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(71) Applicant: **MAHARASHTRA HYBRID SEEDS COMPANY PRIVATE LIMITED (MAHYCO)** [IN/IN]; Jalna - Aurangabad Road, Post Box No. 76, Dawalwadi, Jalna 431203, Maharashtra (IN).

(72) Inventors: **CHAR, Bharat**; RH2-D, Mahyco Housing, Dawalwadi, Jalna-Aurangabad Road, Jalna 431203, Maharashtra (IN). **GANDHI, Ratnapal Popatlal**; RH1, Mirajgave nagri near Mahkse petrol pump Beed by pass Aurangabad, 431001, Maharashtra (IN). **NAIR, Narendran Madhavan**; 2BHK B, Mahyco Housing, Dawalwadi, Jalna-Aurangabad Road, Jalna 431203, Maharashtra (IN).

(74) Agent: **SAURASTRI, Anshul Sunilkumar**; Krishna & Saurastri Associates LLP, 74/F, Venus, Worli Sea Face, Mumbai 400 018, Maharashtra (IN).

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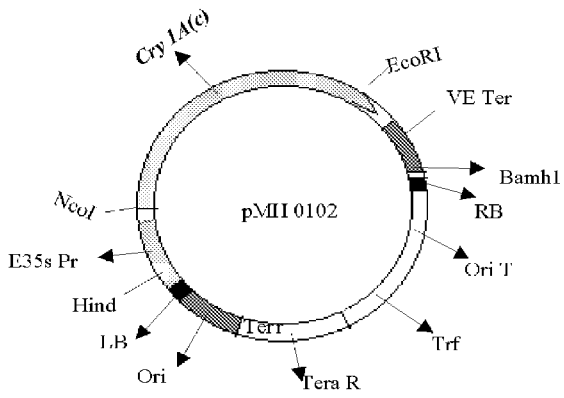


Figure 1

(57) Abstract: The invention provides brinjal event MAH-45151, and plants, plant cells, seeds and plant parts comprising event MAH-45151 which confers resistance to Lepidopteran insect damage. The invention also provides nucleic acid sequences specific for event MAH-45151 and plants, plant cells, seeds and plant parts comprising nucleic acids specific for event MAH-45151. This invention also provides methods for detection the presence of the event MAH-45151 based on DNA sequence of the recombinant construct inserted into the brinjal genome that resulted in the MAH-45151 event and/or the genomic sequences flanking the insertion site.

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BRINJAL (SOLANUM MELONGENA) EVENT MAH-45151 AND COMPOSITION AND METHOD OF DETECTION

FIELD OF INVENTION

This invention relates to a novel transformation event MAH-45151 of brinjal plant, *Solanum melongena* that exhibits tolerance to fruit and shoot borer. The invention also provides nucleic acid molecules that are unique to the event and methods related to the same. Kits and conditions useful in conducting assays for detection of the event MAH-45151 are also provided.

BACKGROUND OF INVENTION

Brinjal has been cultivated in the country for last 4,000 years, although it is often thought of as a Mediterranean or mid-Eastern vegetable. India has been widely considered as a source of origin for brinjal. Amongst the Solanaceous vegetables, brinjal (*Solanum melongena* Linn) is the most common, popular and principal vegetable crops grown in many geographical parts in India. The area under brinjal cultivation is estimated at 0.51 million ha. with total production of 8,200,000 Mt (FAO data, 2004, http://faostat.fao.org/). Brinjal is grown by small farmers and is an important source of income. It is a versatile crop, adapted to different agro-climatic regions and can be grown throughout the year in India. A number of cultivars are grown in the country, consumer preference being dependent upon the yield of the cultivars, fruit color, size and shape. It is a highly productive crop, the fruit are consumed as cooked vegetables in various ways, and rural people in India use dried shoots as fuel. It is a good source of minerals and vitamins and rich in total water, soluble sugars, free reducing sugars and amide proteins etc.

Brinjal plants are susceptible to insect infestation in all areas of the world in which the plants are cultivated. However, in recent years the production of brinjal in the Indian sub-continent has been seriously affected due to a steady increase in the insect pest infestation, especially the fruit and shoot borer, *Leucinodes orbonalis* (Guen). The young larvae of the fruit and shoot borer, bore in to petioles and midribs of large leaves and tender shoots causing shoot tips to wilt and later they bore into flower buds and fruits. The affected fruits lose their market value over and above the considerable reduction in yield. In India, it has been estimated that fruit and shoot borer causes damage to fruits ranging from 25.8 - 92.5 % and yield reduction from 20.7 - 60%.

Farmers use large quantities of chemical insecticides singly or in combination to get blemish free fruits, which fetch premium prices in the market. This practice of indiscriminate use of

insecticides leads to build up of pesticide residues in the produce, destruction of natural enemies, pest resurgence and environmental pollution.

Recombinant DNA technology has been applied to plants for more than a decade to improve varieties due to their economic significance. Brinjal plants which exhibit improved characteristics as a result of the insertion of heterologous DNA sequences have been produced using such recombinant DNA technology and it exhibits following improvement.

To reduce pest-linked damage in brinjal crop as well as to protect the environment from adverse effects of pesticides, deploying the lepidopteran specific *cry1Ac* gene under the control of a suitable promoter for a high level of expression in brinjal may provide an effective built-in control for fruit and shoot borer. This would result in bringing down the cultivation costs of brinjal, as contribution of chemical pesticides to brinjal cultivation is sizable.

The source organism for *cry1Ac* gene is *Bacillus thuringensis* (Bt), which is a Gram positive bacterium synthesizing insecticidal crystalline (Cry) inclusions during sporulation. The *cry1Ac* gene present in Bt brinjal encodes the *cry1Ac* (δ-endotoxins) of 130 kda and is highly specific to Lepidpoteran larvae. *Cry1Ac* protein must be ingested by the insect to exhibit insecticidal activity. The protein in its crystalline form is insoluble in aqueous solution at neutral or acidic pH (Bulla et. al., 1977); however the pH of the larval insect gut is alkaline which favors solubilization of the protein crystal. The solubilized protein is subsequently activated by the proteases in the insect gut. These proteases cleave the carboxy terminal domain from the rest of the protein (Chroma and Kaplan, 1990) as well as approximately 28 amino acids from the amino terminal end of the protein (Bietlot et. al. 1989). The activated protein, which consists of approximately 600 amino acids, diffuses through the peritrophic membrane of the insect to the midgut epithelium. Here it binds to specific high affinity receptors on the surface of the midgut epithelium of target insects (Wolfersberger et. al., 1986; Hofman et. al., 1988; Hofman et. al., 1988a; Van Rie et. al., 1989; Van Rie et. al., 1990). Pores are formed in the membrane leading to leakage of intracellular content (eg. K+) into the gut lumen and water into the epithelial gut cells (Sacchi et. al., 1986; Knowles et. al., 1989). The larval gut epithelial cells swell due to osmotic

pressure and lyse. The gut becomes paralyzed as a consequence of changes in electrolytes and pH in the gut causing the larval insect to stop eating and die.

A number of groups have carried out transformation of brinjal using different methods. The most successful of the methods are Agrobacterium-mediated methods for transformation (Kumar et. al.1998, Nicola et. al.1998, Fári et. al.1995, Rotino et. al.1990).

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 into chromatin regions which may be more transcriptionally active (euchromatin) or less active (heterochromatin). Examples of these are methylated regions in which gene expression is suppressed, or in the proximity of transcriptional regulation elements like enhancers and suppressor, which increase or decrease gene expression respectively. Therefore, it is necessary to screen a, large number of independent transformation event for the expression of the transgene and to identify the event showing desired expression of the heterologous inserted gene. Such selection often requires greenhouse and field trials with many events over multiple years, in multiple locations, and under a variety of conditions so that a significant amount of agronomic, phenotypic, and molecular data may be collected. The resulting data and observations must then be analyzed by teams of scientists and agronomists with the goal of selecting a commercially suitable event. Such an event, once selected, may then be used for introgressing the desirable trait into other genetic backgrounds using plant breeding methods, and thus producing a number of different crop varieties that contain the desirable trait and are suitably adapted to specific local growing conditions.

It is advantageous to be able to detect presence of a particular event in order to determine whether progeny of a sexual cross contain a transgene of interest. In addition, a method for detecting a particular event may be helpful for complying with regulations requiring pre-market approval of sale of seeds to produce transgenic crop plants and foods derived from such plants, for example, or for use in environmental monitoring, monitoring traits in crops in field, or monitoring products derived from a crop harvest, as well as for use in ensuring compliance of parties subject to regulatory or contractual terms.

It is possible to detect the presence of a transgene by any nucleic acid detection method known in the art including but not limited to thermal amplification (PCR) For detection of a particular DNA construct that has been used for transforming various plant varieties, these detection methods generally focus on frequently used genetic elements, such as promoters, terminators, marker genes, etc., because for many DNA constructs, the coding sequence region is interchangeable. As a result, such methods may not be useful for discriminating between separate events produced from the same DNA construct or very similar constructs. These methods can be used, however, if the sequence of chromosomal DNA adjacent to the inserted DNA ("flanking DNA") is known. Specifically, one primer included sequence from within the T-DNA insert and a second primer included sequence from genomic DNA flanking the T- DNA. It would be desirable to have such a method that would detect the presence of the MAH-45151 event, even in the presence of other eggplant events.

SUMMARY OF THE INVENTION

In an aspect, the present invention provides a recombinant DNA comprising at least one junction sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13 and/or complement fragments thereof consisting a transgene insertion and flanking genomic DNA of brinjal event MAH-45151.

The recombinant DNA of the present invention where the nucleic acid sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13, comprises a sequence as set forth in SEQ ID NO: 16, and or the complement thereof, or a portion of SEQ ID NO: 16 or its complement thereof.

The present invention provides recombinant DNA where the nucleic acid sequence as set forth in SEQ ID NO: 7 comprises a primer sequence as set forth in SEQ ID NO: 3. The present invention also provides a recombinant DNA where the nucleic acid sequence as set forth in SEQ ID NO: 13 comprises a primer sequence as set forth in SEQ ID NO: 12.

The present invention further provides a pair of primers comprising at least 10 contiguous nucleotides selected from a sequence as set forth in SEQ ID NO:13, or its complement, where the said DNA molecule is capable of producing an amplicon comprising of sequence as set forth in SEQ ID NO: 16.

In another aspect, a pair of primers comprising of at least 15 contiguous nucleic acids each, wherein a first primer in said pair is selected from a nucleic acid sequence as set forth in SEQ ID NO: 12 and a second primer is selected from a group of sequences as set forth in SEQ ID NO: 3 or SEQ ID NO: 14 or SEQ ID NO: 15. wherein said primer pair is capable of producing an amplicon comprising SEQ ID NO: 16 diagnostic for MAH-45151 event is provided.

In yet another aspect, a method of detecting the presence of MAH-45151 event in a sample comprising the steps of: contacting the sample comprising DNA with a pair of primers wherein a first primer as set forth in SEQ ID NO: 12 or its complement thereof and a second primer can be selected from nucleic acid sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 14 and/or SEQ ID NO: 15 or its complement thereof; performing a nucleic acid amplification reaction thereby producing a diagnostic amplicon comprising SEQ ID NO: 16 for MAH-45151 event; and detecting the diagnostic amplicon as set forth in SEQ ID NO: 16.

