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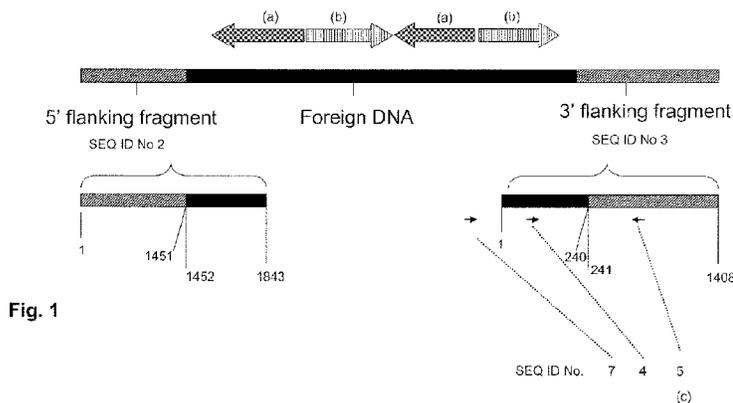
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(54) **Title:** ELITE EVENT EE-GM3 AND METHODS AND KITS FOR IDENTIFYING SUCH EVENT IN BIOLOGICAL SAMPLES



(57) **Abstract:** The invention provides specific transgenic soybean plants, plant material and seeds, characterized in that these products harbor a specific herbicide tolerance transformation event at a specific location in the soybean genome. Tools are also provided which allow rapid and unequivocal identification of the event in biological samples.

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## **Elite Event EE-GM3 and Methods and Kits for Identifying Such Event in Biological Samples**

### **Cross-Reference to Related Applications**

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This application claims priority to U.S. Provisional Patent Application No. 61/367,227, filed July 23, 2010; U.S. Provisional Patent Application No. 61/263,690, filed November 23, 2009; and European Application No. EP 09014564.0, filed November 23, 2009, the disclosures of each of which are hereby incorporated by reference in their entireties.

10

### **Field of the Invention**

This invention relates to transgenic soybean plants, plant material and seeds, characterized by harboring a specific transformation event, particularly by the presence of genes encoding proteins that confer herbicide tolerance, at a specific location in the soybean genome. The soybean plants of the invention combine the herbicide tolerance phenotype with an agronomic performance, genetic stability and functionality in different genetic backgrounds equivalent to the non-transformed soybean line in the absence of herbicide(s). This invention further provides methods and kits for identifying the presence of plant material comprising specifically transformation event EE-GM3 in biological samples.

20

### **Background of the Invention**

The phenotypic expression of a transgene in a plant is determined both by the structure of the gene or genes itself and by its or their location in the plant genome. At the same time the presence of the transgenes or "foreign DNA" at different locations in the genome will influence the overall phenotype of the plant in different ways. The agronomically or industrially successful introduction of a commercially interesting trait in a plant by genetic manipulation can be a lengthy procedure dependent on different factors. The actual transformation and regeneration of genetically transformed plants are only the first in a series of selection steps, which include extensive genetic characterization, breeding, and evaluation in field trials, eventually leading to the selection of an elite event.

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The unequivocal identification of an elite event is becoming increasingly important in view of discussions on Novel Food/Feed, segregation of GMO and non-GMO products and the identification of proprietary material. Ideally, such identification method is both quick and simple, without the need for an extensive laboratory set-up. Furthermore, the method should provide results that allow unequivocal determination of the elite event without expert interpretation, but which hold up under expert scrutiny if necessary. Specific tools for use in the identification of elite event EE-GM3 in biological samples are described herein.

10 In this invention, EE-GM3 has been identified as an elite event from a population of transgenic soybean plants in the development of herbicide tolerant soybean (*Glycine max*) comprising a gene coding for glyphosate tolerance combined with a gene conferring tolerance to 4- hydroxy phenylpyruvate dioxygenase (HPPD) inhibitors, each under control of a plant-expressible promoter.

15 Soybean plants comprising a herbicide tolerance gene have been disclosed in the art. However, none of the prior art disclosures teach or suggest the present invention.

It is known in the art that getting a commercial herbicide tolerant elite transformation event in soybean plants with acceptable agronomic performance, with no yield drag, and providing sufficient herbicide tolerance, certainly to 2 different classes of herbicides, is by no means straightforward.

