTTGTTGAAAAGT

CTCAATAGCCCTTTGGTCTTCTGAGACTGTATCTTTGATATTCTTGGAGTAGACGAG
AGTGTCGTGCTCCACCATGTTATCACATCAATCCACTTGCTTTGAAGACGTGGTTGG
AACGTCTTCTTTTTCCACGATGCTCCTCGTGGGTGGGGGTCCATCTTTGGGACCACT
GTCGGCAGAGGCATCTTGAACGATAGCCTTTCCTTTATCGCAATGATGGCATTTGTA
5 GGTGCCACCTTCCTTTTCTACTGTCCTTTTGATGAAGTGACAGATAGCTGGGCAATG
GAATCCGAGGAGGTTTCCCGATATTACCCTTTGTTGAAAAGTCTCAATAGCCCTTTG
GTCTTCTGAGACTGTATCTTTGATATTCTTGGAGTAGACGAGAGTGTCGTGCTCCAC
CATGTTGGCAAGCTGCTCTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCC
GATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGC
10 GCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTA
TGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGA
AACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGT
CAACAGAGGTGGATGGACAGACCCGTTCTTACACCGGACTGGGCGCGGGATAGGA
TATTCAGATTGGGATGGGATTGAGCTTAAAGCCGGCGCTGAGACCATGCTCAAGGT
15 AGGCAATGTCCTCAGCGTCGAGCCCGGCATCTATGTCGAGGGCATTGGTGGAGCGC
GCTTCGGGGATACCGTGCTTGTAACTGAGACCGGATATGAGGCCCTCACTCCGCTT
GATCTTGGCAAAGATATTTGACGCATTTATTAGTATGTGTTAATTTTCATTTGCAGT
GCAGTATTTTCTATTCGATCTTTATGTAATTCGTTACAATTAATAAATATTCAAATC
AGATTATTGACTGTCATTTGTATCAAATCGTGTTTAATGGATATTTTTATTATAATAT
20 TGATGATATCTCAATCAAAACGTAGATAATAATAATATTTATTTAATATTTTTGCGT
CGCACAGTGAAAATCTATATGAGATTACAAAATACCGACAACATTATTTAAGATAC
ATAGACATTAACCCTGAGACTGTTGGACATCAACGGGTAGATTCCTTCATGCATAG
CACCTCATTCTTGGGGACAAAAGCACGGTTTGGCCGTTCCATTGCTGCACGAACGA
GCTTTGCTATATCCTCGGGTTGGATCATCTCATCAGGTCCAATCAAATTTGTCCAAG
25 AACTCATGTTAGTCGCAACGAAACCGGGGCATATGTCGACCTGCAGGAATTCAGGC
CTCTAGATCTCATTATTCCTCCATCAAGAGAAGCTCCACGCTGTCCACGATGAAGGT
TCCCTCGGTTTCACCGATCTCGATCCACACTTTGTCGGTCTCAGGAAAGTACTCAAG
CTCCTTGGTAACATAGCCAACTGGAAGTGGTGTGTAGTCCCTGTAACCTCTGTTGAA
CTCGCAAGGGTTCTCACGTCTGCCATCTGTGTAGGATTTCTCCTCGTACACGGAGGC
30 ATAGTCAGCAGGAACGGAAGGAGCTTCGTTGTAACCTCTGTTACGGCTAGTGTAGG
CACCTCCGTACTCTTCCTGATTCACAGTGTAGTCGTTGCAAGTAACGGTGTTGTTGG
GATAGATTTCTTCCTCGACGCAGTTGGAGAACTTAAGCTCGTCGGTGTTGTTCTCGA

TCTCGTGGATGGTCACGCAACCCTCACCGTATCCCTCCTTGTAAGCGGTCACACGGA
GAATGTAGCCTCTACCTGGACAGACTCTAACCTCTTGGGACACTTCAGCTTCCCACT
CAGGCACAACCAGGACGGAACGCTGATTGTTCTGTTCCTCCACGTCCACATGACCT
TTCACATTCCAGCAGCTGAGGCCATTGTTGAAGTCACCGTTCTTGATGACGTTTCTG
5 GCATCGTACAAGGAGAATGCGGTAAAGATACGTCCCTCAAGTTCCTCGAAGATGGC
AGCGTTCACACCAGGGATCACGGACAACTCAGGCAAGTAAGCCTCACGAATGCTGT
GCACACGTTTGTCTGCGGCGTGGATCATGGCGATGTTGGTGTCGGCTTGCAACTGAT
CATATTGGGAGTTCACGAACAAAGCATCCACGGACTCTTTGGCCTCCTTGTAAACG
ATGTTAGTTTCCCATTCGAGTTTCTCACGTTTGTCCCTCCACTTCTTCTCTGCTCTCTT
10 CACACGAGCGAGAGCTTCACCGACCAATGGTTTCTCTTCGAGAAACTCAAGGTTGC
CAAGTCTTGCGTGTCCGTCTTGGGTCTTGATCTTGAAGATGACCCAGACTCCGAGGT
CCTCATTCAGGTCAGTACATCCCACATCGATGTCCAAGGAGAAGTGATGAGAATGG
TGGGCACACTTCTCGCCATCCCTGCAGGAGCAGTCCAAGTCAGGATTCCACTCAAG
GTGTGGAGCGCATCTGTTAGGCTCTCCACACTTCCCAATGGGAGATTGGGCAGAAA
15 GTGGCCAGAGGGAACCAGTACCTGGGACATTCACGGTCTCGTGCTTGGCATTGTAC
CTGATCGAGTAGATTTCAAGGTCTTGGCTGTCTTCGATGTAGCCTCTAAGTTGATAC
CTGGTGAAGGCTTTGAGTTTGGACTCATCGATCTTCTGGTACAAGTAGGTAGGGTA
GCACTCGTCGAAAGTTCCGGAGAGGGTGACGTAGTTCTCCTTGAACACATCGTCGC
CTCCTTGGATGGTGATCCCGGTGCTTCCACCCCAACCACGTTCTGGCTGCCTGTTGA
20 TGTCTTTGAAGTTGGAGTCTTGCAAGAGATTCCTCTCGTCGCTGAGACGCTTGGCGT
GTTTAACTTTCTCGGAGAGTTCACGCTTCTCGTCGAGGCAGAACTCATCGCTAAGGT
AGGTGACCAAGTTGGACACTTGGTCAATGTGATAGTCAGTAACGTTAGTTTTCAAG
CCAAGCTGATTGGTGGAGGTAAAGAGGGCGTTCACAGCCTTCTGGGCTCTCTCAAG
GTTGTACTCAGCCTCGAGTGTTGCAGTAACTGGAATGAACTCGAATCTGTCGATAA
25 TCACTCCTGCAGTCCCACTAAAGTTTCTAACACCCACGATGTTACCGAGTGAAGAT
GTAAAAGCATTGGCACTTTCAAAGTAACCGAAATCGCTGGATTGGAGATTATCCAA
GGAGGTAGCTGTAGCTGGAACTGTATTGGAGAAGATGGATGAATTACCCCAATTAA
CGTTGAGGTGAATAGGGGTCACAGAAGCATACCTCACACGAACTCTATATCTGGTA
GATGTGGATGGGAAGTGAATTGGAACTTCAATATACCCTCTATTCTGAATGTTATTT
30 CCACTGCTGTTGAGTCTAACGAGGTCTCCACCAGTGAATCCTGGTCCTGAAATGAC
AGAACCGTTGAAGAGAAAGTTTCCCTTCACTGCAGGGATTTGAGTAATACTATCGG
ATGCGATGATGTTGTTGAACTCAGCACTACGATGTATCCAAGAGAACATAGGAGCT

CTGATGATGCTCACGGAACTGTTGCTGAATCCGGAACGGAACATGGACACGTGGCT
CAACCTGTGGGAGAATCCTTGCCTGGGTGGCACATTGTTGTTCTGTGGTGGGATTTC
GTCCAAGGAATCAACGGTTCCGCTCTTTCTGTAAACAGCGGATGGCAAGTTAGAAG
AGGTTCCATAGGCGAACTCTGTTCCGTCAAGAACGGAAAGTTGCTGGTTGTTGATA
CCGATATTGAAGGGTCTTCTGTACAAGGTGGAAGACAAGGTTCTGTAGACACCCTG
ACCTAGTTGAGCAACGATACGTTGTTGTGGAGCGGCGTTTCCCATAGTTCCATAGA
GAGGAAAGGTAAACTCGGGCCCGCTGAATCCAACTGGAGAGGCCATGATCTGGTG
TCCAGACCAGTAATACTCTCCTCTGTGAGCATCGGTGTAGATAGTTATGCTGTTCAA
GATGTCCATCAAGTGTGGGCTCCTGATGGAGCCTTCGATACCTTGGGCAGAACCAC
GGAAGCTACCGTCGAAGTTCTCAAGAACTGGGTTAGTATAGATTTCTCTGGTAAGT
TGGGACACTGTACGGATAGGGTAGGTTCTGGAGTCATAGTTCGGGAAGAGAGACA
CAATGTCCAAAACTGTGAGGGTCAATTCTCTCCTGAACTGGTTGTATCTAATCCAAT
CTCTAGAATCAGGACCCCAGACACGCTCCAAGCCAGTGTTGTACCAACGAACAGCG
TGGTCGGTGTAGTTTCCAATCAGCCTAGTAAGGTCGTTGTAACGGCTATTGATGGTT
GCAGCATCGAATCCCCACCTTTGCCCAAACACGCTAACGTCTCGAAGCACGCTGAG
GTGAAGATTAGCTGCTTGAACGTACACGGACAAGAGAGGAACTTGGTAGTTCTGGA
CTGCGAACAATGGGATAGCTGTGGTCAAGGCGCTGTTCATGTCGTTGAATTGAATA
CGCATTTCCTCGCGGAGAGCTGGGTTAGTAGGATCGGCTTCCCACTCTCTGAAGCTC
TCTGCATAGATTTGGTAGAGATTGCTCAATCCTTCCAACCTAGAGATGGCCTGGTTC
CTGGCGAACTCTTCGATCCTCTGGTTGATCAACTGCTCAATTTGCACCAGGAATGCA
TCCCATTGAGATGGACCAAAGATACCCCAGATGATGTCAACTAGTCCGAGAACGAA
CCCAGCACCTGGCACGAACTCGCTGAGCAGAAACTGTGTCAAGGACAAGGAGATG
TCGATGGGAGTGTAACCGGTTTCAATGCGTTCTCCACCAAGTACTTCAACTTCTGGG
TTACTCAAGCAGTTGTATGGAATGCATTCGTTGATGTTTGGGTTGTTGTCCAT

[00043] SEQ ID NO: 14. Cauliflower genomic DNA sequence flanking the left border of the inserted transgene.

AAAACGAAAAGATACAGAAAATAAATAAAAAAGGTTGGAGTGATGATGATAAGTC
AAAAGTAGCTGGTTTATGATCACATCTTTCCCGTAGGAAATCAATGGGAATCCGAA
TGATTTCTTTCATCATCCTTACCAACATTTAGCTCCCCTCAATACTTCAAAAACATTC
ATTCTAATAAACGACTAAGAGAGTAAAATCACTAACGATATGTGCCTTCTAATGTA
TCAAAATAAGAATTCCTAAGAGAGAAATTCAGACAAGAAAATCAATGAAAGACGC

GAGATAAGAGCGTTGATGCAAGAAAACGTTGTTTCATTCTTTGTTGGAAGAGGAGA
CAAGAGATGTAATAGTATATATAT

[00044] SEQ ID NO: 15. T-DNA sequence starting with *cry1Ac* gene up to the left border of the transgene.