The method according to the present invention provides that the sample comprising DNA sequence, or a portion of DNA sequence is as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13, complements or fragments thereof. The sample is a brinjal tissue.

In further aspect, a DNA detection kit is provided. The DNA detection kit according to the present invention comprises a first primer comprising at least 15 contiguous nucleic acids as set forth in SEQ ID NO: 12 or complements thereof; and a second primer comprising at least 15 contiguous nucleic acids selected from nucleic acid sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 14 and/or SEQ ID NO: 15 or its complement thereof; wherein detection of amplicon comprising SEQ ID No. 16, is diagnostic for the presence of the MAH-45151 event.

The present invention also provides a method of producing a progeny of an insect resistant brinjal plant. The method comprising modifying a brinjal plant's genome to incorporate nucleotide sequences as set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13 or complements or fragments thereof; crossing said insect resistant brinjal plant comprising the MAH-45151 event, with a brinjal plant without the said event; obtaining at least one progeny of brinjal plant derived from the cross; and selecting a progeny of brinjal plant that is insect resistant and comprises nucleotide sequence of SEQ ID NO:16.

In yet another aspect, an insect resistant brinjal plant, or parts thereof is provided. The brinjal plant according to the present invention comprises at least one nucleic acid sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 13 forms a part of the plant's genome. The present invention provides a seed, nucleus, a brinjal plant or part thereof capable of producing an amplicon comprising SEQ ID NO: 16 that is diagnostic for MAH-45151 event when subjected to PCR amplification reaction. The seed of the brinjal plant of the present invention comprises nucleic acid capable of producing an amplicon comprising SEQ ID NO: 16. and is diagnostic for MAH-45151 event and having an accession number NCIMB 42846.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a map of the construct pMH0102

Figure 2 is a digital image of gel of MAH-45151 event using the event specific primers

Figure 3 is a map of the T-DNA inserted in the MAH-45151 event along with the event specific amplicon size for detection of MAH-45151 event.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO: 1 is the nucleic acid sequence representing the 5' region of T-DNA expression cassette (MHIP-5 primer).

SEQ ID NO: 2 is the adapter primer sequence used for amplification of T-DNA flanking genomic DNA sequence (AP primer).

SEQ ID NO: 3 is the nucleic acid sequence representing the 5' region of T-DNA expression cassette (MHIP-6 primer).

SEQ ID NO: 4 is the adapter primer sequence used for amplification of T-DNA flanking genomic DNA sequence (NAP primer).

SEQ ID NO: 5 is the nucleic acid sequence used for sequencing the amplified fragment cloned in pGEMT vector (T7 primer).

SEQ ID NO: 6 is the nucleic acid sequence used for sequencing the amplified fragment cloned in pGEMT vector (SP6 primer).

SEQ ID NO: 7 represents nucleic acid sequence amplified using SEQ ID: 3 and SEQ ID: 4 primer. SEQ ID: 7 consists of a part of the T-DNA sequence starting with primer MHIP-6 followed by T-DNA sequence adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event from left border side. The flanking genomic DNA sequence is followed by adapter sequence

SEQ ID NO: 8 represents a part of the T-DNA sequence adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event from left border side.

SEQ ID NO: 9 consists of a part of the T-DNA sequence adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event from left border side.

SEQ ID NO: 10 represents a part of the T-DNA sequence adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event from left border side.

SEQ ID NO: 11 consists of a part of the T-DNA sequence adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event from left border side.

SEQ ID NO: 12 represent primer sequence designed in the left border flanking genomic DNA sequence of MAH-45151 event (MHTBJ-13 primer).

SEQ ID NO: 13 consists of nucleic acid sequence starting from SEQ ID No: 12 followed by T-DNA flanking genomic DNA sequence from left border side. Adjacent to the flanking sequence is the integrated transgenic expression cassette sequence till the end of *cry1Ac* gene. This illustrate that any primers pair designed in T-DNA region which when used in combination with SEQ ID No: 12 would act as a diagnostic tool for identification of MAH-45151 event.

SEQ ID NO: 14 is a primer sequence designed in the T-DNA region which when used in combination with SEQ ID No: 12 would act as a diagnostic tool for identification of MAH-45151 event (primer 1).

SEQ ID NO: 15 is a primer sequence designed in the T-DNA region which when used in combination with SEQ ID No: 12 would act as a diagnostic tool for identification of MAH-45151 event (primer 2).

SEQ ID NO:16 consists of a part of the T-DNA sequence adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event from left border side.

DETAILED DISCRIPTION OF THE INVENTION

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the invention. Unless otherwise noted,

terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

Definitions

As used herein, the term "comprising" means "including but not limited to".

The term "event" refers to a DNA molecule comprising the inserted DNA and the flanking brinjal genomic DNA immediately adjacent to either side of the inserted DNA. This DNA molecule is created by the act of inserting the transgenic DNA into the genome of the plant, i.e., by the act of transformation. This DNA molecule therefore comprises a nucleic acid sequence that is both specific to the event and that is unique to the genome of the plant into which the transgenic DNA has been inserted, in that this nucleic acid sequence contains both the sequence of a particular region of plant genomic DNA and of the transgenic DNA insert. The event comprises insertion of transgenic DNA into the chromosome/genome of the plant. An "event" is produced by: (i) transformation of a plant cell with a nucleic acid construct that includes a transgene of interest, (ii) regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and (iii) selection of a particular plant characterized by insertion of the transgene into a particular location in the plant's genome. The term “event” refers to the original transformant and any progeny produced by a sexual outcross between the original transformant or its descendants bearing the heterologous gene, and another brinjal variety.
As used herein, the term "brinjal" means *Solanum melongena* and includes all plant varieties that can be bred with brinjal, including wild brinjal species as well as those plants belonging to Solanum that permit breeding between species.

"Fruit and shoot borer" refers to pests causing damage to brinjal. Larva bores into tender shoots and causes withering of terminal shoots / dead hearts - also bores petioles of leaves, flower buds and developing buds, causes withering of leaves, shedding of buds and make fruits unfit for consumption. Attacked fruits are with boreholes plugged with excreta. Fruits become out of shape also.

As used herein, the term "recombinant" refers to a form of DNA and/or protein and/or an organism that would not normally be found in nature and as such was created by human intervention. Such human intervention may produce a recombinant DNA molecule and/or a recombinant plant.

As used herein, a "recombinant DNA molecule" is a DNA molecule comprising a combination of DNA molecules that would not naturally occur together and is the result of human intervention, e.g., a DNA molecule that is comprised of a combination of at least two DNA molecules heterologous to each other, and/or a DNA molecule that is artificially synthesized and comprises a nucleic acid sequence that deviates from the nucleic acid sequence that would normally exist in nature, and/or a DNA molecule that comprises a transgene artificially incorporated into a host cell's genomic DNA and the associated flanking DNA of the host cell's genome.

As used herein, a "recombinant plant" is a plant that would not normally exist in nature, is the result of human intervention, and contains a transgene and/or heterologous DNA molecule incorporated into its genome. As a result of such genomic alteration, the recombinant plant is distinctly different from the related wild type plant.

As used herein, the term "transgene" refers to a nucleic acid molecule artificially incorporated into a host cell's genome. Such transgene may be heterologous to the host cell. The term "transgenic plant" refers to a plant comprising such a transgene.

As used herein, the term "heterologous" refers to a first molecule not normally found in combination with a second molecule in nature. For example, a molecule may be derived from a first species and inserted into the genome of a second species. The molecule would thus be heterologous to the host and artificially incorporated into a host cell's genome.

As used herein, the term "chimeric" refers to a single DNA molecule produced by fusing a first DNA molecule to a second DNA molecule, where neither first nor second DNA molecule would

normally be found in that configuration, i.e., fused to the other. The chimeric DNA molecule is thus a new DNA molecule not otherwise normally found in nature.

As used herein, the term "DNA", "DNA molecule", "nucleic acid molecule" refers to a DNA molecule of genomic or synthetic origin, i.e., a polymer of deoxyribonucleic acid bases or a nucleic acid molecule, read from the 5 ' (upstream) end to the 3' (downstream) end.

As used herein, the term "DNA sequence", "nucleic acid sequence" or "nucleic acid sequence" refers to the nucleic acid sequence of a DNA molecule.

A "junction sequence" or "junction region" refers to the DNA sequence and/or corresponding DNA molecule that spans the inserted transgenic DNA and the adjacent flanking genomic DNA.

A "primer" is typically a highly purified, nucleic acid that is designed for use in specific annealing or hybridization methods that involve thermal amplification. A pair of primers may be used with template DNA, such as a sample of brinjal genomic DNA, in a thermal amplification, such as polymerase chain reaction (PCR), to produce an amplicon, where the amplicon produced from such reaction would have a DNA sequence corresponding to sequence of the template DNA located between the two sites where the primers hybridized to the template.

As used herein, an "amplicon" is a piece or fragment of DNA that has been synthesized using amplification techniques. The term “amplicon” or “amplified DNA” refers to the product of nucleic acid amplification of a target nucleic acid sequence that is part of nucleic acid template.

The term "specific for (a target sequence)" indicates that a primer hybridizes under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

The term “DNA cassette” refers to the DNA sequence with the promoter for driving the Heterologous Gene/DNA and a appropriate terminator which when present together regulates a gene expression.

As used herein, "progeny" includes any plant, seed, plant cell, and/or regenerable plant part.

As used herein, a "plant part" includes but are not limited to pollen, ovule, pod, flower, and root or stem tissue, fibers, and leaves. Plant parts may be viable, nonviable, regenerable, and/or non-regenerable.

The present invention provides a transgenic brinjal event MAH-45151 that exhibits commercially acceptable tolerance to fruit and shoot borer. Brinjal, *Solanum melongena*, has been genetically modified to resist Lepidopteran pests, thus to remove the negative impact on brinjal production by these pests. This was accomplished by the insertion of the DNA cassette that encodes the insecticidal *Cry 1 Ac* protein from *Bacillus thuringiensis*. This invention further relates to plants, plant parts, progeny plants which contain at least the nucleic acid sequences comprising the said DNA cassette, and to methods and compositions of matter for use in detecting the presence of said sequences in a sample.

The arrangement of the inserted DNA in brinjal event MAH-45151 in relation to the surrounding brinjal plant genomic DNA is specific and unique for brinjal event MAH-45151. This DNA molecule is also an integral part of the brinjal chromosome of event MAH-45151 containing plants and as such is static in the plant and may be passed on to progeny of the plant.

The present invention provides the original transformant that includes the transgene inserted into the particular location in the plant's genome and progeny of the transformant that include the transgene inserted into the particular location in the plant's genome. Such progeny may be produced by a sexual outcross between the transformant, or its progeny, and another plant. Such other plant may be a transgenic plant comprising the same or different transgene and/or a non-transgenic plant, such as one from a different 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 genomic location.

Event MAH-45151 comprises an integrated transgenic expression cassette that confers tolerance to fruit and shoot borer to the brinjal plant.