25 Indeed, it has been reported that the first soybean event (event 40-3-2) released on the market with herbicide tolerance, had a significant yield drag compared to (near-)isogenic lines (Elmore et al. (2001) *Agron. J.* 93:408–412).

Also, Optimum GAT(TM) soybeans were made to combine tolerance to glyphosate with tolerance to ALS herbicides, but it has been reported by their developer that these soybeans were not meeting the standards for glyphosate tolerance by itself (without combination with another

glyphosate tolerance soybean event such as event 40-3-2 (see, e.g., [www.bloomberg.com/apps/news?pid=newsarchive&sid=ad4L0hH9MKWE](http://www.bloomberg.com/apps/news?pid=newsarchive&sid=ad4L0hH9MKWE))).

### **Summary of the Preferred Embodiments of the Invention**

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The present invention relates to a transgenic soybean plant, or seed, cells or tissues thereof, comprising, stably integrated into its genome, an expression cassette which comprises a herbicide tolerance gene comprising the coding sequence of the 2mEPSPS gene and another herbicide tolerance gene comprising the coding sequence of HPPD-Pf W336 (both as described  
10 in Example 1.1 herein and as represented in SEQ ID No 1), which is tolerant to glyphosate and an HPPD inhibitor herbicide such as isoxaflutole, and, in the absence of herbicide(s), has an agronomic performance which is substantially equivalent to the non-transgenic isogenic line. After application of one or more herbicides to which tolerance is provided, the plant will have a superior agronomic phenotype compared to a non-transgenic plant.

15

According to the present invention the soybean plant or seed, cells or tissues thereof comprise elite event EE-GM3.

More specifically, the present invention relates to a transgenic soybean plant, seed, cells or  
20 tissues thereof, the genomic DNA of which is characterized by the fact that, when analyzed in a PCR Identification Protocol as described herein, using two primers directed to the 5' or 3' flanking region of EE-GM3 and the foreign DNA comprising herbicide tolerance genes, respectively, yields a fragment which is specific for EE-GM3. The primers may be directed against the 5' flanking region within SEQ ID NO: 2 and the foreign DNA comprising herbicide  
25 tolerance genes, respectively. The primers may also be directed against the 3' flanking region within SEQ ID NO: 3 and the foreign DNA comprising herbicide tolerance genes, respectively, such as the primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID NO: 5 and SEQ ID NO: 4 or SEQ ID No.: 7 respectively, and yield a DNA fragment of between 100 and 800 bp, such as a fragment of about 263 bp or 706 bp.

30

Reference seed comprising the elite event of the invention has been deposited at the NCIMB under accession number NCIMB 41659. One embodiment of the invention is the seed comprising elite event EE-GM3 deposited as accession number NCIMB 41659, which will grow into a soybean plant tolerant to herbicides, particularly tolerant to glyphosate and/or HPPD inhibitors such as isoxaflutole. The seed of NCIMB deposit number NCIMB 41659, is a seed lot consisting of at least about 95% transgenic seeds homozygous for the transferred DNA, comprising the elite event of the invention, which will grow into herbicide tolerant plants, whereby the plants are glyphosate and/or isoxaflutole tolerant. The seed or progeny seed obtainable or obtained from the deposited seed (e.g., following crossing with other soybean plants with a different genetic background) can be sown and the growing plants can be treated with glyphosate or isoxaflutole as described herein to obtain 100% glyphosate or isoxaflutole tolerant plants, comprising the elite event of the invention. The invention further relates to cells, tissues, progeny, and descendants from a plant comprising the elite event of the invention grown from the seed deposited at the NCIMB having accession number NCIMB 41659. The invention further relates to plants obtainable from (such as by propagation of and/or breeding with) a soybean plant comprising the elite event of the invention (such as a plant grown from the seed deposited at the NCIMB having accession number NCIMB 41659). The invention also relates to soybean plants comprising elite event EE-GM3.

The invention further relates to a method for identifying a transgenic plant, or cells or tissues thereof, comprising elite event EE-GM3 which method is based on identifying the presence of characterizing DNA sequences or amino acids encoded by such DNA sequences in the transgenic plant, cells or tissues. According to a preferred embodiment of the invention, such characterizing DNA sequences are sequences of 15bp or at least 15 bp, preferably 20bp or at least 20 bp, most preferably 30bp or more which comprise the insertion site of the event, i.e. both a part of the inserted foreign DNA comprising herbicide tolerance genes and a part of the soybean genome (either the 5' or 3' flanking region) contiguous therewith, allowing specific identification of the elite event. The invention also relates to plants comprising the event EE-GM3 as identified herein.