5 ACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGA
ATTAACGCCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGG
AATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTT
CTTATATGCTCAACACATGAGCGAAACCCTATAGGAACCCTAATTCCCTTATCTGGG
AACTACTCACACATTATTATGGAGAAACTCGAGCTTGTCGATCGACTCTAGCTAGA
10 GGATCGATCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAG
AAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGT
CAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCC
TGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGC
CATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGTGTCACGACGAGATCC
15 TCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCC
CTGATGCTCTTCGTCCAGATCATCCTGATCGACAAGACCGGCTTCCATCCGAGTACG
TGCTCGCTCGATGCGATGTTTCGCTTGGTGGTCGAATGGGCAGGTAGCCGGATCAA
GCGTATGCAGCCGCCGCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCA
AGGTGAGATGACAGGAGATCCTGCCCCGGCACTTCGCCCAATAGCAGCCAGTCCCT
20 TCCCGCTTCAGTGACAACGTCGAGCACAGCTGCGCAAGGAACGCCCGTCGTGGCCA
GCCACGATAGCCGCGCTGCCTCGTCCTGGAGTTCATTCAGGGCACCGGACAGGTCG
GTCTTGACAAAAAGAACCGGGCGCCCCTGCGCTGACAGCCGGAACACGGCGGCAT
CAGAGCAGCCGATTGTCTGTTGTGCCCAGTCATAGCCGAATAGCCTCTCCACCCAA
GCGGCCGGAGAACCTGCGTGCAATCCATCTTGTTCAATCCCCATGGTCGATCGACA
25 GATCTGCGAAAGCTCGAGAGAGATAGATTTGTAGAGAGAGACTGGTGATTTCAGCG
TGTCCTCTCCAAATGAAATGAACTTCCTTATATAGAGGAAGGTCTTGCGAAGGATA
GTGGGATTGTGCGTCATCCCTTACGTCAGTGGAGATATCACATCAATCCACTTGCTT
TGAAGACGTGGTTGGAACGTCTTCTTTTTCCACGATGCTCCTCGTGGGTGGGGGTCC
ATCTTTGGGACCACTGTCGGCAGAGGCATCTTGAACGATAGCCTTTCCTTTATCGCA
30 ATGATGGCATTTGTAGGTGCCACCTTCCTTTTCTACTGTCCTTTTGATGAAGTGACA
GATAGCTGGGCAATGGAATCCGAGGAGGTTTCCCGATATTACCCTTTGTTGAAAAG
TCTCAATAGCCCTTTGGTCTTCTGAGACTGTATCTTTGATATTCTTGGAGTAGACGA

GAGTGTCGTGCTCCACCATGTTATCACATCAATCCACTTGCTTTGAAGACGTGGTTG
GAACGTCTTCTTTTTCCACGATGCTCCTCGTGGGTGGGGGTCCATCTTTGGGACCAC
TGTCGGCAGAGGCATCTTGAACGATAGCCTTTCCTTTATCGCAATGATGGCATTTGT
AGGTGCCACCTTCCTTTTCTACTGTCCTTTTGATGAAGTGACAGATAGCTGGGCAAT
5 GGAATCCGAGGAGGTTTCCCGATATTACCCTTTGTTGAAAAGTCTCAATAGCCCTTT
GGTCTTCTGAGACTGTATCTTTGATATTCTTGGAGTAGACGAGAGTGTCGTGCTCCA
CCATGTTGGCAAGCTGCTCTAGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGC
CGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAG
CGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTT
10 ATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG
AAACAGCTATGACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAG
TCAACAGAGGTGGATGGACAGACCCGTTCTTACACCGGACTGGGCGCGGGATAGG
ATATTCAGATTGGGATGGGATTGAGCTTAAAGCCGGCGCTGAGACCATGCTCAAGG
TAGGCAATGTCCTCAGCGTCGAGCCCGGCATCTATGTCGAGGGCATTGGTGGAGCG
15 CGCTTCGGGGATACCGTGCTTGTAACTGAGACCGGATATGAGGCCCTCACTCCGCT
TGATCTTGGCAAAGATATTTGACGCATTTATTAGTATGTGTTAATTTTCATTTGCAG
TGCAGTATTTTCTATTCGATCTTTATGTAATTCGTTACAATTAATAAATATTCAAATC
AGATTATTGACTGTCATTTGTATCAAATCGTGTTTAATGGATATTTTTATTATAATAT
TGATGATATCTCAATCAAAACGTAGATAATAATAATATTTATTTAATATTTTTGCGT
20 CGCACAGTGAAAATCTATATGAGATTACAAAATACCGACAACATTATTTAAGATAC
ATAGACATTAACCCTGAGACTGTTGGACATCAACGGGTAGATTCCTTCATGCATAG
CACCTCATTCTTGGGGACAAAAGCACGGTTTGGCCGTTCCATTGCTGCACGAACGA
GCTTTGCTATATCCTCGGGTTGGATCATCTCATCAGGTCCAATCAAATTTGTCCAAG
AACTCATGTTAGTCGCAACGAAACCGGGGCATATGTCGACCTGCAGGAATTCAGGC
25 CTCTAGATCTCATTATTCCTCCATCAAGAGAAGCTCCACGCTGTCCACGATGAAGGT
TCCCTCGGTTTCACCGATCTCGATCCACACTTTGTCGGTCTCAGGAAAGTACTCAAG
CTCCTTGGTAACATAGCCAACTGGAAGTGGTGTGTAGTCCCTGTAACCTCTGTTGAA
CTCGCAAGGGTTCTCACGTCTGCCATCTGTGTAGGATTTCTCCTCGTACACGGAGGC
ATAGTCAGCAGGAACGGAAGGAGCTTCGTTGTAACCTCTGTTACGGCTAGTGTAGG
30 CACCTCCGTACTCTTCCTGATTCACAGTGTAGTCGTTGCAAGTAACGGTGTTGTTGG
GATAGATTTCTTCCTCGACGCAGTTGGAGAACTTAAGCTCGTCGGTGTTGTTCTCGA
TCTCGTGGATGGTCACGCAACCCTCACCGTATCCCTCCTTGTAAGCGGTCACACGGA

GAATGTAGCCTCTACCTGGACAGACTCTAACCTCTTGGGACACTTCAGCTTCCCACT
CAGGCACAACCAGGACGGAACGCTGATTGTTCTGTTCCTCCACGTCCACATGACCT
TTCACATTCCAGCAGCTGAGGCCATTGTTGAAGTCACCGTTCTTGATGACGTTTCTG
GCATCGTACAAGGAGAATGCGGTAAAGATACGTCCCTCAAGTTCCTCGAAGATGGC
5 AGCGTTCACACCAGGGATCACGGACAACTCAGGCAAGTAAGCCTCACGAATGCTGT
GCACACGTTTGTCTGCGGCGTGGATCATGGCGATGTTGGTGTCGGCTTGCAACTGAT
CATATTGGGAGTTCACGAACAAAGCATCCACGGACTCTTTGGCCTCCTTGTAAACG
ATGTTAGTTTCCCATTCGAGTTTCTCACGTTTGTCCCTCCACTTCTTCTCTGCTCTCTT
CACACGAGCGAGAGCTTCACCGACCAATGGTTTCTCTTCGAGAAACTCAAGGTTGC
10 CAAGTCTTGCGTGTCCGTCTTGGGTCTTGATCTTGAAGATGACCCAGACTCCGAGGT
CCTCATTCAGGTCAGTACATCCCACATCGATGTCCAAGGAGAAGTGATGAGAATGG
TGGGCACACTTCTCGCCATCCCTGCAGGAGCAGTCCAAGTCAGGATTCCACTCAAG
GTGTGGAGCGCATCTGTTAGGCTCTCCACACTTCCCAATGGGAGATTGGGCAGAAA
GTGGCCAGAGGGAACCAGTACCTGGGACATTCACGGTCTCGTGCTTGGCATTGTAC
15 CTGATCGAGTAGATTTCAAGGTCTTGGCTGTCTTCGATGTAGCCTCTAAGTTGATAC
CTGGTGAAGGCTTTGAGTTTGGACTCATCGATCTTCTGGTACAAGTAGGTAGGGTA
GCACTCGTCGAAAGTTCCGGAGAGGGTGACGTAGTTCTCCTTGAACACATCGTCGC
CTCCTTGGATGGTGATCCCGGTGCTTCCACCCCAACCACGTTCTGGCTGCCTGTTGA
TGTCTTTGAAGTTGGAGTCTTGCAAGAGATTCCTCTCGTCGCTGAGACGCTTGGCGT
20 GTTTAACTTTCTCGGAGAGTTCACGCTTCTCGTCGAGGCAGAACTCATCGCTAAGGT
AGGTGACCAAGTTGGACACTTGGTCAATGTGATAGTCAGTAACGTTAGTTTTCAAG
CCAAGCTGATTGGTGGAGGTAAAGAGGGCGTTCACAGCCTTCTGGGCTCTCTCAAG
GTTGTACTCAGCCTCGAGTGTTGCAGTAACTGGAATGAACTCGAATCTGTCGATAA
TCACTCCTGCAGTCCCACTAAAGTTTCTAACACCCACGATGTTACCGAGTGAAGAT
25 GTAAAAGCATTGGCACTTTCAAAGTAACCGAAATCGCTGGATTGGAGATTATCCAA
GGAGGTAGCTGTAGCTGGAACTGTATTGGAGAAGATGGATGAATTACCCCAATTAA
CGTTGAGGTGAATAGGGGTCACAGAAGCATACCTCACACGAACTCTATATCTGGTA
GATGTGGATGGGAAGTGAATTGGAACTTCAATATACCCTCTATTCTGAATGTTATTT
CCACTGCTGTTGAGTCTAACGAGGTCTCCACCAGTGAATCCTGGTCCTGAAATGAC
30 AGAACCGTTGAAGAGAAAGTTTCCCTTCACTGCAGGGATTTGAGTAATACTATCGG
ATGCGATGATGTTGTTGAACTCAGCACTACGATGTATCCAAGAGAACATAGGAGCT
CTGATGATGCTCACGGAACTGTTGCTGAATCCGGAACGGAACATGGACACGTGGCT