Brinjal plants were transformed with a gene (*cry1Ac*) from *Bacillus thuringiensis* encoding crystal protein (*Cry1Ac*) that confers resistance to lepidopteran insects by selectively damaging their midgut lining. *Cry1Ac* is δ endotoxins that act as insecticides. *Cry1Ac* is useful for controlling a broad spectrum of pests.

The recombinant DNA described herein results from insertion of transgene into the brinjal genomic DNA, which may ultimately result in the expression of a recombinant RNA and/or protein molecule in that organism. A recombinant plant is a brinjal plant described herein as comprising event MAH-45151.

In an aspect, the present invention thus provides a recombinant DNA molecule comprising at least one junction sequence and their corresponding nucleic acid sequences. The junction sequences of the present invention is as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 or complement thereof and fragments thereof are disclosed with reference to only one strand of the two complementary nucleic acid sequence strands. By implication, the complementary sequences (i.e. the sequences of the complementary strand), also referred to in the art as the reverse complementary sequences, are within the scope of the invention and are expressly intended to be within the scope of the subject matter claimed.

The junction sequences may be arbitrarily represented by the nucleic acid sequence as set forth in SEQ ID NO: 7 representing 959 nucleic acids of the flanking genomic DNA adjacent to and contiguous with the inserted DNA. Alternatively, the junction sequences may be arbitrarily represented by the nucleic acid sequence as set forth in SEQ ID NO: 8 and SEQ ID NO: 9 each representing (330) and (215) nucleic acids respectively of the flanking genomic DNA adjacent to and contiguous with the inserted DNA. Alternatively, the junction sequences may be arbitrarily represented by the nucleic acid sequence nucleic acid sequence as set forth in SEQ ID NO: 10 and SEQ ID NO: 11, each representing (95) and (40) nucleic acids respectively of the flanking genomic DNA adjacent to and contiguous with the inserted DNA. These nucleic acids are present in brinjal MAH-45151 event as part of the genome. The identification of one or more of

nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 16 or SEQ ID NO: 13 in a sample derived from a brinjal plant, seed, or plant part is determinative that the DNA was obtained from brinjal event MAH-45151 and is diagnostic for the presence of brinjal event MAH-45151. The invention thus provides a DNA molecule that contains at least one of the nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13. Any segment of DNA derived from transgenic brinjal event MAH-45151 that is sufficient to include at least one of the sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 and comprises SEQ ID NO: 16 is within the scope of the invention. In addition, any nucleic acid comprising a sequence complementary to any of the sequences described within this paragraph is within the scope of the invention. Figure 3 illustrates the physical arrangement of various SEQ IDs.

The nucleic acid sequence corresponding to the nucleic acid sequence of the inserted transgenic DNA and substantial segments of the brinjal genomic DNA flanking left border end of the inserted transgenic DNA is provided herein as SEQ ID NO: 13 or complement thereof. A subsection of this is the inserted transgenic DNA provided as SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 16 or complement thereof.

The brinjal event MAH-45151 further comprises a region spanning the 5' end where the transgenic DNA is inserted into the genomic DNA, referred to herein as the 5' junction.

The sequence of the heterologous DNA insert, junction sequences, or flanking sequences from brinjal event MAH-45151 can be verified (and corrected if necessary) by amplifying such sequences from the event using primers derived from the sequences provided herein followed by standard DNA sequencing of the amplicon or of the cloned DNA.

The invention provides exemplary DNA molecules that can be used either as primers or probes for diagnosing the presence of DNA derived from a brinjal plant comprising event MAH-45151 in a sample. Such primers or probes are specific for a target nucleic acid sequence and as such

are useful for the identification of brinjal event MAH-45151 nucleic acid sequence by the methods of the invention described herein.

A primer can be typically designed to hybridize to a complementary target DNA strand to form a hybrid between the primer and the target DNA strand, and the presence of the primer is a point of recognition by a polymerase to begin extension of the primer (i.e., polymerization of additional nucleic acids into a lengthening nucleic acid molecule) using as a template the target DNA strand.

The present invention thus provides recombinant DNA where the nucleic acid sequence as set forth in SEQ ID NO: 7 comprises a primer sequence as set forth in SEQ ID NO: 3. The present invention also provides a recombinant DNA where the nucleic acid sequence as set forth in SEQ ID NO: 13 comprises a primer sequence as set forth in SEQ ID NO: 12.

The present invention further provides that a pair of primers comprising at least 10 contiguous nucleotides selected from a sequence as set forth in SEQ ID NO: 13, or its complement, where the said DNA molecule, when used together, in a DNA amplification procedure, is capable of producing an diagnostic amplicon comprising of sequence as set forth in SEQ ID NO: 16.

In another aspect, a pair of primers comprising of at least 15 contiguous nucleic acids each, wherein a first primer in the said pair is selected from a nucleic acid sequence as set forth in SEQ ID NO: 12 and a second primer sequence is selected from a group of sequences as set forth in SEQ ID NO: 3 or SEQ ID NO: 14 or SEQ ID NO: 15. wherein said pair is capable of producing an amplicon comprising SEQ ID NO: 16 diagnostic for MAH-45151 event is provided.

Primer pairs, as used in the invention, are intended to refer to use of two primers binding to the opposite strands of a double stranded nucleic acid segment for the purpose of amplifying linearly the nucleic acid segment between the positions targeted for binding by the individual members of the primer pair, typically in a thermal amplification reaction or other conventional nucleic-acid

amplification methods. Exemplary DNA molecules useful as primers as set forth in SEQ ID NO: 3 or SEQ ID NO: 12 or SEQ ID NO: 14 or SEQ ID NO: 15. In another embodiment, the primer pair as set forth in SEQ ID NO: 12 and SEQ ID NO: 3 or SEQ ID NO: 14 or SEQ ID NO: 15 are useful when used together in a thermal amplification reaction with template DNA derived from brinjal event MAH-45151, to produce an amplicon diagnostic for brinjal event MAH-45151 DNA in a sample.

Any number of methods well known to those skilled in the art can be used to isolate and manipulate a DNA molecule, or fragment thereof, disclosed in the invention. For example, PCR (polymerase chain reaction) technology can be used to amplify a particular DNA molecule and/or to produce variants of the original molecule. DNA molecules, or fragment thereof, can also be obtained by other techniques such as by directly synthesizing the fragment by chemical means, as is commonly practiced by using an automated oligonucleic acid synthesizer.

The DNA molecules and corresponding nucleic acid sequences provided herein are therefore useful for, among other things, identifying brinjal event MAH-45151, selecting plant varieties or hybrids comprising brinjal event MAH-45151, detecting the presence of DNA derived from the transgenic brinjal event MAH-45151 in a sample, and monitoring samples for the presence and/or absence of brinjal event MAH-45151 or plant parts derived from brinjal plants comprising event MAH-45151.

In another aspect, a method of detecting the presence of MAH-45151 event in a sample comprising the steps of: contacting the sample comprising DNA with a pair of primers comprising a first primer as set forth in SEQ ID NO: 12 or its complement thereof and a second primer can be selected from nucleic acid sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 14 and/or SEQ ID NO: 15 or its complement thereof; performing a nucleic acid amplification reaction thereby producing a diagnostic amplicon comprising SEQ ID NO: 16 diagnostics for MAH-45151 event; and detecting the diagnostic amplicon as set forth in SEQ ID NO: 16.

The detection of a nucleic acid sequence specific for event MAH-45151 in the amplicon is determinative and/or diagnostic for the presence of the brinjal event MAH-45151 specific DNA

in the sample. An example of a primer pair that is capable of producing an amplicon from event MAH-45151 DNA under conditions appropriate for DNA amplification of nucleic acid sequence as set forth in SEQ ID NO: 13. Other primer pairs may be readily designed by one of skill in the art and would comprise at least one fragment of nucleic acid sequence as set forth in SEQ ID NO: 13.

The method according to the present invention provides that the sample comprising DNA sequence or a portion of DNA sequence is as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13, complements or fragments thereof. The sample is a brinjal tissue.

In further aspect, a DNA detection kit is provided. The DNA detection kit according to the present invention comprises a first primer comprising at least 15 contiguous nucleic acids as set forth in SEQ ID NO: 12 or complements thereof; and a second primer comprising at least 15 contiguous nucleic acids selected from nucleic acid sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 14 and/or SEQ ID NO: 15 or its complement thereof; wherein detection of said amplicon comprising SEQ ID No. 16 is diagnostic for the presence of the MAH-45151 event.

DNA detection kits are provided that are useful for the identification of brinjal event MAH-45151 DNA in a sample and can also be applied to methods for breeding brinjal plants containing the appropriate event DNA. Such kits contain DNA primers and/or probes comprising fragments of nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 or complement thereof.

One example of such a kit comprises a primer pair useful for producing an amplicon comprising SEQ ID No. 16 useful for detecting the presence and/or absence of DNA derived from transgenic brinjal event MAH-45151 in a sample. Such a kit would employ a method comprising contacting a target DNA sample with a primer pair as described herein, then performing a nucleic acid amplification reaction sufficient to produce an amplicon comprising a DNA molecule having a

SEQ ID NO:16; having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 or complement thereof, and then detecting the presence and/or absence of the amplicon. Such a method may also include sequencing the amplicon or a fragment thereof, which would be determinative of, i.e. diagnostic for, the presence of the brinjal event MAH-45151 specific DNA in the target DNA sample. Other primer pairs may be readily designed by one of skill in the art from nucleic acids sequence as set froth in SEQ ID NO: 13 and be sufficiently unique to brinjal event MAH-45151 DNA in order to identify DNA derived from the event.

Nucleic-acid amplification can be accomplished by any of the various nucleic- acid amplification methods known in the art, including thermal amplification methods. Many techniques are known in the art for detecting, quantifying, and/or sequencing the amplicon produced by these methods.

The kits and detection methods of the invention are useful for, among other things, identifying brinjal event MAH-45151, selecting plant varieties or hybrids comprising brinjal event MAH-45151, detecting the presence of DNA derived from the transgenic brinjal plants comprising event MAH-45151 in a sample, and monitoring samples for the presence and/or absence of brinjal plants comprising event MAH-45151 or plant parts derived from brinjal plants comprising event MAH-45151.

The present invention also provides a method of producing an insect resistant progeny of brinjal plant. The method of producing a transgenic brinjal plant resistant to insect pests comprising transforming a brinjal cell with the DNA construct pMHO102.

The method comprising modifying a brinjal plant's genome to incorporate nucleotide sequences as set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13 or complements or fragments thereof; crossing said insect resistant brinjal plant comprising the MAH-45151 event, with a brinjal plant without the said event; obtaining at least one progeny of brinjal plant derived from the cross; and selecting a progeny of brinjal plant that is insect resistant and comprises nucleotide sequence of SEQ ID NO:16.

In yet another aspect, an insect resistant brinjal plant, or parts thereof is provided. The present invention also provides an insect resistant transgenic brinjal plant comprising an event MAH-45151. The present invention relates to an insect resistant transgenic brinjal plant comprising an elite event MAH-45151. The transgenic plant is characterized by harboring the *cry1Ac* gene under the control of CaMV 35S promoter at a specific locus in the brinjal genome. Further, the invention discloses a method for detection of an event MAH-45151 in transgenic brinjal plant. The invention further provides a kit for identification of the transgenic plants comprising the event MAH-45151.