30

The present invention further relates to methods for identifying elite event EE-GM3 in biological samples, which methods are based on primers or probes which specifically recognize the 5' and/or 3' flanking sequence of the foreign DNA comprising the herbicide tolerance genes in EE-GM3.

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More specifically, the invention relates to a method comprising of amplifying a sequence of a nucleic acid present in biological samples, using a polymerase chain reaction with at least two primers, one of which recognizes the 5' or 3' flanking region of foreign DNA comprising the herbicide tolerance genes in EE-GM3, the other which recognizes a sequence within the foreign  
10 DNA comprising the herbicide tolerance genes, preferably to obtain a DNA fragment of between 100 and 800 bp. The primers may recognize a sequence within the 5' flanking region of EE-GM3 (SEQ ID No. 2, from position 1 to position 1451) or within the 3' flanking region of EE-GM3 (complement of SEQ ID No 3 from position 241 to position 1408) and a sequence within the foreign DNA comprising herbicide tolerance genes (complement of SEQ ID No 2 from  
15 position 1452 to 1843 or SEQ ID No 3 from position 1 to position 240), respectively. The primer recognizing the 3' flanking region may comprise the nucleotide sequence of SEQ ID No. 5 and the primer recognizing a sequence within the foreign DNA comprising herbicide tolerance genes may comprise the nucleotide sequence of SEQ ID No. 4 or SEQ ID No. 7 described herein. This invention also relates to the specific primers and the specific DNA amplified using  
20 such primers, as described herein.

The present invention more specifically relates to a method for identifying elite event EE-GM3 in biological samples, which method comprises amplifying a sequence of a nucleic acid present in a biological sample, using a polymerase chain reaction with two primers comprising or  
25 consisting (essentially) of the nucleotide sequence of SEQ ID No. 4 and SEQ ID No. 5 respectively, to obtain a DNA fragment of about 263 bp or with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 5 and SEQ ID No. 7 respectively, to obtain a DNA fragment of about 706 bp. Also plants comprising the thus-identified elite event EE-GM3 are included in this invention.

30

The present invention further relates to the specific flanking sequences of EE-GM3 described herein, which can be used to develop specific identification methods for EE-GM3 in biological samples. Such specific flanking sequences may also be used as reference control material in identification assays. More particularly, the invention relates to the 5' and/or 3' flanking regions of EE-GM3 which can be used for the development of specific primers and probes as further described herein. Also suitable as reference material are nucleic acid molecules, preferably of about 150-850 bp, comprising the sequence which can be amplified by primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 7 and SEQ ID No. 5 or of SEQ ID No. 4 and SEQ ID No. 5.

10

The invention further relates to identification methods for the presence of EE-GM3 in biological samples based on the use of such specific primers or probes. Primers may comprise, consist or consist essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451 or the complement of the nucleotide sequence of SEQ ID 3 from nucleotide 241 to nucleotide 1408, combined with primers comprising, consisting, or consisting essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 2, such as a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240. Primers may also comprise these nucleotide sequences located at their extreme 3' end, and further comprise unrelated sequences or sequences derived from the mentioned nucleotide sequences, but comprising mismatches.

25

The invention further relates to kits for identifying elite event EE-GM3 in biological samples, said kits comprising at least one primer or probe which specifically recognizes the 5' or 3' flanking region of the foreign DNA comprising herbicide tolerance genes in EE-GM3.

30

The kit of the invention may comprise, in addition to a primer which specifically recognizes the 5' or 3' flanking region of EE-GM3, a second primer which specifically recognizes a sequence within the foreign DNA comprising herbicide tolerance genes of EE-GM3, for use in a PCR

Identification Protocol. The kits of the invention may comprise at least two specific primers, one of which recognizes a sequence within the 5' flanking region of EE-GM3, and the other which recognizes a sequence within the foreign DNA comprising herbicide tolerance genes. The primer recognizing the 3' flanking region may comprise the nucleotide sequence of SEQ ID No. 5 and the primer recognizing the transgenes or foreign DNA comprising herbicide tolerance genes may comprise the nucleotide sequence of SEQ ID Nos. 4 or 7, or any other primer or primer combination as described herein.