CAACCTGTGGGAGAATCCTTGCCTGGGTGGCACATTGTTGTTCTGTGGTGGGATTTC
GTCCAAGGAATCAACGGTTCCGCTCTTTCTGTAAACAGCGGATGGCAAGTTAGAAG
AGGTTCCATAGGCGAACTCTGTTCCGTCAAGAACGGAAAGTTGCTGGTTGTTGATA
CCGATATTGAAGGGTCTTCTGTACAAGGTGGAAGACAAGGTTCTGTAGACACCCTG
5 ACCTAGTTGAGCAACGATACGTTGTTGTGGAGCGGCGTTTCCCATAGTTCCATAGA
GAGGAAAGGTAAACTCGGGCCCGCTGAATCCAACTGGAGAGGCCATGATCTGGTG
TCCAGACCAGTAATACTCTCCTCTGTGAGCATCGGTGTAGATAGTTATGCTGTTCAA
GATGTCCATCAAGTGTGGGCTCCTGATGGAGCCTTCGATACCTTGGGCAGAACCAC
GGAAGCTACCGTCGAAGTTCTCAAGAACTGGGTTAGTATAGATTTCTCTGGTAAGT
10 TGGGACACTGTACGGATAGGGTAGGTTCTGGAGTCATAGTTCGGGAAGAGAGACA
CAATGTCCAAAACTGTGAGGGTCAATTCTCTCCTGAACTGGTTGTATCTAATCCAAT
CTCTAGAATCAGGACCCCAGACACGCTCCAAGCCAGTGTTGTACCAACGAACAGCG
TGGTCGGTGTAGTTTCCAATCAGCCTAGTAAGGTCGTTGTAACGGCTATTGATGGTT
GCAGCATCGAATCCCCACCTTTGCCCAAACACGCTAACGTCTCGAAGCACGCTGAG
15 GTGAAGATTAGCTGCTTGAACGTACACGGACAAGAGAGGAACTTGGTAGTTCTGGA
CTGCGAACAATGGGATAGCTGTGGTCAAGGCGCTGTTCATGTCGTTGAATTGAATA
CGCATTTCCTCGCGGAGAGCTGGGTTAGTAGGATCGGCTTCCCACTCTCTGAAGCTC
TCTGCATAGATTTGGTAGAGATTGCTCAATCCTTCCAACCTAGAGATGGCCTGGTTC
CTGGCGAACTCTTCGATCCTCTGGTTGATCAACTGCTCAATTTGCACCAGGAATGCA
20 TCCCATTGAGATGGACCAAAGATACCCCAGATGATGTCAACTAGTCCGAGAACGAA
CCCAGCACCTGGCACGAACTCGCTGAGCAGAAACTGTGTCAAGGACAAGGAGATG
TCGATGGGAGTGTAACCGGTTTCAATGCGTTCTCCACCAAGTACTTCAACTTCTGGG
TTACTCAAGCAGTTGTATGGAATGCATTCGTTGATGTTTGGGTTGTTGTCCAT

[00045] SEQ ID NO: 16. DNA sequence of the left junction.

25 GATGTAATAGTATATATATACAAATTGACGCTTAGAC

[00046] SEQ ID NO: 17. DNA sequence of the left junction.

GAGGAGACAAGAGATGTAATAGTATATATATACAAATTGACGCTTAGACAACTTAA
TAACAC

[00047] SEQ ID NO: 18. DNA sequence of the left junction.

GATGCAAGAAAACGTTGTTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATA
GTATATATATACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTA
ATGTACTGAATTAACGCC

[00048] SEQ ID NO: 19. DNA sequence of the amplicon generated using forward
5 primer of SEQ ID NO: 8 and reverse primer of SEQ ID NO: 10.

GTAGGAAATCAATGGGAATCCGAATGATTTCTTTCATCATCCTTACCAACATTTAGC
TCCCCTCAATACTTCAAAAACATTCATTCTAATAAACGACTAAGAGAGTAAAATCA
CTAACGATATGTGCCTTCTAATGTATCAAAATAAGAATTCCTAAGAGAGAAATTCA
GACAAGAAAATCAATGAAAGACGCGAGATAAGAGCGTTGATGCAAGAAAACGTTG
10 TTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATAGTATATATATACAAATT
GACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAATTAACG
CCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGGAATTAGA
AATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTATAT
GCTCAACACATGAGCGAAACCCTATAGGAACCCTAATTCCCTTATCTGGGAACTAC
15 TCACACATTATTATGGAGAAACTCGAGCTTGTCGATCGACTCTAGCTAGAGGATCG
ATCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCG
ATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCC
ATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTG

[00049] SEQ ID NO: 20. DNA sequence of the amplicon generated using forward
20 primer of SEQ ID NO: 8 and reverse primer of SEQ ID NO: 11.

GTAGGAAATCAATGGGAATCCGAATGATTTCTTTCATCATCCTTACCAACATTTAGC
TCCCCTCAATACTTCAAAAACATTCATTCTAATAAACGACTAAGAGAGTAAAATCA
CTAACGATATGTGCCTTCTAATGTATCAAAATAAGAATTCCTAAGAGAGAAATTCA
GACAAGAAAATCAATGAAAGACGCGAGATAAGAGCGTTGATGCAAGAAAACGTTG
25 TTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATAGTATATATATACAAATT
GACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAATTAACG
CCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGGAATTAGA
AATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTATAT
GCTCAACACATGAGCGAAACCCTATAGGAACCCTAATTCCCTTATCTGGGAACTAC
30 TCACACATTATTATGGAGAAACTCGAGCTTGTCGATCGACTCTAGCTAGAGGATCG
ATCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAGAAGGCG

ATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGTCAGCCC
ATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCCTGATAG
CGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGCCATTTTC
CACCATGATATTCGGCAAGCAGGCATCGCCATGTGTCACGACGAGATCCTCGCCGT
5 CGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCCCTGATGC
TCTTCG

[00050] SEQ ID NO: 21. DNA sequence of the amplicon generated using forward primer of SEQ ID NO: 8 and reverse primer of SEQ ID NO: 5.

GTAGGAAATCAATGGGAATCCGAATGATTTCTTTCATCATCCTTACCAACATTTAGC
10 TCCCCTCAATACTTCAAAAACATTCATTCTAATAAACGACTAAGAGAGTAAAATCA
CTAACGATATGTGCCTTCTAATGTATCAAAATAAGAATTCCTAAGAGAGAAATTCA
GACAAGAAAATCAATGAAAGACGCGAGATAAGAGCGTTGATGCAAGAAAACGTTG
TTTCATTCTTTGTTGGAAGAGGAGACAAGAGATGTAATAGTATATATATACAAATT
GACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGAATTAACG
15 CCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGGAATTAGA
AATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTTCTTATAT
GCTCAACACATGAGCGAAACC

[00051] SEQ ID NO: 22. T-DNA internal complementary sequence comprising SEQ ID NO: 5, 3, 10 and 11.

20 ACAAATTGACGCTTAGACAACTTAATAACACATTGCGGACGTTTTTAATGTACTGA
ATTAACGCCGAATTAATTCGGGGGATCTGGATTTTAGTACTGGATTTTGGTTTTAGG
AATTAGAAATTTTATTGATAGAAGTATTTTACAAATACAAATACATACTAAGGGTTT
CTTATATGCTCAACACATGAGCGAAACCCTATAGGAACCCTAATTCCCTTATCTGGG
AACTACTCACACATTATTATGGAGAAACTCGAGCTTGTCGATCGACTCTAGCTAGA
25 GGATCGATCCGAACCCCAGAGTCCCGCTCAGAAGAACTCGTCAAGAAGGCGATAG
AAGGCGATGCGCTGCGAATCGGGAGCGGCGATACCGTAAAGCACGAGGAAGCGGT
CAGCCCATTCGCCGCCAAGCTCTTCAGCAATATCACGGGTAGCCAACGCTATGTCC
TGATAGCGGTCCGCCACACCCAGCCGGCCACAGTCGATGAATCCAGAAAAGCGGC
CATTTTCCACCATGATATTCGGCAAGCAGGCATCGCCATGTGTCACGACGAGATCC
30 TCGCCGTCGGGCATGCGCGCCTTGAGCCTGGCGAACAGTTCGGCTGGCGCGAGCCC
CTGATGCTCTTCGTCCAGATCATCCTGATCGACA

[00052] **SEQ ID NO: 23**. Forward primer sequence in the cauliflower genomic region adjacent to the left border of the inserted transgene useful for detection of transgenic cauliflower event CFE-4.

CATCATCCTTACCAACATTTA

5 **[00053]** **SEQ ID NO: 24**. Forward primer sequence in the cauliflower genomic region adjacent to the left border of the inserted transgene useful for detection of transgenic cauliflower event CFE-4.

GTAGCTGGTTTATGACACATC

[00054] **SEQ ID NO: 25**. Forward primer sequence within the T-DNA region adjacent 10 to the right border of the inserted transgene useful for detection of the transgenic cauliflower event CFE-4.

CCAACAGTTGCGCAGCCTGAATG

[00055] **SEQ ID NO: 26**. Reverse primer sequence complementary to the cauliflower plant genomic DNA sequence flanking the right border region of T-DNA of the inserted 15 transgene and used for determining zygosity of the CFE-4 event.

CGTTCCGGAAGTAGACCAATC

[00056] **SEQ ID NO**: **27**. DNA sequence of the amplicon generated using forward primer of SEQ ID NO: 25 and reverse primer of SEQ ID NO: 26.

CGTTCCGGAAGTAGACCAATCGACCAAACTGATGGCCCATGAAGAAAAGGT
20 CCAGGCTATAAACTAGTGACCACATAGATAGCCCATGAGCCCTCATTAGGG
TCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATCTGATCCAAG
CTCAAGCTGCTCTAGCATTCGCCATTCAGGCTGCGCAACTGTTGG

[00057] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 in a biological sample, said method 25 comprising of (a) obtaining a biological sample comprising of cauliflower DNA; (b) contacting said biological sample with a first DNA primer and a second DNA primer; (c) performing a DNA amplification reaction to produce a DNA amplicon molecule; and (d) detecting the presence of said DNA amplicon molecule, wherein the detection of presence of said DNA amplicon molecule is diagnostic for said CFE-4 event.

[00058] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 as described herein, wherein the first DNA primer is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 26, and wherein the second DNA primer is selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 25.

[00059] In an embodiment of the present disclosure, there is provided a method of detection of transgenic cauliflower event CFE-4 as described herein, wherein said DNA amplicon comprises of at least 50 contiguous nucleotides selected from the group of sequences consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 27, and complements thereof.

[00060] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4, said method comprising of (a) obtaining a biological sample comprising of cauliflower DNA; (b) contacting said biological sample with a first DNA primer, a second DNA primer, and a third DNA primer, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule; and (d) detecting the presence of said DNA amplicon molecule, wherein detection of presence of more than one DNA amplicon having different nucleotide sequences is indicative of heterozygosity of the transgenic cauliflower event CFE-4, while detection of presence of one or more DNA amplicon with identical nucleotide sequence is indicative of homozygosity of the transgenic event CFE-4.

[00061] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 as described herein, wherein the first DNA primer is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 26.

[00062] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 as described herein, wherein the second DNA primer is selected from the group

consisting of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 25.

[00063] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 as described herein, wherein the third DNA primer is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 26.

[00064] In an embodiment of the present disclosure, there are provided synthetic DNA molecules comprising of any of the DNA amplicons obtained from the method of detection of transgenic cauliflower event CFE-4 as described herein.

[00065] In an embodiment of the present disclosure, there is provided a kit for detection of transgenic cauliflower event CFE-4 comprising of forward and reverse primers having at least 10 contiguous nucleotides selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 22, SEQ ID NO: 15, and SEQ ID NO: 27.