The brinjal plant according to the present invention comprises at least one nucleic acid sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 13 forms a part of the plant's genome. The present invention provides a seed, nucleus, or part thereof capable of producing an amplicon comprising SEQ ID NO: 16 that is diagnostic for MAH-45151 event. The seed according to the present invention is capable of producing an amplicon comprising SEQ ID NO: 16 and is diagnostic for MAH-45151 event and having an accession number NCIMB 42846.

The invention thus provides brinjal plants, progeny, seeds, plant cells, and plant parts (such as pollen, ovule, pod, flower tissue, root tissue, stem tissue, and leaf tissue). These plants, progeny, seeds, plant cells, plant parts, and commodity products contain a detectable amount of a nucleic acid of the invention, i.e., such as a nucleic acid having at least one of the nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13. Plants, progeny, seeds, plant cells, and plant parts of the invention may also contain one or more additional transgenes. Such transgene may be any nucleic acid sequence encoding a protein or RNA molecule conferring a desirable trait including but not limited to increased insect resistance, increased water use efficiency, increased yield performance, increased drought resistance, increased seed quality, improved nutritional quality, and/or increased herbicide tolerance, in which the desirable trait is measured with respect to a brinjal plant lacking such additional transgene.

The invention provides brinjal plants, progeny, seeds, plant cells, and plant part such as pollen, ovule, pod, flower, root or stem tissue, and leaves derived from a transgenic brinjal plant comprising event MAH-45151. A representative sample of brinjal seed comprising event MAH-45151 has been deposited according to the Budapest Treaty with the National Collection of Industrial, Food and Marine Bacteria (NCIMB). The NCIMB repository has assigned the Patent Deposit Designation 42846 to the event MAH-45151 comprising seed.

Plants of the invention may pass along the event DNA, including the transgene, to progeny. The progeny comprising the event DNA derived from an ancestor plant and/or comprising a DNA molecule having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13. Plants, progeny, and seeds may be homozygous or heterozygous for the transgene. Progeny may be grown from seeds produced by a brinjal event MAH-45151 containing plant and/or from seeds produced by a plant fertilized with pollen from a brinjal event MAH-45151 containing plant.

A varietal or hybrid seed or plant of the present invention may thus be derived by crossing a first parent that lacks the specific and unique DNA of the brinjal event MAH-45151 with a second parent comprising brinjal event MAH-45151, resulting in a hybrid comprising the specific and unique DNA of the brinjal event MAH-45151. Each parent can be a hybrid or an inbred/varietal, so long as the cross or breeding results in a plant or seed of the invention, i.e., a seed having at least one allele containing the DNA of brinjal event MAH-45151 and/or a DNA molecule having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13. Two different transgenic plants may thus be crossed to produce hybrid offspring that contain two independently segregating, added, exogenous genes. For example, the MAH-45151 containing fruit and shoot borer tolerant brinjal can be crossed with other transgenic brinjal plants to produce a plant having the characteristics of both transgenic parents. One example of this would be a cross of MAH-45151 containing fruit and shoot borer tolerant brinjal with a plant having one or more additional traits such as herbicide tolerance and/or insect control, resulting in a progeny plant or seed that is tolerant to fruit and shoot borer and has at least one or more additional traits. Back-crossing to a

parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation.

The invention provides methods for controlling insects and methods for producing plants with brinjal event MAH-45151. A method for controlling insects in a field is provided and consists of planting brinjal event MAH-45151 containing varietal or hybrid plants in a field and exposing plants to fruit and shoot borer in the field without injuring the MAH-45151 containing plants.
A brinjal plant that tolerates fruit and shoot borer may be produced by sexually crossing an event MAH-45151 containing plant comprising a DNA molecule having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 with another brinjal plant and thereby producing seed, which is then grown into progeny plants. These progeny plants may then be exposed to fruit and shoot borer to select the progeny plants that are tolerant to fruit and shoot borer insects. Alternatively, these progeny plants may be analyzed using diagnostic methods to select the progeny plants that contain the event MAH-45151 DNA. The other plant used in the crossing may or may not be tolerant to fruit and shoot borer and may or may not be transgenic. The progeny plant and/or seed produced may be varietal or hybrid seed. In practicing this method, the step of sexually crossing one plant with another plant, i.e., cross-pollinating, may be accomplished or facilitated by human intervention, for example: by human hands collecting the pollen of one plant and contacting this pollen with the style or stigma of a second plant; by human hands and/or actions removing, destroying, or covering the stamen or anthers of a plant (e.g., by detasseling or by application of a chemical gametocide) so that natural self-pollination is prevented and cross-pollination would have to take place in order for fertilization to occur; by human placement of pollinating insects in a position for "directed pollination" (e.g., by placing beehives in orchards or fields or by caging plants with pollinating insects); by human opening or removing of parts of the flower to allow for placement or contact of foreign pollen on the style or stigma (e.g., in soy which naturally has flowers that hinder or prevent cross-pollination, making them naturally obligate self-pollinators without human intervention); by selective placement of plants (e.g., intentionally planting plants in pollinating proximity); and/or by application of chemicals to precipitate flowering or to foster receptivity (of the stigma for pollen).

A brinjal plant that is tolerant to brinjal fruit and shoot borer may be produced by selfing an event MAH-45151 containing plant comprising a DNA molecule having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 and thereby producing seeds, which is then grown into progeny plants. These progeny plants may then be exposed to fruit and shoot borer to select for progeny plants that are tolerant to fruit and shoot borer. Alternatively, these progeny plants may be analyzed using diagnostic methods to select for progeny plants that contain the event MAH-45151 DNA. In practicing this method, the step of sexually crossing one plant with itself, i.e., self-pollinating or selfing, may be accomplished or facilitated by human intervention, for example: by human hands collecting the pollen of the plant and contacting this pollen with the style or stigma of the same plant and then optionally preventing further fertilization of the plant; by human hands and/or actions removing, destroying, or covering the stamen or anthers of other nearby plants (e.g., by detasseling or by application of a chemical gametocide) so that natural cross-pollination is prevented and self-pollination would have to take place in order for fertilization to occur; by human placement of pollinating insects in a position for "directed pollination" (e.g., by caging a plant alone with pollinating insects); by human manipulation of the flower or its parts to allow for self-pollination; by selective placement of plants (e.g., intentionally planting plants beyond pollinating proximity); and/or by application of chemicals to precipitate flowering or to foster receptivity (of the stigma for pollen).

Progeny of brinjal plants and seeds encompassed by these methods and produced by using these methods will be distinct from other brinjal plants, for example because the progeny of brinjal plants and seeds: are recombinant and as such created by human intervention; are fruit and shoot borer tolerant; contain at least one allele that consists of the transgene DNA of the invention; and/or contain a detectable amount of a DNA molecule comprising at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13. A seed may be selected from an individual progeny plant, and so long as the seed comprises a DNA molecule having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13, it will be within the scope of the invention.

The invention provides a transgenic plant comprising a DNA molecule having at least one nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13 present in its genome. In this process, recombinant DNA is inserted into a plant cell's genome to create a transgenic plant cell that is separate and unique from naturally occurring plant cells. This transgenic plant cell can then be cultured using modern techniques. The new plant cell's genetic composition and phenotype is a technical effect created by the integration of the heterologous DNA into the genome of the cell. Another aspect of the invention is a method using modern plant tissue culture techniques to produce transgenic plants

The methods of the invention are therefore useful for, among other things, controlling insects in a field while growing plants for the purpose of producing seed and/or plant parts comprising brinjal event MAH-45151 for agricultural or research purposes, selecting for progeny comprising brinjal event MAH-45151 for plant breeding or research purposes, and producing progeny plants and seeds comprising brinjal event MAH-45151.

The plants, progeny, seeds, plant cells, plant parts (such as pollen, ovule, pod, flower, root or stem tissue, and leaves), and commodity products of the invention may be evaluated for DNA composition, gene expression, and/or protein expression. Such evaluation may be done by using any standard method such as PCR, for detection and/or the detection kits provided herein.

The present invention provides efficient method for transforming plant, plant cells and tissues of brinjal (*Solanum melongena*) using *Agrobacterium* mediated method for conferring resistance to insect pests. In one aspect, *Agrobacterium tumefaciens* strain LBA4404 can be used. The strain of *Agrobacterium tumefaciens* can be obtained from commercial sources known to a person skilled in the art. Some commonly available commercial strains are EHA 101, EHA 105, LBA 4404 and the like.

The DNA cassette was inserted into the genome of brinjal through *Agrobacterium* transformation using a DNA fragment derived from vector pMH0102 to produce brinjal event MAH-45151, which has been bred with many different brinjal varieties.

The method of producing a transgenic Brinjal plant resistant to insect pests according to the present invention comprising transforming a brinjal cell with the DNA construct pMHO102. The fertile brinjal plant obtained from the said brinjal cell can be self pollinated or crossed with compatible brinjal varieties to produce insect resistant brinjal plant.

Insecticidal *cry1Ac* gene from *Bacillus thuringiensis* has been transferred into brinjal line 60208 developed by MAHYCO. The present invention provides an efficient method for transforming plant, plant cells and tissues of brinjal (*Solanum melongena*) plant using *Agrobacterium*-mediated transformation method for conferring resistance to insect pests.

The vector pMHO102 (Figure 1) containing *crylAc* gene under the control of *CaMVe35S* promoter and *ve10518* terminator; was transformed in the *Agrobacterium tumefaciens* cells. The recombinant *Agrobacterium tumefaciens* was inoculated into a suitable medium for its growth, *Agrobacterium* cells were inoculated into 25 ml of sterile 2YT medium (pH 7) in a flask. 2YT medium contains 1% Yeast extract, 1.6% Tryptone and 0.5% NaCl. Suitable antibiotics were added to this medium before inoculating bacteria for the selective growth of *Agrobacterium* with the plasmid pMHO102. The bacteria were inoculated in 2YT medium in flask and kept on a shaker to get Optical Density (600nm) in the range of 0.01 to 2, preferably 1.8.

The explants for transformation are 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. The details of the transformation of the brinjal (Solanum melongena) plant are provided in Example 1. Explants were inoculated in recombinant *Agrobacterium* suspension (preferably 15 minutes), blotted dry on sterile filter paper and later transferred to petri plates containing suitable growth medium for co-cultivation.

After the co-cultivation (2 to 5 days preferably 2 days of co-cultivation), these explants were washed in liquid MSO medium with 500mg/l Cefotaxime to inhibit the growth of *Agrobacterium* and were transferred on selection medium.

Transformants regenerated on the selection medium were transferred to rooting medium and the rooted plants were hardened and established in green house.

Detailed procedure of transformation of brinjal plant with the pMHO102 construct is provided in the Example 1.

Another embodiment of the present invention is to provide a method of identification of the flanking sequence around the transgenic insertion site for the event MAH-45151 by PCR amplification. Nucleic acid amplification can be accomplished by any of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR).

Transgenic insertion and neighboring flanking brinjal DNA were purified by agarose gel electrophoresis and cloned. The cloned fragment was sequenced by methods known in the art. The sequence flanking the junction of the insertion is shown in nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13. Details of identification of the flanking sequence of junction are given in Example 3.