The invention further relates to a kit for identifying elite event EE-GM3 in biological samples, said kit comprising the PCR primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 5 and SEQ ID No. 4 for use in the EE-GM3 PCR Identification Protocol described herein.

The invention also relates to a kit for identifying elite event EE-GM3 in biological samples, which kit comprises a specific probe comprising or consisting (essentially) of a sequence which corresponds (or is complementary to) a sequence having between 80% and 100% sequence identity with a specific region of EE-GM3. Preferably, the sequence of the probe corresponds to a specific region comprising part of the 5' or 3' flanking region of EE-GM3. Most preferably the specific probe comprises or consists (essentially) of (or is complementary to) a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 1431 to 1472 of SEQ ID No 2 or a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 220 to 260 of ID No. 3.

According to another aspect of the invention, DNA sequences are disclosed comprising the insertion site of the event and sufficient length of polynucleotides of both the soybean genomic DNA and the foreign DNA comprising herbicide tolerance genes (transgene), so as to be useful as primer or probe for the detection of EE-GM3, and to characterize plants comprising event EE-GM3. Such sequences may comprise at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising the herbicide tolerance genes of EE-GM3, at each side of the junction site respectively. Most preferably, such DNA sequences comprise at least 9 nucleotides of the soybean genomic DNA and a similar number of

nucleotides of the foreign DNA comprising herbicide tolerance genes contiguous with the insertion site in SEQ ID NO: 2 or SEQ ID NO: 3. In one aspect of the invention, soybean plants are provided comprising such specific DNA sequences.

- 5 The methods and kits encompassed by the present invention can be used for different purposes such as, but not limited to the following: to identify the presence or determine the (lower) threshold of EE-GM3 in plants, plant material or in products such as, but not limited to food or feed products (fresh or processed) comprising or derived from plant material; additionally or alternatively, the methods and kits of the present invention can be used to identify transgenic  
10 plant material for purposes of segregation between transgenic and non-transgenic material; additionally or alternatively, the methods and kits of the present invention can be used to determine the quality (i.e. percentage pure material) of plant material comprising EE-GM3.

The invention further relates to the 5' and/or 3' flanking regions of EE-GM3 as well as to the  
15 specific primers and probes developed from the 5' and/or 3' flanking sequences of EE-GM3.

The invention also relates to genomic DNA obtained from plants comprising elite event EE-GM3. Such genomic DNA may be used as reference control material in the identification assays herein described.

20

Also provided herein is a transgenic herbicide tolerant soybean plant, or cells, parts, seeds or progeny thereof, each comprising at least one elite event, said elite event comprises a foreign DNA comprising:

- 25 i) a first chimeric gene which comprises a modified epsps gene from *Zea mays* encoding a glyphosate tolerant EPSPS enzyme under the control of a plant-expressible promoter, and  
ii) a second chimeric gene which comprises a modified hppd gene from *Pseudomonas fluorescens* encoding an HPPD inhibitor herbicide tolerant enzyme under the control of a plant-expressible promoter.

In one embodiment, said elite event comprises nucleotides 1 to 1451 of SEQ ID No 2 immediately upstream of and contiguous with said foreign DNA and nucleotides 241 to 1408 of SEQ ID No 3 immediately downstream of and contiguous with said foreign DNA.

In a further embodiment, said elite event is obtainable by breeding with a soybean plant grown from reference seed comprising said event having been deposited at the NCIMB under deposit  
5 number NCIMB 41659.

In another embodiment, the genomic DNA of said soybean plant, or cells, parts, seeds or progeny thereof when analyzed using the elite event identification protocol for said elite event with two primers comprising the nucleotide sequence of SEQ ID No 4 and SEQ ID No 5  
10 respectively, yields a DNA fragment of (about) 263 bp.

Also provided herein is a method for identifying a transgenic soybean plant, or cells, parts, seed or progeny thereof tolerant to glyphosate and/or an HPPD inhibitor herbicide, such as isoxaflutole, in biological samples, said method comprising amplifying a DNA fragment of  
15 between 100 and 500 bp from a nucleic acid present in biological samples using a polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of the elite event specified above, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451, or the 3' flanking region of said elite event, said 3' flanking region comprising or the nucleotide sequence of the complement of SEQ ID No  
20 3 from nucleotide 241 to nucleotide 1408, the other primer of said primers recognizing a sequence within the foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240.