[00066] The present disclosure provides a transgenic cauliflower plant comprising an event designated as CFE-4, progeny of the plant, and cells of the plant, as well as seeds produced from the plant. Representative seed for growing the plant, for producing progeny, for obtaining cells, or for producing a crop of said seed comprising the transgenic event have been deposited with the National Collection of Industrial, Food and Marine Bacteria (NCIMB) on February 08, 2011 and has the accession number NCIMB 41809.

[00067] The present disclosure relates to transformation of cauliflower plant with the *cry1Ac* gene for conferring lepidopteron resistance. The invention pertains to transforming cauliflower plants with the plant expression vector pC2300ve10518 by *Agrobacterium*-mediated transformation method. Events having a single copy insert were identified and bioassay were performed for the identification of a transgenic line, namely cauliflower event CFE-4. After selection of said transgenic event, the plant sequences flanking the inserted heterologous region were obtained using PCR walking and characterized. The sequence analysis of the plant region flanking the T-DNA heterologous region revealed the sequence of the junction sequence specific for the

event. Further, primers were designed for the amplification of the junction sequence characteristic for the transgenic cauliflower event CFE-4.

[00068] The transgenic cauliflower plants were produced by an *Agrobacterium*-mediated transformation of a cauliflower line (*Brassica oleracea* var. *botrytis*) with the plasmid construct comprising of *cry1Ac* and *nptII* genes in the T-DNA. The plasmid construct pC2300ve10518 contains plant expression cassettes with the genetic regulatory elements necessary for expression of the *cry1Ac* gene in cauliflower plant cells wherein the *cry1Ac* gene expression is driven by the e35S Cauliflower Mosaic Virus promoter. Transgenic cauliflower cells were regenerated into cauliflower plants expressing the *cry1Ac* gene. Multiple independent events were generated using the same gene construct. Transgenic cauliflower plants were obtained which were hardened and grown in a green house. Each of these transgenic insertions in the individual transgenic plants can be considered as distinct events since the insertion of the heterologous DNA is likely in different locations and copy numbers within the plant genome. The transgenic plants were characterized for the presence of single or multiple transgene insertions in the heterologous DNA in the plant genome. Transgenic plants having single transgene inserts were identified. Individual transgenic cauliflower events (plants) were selected based on insect bioassays, and resistance to lepidopteran insect infestation. Plants from insect-resistant events expressing Cry1Ac protein were selected for further analysis. A number of transgenic cauliflower plants expressing Cry1Ac protein were confirmed using ELISA method. ELISA positive lines were subjected to the insect bio-assays using DBM larvae. Based on the ELISA and bioassay analysis, a transgenic cauliflower event was identified having high expression of the Cry1Ac protein and showing resistance to insects. This event was named as CFE-4 which was further used for the molecular characterization of the junction sequence characteristic for the event.

[00069] In an embodiment of the present disclosure, the portion of the pC2300ve10518 plasmid DNA inserted in to the cauliflower genome, giving rise to the transgenic cauliflower plant event CFE-4, consists of the left and right border segments

and the plant expression cassettes (encoding *cry1Ac*, and *nptII*) was characterized by detailed molecular analysis.

[00070] Nucleic acid amplification can be accomplished by any one of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described in PCR protocols: (Innis *et al*., *Academic Press, San Diego, CA, USA*, 1990.

[00071] In an embodiment of the present disclosure, backcrossing was performed to move a transgene from a donor parent (transformed cauliflower plant containing CFE-4 event) to a recurrent parent (a desired cauliflower line). The backcross method was used for transferring the CFE-4 event into other lines/hybrids of cauliflower. The progeny of this cross, which had the CFE-4 event, was then back-crossed repeatedly to the recurrent parent line to obtain a stable line with desired properties of the recurrent parent and the CFE-4 event.

[00072] In an embodiment of the present disclosure, four ELISA positive primary transformants that express the *cry1Ac* gene were regenerated. Out of the four lines, Mendelian segregation pattern i.e. 3:1 ratio of the transgene (*cry1Ac* gene) inheritance in two lines (T1 generation seedlings) indicated that integration of the *cry1Ac* gene was confined to a single locus in the genome.

[00073] In an embodiment of the present disclosure, leaves of the transgenic cauliflower, harbouring event CFE-4, expressing the *cry1Ac* gene showed 100% larval mortality in insect bioassays using target pest whereas no larval mortality observed in bioassays using leaves from non-transgenic cauliflower leaf not having event CFE-4.

[00074] In an embodiment of the present disclosure, bioassays using target pets were carried out using leaves from transgenic cauliflower, harbouring events independent from event CFE-4.

[00075] In an embodiment of the present disclosure, representative seed for growing the plant, for producing progeny, for obtaining cells, or for producing a crop of said seed comprising the transgenic event have been deposited with NCIMB on February 08, 2011 having accession number NCIMB 41809.

[00076] An embodiment of the present disclosure is to provide a method for detecting the presence of a cauliflower event CFE-4 or a progeny thereof in a biological sample, the method comprises: obtaining a biological sample comprising of cauliflower DNA; contacting the biological sample with a first DNA primer and a second DNA
5 primer wherein first DNA primer recognizes a sequence within SEQ ID NO: 14 and a second DNA primer, which recognizes a sequence within SEQ ID NO: 15 or SEQ ID NO: 22; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of said amplicon is diagnostic for said CFE-4 event in the sample.

10 **[00077]** Another embodiment of the disclosure provides a method for detecting the presence of a cauliflower event CFE-4 or a progeny thereof in a biological sample, the method comprises: obtaining a biological sample comprising of cauliflower DNA; contacting the biological sample with a first DNA primer and a second DNA primer, wherein the first DNA primer is selected from the group consisting of SEQ ID NO: 23,
15 SEQ ID NO: 8, SEQ ID NO: 24, and SEQ ID NO:26 and a second DNA primer selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 3, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 25 ; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of said amplicon is diagnostic for said CFE-4 event in the sample.

20 **[00078]** Yet another embodiment of the disclosure provides a method for detecting the presence of a cauliflower event CFE-4 or a progeny thereof in a biological sample, the method comprises: obtaining a biological sample comprising of cauliflower DNA; contacting the biological sample with a first DNA primer of SEQ ID NO: 8 and a second DNA primer of SEQ ID NO: 5; performing a DNA amplification reaction to
25 produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of said amplicon of size 471bp is diagnostic for event CFE-4 in the sample. The nucleotide sequence of the 471bp DNA amplicon molecule is as set forth in SEQ ID NO: 21 or its complement.

[00079] In still another embodiment the present disclosure provides a method for detecting the presence of a cauliflower event CFE-4 or a progeny thereof in a biological sample, the method comprising: obtaining a biological sample comprising of cauliflower DNA; contacting the biological sample with a first DNA primer of SEQ ID NO: 8 and a second DNA primer of SEQ ID NO: 10; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of said amplicon of size 724bp is diagnostic for event CFE-4 in a said sample. The nucleotide sequence of the 724bp DNA amplicon molecule is as set forth in SEQ ID NO: 19 or its complement.

[00080] In still another embodiment, the disclosure provides a method for detecting the presence of a cauliflower event CFE-4 or a progeny thereof in a biological sample, the method comprising: obtaining a biological sample comprising of cauliflower DNA; contacting the biological sample with a first DNA primer of SEQ ID NO: 8 and a second DNA primer of SEQ ID NO: 11; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of said amplicon of size 902bp is diagnostic for event CFE-4 in a said sample. The nucleotide sequence of the 902bp DNA amplicon molecule is as set forth in SEQ ID NO: 20 or its complement.

[00081] In an embodiment of the present disclosure, there is provided a method for detecting the presence of a cauliflower event CFE-4 or a progeny thereof in a biological sample, the method comprising: obtaining a biological sample comprising of cauliflower DNA; contacting the biological sample with a first DNA primer of SEQ ID NO: 25 and a second DNA primer of SEQ ID NO: 26; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of said amplicon of size 198bp is diagnostic for event CFE-4 in a said sample. The nucleotide sequence of the 198bp DNA amplicon molecule is as set forth in SEQ IDNO: 27 or its complement.

[00082] Another embodiment of the disclosure provides a method for determining the zygosity of a cauliflower plant comprising cauliflower event CFE-4 DNA in a

biological sample, the method comprising: obtaining a sample of cauliflower DNA; contacting the sample having cauliflower DNA with a first DNA primer of SEQ ID NO: 8, a second DNA primer of SEQ ID NO: 5 and a third DNA primer of SEQ ID NO: 12 ; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of two different DNA amplicon molecules of sizes 471bp and 326bp indicates heterozygosity of the cauliflower event CFE-4. Detection of only a DNA amplicon molecule of 471bp indicates homozygosity of the CFE-4 event.

[00083] Another embodiment of the disclosure provides a method for determining the zygosity of a cauliflower plant comprising cauliflower event CFE-4 DNA in a biological sample, the method comprising: obtaining a sample of cauliflower DNA; contacting the sample having cauliflower DNA with a first DNA primer of SEQ ID NO: 8, a second DNA primer of SEQ ID NO: 5 and a third DNA primer of SEQ ID NO: 26; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of two different DNA amplicon molecules of sizes 471bp and 407 indicates heterozygosity of the cauliflower event CFE-4. Detection of only a DNA amplicon molecule of 471bp indicates homozygosity of the CFE-4 event.

[00084] Yet another embodiment of the disclosure provides a method for determining the zygosity of a cauliflower plant comprising cauliflower event CFE-4 DNA in a biological sample, the method comprising: obtaining a sample of cauliflower DNA; contacting the sample having cauliflower DNA with a first DNA primer of SEQ ID NO: 8, a second DNA primer of SEQ ID NO: 10 and a third DNA primer of SEQ ID NO: 12; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting the said DNA amplicon molecule; wherein the presence of two different DNA amplicon molecules of sizes 724bp and 326bp indicates heterozygosity of the cauliflower event CFE-4. Detection of a single DNA amplicon molecule of 724bp indicates homozygosity of the CFE-4 event.

[00085] Another embodiment of the disclosure provides a method for determining the zygosity of a cauliflower plant comprising cauliflower event CFE-4 DNA in a biological sample, the method comprising: obtaining a sample of cauliflower DNA; contacting the sample having cauliflower DNA with a first DNA primer of SEQ ID NO: 8, a second DNA primer of SEQ ID NO: 10 and a third DNA primer of SEQ ID NO: 26; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of two different DNA amplicon molecules of sizes 724bp and 407 indicates heterozygosity of the cauliflower event CFE-4. Detection of only a DNA amplicon molecule of 724bp indicates homozygosity of the CFE-4 event

[00086] Still another embodiment of the disclosure provides a method of determining the zygosity of a cauliflower plant comprising cauliflower event CFE-4 DNA in a biological sample, the method comprising: obtaining a sample of cauliflower DNA; contacting the sample having cauliflower DNA with a first DNA primer of SEQ ID NO: 8, a second DNA primer of SEQ ID NO: 11 and a third DNA primer of, SEQ ID NO: 12; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting the said DNA amplicon molecule; wherein the detection of two DNA amplicon molecules of sizes 902bp and 326bp indicates heterozygosity of the cauliflower event CFE-4. Detection of a single DNA amplicon molecule of 902bp indicates homozygosity of the CFE-4 event.