Another embodiment of the present invention is to provide diagnostic methods for identification of the MAH-45151 event. Details of PCR method of identification of the MAH-45151 event are given in Example 4.

The present invention also provides a synthetic oligo nucleic acid for the detection of the presence of brinjal plant MAH-45151 event, wherein the sequence of said oligo nucleic acid is selected from a group consisting nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 13

The present invention further relates to a transgenic plant or seed having the brinjal plant MAH-45151 event, wherein the genome of said MAH-45151 event comprises of nucleic acid sequence as set forth in SEQ ID NO: 7 or SEQ ID NO: 8 or SEQ ID NO: 9 or SEQ ID NO: 10 or SEQ ID NO: 11 or SEQ ID NO: 16 or SEQ ID NO: 13 or complement thereof.

While the invention is broadly as defined above, it will be appreciated by those persons skilled in the art that it is not limited thereto and that it also includes embodiments of which the following description gives examples. The following examples are included to demonstrate examples of certain preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches the inventors have found function well in the practice of the invention, and thus can be considered to constitute examples of preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Examples:

In the present invention the *crylAc* gene of *Bacillus thruingiensis* has been transferred into brinjal line 60208 developed by MAHYCO which has the following characters:
Fruit color: Purple, white variegated Fruit shape: Oval
Plant habit: Bushy
Fruit development: In clusters
Calyx: Spiny
Over 50 independent transformation events were screened to identify a brinjal MAH-45151 event. All events underwent transgene segregation analysis and protein expression evaluation to determine the optimum event for commercialization. Details are provided in the Example 2.

Molecular characterization of the brinjal plant MAH-45151 event was carried out. Details are provided in the Example 3. Further diagnostic methods for identification of the brinjal plant MAH-45151 event was carried out details are provided in Example 4.

The brinjal event MAH-45151 was chosen on the basis of a number of criteria. Segregation analysis over three generations indicated that there is a single locus of insertion of the *cry 1Ac* gene in this line. This was confirmed by DNA blot analysis. Protein quantification studies using

quantitative ELISA were performed on a number of brinjal single insertion events. These studies indicated that the MAH-45151 line expressed the *Cry1Ac* protein, and that the expression of the inserted gene was stable in a number of different genetic backgrounds, over multiple generations. Phenotypic analysis of the MAH-45151 event bearing line showed that it was morphologically indistinguishable from the non-transformed parent line from which it was derived, and therefore most suitable for further backcross breeding. The elite plant was employed to transfer the MAH-45151 elite event in other brinjal cultivars.

Nucleic acid amplification can be accomplished by any 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: a guide to methods and applications (ed. Innis et al., Academic Press, San Diego, 1990). Transgenic insertion and neighboring flanking brinjal DNA were purified by agarose gel electrophoresis and cloned. The cloned fragment was sequenced by methods known in the art.

Example 1

Transformation of Brinjal

Sterilization and inoculation of seeds

Seeds from a proprietary brinjal line of Maharashtra Hybrid Seeds Company Ltd. were surface sterilized in a 50 ml plastic centrifuge tube with 1.5 % NaOCl for 10 min. with vigorous shaking (20 ml NaOCl for 500 seeds). After 5 min the solution was decanted and the seeds were washed 5 times with sterile distilled water. The seeds were blotted dry on sterile filter paper for 1 hr and inoculated on MS0 medium (Table 1) in bottles at 10 seeds/bottle. The seeds were maintained at 25°C for 12-15 days to germinate with a photoperiod regime of 16 hrs light and 8 hrs darkness.

Agrobacterium cultures

A day before co-cultivation was to be done, a culture of the *Agrobacterium* strain LBA404 harbouring the transformation vector was grown overnight in 25 ml liquid LB medium (Table 1) at 28°C with shaking at 175 rpm with the respective antibiotics. This overnight culture was

started with a loopful of bacterial cells taken from a freshly-streaked solid medium plate containing the same antibiotics.

Explant preparation

On the day of co-cultivation, cotyledons from 2-15 days old seedlings were removed and cut longitudinally in half through the midrib and used as explants. The explants were kept in petridishes on sterile filter papers soaked in liquid MS medium (Table 1) to prevent the explants from drying out.

Co-cultivation

The overnight grown bacterial culture was centrifuged at 10000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in liquid. MS medium (25 ml) and mixed well (Table 1). 25 ul of 100mM acetosyringone was added to the bacterial culture which was then placed in the incubator for growth with shaking for a further 2 hr. The optical density (OD) of the bacterial culture was measured at 600 nm until an OD of 1.5 to 1.8 was reached. The explants were incubated in the bacterial culture in a petridish or a glass beaker for 10 min with slow stirring. The explants were then blotted on a sterile filter paper to remove excess bacteria and placed on co-cultivation medium B1AsP (Please find the media composition in table) for the three days for co-cultivation. (15 explants/ plate). The plates were incubated under light at 25°C for three days with a photoperiod regime of 16 hrs light + 8 hrs darkness.

Positive and negative controls were also maintained in each experiment. Positive controls were explants regenerated on medium without antibiotics to check the tissue culture regeneration, whereas negative controls are explants maintained on antibiotic-containing media to make sure the antibiotic is checking the growth.

Selection (B1KC medium)

After 3 days the explants were transferred on selection medium B1KC (Table 1) medium with kanamycin 50 mg/l and cefatoxime 250 mg/l for a period of 2 weeks. Transformed explants produce calli at the cut end of explants, and non- transformed explants bleach. Putative transformed explants were maintained on fresh selection medium B1KC again for two weeks.

This was repeated for a total period of 6 weeks on selection medium. At the end of the sixth week, green meristematic tissues/calli develops.

Shoot Regeneration (B2KC medium)

Green meristematic tissues were transferred onto regeneration medium, B2 KC (Table 1), with kanamycin (50 mg/l) and cefotaxime (250mg/l) for a period 2 weeks. The calli were subcultured three times every 2 weeks (3 selections) on fresh medium resulting in small green shoot buds developing from the calli.

Shoot Elongation (B3KC medium)

The shoot buds were transferred onto B3KC medium (Table 1) with kanamycin (50 mg/l) and cefotaxime 250mg/l for a period of 2 weeks. The shoot buds were twice subcultured every 2 weeks (2 selections) on fresh medium. At the end of the fourth week, the shoot buds elongate and are ready for rooting. Sometimes on the elongation medium rooting may begin. If rooting starts on the elongation medium the plantlets were left undisturbed in order to avoid damage to the roots.

Rooting (B4KC medium)

The shoots were transferred onto rooting medium B4KC (Table 1) with kanamycin (50 mg/l) and cefotaxime (250mg/l) for a period of 2 weeks. The shoots were subcultured every 2 weeks till rooting occurred.

Hardening

The rooted plants were washed with sterile distilled water thoroughly to remove the gelling agent (agar or phytagel). The plants were treated with 0.1 % Bavistin for at least 1 hr before transferring to cups containing mixture of promix (60%) and soil (40%). The plants were covered with polythene bags for 7 days. After 7 days, the polythene bags were cut from the corners to allow the hardening process to begin, which is completed in about 2 weeks.

Table 1: Composition of media used in brinjal transformation

Sr. No.	Medium	Composition	Purpose
1	LB Solid	Trytone 10gm/L, Yeast extract 5gm/L, NaCl 10 gm/L, pH 7.0, Agar 15gm/L	Bacterial culture
2	LB liquid	Trytone 10gm/L, Yeast extract 5gm/L, NaCl 10 gm/L, pH 7.0	Bacteria inoculation.
3	MS liquid	MS Salts (Murashige and Skoog 1962), , MS Vitamins, Sucrose 3%, pH 5.8	Bacteria resuspension
4	MS0	MS Salts B5 Vitamins B5 vitamins (Gamborg et al. 1968), Sucrose 3%, pH 5.8, Agar 0.8%	Seed inoculation
5	B1As	MS Salts, B5 Vitamins, NAA 0.05mg/L, Zeatin 2mg/L, Glucose 1.5%, Acetosyringone 100uM pH 5.8, Phytagel 0.35%	Co-cultivation
6	B1KC	MS Salts, B5 Vitamins, NAA 0.05mg/L, Zeatin 2mg/L, Glucose 1.5%, Kanamycin 50 mg/l, Cefotaxime 250 mg/l , pH 5.8, Agar 0.8%	Selection.
7	B2KC	MS Salts, B5 Vitamins, BAP 2.5mg/L, Kinetin 1mg/l ,l Adenine(freebase) 2mg/L, IAA 0.5mg/l, Sucrose 3%, Kanamycin 50 mg/l, Cefotaxime 250 mg/l , pH 5.8, Agar 0.8%	Shoot Bud Regeneration
8	B3KC	MS Salts, MS Vitamins, IAA 0.5mg/l , Glucose 1.5%, Kanamycin 50 mg/l, Cefotaxime 250 mg/l , pH 5.8, Agar 0.8%	Shoot Elongation.
9	B4KC	MS Salts, B5 Vitamins, Glucose 0.75%, Kanamycin 50mg/L, Cefotaxime 250mg/L, pH 5.8, Phytagel 0.35%.	Rooting.

5

Example 2

Identification of brinjal plant MAH-45151elite event

A large number (>50) of independent transformation events were generated in order to maximize the chance of a high-transgene-expressing, genetically stable event for production of commercial transgenic brinjal lines. All brinjal plants coming out of the transformation experiments were analyzed for presence of the *cry1Ac* gene by PCR, and the positive plants subjected to ELISA for determining the expressivity of the transgene. The initial transformants (T0) were advanced to the next generation by selfing, and the T1 progeny plants were checked by PCR to determine the segregation of the transgene. The expected T1 segregation ratio for the transgene in a line with a single *cry1Ac* gene insertion is 3:1 based on Mendelian genetics. Further, as *cry1Ac* acts as a dominant gene when introduced as a transgene, the expression of the gene was monitored by ELISA in the T1 generation. Again, in a single insertion event, the expected ratio of *Cry1Ac* expressing plants to non-expressing plants is 3:1. Insect bioassays were carried out on tissue from selected lines in order to determine which lines would have better efficacy against the fruit and shoot borer pest. Southern blot analysis of selected individual transformation events was carried out to confirm the number of loci (insert copy number) at which the transgene integrated in the brinjal genome.

Based on the above criteria, transformed lines were selected which displayed segregation characteristics of single locus insertion events and showed effective tolerance to fruit and shoot borer. Conversely, those lines that were found to have abnormal segregation ratios and/or low efficacy against the pest were not taken further. The lines selected for advancement were grown in the greenhouse and *Cry1Ac* protein was estimated through the life of the crop by quantitative ELISA, which enables determination of the highest protein expressing lines. The tissues analyzed were leaf, shoot, stem, flower and root. After a careful analysis of the above parameters, event MAH-45151 was found to be the best available event, in terms of *Cry1Ac* expression, efficacy against the pest and genetic stability over three generations. Marker-free status of the selected event was confirmed by various analyses such as PCR, GUS assays and kanamycin sensitivity test. The MAH-45151 elite event was used for further breeding for developing fruit and shoot borer tolerant brinjal.