25

Also provided herein is a kit for identifying a transgenic soybean plant, or cells, parts, seed or progeny thereof tolerant to glyphosate and/or an HPPD inhibitor herbicide, such as isoxaflutole, in biological samples, said kit comprising one primer recognizing the 5' flanking region of the elite event specified above, said 5' flanking region comprising the nucleotide sequence of SEQ  
30 ID No 2 from nucleotide 1 to nucleotide 1451, or one primer recognizing the 3' flanking region of said elite event, said 3' flanking region comprising the nucleotide sequence of the complement

of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, and one primer recognizing a sequence within the foreign DNA, said foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240.

5

In one embodiment of the invention, the foreign DNA of elite event EE-GM3, as used herein, comprises the nucleotide sequence of SEQ ID No 11 from nucleotide position 1452 to nucleotide position 16638 or its complement, or comprises a sequence with at least 95, 98, 99, or 99.5 % sequence identity to the nucleotide sequence of SEQ ID No 11 from nucleotide position 1452 to  
10 nucleotide position 16638 or its complement.

Also provided herein is a soybean plant, plant cell, tissue, or seed, comprising in their genome a nucleic acid molecule comprising a nucleotide sequence with at least 97, 98, or at least 99 % sequence identity to the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to  
15 nucleotide position 16638 or the complement thereof, or a nucleotide sequence with at least 97, 98, or at least 99 % sequence identity to SEQ ID No. 11 or the complement thereof.

One embodiment of this invention provides a soybean plant, plant cell, tissue, or seed, comprising in their genome a nucleic acid molecule hybridizing to the nucleotide sequence of  
20 SEQ ID No 1 or the complement thereof, or hybridizing to the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or the complement thereof, or hybridizing to the nucleotide sequence of SEQ ID No. 11 or the complement thereof.

Also provided herein is an isolated nucleic acid molecule comprising a nucleotide sequence with  
25 at least 99 % sequence identity to the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or the complement thereof, or a nucleotide sequence with at least 99 % sequence identity to SEQ ID No. 11 or the complement thereof, or an isolated nucleic acid molecule comprising a nucleotide sequence hybridizing to the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or the complement  
30 thereof, or hybridizing to the nucleotide sequence of SEQ ID No. 11 or the complement thereof.

### **Brief Description of the Drawings**

The following Examples, not intended to limit the invention to specific embodiments described,  
5 may be understood in conjunction with the accompanying Figures, incorporated herein by  
reference, in which:

#### **Fig. 1: Schematic representation of the relationship between the cited nucleotide**

**sequences and primers.** black bar: foreign DNA; hatched bar: DNA of plant origin;  
10 checkered arrow (a): chimeric HPPD Pf W366- encoding gene (see Table 1 for  
composition of the chimeric gene); hatched arrow (b): chimeric 2mEPS- encoding  
gene (see Table 1 for composition of the chimeric gene); black arrows: oligonucleotide  
primers, the figures under the bars represent nucleotide positions; (c) refers to  
complement of the indicated nucleotide sequence; Note: the scheme is not drawn to scale.

15

#### **Fig. 2: Results obtained by the PCR Identification Protocol developed for EE-GM3.**

Loading sequence of the gel: Lane1: Molecular weight marker (100 bp ladder); lanes 2  
and 3 : DNA samples from soybean plants comprising the transgenic event EE-GM3;  
lanes 4-7: DNA samples from transgenic soybean plants not comprising elite event EE-  
20 GM3, but comprising the same herbicide tolerance genes (other transformation events);  
lane 8: DNA sample from wild type soybean; lane 9: no-template DNA control; lane 10:  
molecular weight marker.

#### **Fig. 3: Results obtained by the zygosity scoring PCR protocol developed for EE-GM3.**

Loading sequence of the gel: Lane1: Molecular weight marker (100 bp ladder); lanes 2  
25 and 5: DNA samples from soybean plants comprising the transgenic event EE-GM3 in  
homozygous form; lanes 3, 8 and 9: DNA samples from soybean plants comprising the  
transgenic event EE-GM3 in heterozygous form; lanes 4, 6 and 7: control DNA sample  
from azygous soybean plant; lane 10: no-template DNA control; lane 11: molecular  
30 weight marker.