[00087] Another embodiment of the disclosure provides a method for determining the zygosity of a cauliflower plant comprising cauliflower event CFE-4 DNA in a biological sample, the method comprising: obtaining a sample of cauliflower DNA; contacting the sample having cauliflower DNA with a first DNA primer of SEQ ID NO: 8, a second DNA primer of SEQ ID NO: 11 and a third DNA primer of SEQ ID NO: 26; performing a DNA amplification reaction to produce a DNA amplicon molecule; detecting said DNA amplicon molecule; wherein the detection of two different DNA amplicon molecules of sizes 902bp and 407 indicates heterozygosity of the cauliflower

event CFE-4. Detection of only a DNA amplicon molecule of 902bp indicates homozygosity of the CFE-4 event

[00088] In an embodiment of the disclosure, there is provided isolated DNA molecules as disclosed in the present invention comprising a sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18 and complements thereof .

[00089] Another embodiment of the invention provides an isolated DNA molecule selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 27, which is a junction sequence amplified by the pair of DNA primers SEQ ID NO: 8 and 5, SEQ ID NO: 8 and 10, SEQ ID NO: 8 and 11, and SEQ ID NO: 25 and 26 respectively.

[00090] In an embodiment of the present disclosure, there is provided DNA primer sequences for detection of the CFE-4 event in cauliflower. The DNA primer sequence as disclosed in the present invention having a nucleotide sequence selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 5, SEQ ID NO: 3, SEQ ID NO: 10, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 12, and SEQ ID NO: 11.

[00091] Another embodiment of the present disclosure provides an insect resistant transgenic cauliflower plant or part thereof containing Bt gene comprising event CFE-4. Representative cauliflower seed comprising event CFE-4 have been deposited under NICMB accession number NCIMB 41809.

[00092] Yet another embodiment of the present disclosure provides a cauliflower plant or part thereof, the part of the plant as disclosed in the invention is selected from the group consisting of cell, flower, shoot, inflorescence meristem, root, leaf, seed and stem.

[00093] In an embodiment of the present disclosure provides cauliflower plant or part thereof as disclosed in the disclosure is a progeny of a cauliflower plant comprising said event CFE-4.

[00094] Another embodiment of the present disclosure provides a plant or part thereof as disclosed in the present disclosure comprises a junction sequence amplified using a pair of DNA primers wherein said pair is selected from the group consisting of SEQ ID NO: 8 and 5, SEQ ID NO: 8 and 10, SEQ ID NO: 8 and 11, and SEQ ID NO: 25 and 26. DNA amplicon molecules thus produced are represented by SEQ ID NO: 21, SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 27 respectively or complements thereof.

[00095] Another embodiment of the present disclosure provides a cauliflower plant or part thereof as disclosed in the present disclosure as resistant to insect infestation. A method of producing a cauliflower plant resistant to insect infestation is provided, wherein the cauliflower plant comprises of Bt protein encoding DNA sequences.

[00096] In an embodiment the present disclosure provides a cauliflower commodity product produced from the plant, wherein said commodity product comprises said event CFE-4 and produces a DNA amplicon molecule diagnostic for the event.

[00097] Another embodiment of the present disclosure provides a method as disclosed in the present disclosure comprises crossing a plant comprising event CFE-4 with a second plant and selecting progeny comprising the CFE-4 event.

[00098] In an embodiment the present disclosure, there is provided a hybrid cauliflower plant produced from plant comprising cauliflower event CFE-4.

[00099] Another embodiment of the present disclosure provides a biological sample as disclosed in the present disclosure selected from a group consisting of cotyledon with petiole, hypocotyls, embryo, immature embryo, leaf lamina, cotyledonary axil, shoot tip, anther, root and callus or any other suitable explant.

[000100] In an embodiment of the present disclosure, there is provided a kit for detection of the CFE-4 event, said kit comprising a first primer (SEQ ID NO: 23, SEQ ID NO: 8, SEQ ID NO: 24, SEQ ID NO: 26) and a second primer (SEQ ID NO: 5, SEQ ID NO: 3, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 25), reagents, reaction buffers, detection reagents and control DNA.

[000101] Another embodiment of the present disclosure provides a kit for detection of zygosity said kit has a first primer, a second primer, and a third primer. The first primer

as disclosed in present invention has sequence as set forth in SEQ ID NO: 23, or SEQ ID NO: 8, or SEQ ID NO: 24, the second primer has sequence as set forth in SEQ ID NO: 5, or SEQ ID NO: 3, or SEQ ID NO: 10, or SEQ ID NO: 11 and the third primer has sequence as set forth in SEQ ID NO: 12.

5 **[000102]** Another embodiment of the present disclosure provides a kit for detection of zygosity said kit has a first primer, a second primer, and a third primer. The first primer as disclosed in present invention has sequence as set forth in SEQ ID NO: 23, or SEQ ID NO: 8, or SEQ ID NO: 24, the second primer has sequence as set forth in SEQ ID NO: 5, or SEQ ID NO: 3, or SEQ ID NO: 10, or SEQ ID NO: 11 and the third primer
10 has sequence as set forth in SEQ ID NO: 26 or SEQ ID NO: 12.

[000103] In an embodiment of the present disclosure, there is provided DNA amplicons that can be produced from the sequences described herein that are diagnostic for the presence of the transgenic cauliflower event CFE-4 DNA in a biological sample. An amplicon diagnostic of transgenic cauliflower event CFE-4 DNA in a biological sample
15 comprises a junction sequence having nucleotide sequence as set forth in SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, and SEQ ID NO: 27. These amplicons can be produced using DNA primer sequences as described herein below from any biological sample of DNA derived from a transgenic cauliflower, which harbours event CFE-4. Such biological sample sources
20 of DNA corresponding to the transgenic cauliflower event CFE-4 can be cauliflower pollen, cauliflower ovule, cauliflower seed, cauliflower roots, or cauliflower leaves.

[000104] In an embodiment of the present disclosure, there is provided a method of protecting cauliflower plant from lepidopteran insect infestation, said method comprising providing in the diet of the lepidopteran insect one or more transgenic
25 cauliflower plant cells, or tissues, wherein each transgenic cauliflower plant cell genome comprises a polynucleotide sequence as set forth in SEQ ID NO: 16 or its complement, SEQ ID NO: 17 or its complement, SEQ ID NO: 18 or its complement, and SEQ ID NO: 27 or its complement.

[000105] In an embodiment of the present disclosure, lepidopteran insect that feeds on transgenic cauliflower plant cells is inhibited from further feeding on the transgenic cauliflower plant from which the transgenic cauliflower plant cells are derived.

[000106] In an embodiment of the present disclosure, there is provided a variety of cauliflower comprising DNA diagnostic for the presence of a transgenic event, CFE-4. Transgenic event, CFE-4 DNA can be obtained by breeding a cauliflower plant comprising transgenic cauliflower event CFE-4 with a cauliflower plant devoid of event CFE-4 to produce a hybrid cauliflower plant comprising DNA diagnostic for said CFE-4 event. Such a hybrid cauliflower plant comprising DNA diagnostic for the transgenic cauliflower event CFE-4 is within the scope of the present disclosure, including seeds produced from the hybrid, pollen, ovule, seed, roots, and leaves of the hybrid cauliflower plant harbouring event CFE-4.

[000107] Cauliflower plants grown from seed that are homozygous for the transgenic cauliflower event CFE-4 are also within the scope of the present invention. Cauliflower plants grown from seed that are heterozygous for the transgenic cauliflower event CFE-4 are also within the scope of the present invention so long as these seed also comprise the diagnostic DNA sequences. Cells, seed, tissue and hybrid produced from such plants comprising the diagnostic DNA are also within the scope of the present invention.

[000108] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 or a progeny thereof in a biological sample, said method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the biological sample with a first DNA primer and a second DNA primer, wherein the first DNA primer recognizes a sequence within SEQ ID: 14 and the second DNA primer recognizes a sequence within SEQ ID: 15 or SEQ ID: 22, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and detecting the presence of said DNA amplicon molecule,
wherein the presence of said DNA amplicon molecule is diagnostic for said CFE-4 event in the said sample.

[000109] Having illustrated and described the principles of the present invention, it should be apparent to a person skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications that are within the spirit and scope of the appended embodiments.

5 **[000110]** In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 or a progeny thereof in a biological sample, said method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the biological sample with the first DNA primer and the second DNA primer, wherein the first DNA primer is selected from the group
10 consisting of SEQ ID: 24, SEQ ID: 8, SEQ ID: 23, and SEQ ID NO: 26, and a second DNA primer is selected from the group consisting of SEQ ID: 5, SEQ ID: 3, SEQ ID: 10, SEQ ID: 11, and SEQ ID NO: 25, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of said DNA amplicon molecule is diagnostic
15 for said event in the said sample.

[000111] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 or a progeny thereof in a biological sample, said method comprising (a) contacting the biological sample with a first DNA primer having nucleotide sequence as set forth in SEQ ID NO: 8, and a second DNA
20 primer having nucleotide sequence as set forth in SEQ ID NO: 5, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of said DNA amplicon molecule is diagnostic for said event in the said sample.

[000112] In an embodiment of the present disclosure, there is provided a method of
25 detecting transgenic cauliflower event CFE-4 using DNA primers as set forth in SEQ ID NO: 8 and SEQ ID NO: 5, wherein the length of the DNA amplicon is 471bp and the nucleotide sequence of the DNA amplicon is as set forth in SEQ ID: 21 or its complement.

[000113] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 or a progeny thereof in a biological sample, said method comprising (a) contacting the biological sample with a first DNA primer having nucleotide sequence as set forth in SEQ ID NO: 8, and a second DNA
5 primer having nucleotide sequence as set forth in SEQ ID NO: 10, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of said DNA amplicon molecule is diagnostic for said event in the said sample.

[000114] In an embodiment of the present disclosure, there is provided a method of
10 detecting transgenic cauliflower event CFE-4 using DNA primers as set forth in SEQ ID NO: 8 and SEQ ID NO: 10, wherein the length of the DNA amplicon is 724bp and the nucleotide sequence of the DNA amplicon is as set forth in SEQ ID: 19 or its complement.

[000115] In an embodiment of the present disclosure, there is provided a method of
15 detecting transgenic cauliflower event CFE-4 or a progeny thereof in a biological sample, said method comprising (a) contacting the biological sample with a first DNA primer having nucleotide sequence as set forth in SEQ ID NO: 8, and a second DNA primer having nucleotide sequence as set forth in SEQ ID NO: 11, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting
20 the presence of said DNA amplicon molecule, wherein the presence of said DNA amplicon molecule is diagnostic for said event in the said sample.

[000116] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 using DNA primers as set forth in SEQ ID NO: 8 and SEQ ID NO: 11, wherein the length of the DNA amplicon is 902bp and
25 the nucleotide sequence of the DNA amplicon is as set forth in SEQ ID: 20 or its complement.

[000117] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 or a progeny thereof in a biological sample, said method comprising (a) contacting the biological sample with a first DNA

primer having nucleotide sequence as set forth in SEQ ID NO: 26, and a second DNA primer having nucleotide sequence as set forth in SEQ ID NO: 25, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of said DNA
5 amplicon molecule is diagnostic for said event in the said sample.