Example 3

Molecular characterization of the event MAH-45151

The brinjal transgenic MAH-45151 event was analyzed to identify brinjal genomic DNA sequences flanking the *cry1Ac* expression cassette using the method of Cottage *et al.*, 2001. (Plant Molecular Biology Reporter, December 2001. Plant genomic DNA was extracted from fresh young leaves of MAH-45151 event bearing plants, (Dellaporta et al., 1983). Genomic DNA (2µg) was digested with *DraI* enzyme in 20 µl of reaction volume using standard buffers. The digestion reaction was incubated at 37^0C overnight. The digestion product was then incubated at 65^0C for enzyme inactivation and was precipitated with 3M sodium acetate and ethanol. DNA was air dried and dissolved in 25 µl sterile distilled water. Digested DNA was ligated to the annealed adapter in ligase buffer supplied by the manufacturer. The sequences of the adapters are as below

ADAP 1: 5' – cta ata cga ctc act ata ggg ctc gag cgg ccg ccc ggg cag gt – 3'

ADAP 2: 5'- P-acc tgc cc-H_2N -3'

Both the adapters were at first annealed to each other and then ligated to the digested genomic DNA of MAH-45151 event.

The ligation mixture was incubated at 16^0C overnight to obtain adapter library. The adapter library was diluted to 100 µl, and first round amplification was carried out using the following primer combination: forward primer

MHIP-5 - 5′- GAC CTG CAG CCA AGC TTC G -3′ SEQ ID NO: 1

AP - 5′- GGA TCC TAA TAC GAC TCA CTA TAG GGC-3′ SEQ ID NO: 2

The details of restriction digestion, ligation and PCR are given below:

Restriction digestion:

Genomic DNA	12.0 µl (2 µg)
10X Reaction buffer	2.0 µl (final concentration 1x)
Dra-I enzyme	1.0 µl (10units/ µl)
Sterile water	Make up the volume to 20.0 µl.

Ligation:

Digested Genomic DNA	23.0 µl (2 µg)
Annealed adapters	3.0 µl (100 ng/µl)
10X Reaction buffer	3.0 µl (final concentration 1x)
T4-ligase enzyme	1.0 µl (5units/ µl)
Sterile water	Make up the volume to 30.0 µl

First round PCR:

Reagents	**Amount to be added**
Nuclease-free water	Make up to 25 µl
10X reaction buffer (with $MgCl_2$)	2.5 µl
10mM dNTPs	0.5 µl
Primer MHIP-5 (100ng/µl)	1.0 µl
Primer AP (100 ng/µl)	1.0 µl
Taq DNA polymerase (5 units/µl)	0.5 µl
DNA template	3.0 µl

Thermal Cycler program:

Temperature	Time	Cycles
95^0c	5 minutes	1
94^0c 58^0c 68^0c	30 seconds 30 seconds 4 minutes	40
68^0c	10 minutes	1
4^0c	Hold	

The second round of PCR was carried out to obtain the specific flanking region adjacent to the inserted heterologous gene. The template used for second round PCR was first round PCR product which was diluted 5 times. The details of the PCR are given below:

MHIP-6 - 5'- CAA GCT TCG AAT TAA TTC AGT AC -3' (SEQ ID NO: 3)

NAP - 5'- TAT AGG GCT CGA GCG GC-3' (SEQ ID NO: 4)

Second PCR:

Reagents	Amount to be added
Nuclease-free water	Make up to 25 µl
10X reaction buffer (with $MgCl_2$)	2.5 µl
10mM dNTPs	0.5 µl
Primer MHIP-6 (100ng/µl)	1.0 µl
Primer NAP (100 ng/µl)	1.0 µl
Taq DNA polymerase (5 units/µl)	0.5 µl
DNA template	3.0 µl

Thermal Cycler program:

Temperature	Time	Cycles
95^0c	5 minutes	1
94^0c 58^0c 68^0c	30 seconds 30 seconds 4 minutes	40
68^0c	10 minutes	1
4^0c	Hold	

The PCR product was analyzed on a 1% agarose gel, and the amplified fragment was eluted from the agarose gel using a Qiagen DNA gel elution kit. A DNA fragment of 1049 base pair amplified from the left border region of the T-DNA after two rounds of PCR (using primers MHIP-6 and NAP). The amplified fragment was cloned into pGEM-T Easy vector to obtain a recombinant clone. The clone selected for analyzing the sequence was designated MAH-45151 Dra-I-5, and plasmid DNA from this clone was isolated using standard methods known in the art. The cloned fragment was sequenced using T7 (SEQ ID NO: 5) and SP6 (SEQ ID NO: 6) primers. The sequences of T7 and SP6 primers are as below;

T7:- 5'- TAA TAC GAC TCA CTA TAG GG - 3' SEQ ID NO: 5

SP6:- 5'- TAT TTA GGT GAC ACT ATA G – 3' SEQ ID NO: 6

The sequence obtained after sequencing the 1049 bp fragment using T7 and Sp6 primers is provided in SEQ ID NO: 7

SEQ ID NO. 7:

CAAGCTTCGAATTAATTCAGTACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAATTCT
CTCAAACTTAGTTGGCTATACAAATGTCGATTCTTGTTAAACAAGCACGGAATGTTGAAATTTA
CAGTTTTAATCTTATTGCGGAACAATACTTAACTACTTAAACTTTTTCTTATTATTTGCACTCA
ATGATAGAATATAAGACTAAGAAAGTTGTTTTAGTTGCAAAGCAAATTACTAACAAACTTTGTG
TTTTTTGGTCTCTGCTTTATAATTGTAATAAATATAGCTTTTACCAGACATCATCTATGCTTTT
ACTATTGATAAGTTCAAGTTACCAACTTAGATTCCCTATGTTCCTTTTCTTTATTCCCCATTAT
TCTTGTTGCAGCCTAATAAGCATATCAGATACTGAGATGAAGACTATAAAGTTCCTTGTTGCTA
TTATTCACAAGTACAGAGATTGTTCAGACATTAAAATAAAGGATATATACCGCAGGCTTCTTAG
TGAGACGCTAGGAATCATTTCTAACATGAAACATTTGTATGCTTCCAATGATATGGAAGAAGTC
ATTTTGGCCCTTCAAACCTCTTCATCTCTGGATCAGCAGTGTCAGATGGAAAGTTATTTCAATG
CAAGCCAAACTTGTCATCTTTTATGGCTGGCCTTGGTGATATTGAAGTGGAAGACCGAGAGGAC
AATGGAATGAGTTCAGCCGTATGGGAGCTATATCAAATGTTGCTTAGAGAGCAACATTGGGCAC
TTGTTCACCTCGCTATCGCAGCCTTTGCATATTTTGCTGCCCACAGTAGTTGTAATCAGCTCTG
GAGATATTTACCTCTAGATGCTGCTCTTTCTTTTGATCTAGCAACAGGAAAAGAAGCTGATGAG
GGGAGATTTATGTCTGAACTGAAAGCATTTCTTGATAAGGAACCAGCATGTCCAAAGATAAAAT
CTTGTCCGAACACAGTTAACATGTTTGCTATGGATGGCCAAATGCTGAAAGAGACCTTT**ACCTG**
CCCGGGCGGCCGCTCGAGCCCTATA

Note: Sequence in bold font at the start is the T-DNA vector sequence, and in bold at the end is the adapter sequence; the sequence in regular font represents the brinjal genomic DNA sequence flanking the MAH-45151 event T-DNA region from left border side.

Sequence ID NO: 7 consists of a part of the T-DNA sequence starting with primer MHIP-6 (SEQ ID NO: 3, base pair 1-23) followed by T-DNA sequence (base pairs 24-60) adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event (base pairs 61 to 1019) from left border side. The flanking genomic DNA sequence is followed by adapter sequence (base pairs 1020 to 1049).

SEQ ID NO. 8:

ATTAATTCAGTACATTAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAATTCTCTCAAACTTA
GTTGGCTATACAAATGTCGATTCTTGTTAAACAAGCACGGAATGTTGAAATTTACAGTTTTAAT
CTTATTGCGGAACAATACTTAACTACTTAAACTTTTTCTTATTATTTGCACTCAATGATAGAAT
ATAAGACTAAGAAAGTTGTTTTAGTTGCAAAGCAAATTACTAACAAACTTTGTGTTTTTTGGTC
TCTGCTTTATAATTGTAATAAATATAGCTTTTACCAGACATCATCTATGCTTTTACTATTGATA
AGTTCAAGTTACCAACTTAGATTCCCTATGTTCCTTTTCTTTATTCCCCATTATTCTTGT

Sequence ID NO: 8 consists of a part of the T-DNA sequence (base pairs 1-50) adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event (base pairs 51 to 380) from left border side.

SEQ ID NO. 9:

TAAAAACGTCCGCAATGTGTTATTAAGTTGTCTAATTCTCTCAAACTTAGTTGGCTATACAAAT
GTCGATTCTTGTTAAACAAGCACGGAATGTTGAAATTTACAGTTTTAATCTTATTGCGGAACAA
TACTTAACTACTTAAACTTTTTCTTATTATTTGCACTCAATGATAGAATATAAGACTAAGAAAG
TTGTTTTAGTTGCAAAGCAAATTACTAACAAACTTTGTGTTTTTTGGTCTCTGCTTTA

Sequence ID NO: 9 consists of a part of the T-DNA sequence (base pairs 1-35) adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event (base pairs 36 to 250) from left border side.

SEQ ID NO.10:

ACGTCCGCAATGTGTTATTAAGTTGTCTAATTCTCTCAAACTTAGTTGGCTATACAAATGTCGA
TTCTTGTTAAACAAGCACGGAATGTTGAAATTTACAGTTTTAATCTTATTGCGGAACAATA

Sequence ID NO:10 consists of a part of the T-DNA sequence (base pairs 1-30) adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event (base pairs 31 to 125) from left border side.

SEQ ID NO.11:

TGTGTTATTAAGTTGTCTAATTCTCTCAAACTTAGTTGGCTATACAAATGTCGATTCTTG

Sequence ID NO:11 consists of a part of the T-DNA sequence (base pairs 1-20) adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event (base pairs 21 to 60) from left border side.

SEQ ID NO.16: **AGTTGTCTAA**TTCTCTCAAA

Sequence ID NO:16 consists of a part of the T-DNA sequence (base pairs 1-10) adjacent to which is brinjal T-DNA flanking genomic DNA sequence of MAH-45151 event (base pairs 11 to 20) from left border side.

Example 4

Diagnostic methods for identification of the MAH-45151 event

To detect the presence or absence of the brinjal MAH-45151 event, a molecular method was developed**.** The sequence analysis of the fragment shown as SEQ ID NO: 7 were carried out and primers were designed to amplify the transgenic insertion locus for use as a diagnostic tool. The two were used to amplify the transgenic insertion locus,

The forward primer was designed in the MAH45151 T-DNA flanking genomic DNA and was named as MHTBJ-13 and the reverse primer used was MHIP-6 (SEQ ID No.3). The sequence of MHTBJ-13 primer is provided below as SEQ ID NO.12.