### Detailed Description of the Preferred Embodiments of the Invention

The incorporation of a recombinant DNA molecule in the plant genome typically results from transformation of a cell or tissue. The particular site of incorporation is usually due to random  
5 integration.

The DNA introduced into the plant genome as a result of transformation of a plant cell or tissue with a recombinant DNA or “transforming DNA”, and originating from such transforming DNA is hereinafter referred to as “foreign DNA” comprising one or more “transgenes”. The transgenes  
10 of EE-GM3 are the glyphosate and HPPD inhibitor herbicide tolerance genes. “Plant DNA” in the context of the present invention will refer to DNA originating from the plant which is transformed. Plant DNA will usually be found in the same genetic locus in the corresponding wild-type plant. The foreign DNA can be characterized by the location and the configuration at the site of incorporation of the recombinant DNA molecule in the plant genome. The site in the  
15 plant genome where a recombinant DNA has been inserted is also referred to as the “insertion site” or “target site”. Insertion of the recombinant DNA into the region of the plant genome referred to as “pre-insertion plant DNA” can be associated with a deletion of plant DNA, referred to as “target site deletion”. A “flanking region” or “flanking sequence” as used herein refers to a sequence of at least 20 bp, preferably at least 50 bp, and up to 5000 bp of DNA different from  
20 the introduced DNA, preferably DNA from the plant genome which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the foreign DNA. Transformation procedures leading to random integration of the foreign DNA will result in transformants with different flanking regions, which are characteristic and unique for each transformant. When the recombinant DNA is introduced into a plant through  
25 traditional crossing, its insertion site in the plant genome, or its flanking regions will generally not be changed.

An “isolated nucleic acid (sequence)” or “isolated DNA (sequence)”, as used herein, refers to a nucleic acid or DNA (sequence) which is no longer in the natural environment it was isolated  
30 from, e.g., the nucleic acid sequence in another bacterial host or in a plant genome, or a nucleic

acid or DNA fused to DNA or nucleic acid from another origin, such as when contained in a chimeric gene under the control of a plant-expressible promoter.

5 An event is defined as a (artificial) genetic locus that, as a result of genetic engineering, carries a foreign DNA or transgene comprising at least one copy of a gene of interest or of the genes of interest. The typical allelic states of an event are the presence or absence of the foreign DNA. An event is characterized phenotypically by the expression of the transgene. At the genetic level, an event is part of the genetic make-up of a plant. At the molecular level, an event can be characterized by the restriction map (e.g., as determined by Southern blotting), by the upstream  
10 and/or downstream flanking sequences of the transgene, the location of molecular markers and/or the molecular configuration of the transgene. Usually transformation of a plant with a transforming DNA comprising at least one gene of interest leads to a population of transformants comprising a multitude of separate events, each of which is unique. An event is characterized by the foreign DNA and at least one of the flanking sequences.

15

An elite event, as used herein, is an event which is selected from a group of events, obtained by transformation with the same transforming DNA, based on the expression and stability of the transgene(s) and its compatibility with optimal agronomic characteristics of the plant comprising it. Thus the criteria for elite event selection are one or more, preferably two or more,  
20 advantageously all of the following:

- a) that the presence of the foreign DNA does not compromise other desired characteristics of the plant, such as those relating to agronomic performance or commercial value;
- b) that the event is characterized by a well defined molecular configuration which is  
25 stably inherited and for which appropriate tools for identity control can be developed;
- c) that the gene(s) of interest show(s) a correct, appropriate and stable spatial and temporal phenotypic expression, both in heterozygous (or hemizygous) and homozygous condition of the event, at a commercially acceptable level in a range of environmental conditions in which the plants carrying the event are likely to be exposed in normal agronomic use.

30

It is preferred that the foreign DNA is associated with a position in the plant genome that allows easy introgression into desired commercial genetic backgrounds.

5 The status of an event as an elite event is confirmed by introgression of the elite event in different relevant genetic backgrounds and observing compliance with one, two or all of the criteria e.g. a), b) and c) above.

An “elite event” thus refers to a genetic locus comprising a foreign DNA, which meets the above-described criteria. A plant, plant material or progeny such as seeds can comprise one or  
10 more elite events in its genome.