[000118] In an embodiment of the present disclosure, there is provided a method of detecting transgenic cauliflower event CFE-4 using DNA primers as set forth in SEQ ID NO: 26 and SEQ ID NO: 25, wherein the length of the DNA amplicon is 198 bp and the nucleotide sequence of the DNA amplicon is as set forth in SEQ ID: 27 or its
10 complement.

[000119] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 in a biological sample, said method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the sample having cauliflower DNA
15 with a first DNA primer, a second DNA primer, and a third DNA primer, wherein the first DNA primer recognizes a sequence as set forth in SEQ ID: 8, the second DNA primer recognizes a sequence as set forth in SEQ ID: 5 and the third DNA primer recognizes a sequence as set forth in SEQ ID: 12, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said
20 DNA amplicon molecule, wherein the presence of two DNA amplicons of sizes 471bp and 326bp indicates heterozygosity of the transgenic cauliflower event CFE-4 and presence of a single amplicon of size 471bp indicates homozygosity of the said event.

[000120] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event
25 CFE-4 in a biological sample, said method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the sample having cauliflower DNA with a first DNA primer, a second DNA primer, and a third DNA primer, wherein the first DNA primer recognizes a sequence as set forth in SEQ ID: 8, the second DNA primer recognizes a sequence as set forth in SEQ ID: 5 and the third DNA primer

recognizes a sequence as set forth in SEQ ID: 26, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of two DNA amplicons of sizes 471bp and 407 indicates heterozygosity of the transgenic cauliflower event CFE-4 and presence of a single amplicon of size 471bp indicates homozygosity of the said event

[000121] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 in a biological sample, the method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the sample having cauliflower DNA with a first DNA primer, a second DNA primer and a third DNA primer, wherein the first DNA primer recognizes a sequence as set forth in SEQ ID: 8, the second DNA primer recognizes a sequence as set forth in SEQ ID: 10 and the third DNA primer recognizes a sequence as set forth in SEQ ID: 12, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of two DNA amplicons of sizes 724bp and 326bp indicates heterozygosity of the transgenic cauliflower event CFE-4 and presence of a single amplicon of size 724bp indicates homozygosity of the said event.

[000122] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 in a biological sample, said method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the sample having cauliflower DNA with a first DNA primer, a second DNA primer, and a third DNA primer, wherein the first DNA primer recognizes a sequence as set forth in SEQ ID: 8, the second DNA primer recognizes a sequence as set forth in SEQ ID: 12 and the third DNA primer recognizes a sequence as set forth in SEQ ID: 26, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of two DNA amplicons of sizes 724bp and 407 indicates heterozygosity of the transgenic cauliflower event CFE-4 and presence of a single amplicon of size 724bp indicates homozygosity of the said event.

[000123] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 in a biological sample, the method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b) contacting the sample having cauliflower DNA with a first DNA primer, a second DNA primer and a third DNA primer, wherein the first DNA primer recognizes a sequence as set forth in SEQ ID: 8, the second DNA primer recognizes a sequence as set forth in SEQ ID: 11 and the third DNA primer recognizes a sequence as set forth in SEQ ID: 12, (c) performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d) detecting the presence of said DNA amplicon molecule, wherein the presence of two DNA amplicons of sizes 902bp and 326bp indicates heterozygosity of the transgenic cauliflower event CFE-4 and presence of a single amplicon of size 902bp indicates homozygosity of the said event.

[000124] In an embodiment of the present disclosure, there is provided a method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4 in a biological sample, said method comprising (a) obtaining a biological sample comprising of cauliflower DNA, (b)contacting the sample having cauliflower DNA with a first DNA primer, a second DNA primer, and a third DNA primer, wherein the first DNA primer recognizes a sequence as set forth in SEQ ID: 8, the second DNA primer recognizes a sequence as set forth in SEQ ID: 11, and the third DNA primer recognizes a sequence as set forth in SEQ ID: 26, (c)performing a DNA amplification reaction to produce a DNA amplicon molecule, and (d)detecting the presence of said DNA amplicon molecule, wherein the presence of two DNA amplicons of sizes 902bp and 407indicates heterozygosity of the transgenic cauliflower event CFE-4 and presence of a single amplicon of size 902bp indicates homozygosity of the said event.

[000125] In an embodiment of the present disclosure, there is provided an isolated DNA molecule comprising any one of the DNA amplicon molecules having sequence as set forth in SEQ ID NO: 21, or SEQ ID NO: 19, or SEQ ID NO: 20, or SEQ ID NO: 27..

[000126] In an embodiment of the present disclosure, there is provided DNA amplicon molecules, wherein the sequence of the said DNA amplicon molecules is selected from the group consisting of SEQ ID: 16, SEQ ID: 17, SEQ ID: 18, SEQ ID: 19, SEQ ID: 20, SEQ ID: 21, SEQ ID NO: 27, and complements thereof.

5 **[000127]** In an embodiment of the present disclosure, there is provided an isolated DNA molecule comprising of junction sequence amplified by a primer pair that recognizes the nucleotide sequence as set forth in SEQ ID: 21, SEQ ID: 19, SEQ ID: 20 and SEQ ID NO: 27.

[000128] In an embodiment of the present disclosure, DNA primers having nucleotide 10 sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 5 are used to produce a polynucleotide fragment having sequence as set forth in SEQ ID NO: 21, wherein said polynucleotide fragment is indicative of transgenic event CFE-4.

[000129] In an embodiment of the present disclosure, DNA primers having nucleotide sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 10 are used to produce a 15 polynucleotide fragment having sequence as set forth in SEQ ID NO: 19, wherein said polynucleotide fragment is indicative of transgenic event CFE-4.

[000130] In an embodiment of the present disclosure, DNA primers having nucleotide sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 11 are used to produce a polynucleotide fragment having sequence as set forth in SEQ ID NO: 20, wherein said 20 polynucleotide fragment is indicative of transgenic event CFE-4.

[000131] In an embodiment of the present disclosure, DNA primers having nucleotide sequence as set forth in SEQ ID NO: 25 and SEQ ID NO: 26 are used to produce a polynucleotide fragment having sequence as set forth in SEQ ID NO: 27, wherein said polynucleotide fragment is indicative of transgenic event CFE-4.

25 **[000132]** In an embodiment of the present disclosure, there is provided primer DNA sequences for detection of the transgenic cauliflower event CFE-4, wherein the primer DNA sequences are selected from the group consisting of SEQ ID: 24, SEQ ID: 8. SEQ ID: 23, SEQ ID: 5, SEQ ID: 3, SEQ ID: 10, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID: 11.

[000133] In an embodiment of the present disclosure, there is provided an insect resistant transgenic cauliflower plant or part thereof expressing Bt gene comprising event CFE-4, wherein representative cauliflower seeds comprising event CFE-4 have been deposited under accession number NCIMB 41809.

5 **[000134]** In an embodiment of the present disclosure, there is provided a transgenic plant or parts thereof comprising a junction sequence amplified using a pair of primers as set forth in SEQ ID: 8 and SEQ ID: 5, or SEQ ID: 8 and SEQ ID: 10, or SEQ ID: 8 and SEQ ID: 11 or SEQ ID NO: 25 and SEQ ID NO: 26.

[000135] In an embodiment of the present disclosure, there is provided a cauliflower 10 commodity product, wherein said commodity product comprises a transgenic event CFE-4, and produces a DNA amplicon molecule diagnostic for said event.

[000136] In an embodiment of the present disclosure, there is provided a hybrid cauliflower plant produced from a plant comprising a transgenic cauliflower event CFE-4.

15 **[000137]** In an embodiment of the present disclosure, there is provided a biological sample for detection of transgenic event CFE-4, wherein the biological sample is selected from the group consisting of cotyledon with petiole, hypocotyls, embryo, immature embryo, leaf lamina, cotyledonay axil, shoot tip, anther, root, and callus.

[000138] In an embodiment of the present disclosure, there is provided a kit for 20 detection of transgenic cauliflower event CFE-4, said kit comprises of a first DNA primer of nucleotide sequence selected from the group consisting of SEQ ID: 24, SEQ ID: 8, SEQ ID NO: 26, and SEQ ID: 23 and a second DNA primer of nucleotide sequence selected from the group consisting of SEQ ID: 5, SEQ ID: 3, SEQ ID: 10, SEQ ID NO: 25, and SEQ ID: 11, reagents, reaction buffers, detection reagents and 25 control DNA molecule.

[000139] In an embodiment of the present disclosure, there is provided a kit for detection of zygosity of transgenic cauliflower event CFE-4, said kit comprises of a first primer of nucleotide sequence selected from the group consisting of SEQ ID: 24, SEQ ID: 8, and SEQ ID: 23, a second primer of nucleotide sequence selected from the group

consisting of SEQ ID: 5, SEQ ID: 3, SEQ ID: 10, and SEQ ID: 11 and a third primer of nucleotide sequence selected from the group consisting of SEQ ID: 12, and SEQ ID NO: 26, reagents, reaction buffers, detection reagents and control DNA molecule.

[000140] In an embodiment of the present disclosure, there is provided an insect resistant transgenic cauliflower plant harbouring event CFE-4.

[000141] In an embodiment of the present disclosure, there is provided an insect resistant transgenic cauliflower plant or parts thereof, including seeds, harbouring event CFE-4.

EXAMPLES

[000142] The disclosure will now be illustrated with working examples, which is intended to illustrate the working of disclosure and not intended to take restrictively to imply any limitations on the scope of the present disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

Example 1

Transformation of Cauliflower

[000143]Plant material: Cauliflower seeds (*Brassica oleracea* L., variety SUNGRO ES-67) were surface sterilized in 0.1% (weight/volume) Mercury II Chloride ($HgCl_2$) in distilled water for five-minutes at room temperature. Sterilized seeds were washed five times in sterile water and placed on CC0 medium for germination. The cotyledons were excised from four-day old seedlings and were used in transformations. Table 1 provides details and the medium used

Table 1: Media code with composition

MEDIA CODE	MEDIA COMPOSITION
CC0	MS salts (Murashige and Skoog 1962), phytagel 0.7%

MEDIA CODE	MEDIA COMPOSITION
CC1	MS salts (Murashige and Skoog 1962), B5 vitamin (Gamborg *et al*. 1968), phytagel 0.7%, sucrose 2%
CC2	CC1 + BAP 3mg/l, NAA 0.02 mg/l, GA3 0.01 mg/l, Silver nitrate 0.5 mg/l, cefotaxime 500mg/l
CC2 (S-1)	CC2 with kanamycin 30 mg/l
CC2 (S-2)	CC2 with kanamycin 30 mg/l, phytagel 0.3%
MSI	MS salts, B5 vitamins, sucrose 3% phytagel 0.3%, IBA 2mg/l, cefotaxime 250 mg/l, kanamycin 30 mg/l
pH of all the above media were adjusted to 6 except MSI to 5.8	

Transformation using *cry1Ac* and *nptII* genes

[000144] Cauliflower transformations were performed using *Agrobacterium tumefaciens* strain EHA105 carrying plasmid pC2300ve10518, wherein the *cry1Ac* gene
5 expression is driven by the e35S Cauliflower Mosaic Virus promoter. The *cry1Ac* gene cloned into the vector pCAMBIA2300 plasmid and introduced into the *Agrobacterium tumefaciens* strain EHA105 was used for transformations. Transformed *Agrobacterium tumefaciens* was inoculated in 25mL of 2YT medium (pH 7.0) in a flask with respective antibiotics (kanamycin & chloramphenicol) for the selective growth of transformed
10 *Agrobacterium tumefaciens* with plasmid pC2300ve10518. The culture was kept on a shaker at 28^0C with 180RPM to get optical density of 0.3 (measured at 600nm).