MHTBJ-13 - 5'-CTT CAT CTC AGT ATC TGA TAT GC-3' (SEQ ID NO: 12)

These primer pairs include, but are not limited to, SEQ ID NO: 12 and SEQ ID NO: 3. For the amplification of the 5'region, any primer pair derived from SEQ ID NO: 7 that when used in

DNA amplification reaction produces a DNA amplicon diagnostic for MAH-45151 event is an aspect of the present invention. Below is provided SEQ ID No.13 consisting of MAH45151 event T-DNA flanking genomic DNA starting form SEQ ID No.12 from to *cry1Ac* gene.

SEQ ID NO.13

CTTCATCTCAGTATCTGATATGCTTATTAGGCTGCAACAAGAATAATGGGGAATAAAGAAAAGG
AACATAGGGAATCTAAGTTGGTAACTTGAACTTATCAATAGTAAAAGCATAGATGATGTCTGGT
AAAAGCTATATTTATTACAATTATAAAGCAGAGACCAAAAAACACAAAGTTTGTTAGTAATTTG
CTTTGCAACTAAAACAACTTTCTTAGTCTTATATTCTATCATTGAGTGCAAATAATAAGAAAAA
GTTTAAGTAGTTAAGTATTGTTCCGCAATAAGATTAAAACTGTAAATTTCAACATTCCGTGCTT
GTTTAACAAGAATCGACATTTGTATAGCCAACTAAGTTTGAGAGAATTAGACAACTTAATAACA
CATTGCGGACGTTTTTAATGTACTGAATTAATTCGAAGCTTGGCTGCAGGTCCGATTGAGACTT
TTCAACAAAGGGTAATATCCAGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACTTTAT
TGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAAGGCC
ATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGCATCG
TGGAAAAAGAAGACGTTCCAACCCGTCTTCAAAGCAAGTGGATTGACGTGATGGTCCGATTGAG
ACTTTTCAACAAAGGGTAATATCCGGAAACCTCCTCGGATTCCATTGCCCAGCTATCTGTCACT
TTATTGTGAAGATAGTGGAAAAGGAAGGTGGCTCCTACAAATGCCATCATTGCGATAAAGGAAA
GGCCATCGTTGAAGATGCCTCTGCCGACAGTGGTCCCAAAGATGGACCCCCACCCACGAGGAGC
ATCGTGGAAAAAGAAGACGTTCCAACCACGTCTTCAAAGCAAGTGGATTGATGTGATATCTCCA
CTGACGTAAGGGATGACGCACAATCCCACTATCCTTCGCAAGACCCTTCCTCTATATAAGGAAG
TTCATTTCATTTGGAGAGGACACGCTGACAAGCTGACTCTAGCAGATCTCCATGGACAACAACC
CAAACATCAACGAATGCATTCCATACAACTGCTTGAGTAACCCAGAAGTTGAAGTACTTGGTGG
AGAACGCATTGAAACCGGTTACACTCCCATCGACATCTCCTTGTCCTTGACACAGTTTCTGCTC
AGCGAGTTCGTGCCAGGTGCTGGGTTCGTTCTCGGACTAGTTGACATCATCTGGGGTATCTTTG
GTCCATCTCAATGGGATGCATTCCTGGTGCAAATTGAGCAGTTGATCAACCAGAGGATCGAAGA
GTTCGCCAGGAACCAGGCCATCTCTAGGTTGGAAGGATTGAGCAATCTCTACCAAATCTATGCA
GAGAGCTTCAGAGAGTGGGAAGCCGATCCTACTAACCCAGCTCTCCGCGAGGAAATGCGTATTC
AATTCAACGACATGAACAGCGCCTTGACCACAGCTATCCCATTGTTCGCAGTCCAGAACTACCA
AGTTCCTCTCTTGTCCGTGTACGTTCAAGCAGCTAATCTTCACCTCAGCGTGCTTCGAGACGTT
AGCGTGTTTGGGCAAAGGTGGGGATTCGATGCTGCAACCATCAATAGCCGTTACAACGACCTTA
CTAGGCTGATTGGAAACTACACCGACCACGCTGTTCGTTGGTACAACACTGGCTTGGAGCGTGT

CTGGGGTCCTGATTCTAGAGATTGGATTAGATACAACCAGTTCAGGAGAGAATTGACCCTCACA
GTTTTGGACATTGTGTCTCTCTTCCCGAACTATGACTCCAGAACCTACCCTATCCGTACAGTGT
CCCAACTTACCAGAGAAATCTATACTAACCCAGTTCTTGAGAACTTCGACGGTAGCTTCCGTGG
TTCTGCCCAAGGTATCGAAGGCTCCATCAGGAGCCCACACTTGATGGACATCTTGAACAGCATA
ACTATCTACACCGATGCTCACAGAGGAGAGTATTACTGGTCTGGACACCAGATCATGGCCTCTC
CAGTTGGATTCAGCGGGCCCGAGTTTACCTTTCCTCTCTATGGAACTATGGGAAACGCCGCTCC
ACAACAACGTATCGTTGCTCAACTAGGTCAGGGTGTCTACAGAACCTTGTCTTCCACCTTGTAC
AGAAGACCCTTCAATATCGGTATCAACAACCAGCAACTTTCCGTTCTTGACGGAACAGAGTTCG
CCTATGGAACCTCTTCTAACTTGCCATCCGCTGTTTACAGAAAGAGCGGAACCGTTGATTCCTT
GGACGAAATCCCACCACAGAACAACAATGTGCCACCCAGGCAAGGATTCTCCCACAGGTTGAGC
CACGTGTCCATGTTCCGTTCCGGATTCAGCAACAGTTCCGTGAGCATCATCAGAGCTCCTATGT
TCTCTTGGATACATCGTAGTGCTGAGTTCAACAACATCATCGCATCCGATAGTATTACTCAAAT
CCCTGCAGTGAAGGGAAACTTTCTCTTCAACGGTTCTGTCATTTCAGGACCAGGATTCACTGGT
GGAGACCTCGTTAGACTCAACAGCAGTGGAAATAACATTCAGAATAGAGGGTATATTGAAGTTC
CAATTCACTTCCCATCCACATCTACCAGATATAGAGTTCGTGTGAGGTATGCTTCTGTGACCCC
TATTCACCTCAACGTTAATTGGGGTAATTCATCCATCTTCTCCAATACAGTTCCAGCTACAGCT
ACCTCCTTGGATAATCTCCAATCCAGCGATTTCGGTTACTTTGAAAGTGCCAATGCTTTTACAT
CTTCACTCGGTAACATCGTGGGTGTTAGAAACTTTAGTGGGACTGCAGGAGTGATTATCGACAG
ATTCGAGTTCATTCCAGTTACTGCAACACTCGAGGCTGAGTACAACCTTGAGAGAGCCCAGAAG
GCTGTGAACGCCCTCTTTACCTCCACCAATCAGCTTGGCTTGAAAACTAACGTTACTGACTATC
ACATTGACCAAGTGTCCAACTTGGTCACCTACCTTAGCGATGAGTTCTGCCTCGACGAGAAGCG
TGAACTCTCCGAGAAAGTTAAACACGCCAAGCGTCTCAGCGACGAGAGGAATCTCTTGCAAGAC
TCCAACTTCAAAGACATCAACAGGCAGCCAGAACGTGGTTGGGGTGGAAGCACCGGGATCACCA
TCCAAGGAGGCGACGATGTGTTCAAGGAGAACTACGTCACCCTCTCCGGAACTTTCGACGAGTG
CTACCCTACCTACTTGTACCAGAAGATCGATGAGTCCAAACTCAAAGCCTTCACCAGGTATCAA
CTTAGAGGCTACATCGAAGACAGCCAAGACCTTGAAATCTACTCGATCAGGTACAATGCCAAGC
ACGAGACCGTGAATGTCCCAGGTACTGGTTCCCTCTGGCCACTTTCTGCCCAATCTCCCATTGG
GAAGTGTGGAGAGCCTAACAGATGCGCTCCACACCTTGAGTGGAATCCTGACTTGGACTGCTCC
TGCAGGGATGGCGAGAAGTGTGCCCACCATTCTCATCACTTCTCCTTGGACATCGATGTGGGAT
GTACTGACCTGAATGAGGACCTCGGAGTCTGGGTCATCTTCAAGATCAAGACCCAAGACGGACA
CGCAAGACTTGGCAACCTTGAGTTTCTCGAAGAGAAACCATTGGTCGGTGAAGCTCTCGCTCGT
GTGAAGAGAGCAGAGAAGAAGTGGAGGGACAAACGTGAGAAACTCGAATGGGAAACTAACATCG

TTTACAAGGAGGCCAAAGAGTCCGTGGATGCTTTGTTCGTGAACTCCCAATATGATCAGTTGCA
AGCCGACACCAACATCGCCATGATCCACGCCGCAGACAAACGTGTGCACAGCATTCGTGAGGCT
TACTTGCCTGAGTTGTCCGTGATCCCTGGTGTGAACGCTGCCATCTTCGAGGAACTTGAGGGAC
GTATCTTTACCGCATTCTCCTTGTACGATGCCAGAAACGTCATCAAGAACGGTGACTTCAACAA
TGGCCTCAGCTGCTGGAATGTGAAAGGTCATGTGGACGTGGAGGAACAGAACAATCAGCGTTCC
GTCCTGGTTGTGCCTGAGTGGGAAGCTGAAGTGTCCCAAGAGGTTAGAGTCTGTCCAGGTAGAG
GCTACATTCTCCGTGTGACCGCTTACAAGGAGGGATACGGTGAGGGTTGCGTGACCATCCACGA
GATCGAGAACAACACCGACGAGCTTAAGTTCTCCAACTGCGTCGAGGAAGAAATCTATCCCAAC
AACACCGTTACTTGCAACGACTACACTGTGAATCAGGAAGAGTACGGAGGTGCCTACACTAGCC
GTAACAGAGGTTACAACGAAGCTCCTTCCGTTCCTGCTGACTATGCCTCCGTGTACGAGGAGAA
ATCCTACACAGATGGCAGACGTGAGAACCCTTGCGAGTTCAACAGAGGTTACAGGGACTACACA
CCACTTCCAGTTGGCTATGTTACCAAGGAGCTTGAGTACTTTCCTGAGACCGACAAAGTGTGGA
TCGAGATCGGTGAAACCGAGGGAACCTTCATCGTGGACAGCGTGGAGCTTCTCTTGATGGAGGA
ATAATGA.

However, any modification of these methods that use DNA molecules or complements thereof to produce an amplicon DNA molecule diagnostic for MAH-45151 can be apparent to the person ordinarily skilled of the art. For example, if SEQ ID: 12 primer if used in combination with primer1 will produce an amplicon of 1250 base pair, or in combination with primer 2 will amplify 1500 base pair from MAH-45151 event. The sequences of primer 1 and 2 are as below.

Primer 1:- 5'-CGA GAA CGA ACC CAG CAC CTG-3' SEQ ID NO.14
Primer 2:- 5'-GTC AAG GCG CTG TTC ATG TCG– 3' SEQ ID NO.15

For the analysis it is important to have positive and negative controls. The PCR method was designed in order to distinguish the MAH-45151 event from the other brinjal transgenic events and non-transgenic lines. Genomic DNA from brinjal MAH-45151 event was isolated from leaves using the method described by Dellaporta *et al;* (1983). Genomic DNA was also isolated from other brinjal transgenic events and non-transgenic brinjal lines as controls for the PCR detection method. A control reaction having no DNA in the reaction mixture was also included.