The tools developed to identify an elite event or the plant or plant material comprising an elite event, or products which comprise plant material comprising the elite event, are based on the specific genomic characteristics of the elite event, such as, a specific restriction map of the  
15 genomic region comprising the foreign DNA, molecular markers or the sequence of the flanking region(s) of the foreign DNA.

Once one or both of the flanking regions of the foreign DNA have been sequenced, primers and probes can be developed which specifically recognize this (these) sequence(s) in the nucleic acid  
20 (DNA or RNA) of a sample by way of a molecular biological technique. For instance a PCR method can be developed to identify the elite event in biological samples (such as samples of plants, plant material or products comprising plant material). Such a PCR is based on at least two specific “primers”, one recognizing a sequence within the 5’ or 3’ flanking region of the elite event and the other recognizing a sequence within the foreign DNA. The primers preferably  
25 have a sequence of between 15 and 35 nucleotides which under optimized PCR conditions “specifically recognize” a sequence within the 5’ or 3’ flanking region of the elite event and the foreign DNA of the elite event respectively, so that a specific fragment (“integration fragment” or discriminating amplicon) is amplified from a nucleic acid sample comprising the elite event. This means that only the targeted integration fragment, and no other sequence in the plant  
30 genome or foreign DNA, is amplified under optimized PCR conditions.

PCR primers suitable for the invention may be the following:

- 5 - oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the plant DNA in the 5' flanking sequence (SEQ ID No 2 from nucleotide 1 to nucleotide 1451) at their 3' end (primers recognizing 5' flanking sequences); or
- 10 - oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the plant DNA in the 3' flanking sequence (complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408) at their 3' end (primers recognizing 3' flanking sequences); or
- oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the inserted DNA sequences (complement of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843) at their 3' end (primers recognizing foreign DNA); or
- 15 - oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the inserted DNA sequences (SEQ ID No 3 from nucleotide 1 to nucleotide 240); or
- 20 - suitable oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the nucleotide sequence of the inserted DNA fragment or its complement (SEQ ID No 1 or SEQ ID No 11 from nucleotide position 1452 to 16638).

The primers may of course be longer than the mentioned 17 consecutive nucleotides, and may, 25 e.g., be 20, 21, 30, 35, 50, 75, 100, 150, 200 nt long or even longer. The primers may entirely consist of nucleotide sequence selected from the mentioned nucleotide sequences of flanking sequences and foreign DNA sequences. However, the nucleotide sequence of the primers at their 5' end (i.e. outside of the 3'-located 17 consecutive nucleotides) is less critical. Thus, the 5' sequence of the primers may comprise or consist of a nucleotide sequence selected from the 30 flanking sequences or foreign DNA, as appropriate, but may contain several (e.g., 1, 2, 5, or 10) mismatches. The 5' sequence of the primers may even entirely be a nucleotide sequence

unrelated to the flanking sequences or foreign DNA, such as, e.g., a nucleotide sequence representing one or more restriction enzyme recognition sites. Such unrelated sequences or flanking DNA sequences with mismatches should preferably be not longer than 100, more preferably not longer than 50 or even 25 nucleotides.

5

Moreover, suitable primers may comprise or consist (essentially) of a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences (located at nucleotides 1451-1452 in SEQ ID No 2 and nucleotides 240-241 in SEQ ID No 3) provided the mentioned 3'-located 17 consecutive nucleotides are not  
10 derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos 2 or 3.

It will also be immediately clear to the skilled artisan that properly selected PCR primer pairs should also not comprise sequences complementary to each other.

15

For the purpose of the invention, the "complement of a nucleotide sequence represented in SEQ ID No: X" is the nucleotide sequence which can be derived from the represented nucleotide sequence by replacing the nucleotides with their complementary nucleotide according to Chargaff's rules ( $A \leftrightarrow T$ ;  $G \leftrightarrow C$ ) and reading the sequence in the 5' to 3' direction, i.e., in  
20 opposite direction of the represented nucleotide sequence.

Examples of suitable primers are the oligonucleotide sequences of SEQ ID No 5 (3' flanking sequence recognizing primer), SEQ ID No 4 (foreign DNA recognizing primer for use with the 3' flanking sequence recognizing primers), or SEQ ID No 7 (foreign DNA recognizing primer  
25 for use with the 3' flanking sequence recognizing primers).