Incubation of explants with transformed *Agrobacterium tumefaciens* and shoot bud induction

[000145] Cotyledons excised from four-day old seedlings were incubated in a
15 suspension containing transformed *Agrobacterium tumefaciens* having recombinant plasmid, pC2300ve10518 for 15 minutes, and blotted dry on sterile filter paper. The dried cotyledons were subsequently transferred to CC1 medium for co-cultivation. No

more than 20 cotyledons per petri dish were plated. The petri dishes were incubated at 25±1°C for four days in a tissue culture incubation room.

[000146] After four days of cultivation period the explants were transferred to post-culture medium (CC2) with no more than 10 cotyledons per plate. After seven days of post-culturing the cotyledons at 25±1°C, the cotyledons were transferred on to CC2 (S-1) for selection I. (See Table 1).

[000147] The cotyledons obtained from selection I were transferred on to selection II medium CC2 (S-2) as indicated in (Table 1) and after 3-4 weeks of growth at 25±1°C, Kanamycin resistant putative transgenic shoots were developed. The shoots thus obtained were subcultured on MSI medium wherein shoots were rooted. The rooted plants were transplanted from MSI medium and allowed to grow further in sterile potting mixture. After the establishment of the plants, the plants were subsequently shifted to larger earthen pots. The tissue samples obtained from rooted plants were used in various assays.

ELISA analysis for Cry1Ac protein

[000148] The putative transgenic plants carrying the *cry1Ac* gene were tested for the expression of Cry1Ac protein using ELISA method. The ELISA plates, supplied by Desigen Diagnostics, Maharashtra, India were coated with monoclonal antibodies specific to the Cry1Ac protein. Tissue samples from non-transgenic plants were used as negative control for the ELISA assay.

Example 2

Identification of cauliflower plant harbouring the CFE-4 event and having insect resistance

A subset of the ELISA positive lines were subjected to the insect bio-assays using DBM larvae.

[000149] Four ELISA positive primary transformants were regenerated that express the *Cry1Ac* gene. Out of the four lines analysed, Mendelian segregation pattern i.e. 3:1 ratio (Table 2) of transgene (*cry1Ac* gene) inheritance in two lines (T_1 generation seedlings) indicated that the integration of the *cry1Ac* gene was confined to a single

locus in the plant host genome. The transgenic leaves expressing the *cry1Ac* gene showed complete protection from the target pest in the bioassays and 100% susceptibility was observed in the case of non-transgenic cauliflower leaf discs as indicated in Table 3.

Table 2: Segregation analysis of *cry1Ac* gene expression in transgenic T_1 plants
5 **obtained from selfed four T_0 lines**

S.NO.	PLANT /EVENT	NUMBER OF SEEDLINGS			χ^2 VALUE (3:1)
	Total	Total	Cry1Ac +	Cry1Ac -	
1.	CFE -1	19	06	13	19.09
2.	CFE-2	09	03	06	8.24
3.	CFE -3	09	05	04	1.83
4.	CFE -4	10	07	03	0.04

[000150] It can be inferred from Table 2 that event CFE-4 represents a transgenic cauliflower with a single locus insert of the transgene in the transformed host.

Table 3: Cauliflower CFE-4 Bioassay Results

Transgenic Plant : Cauliflower
10 Name of the Insect : *Plutella xylostella*
Stage of Insects : 1st instar larva
No. of Insects : 4 larvae/ replication

PLANT CODE	REPLICATION	FEEDING AREA (Sq mm)	STATUS OF LARVAE	
			LIVE	DEAD
Control (non Bt)	1	50	4	0
	2	35	4	0
	3	30	4	0
CFE-4	1	2	0	4

PLANT CODE	REPLICATION	FEEDING AREA (Sq mm)	STATUS OF LARVAE	
			LIVE	DEAD
	2	2	0	4
	3	2	0	4
CFE-4	1	2	0	4
	2	2	0	4
	3	2	0	4

Example 3

Molecular characterization of the event CFE-4

[000151] Transgenic cauliflower containing CFE-4 event was analysed to identify the cauliflower genomic DNA sequences flanking the *cry1Ac* gene expression cassette. The identification of genomic DNA sequences flanking the *cry1Ac* gene expression cassette was done using the method as described in Cottage *et al.*, *Plant Molecular Biology Reporter*, 2001, 19:321-327. Plant genomic DNA was extracted from fresh young (2-3 weeks old) leaves containing event CFE-4 (Dellaporta *et al.*, *Plant Mol. Bio. Rep.*, 1983, *1*, 19-22). The extracted genomic DNA (2µg) was digested with *Dra-I* enzyme in a total volume of 20µl as indicated in Table 4. The digestion reaction was incubated at 37ºC for 18-20 hours. The digestion reaction was subsequently incubated at 65ºC for 30 minutes for enzyme inactivation and the digested DNA was precipitated with 3M sodium acetate and ethanol. Purified DNA was air-dried and dissolved in 12µl of sterile distilled water. Adapters having complementary nucleotide sequences were first annealed to each other and then the annealed adapters were further ligated to the digested genomic DNA fragments as per manufacturer's (NEB) guidelines. The ligation mixture was incubated at 14^0C for 16-18 hours for the ligation of digested genomic DNA to the annealed adapters. The ligation mixture was diluted to 100 µl for obtaining adapter library.

Table 4: Components of Restriction Digestion:

COMPONENTS	AMOUNT
Reaction buffer (10X) (final concentration 1x)	2.0 µl
Dra-I enzyme (10units/ µl)	1.0 µl
Genomic DNA (isolated from plant sample) (2 µg)	12.0 µl
Sterile water	5 µl.
Total Amount of Reaction Mixture	20 µl

Table 5: Components of Ligation Reaction

COMPONENTS	AMOUNT
Digested Genomic DNA (heat inactivated) (2 µg)	12.0 µl
Annealed adapters(100 ng/µl)	2.0 µl
10X Reaction buffer (final concentration 1x)	3.0 µl
T4-ligase enzyme(5units/µl)	1.0 µl
Sterile water	12 µl
Total Amount of Reaction Mixture	30 µl

[000152] The adapter library obtained was used to identify the plant genomic sequence flanking the transgenic insertion site by PCR method. A first round of PCR
5 amplification was carried out using a forward and a reverse primer combination. The forward primer has nucleotide sequence as set forth in SEQ ID NO: 3 and is complementary to the inserted transgene sequence and the reverse primer has nucleotide sequence as set forth in SEQ ID NO: 4. Components used for the first PCR reaction and thermal cycler details are indicated in Table 6 & 7.

10 **Table: 6 Components of First PCR Reaction:**

COMPONENTS	AMOUNT
10X reaction buffer (with $MgCl_2$)	2.5 µl
10mM dNTP's	0.5 µl

COMPONENTS	AMOUNT
Forward Primer (SEQ ID NO:3)(100ng/µl)	1.0 µl
Reverse Primer (SEQ ID NO: 4)(100 ng/µl)	1.0 µl
Taq DNA polymerase (5 units/µl)	0.5 µl
DNA template	3.0 µl
Nuclease-free water	11.5 µl
Total Amount of Reaction Mixture	25 µl

Table 7: Thermal Cycler program

<table>
<tr><th>TEMPERATURE</th><th>TIME</th><th>CYCLES</th></tr>
<tr><td>95 °C</td><td>5 min</td><td>1</td></tr>
<tr><td>95 ^{0}C</td><td>30 seconds</td><td rowspan="3">40</td></tr>
<tr><td>58 ^{0}C</td><td>30 seconds</td></tr>
<tr><td>68 ^{0}C</td><td>4 minutes</td></tr>
<tr><td>68° C</td><td>10 minutes</td><td>1</td></tr>
<tr><td>4 °C</td><td>hold</td><td></td></tr>
</table>

[000153] The second round of PCR reaction was carried out to obtain the specific plant genomic flanking region adjacent to the left border of the inserted heterologous gene. The PCR reaction was carried out using forward primer having nucleotide sequences as set forth in SEQ ID NO: 5 and a reverse primer having nucleotide sequences as set forth in SEQ ID NO: 6. Components used for the second PCR reaction and thermal cycler details are indicated in Table 8 & 9

Table 8: Components of second PCR Reaction:

COMPONENTS	AMOUNT
10X reaction buffer (with $MgCl_2$)	2.5 µl
10mM dNTP's	0.5 µl
Forward Primer (SEQ ID NO:5)(100ng/µl)	1.0 µl
Reverse Primer (SEQ ID NO: 6)(100 ng/µl)	1.0 µl

COMPONENTS	AMOUNT
Taq DNA polymerase (5 units/µl)	0.5 µl
DNA template	2.0 µl
Nuclease-free water	12.5 µl
Total Amount of Reaction Mixture	25 µl

Table 9: Thermal Cycler program:

TEMPERATURE	TIME	CYCLES
95^0c	5 minutes	1
95^0c 58^0c 68^0c	30 seconds 30 seconds 4 minutes	40
68^0c	10 minutes	1
4^0c	hold	

[000154] A small amount of PCR product was analysed on a 1% agarose gel, and the amplified fragment was eluted from the gel by using methods well known in the art. A DNA fragment (amplicon) of 588bp was amplified from the left border region of the T-DNA after two rounds of PCR (using a forward primer as set forth in SEQ ID NO: 6 and a reverse primer as set forth in SEQ ID NO: 5). The amplified fragment (amplicon) was cloned into pGEM-T Easy vector to obtain a recombinant vector. The recombinant vector was transformed into a strain of *E.coli* (e.g. DH5α) by using methods well-known in the art. The clone comprising this recombinant vector was selected for analysing the sequence of the flanking nucleotide regions and was designated as CFE-4 -Clone 1*Dra I.* Plasmid DNA from the clone CFE-4-Clone 1*Dra I* was isolated using standard methods known in the art. The cloned fragment (amplicon) was sequenced using SP-6 primer. The polynucleotide sequence of the cloned fragment is represented by sequence as set forth in SEQ ID NO: 7. The SEQ ID NO: 7 contains the a part of the adapter sequence, cauliflower genomic DNA sequence flanking the left border of the inserted transgene and T-DNA from the left border up to SEQ ID NO: 5.

[000155] Sequence ID NO: 7 consists of a part of the adapter starting with primer

sequence as set forth in SEQ ID NO: 6 followed by cauliflower genomic DNA sequence of flanking the left side of the CFE-4 event, followed by T-DNA sequence terminating in sequence as set forth in SEQ ID NO: 5. The junction sequence of the cauliflower CFE-4 event has been characterized by sequence analysis. The junction nucleotide sequence is represented by the DNA sequence as set forth in SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18. The plant flanking sequences are represented by DNA sequence as set forth in SEQ ID NO: 14. SEQ ID NO: 15 represents the T-DNA heterologous insert sequence starting from the left border up to the *Cry1Ac* gene start sequence.