The genomic DNA from different plants were subjected to amplification using two primers namely SEQ ID NO: 12 and SEQ ID NO: 3. the details are as follows.

PRC reaction:

Reagents	**Amount to be added**
Nuclease-free water	Make up to 20 µl
10X reaction buffer (with $MgCl_2$)	2.0 µl
10mM dNTPs	0.4 µl
Primer MHIP-6 (100 ng/µl)	0.5 µl
Primer MHTBJ-13 (100 ng/µl)	0.5 µl
Taq DNA polymerase (5 units/µl)	0.3 µl
DNA template	2.0 µl

Thermal Cycler program:

Temperature	**Time**	**Cycles**
95^0c	5 minutes	1
94^0c 58^0c 72^0c	30 seconds 30 seconds 1minute	35
72^0c	5 minutes	1
4^0c	Hold	---

The amplified product was analyzed on 1% agarose gel electrophoresis. The results obtained are shown Figure 2. For the figure it is evident that 426 bp fragment amplified only from the brinjal MAH-45151 event where as no amplification was observed in other transgenic events and non-transgenic brinjal plants.

Claims :

1. A recombinant DNA comprising at least one junction sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13, complements or fragments thereof consisting a transgene insertion and flanking genomic DNA of brinjal event MAH-45151.
2. The recombinant DNA as claimed in claim 1, wherein the nucleic acid sequence as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13, comprises a sequence as set forth in SEQ ID NO: 16, and or the complement thereof,or a portion of SEQ ID NO: 16 or its complement thereof.
3. The recombinant DNA as claimed in claim 1, wherein the nucleic acid sequence as set forth in SEQ ID NO: 7 comprises a primer sequence as set forth in SEQ ID NO: 3.
4. The recombinant DNA as claimed in claim 1, wherein the nucleic acid sequence as set forth in SEQ ID NO: 13 comprises a primer sequence as set forth in SEQ ID NO: 12.
5. A pair of primers comprising at least 10 contiguous nucleotides selected from a sequence as set forth in SEQ ID NO:13, or its complement, wherein the said DNA molecule is capable of producing an amplicon comprising of sequence as set forth in SEQ ID NO: 16.
6. A pair of primers comprising of at least 15 contiguous nucleic acids each, wherein a first primer in said pair is selected from a nucleic acid sequence as set forth in SEQ ID NO: 12 and a second primer sequence selected from a group of sequences as set forth in SEQ ID NO: 3 or SEQ ID NO: 14 or SEQ ID NO: 15. wherein said primer pair is capable of producing an amplicon comprising SEQ ID NO: 16 diagnostic for MAH-45151 event.
7. A method of detecting the presence of MAH-45151 event in a sample comprising the steps of:

a. contacting the sample comprising DNA with a pair of primers wherein a first primer is as set forth in SEQ ID NO: 12 or its complement thereof and a second primer is selected from nucleic acid sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 14 and/or SEQ ID NO: 15 or its complement thereof;

b. performing a nucleic acid amplification reaction thereby producing a diagnostic amplicon comprising SEQ ID NO: 16 for MAH-45151 event; and

c. detecting the diagnostic amplicon as set forth in SEQ ID NO: 16.

8. The method as claimed in claim 7, wherein the sample comprising DNA sequence or a portion of DNA sequence is as set forth in SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13, complements or fragments thereof.

9. The method as claimed in claim 7 or 8, wherein the sample is a brinjal tissue.

10. A DNA detection kit comprising:

a. a first primer comprising at least 15 contiguous nucleic acids as set forth in SEQ ID NO: 12 or complements thereof; and

b. a second primer comprising at least 15 contiguous nucleic acids selected from nucleic acid sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 14 and/or and/or SEQ ID NO: 15 or its complement thereof;

wherein detection of amplicon comprising SEQ ID No.16 is diagnostic for the presence of the MAH-45151 event.

11. A method of producing a progeny of an insect resistant brinjal plant comprising:

(a) modifying a brinjal plant's genome to incorporate nucleotide sequences as set forth in SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO: 10, SEQ ID NO: 11 and/or SEQ ID NO: 13 or complements or fragments thereof;

(b) crossing said insect resistant brinjal plant comprising the MAH-45151 event, with a brinjal plant without the said event;

(c) obtaining at least one progeny of brinjal plant derived from the cross of (b); and

(d) selecting a progeny of brinjal plant that is insect resistant and comprises nucleotide sequence of SEQ ID NO:16.

12. An insect resistant brinjal plant, or parts thereof, wherein comprising at least one nucleic acid sequence selected from the group of SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 13 forms a part of the plant's genome.

13. The brinjal plant as claimed in claim 12 comprising a seed, nucleus, or parts thereof capable of producing an amplicon comprising SEQ ID NO: 16 and diagnostic for MAH-45151 event.

14. The seed as claimed in claim 13, wherein said seed comprises nucleic acid capable of producing an amplicon having SEQ ID NO: 16 diagnostic for MAH-45151 event and having an accession number NCIMB 42846.

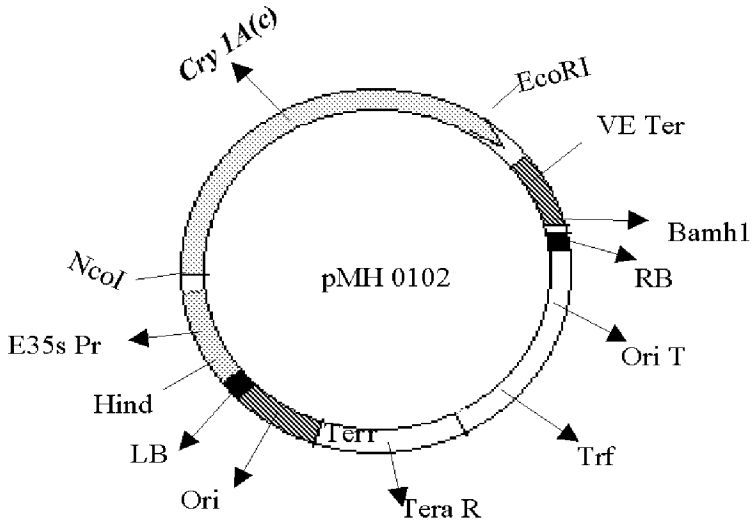

Cry 1A(c)
EcoR1
VE Ter
Bamh1
RB
Ori T
Trf
Tera R
Terr
Ori
LB
Hind
E35s Pr
NcoI
pMH 0102

Figure 1

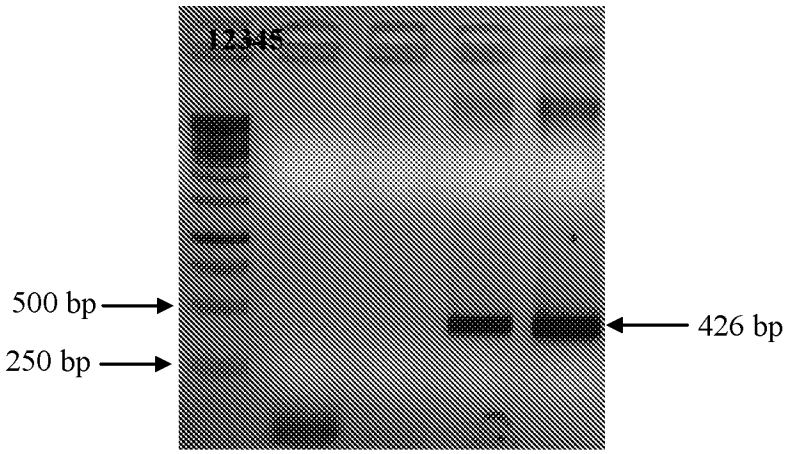


Figure 2

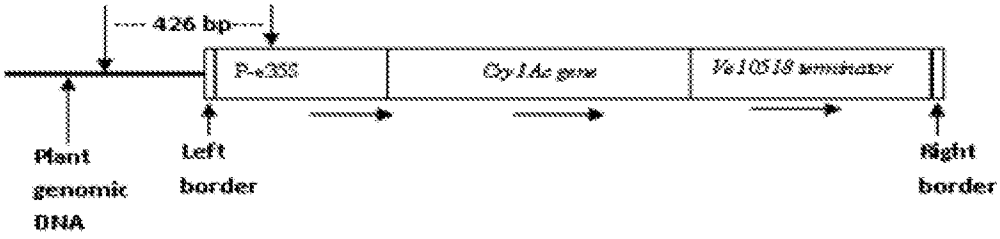

426 bp
P-e35S
Cry1Ac gene
Vs1OS18 terminator
Plant genomic DNA
Left border
Right border

Figure 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2020/050196

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C07K14/325 C12Q1/6895
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C12R C07K C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/091277 A2 (MAHARASHTRA HYBRID SEEDS COMPA [IN]; CHAR BHARAT RAGHUNATH [IN] ET AL.) 16 August 2007 (2007-08-16) examples -----	1-14
X	M. Z. H. PRODHAN ET AL: "Bt eggplant (Solanum melongena L.) in Bangladesh: Fruit production and control of eggplant fruit and shoot borer (Leucinodes orbonalis Guenee), effects on non-target arthropods and economic returns", PLOS ONE, vol. 13, no. 11, 21 November 2018 (2018-11-21), page e0205713, XP055702659, DOI: 10.1371/journal.pone.0205713 the whole document -----	1-14

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 June 2020	19/06/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Kania, Thomas

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DESIREE M. HAUTEA ET AL: "Field Performance of Bt Eggplants (Solanum melongena L.) in the Philippines: Cry1Ac Expression and Control of the Eggplant Fruit and Shoot Borer (Leucinodes orbonalis Guenée)", PLOS ONE, vol. 11, no. 6, 1 January 2016 (2016-01-01), page e0157498, XP055702668, DOI: 10.1371/journal.pone.0157498 the whole document	1-14
A	GURINDER JIT RANDHAWA ET AL: "Qualitative and event-specific real-time PCR detection methods for Bt brinjal event EE-I", JOURNAL OF AOAC INTERNATIONAL, vol. 95, no. 6, 1 November 2012 (2012-11-01), pages 1733-1739, XP55702671, US ISSN: 1060-3271, DOI: 10.5740/jaoacint.11-478	1-14
A	RAJASHEKHAR V BALLARI ET AL: "Detection and identification of genetically modified EE-1 brinjal (Solanum melongena) by single, multiplex and SYBR real-time PCR : Detection and identification of genetically modified EE-1 brinjal", JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE, vol. 93, no. 2, 30 January 2013 (2013-01-30), pages 340-347, XP055702679, GB ISSN: 0022-5142, DOI: 10.1002/jsfa.5764	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2020/050196

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2007091277 A2	16-08-2007	CN 101421404 A	29-04-2009
		EP 1989313 A2	12-11-2008
		JP 5164862 B2	21-03-2013
		JP 2009525748 A	16-07-2009
		TR 200805941 T2	23-02-2009
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