Example 4

Diagnostic methods for identification of the CFE-4 event:

[000156] To detect the presence or absence of the transgenic cauliflower event CFE-4, a molecular diagnostic method was developed**.** The sequence of the polynucleotide fragment as set forth in SEQ ID NO: 7 was used to design primers that amplify the transgenic insertion locus. Primers were developed to amplify the left junction sequence in the CFE-4 event. Amplification of the polynucleotide sequence in the CFE-4 event requires two primers; the first primer in the plant flanking region and a second primer in the heterologous insert region comprising the *cry1Ac* gene. The first primer having nucleotide sequence as set forth in SEQ ID NO: 8 and the second primer having nucleotide as set forth in SEQ ID NO: 5 were used to amplify the transgenic insertion locus left junction sequence from event CFE-4 genomic DNA. The DNA amplicon thus obtained from the primers comprises the junction sequence represented by nucleotide sequences as set forth in SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18. Detection of said amplicon is used as diagnostic tool for the detection of the presence of a transgenic cauliflower CFE-4 event.

[000157] The primer pair used for the amplification of sequences specific to cauliflower event CFE-4 are the nucleotide sequences as set forth in SEQ ID NO: 8 and SEQ ID NO: 5, but the amplicon obtained from the primer pair is not limited to nucleotide sequences as set forth in SEQ ID NO: 8 and SEQ ID NO: 5. For the

amplification of the 5’region, any primer pair derived from nucleotide sequences as set forth in SEQ ID NO: 14 and SEQ ID NO: 15 or SEQ ID NO: 22 can be used and wherein the DNA amplification reaction produces a DNA amplicon molecule diagnostic for said event CFE-4 is an embodiment of the present invention.

5 **[000158]** Similar to amplification of left border junction sequences as discussed above, primers having SEQ ID NO: 25 and SEQ ID NO: 26 were used to amplify right border junction sequences (amplicon sequence is as set forth in SEQ ID NO: 27).

[000159] SEQ ID NO: 9 consists of a part of the adapter followed by cauliflower genomic DNA sequence flanking the left side of the CFE-4 event, followed by partial

10 T-DNA sequence.

[000160] However, any modification of the above said methods that use DNA molecules or complements thereof to produce a DNA amplicon molecule diagnostic for the event CFE-4 is within the ordinary skill of the art. For example, primers as set forth in SEQ ID: 8 when used in combination with a primer having nucleotide sequence as

15 set forth in SEQ ID NO: 10, will produce a DNA amplicon molecule of 724 bp, or in combination with a primer having nucleotide sequence as set forth in SEQ ID NO: 11, will amplify a 902 bp DNA amplicon molecule from event CFE-4.

[000161] For the analysis of a DNA amplicon molecule diagnostic for event CFE-4, it is important to have both positive and negative controls. The PCR method was designed

20 in order to distinguish the CFE-4 event from the other cauliflower transgenic and non-transgenic events. Genomic DNA from cauliflower event CFE-4 was isolated from leaves using the method described by Dellaporta *et al.*, *(Plant Mol. Bio. Rep.*, 1983, 1:19-22). Genomic DNA isolated from other cauliflower transgenic events and non-transgenic cauliflower lines serve as negative controls for the PCR detection method. A

25 control reaction having no DNA in the reaction mixture was also included. The genomic DNA from different plants was subjected to amplification.

Table: 10 Components of Event ID PCR

COMPONENTS	AMOUNT
PCR Reaction buffer (with $MgCl_2$)	2.5 µl

COMPONENTS	AMOUNT
(10X)	
dNTP mix (10 mM)	0.5 µl
Primer (SEQ ID NO: 5) (100 ng/µl)	1.0 µl
Primer (SEQ ID NO: 8) (100 ng/µl)	1.0 µl
Taq DNA polymerase (5 units/µl)	0.5 µl
Genomic DNA (isolated from plant sample)	2.0 µl
Nuclease-free water	Make up to 25 µl
Total Amount of Reaction Mixture	25 µl

Table 11: Thermal Cycler program

TEMPERATURE	TIME	CYCLES
95 °C	5 min	1
95^0 C	30 seconds	35
56^0 C	30 seconds	
72^0 C	40 seconds	
72 °C	5 min	1
4 °C	Hold	---

[000162] The amplified product was analyzed on 1% agarose gel as shown in Figure 3.

Lane 1: molecular weight marker.

Lane 2: no DNA sample.

Lane 3: DNA from non-transgenic cauliflower plant.

Lane 4: DNA from transgenic plants that do not contain event CFE-4.

Lane 5: DNA from event CFE-4.

From figure 3, it is inferred that the 471bp fragment is amplified only from the cauliflower CFE-4 event but not from other transgenic cauliflower events or non-transgenic cauliflower plants. The sequence of the 471bp DNA amplicon molecule is set forth in SEQ ID NO: 21.

[000163] Similarly, PCR was carried out with the combination of primers having nucleotide sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 10 to yield a DNA amplicon molecule of size 724bp having nucleotide sequence as set forth in SEQ ID NO: 19.

[000164] PCR carried out with the combination of primers having nucleotide sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 11 to yield a DNA amplicon molecule of size 902bp having nucleotide sequence as set forth in SEQ ID NO: 20.

[000165] PCR carried out with the combination of primers having nucleotide sequence as set forth in SEQ ID NO: 25 and SEQ ID NO: 26 yield a DNA amplicon molecule of size 198bp having nucleotide sequence as set forth in SEQ ID NO: 27.

Example 5

Zygosity assay for cauliflower CFE-4 event

[000166] Cauliflower genomic DNA sequence from the right border of the T-DNA from event CFE-4 was analyzed and a reverse primer was designed having nucleotide sequence as set forth in SEQ ID NO: 12. The primer having nucleotide sequence as set forth in SEQ ID NO: 8, when used in combination with a primer having nucleotide sequence as set forth in SEQ ID NO: 5, will amplify a nucleotide sequence of 471bp (event CFE-4 specific band). The primer having nucleotide sequence as set forth in SEQ ID NO: 8, when used in combination with the primer having nucleotide sequence as set forth in SEQ ID NO: 12, will amplify a nucleotide sequence of 326bp (non-transgenic allele/plant band). The details of the PCR conditions for conducting zygosity PCR for event CFE-4 event is indicated in Table 12 and Table 13.

[000167] Primer having sequence as set forth in SEQ ID NO: 26 can also be used in place of SEQ ID NO: 12. The amplicon generated by PCR using primers having

sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 26 yield an amplicon of length 407 in a non-transgenic plant.

Table 12: The PCR conditions for conducting zygosity PCR for CFE-4 event

COMPONENTS	AMOUNT
10X reaction buffer (with $MgCl_2$)	2.5 µl
10mM dNTP's	0.5 µl
Primer (SEQ ID NO: 8)(100 ng/µl)	1.0 µl
Primer (SEQ ID NO: 5)(100 ng/µl)	1.0 µl
Primer (SEQ ID NO: 12/26)(100 ng/µl)	1.0 µl
Taq DNA polymerase (5 U/µl)	0.5 µl
DNA template	2.0 µl
Nuclease-free water	Make up to 25 µl
Total Amount of Reaction Mixture	25 µl

5 **Table 13: Thermal Cycler program:**

STEP	TEMPERATURE	TIME	CYCLES
1	95^0c	5 minutes	1
2	95^0c 55^0c 72^0c	30 seconds 30 seconds 90 seconds	35
3	72^0c	5 minutes	1
4	4^0c	Hold	---

[000168] On using primers having nucleotide sequence as set forth in SEQ ID NO: 8, SEQ ID NO: 5, and SEQ ID NO: 12 the presence of two bands of sizes 471bp and 326bp is indicative of heterozygosity of said transgenic CFE-4 event, while presence of

a single 471bp band is indicative of homozygosity of said transgenic event CFE-4. Absence of the 471bp band is indicative of absence of the said transgenic event CFE-4.

[000169] Similarly, primers having nucleotide sequence as set forth in SEQ ID NO: 24 or SEQ ID NO: 23 and SEQ ID NO: 3 or SEQ ID NO: 10 or SEQ ID NO: 11 and
5 SEQ ID NO: 12 can be used to determine the zygosity of the said transgenic event CFE-4.

I/We claim:

1. A method of detecting transgenic cauliflower event CFE-4 in a biological sample, said method comprising:

5 **a.** obtaining a biological sample comprising of cauliflower DNA;

b. contacting said biological sample with a first DNA primer and a second DNA primer;

c. performing a DNA amplification reaction to produce a DNA amplicon molecule; and

10 **d.** detecting the presence of said DNA amplicon molecule,

wherein the detection of presence of said DNA amplicon molecule is diagnostic for said CFE-4 event.

2. The method as claimed in claim 1, wherein the first DNA primer is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 24, and
15 SEQ ID NO: 26, and wherein the second DNA primer is selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 25.

3. The method as claimed in claim 1, wherein said DNA amplicon comprises of at least 50 contiguous nucleotides selected from the group of sequences consisting of SEQ
20 ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 27, and complements thereof.

4. A method of determining the zygosity of DNA of a transgenic cauliflower plant comprising of event CFE-4, said method comprising:

a. obtaining a biological sample comprising of cauliflower DNA;

25 **b.** contacting said biological sample with a first DNA primer, a second DNA primer, and a third DNA primer;

c. performing a DNA amplification reaction to produce a DNA amplicon molecule; and

d. detecting the presence of said DNA amplicon molecule,

wherein, detection of presence of more than one DNA amplicon having different nucleotide sequences is indicative of heterozygosity of the transgenic cauliflower event CFE-4, while detection of presence of one or more DNA amplicon with identical nucleotide sequence is indicative of homozygosity of the transgenic event CFE-4.

5 **5.** The method as claimed in claim 4, wherein the first DNA primer is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 26.

6. The method as claimed in claim 4, wherein the second DNA primer is selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 10, SEQ ID
10 NO: 11, and SEQ ID NO: 25.

7. The method as claimed in claim 4, wherein the third DNA primer is selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 23, SEQ ID NO: 24, and SEQ ID NO: 26.

8. A synthetic DNA molecule comprising of any of the DNA amplicons obtained
15 from a method as claimed in claim 1.

9. The method as claimed in claim 1 or claim 4, wherein the biological sample is selected from the group consisting of cotyledon with petiole, hypocotyls, embryo, immature embryo, leaf lamina, cotyledonary axil, shoot tip, anther, root and callus or any other suitable explants.

20 **10.** A kit for detection of transgenic cauliflower event CFE-4 comprising of forward and reverse primers having at least 10 contiguous nucleotides selected from the group of sequences consisting of SEQ ID NO: 14, SEQ ID NO: 22, SEQ ID NO: 15, and SEQ ID NO: 27.

Dated this 25 October 2013

25 Malathi Lakshmikumaran
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Agent for the Applicant

To,
The Controller of Patents,
30 The Patent Office, at New Delhi

ABSTRACT

INSECT RESISTANT TRANSGENIC CAULIFLOWER PLANT COMPRISING EVENT CFE-4 AND METHODS OF DETECTION THEREOF

5

The present disclosure relates to method of producing an insect resistant transgenic cauliflower plant comprising of event CFE-4. The present disclosure also provides methods of detection of event CFE-4, and zygosity of the said event in plant genome. Further, also provided are primers, and DNA fragments that are useful in production
10 and detection of the CFE-4 event in cauliflower plants.

15