Other examples of suitable oligonucleotide primers comprise at their 3' end the following sequences or consist (essentially) of such sequences:

a. 5' flanking sequence recognizing primers:

- 30 - the nucleotide sequence of SEQ ID No 2 from nucleotide 264 to nucleotide 283  
- the nucleotide sequence of SEQ ID No 2 from nucleotide 266 to nucleotide 285

- the nucleotide sequence of SEQ ID No 2 from nucleotide 1240 to nucleotide 1259
- the nucleotide sequence of SEQ ID No 2 from nucleotide 265 to nucleotide 285
- the nucleotide sequence of SEQ ID No 2 from nucleotide 265 to nucleotide 283
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1239 to nucleotide 1259
- 5 - the nucleotide sequence of SEQ ID No 2 from nucleotide 1241 to nucleotide 1259
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1244 to nucleotide 1263
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1248 to nucleotide 1267
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1250 to nucleotide 1269
- the nucleotide sequence of SEQ ID No 2 from nucleotide 262 to nucleotide 279
- 10 - the nucleotide sequence of SEQ ID No 2 from nucleotide 263 to nucleotide 279
- the nucleotide sequence of SEQ ID No 2 from nucleotide 264 to nucleotide 285
- the nucleotide sequence of SEQ ID No 2 from nucleotide 266 to nucleotide 283
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1238 to nucleotide 1259
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1242 to nucleotide 1259
- 15 - the nucleotide sequence of SEQ ID No 2 from nucleotide 1243 to nucleotide 1263
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1245 to nucleotide 1263
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1247 to nucleotide 1267
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1249 to nucleotide 1269
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1249 to nucleotide 1267
- 20 - the nucleotide sequence of SEQ ID No 2 from nucleotide 263 to nucleotide 285
- the nucleotide sequence of SEQ ID No 2 from nucleotide 267 to nucleotide 283
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1242 to nucleotide 1263
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1243 to nucleotide 1259
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1246 to nucleotide 1267
- 25 - the nucleotide sequence of SEQ ID No 2 from nucleotide 1246 to nucleotide 1263
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1248 to nucleotide 1269
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1250 to nucleotide 1271
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1250 to nucleotide 1267
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1241 to nucleotide 1263
- 30 - the nucleotide sequence of SEQ ID No 2 from nucleotide 1245 to nucleotide 1267
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1247 to nucleotide 1269

- the nucleotide sequence of SEQ ID No 2 from nucleotide 1247 to nucleotide 1263
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1249 to nucleotide 1271
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1242 to nucleotide 1261
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1241 to nucleotide 1261
- 5 - the nucleotide sequence of SEQ ID No 2 from nucleotide 1243 to nucleotide 1261
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1240 to nucleotide 1261
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1244 to nucleotide 1261
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1239 to nucleotide 1261
- the nucleotide sequence of SEQ ID No 2 from nucleotide 1245 to nucleotide 1261

10

b. foreign DNA sequence recognizing primers for use with 5' flanking sequence recognizing primers:

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1751
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1735 to nucleotide 1754
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1750
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1750
- 20 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1752
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1749
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1749
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1751
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1753
- 30

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1748
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1748
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1735 to nucleotide 1751
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1752
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to  
10 nucleotide 1754
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1747
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1753
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1746
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1745
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to  
20 nucleotide 1747
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1744
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1748
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1749
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1745
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to  
30 nucleotide 1744

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1746
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1747
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1748
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1724 to nucleotide 1744
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1724 to  
10 nucleotide 1745
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1724 to nucleotide 1746
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1461 to nucleotide 1478
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1686
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1486
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1508 to  
20 nucleotide 1527
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1686
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1687
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1689
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1704
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to  
30 nucleotide 1705

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1709
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1486
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1497
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1498
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to  
10 nucleotide 1507
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1508
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1688
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1690
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1691
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to  
20 nucleotide 1706
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1707
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1708
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1487
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1499
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to  
30 nucleotide 1505

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1506
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1507
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1508
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1666 to nucleotide 1686
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to  
10 nucleotide 1687
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1688
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1689
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1707
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1709
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to  
20 nucleotide 1710
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1500
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1509
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1689
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1690
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to  
30 nucleotide 1691

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1705
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1706
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1710
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1488
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to  
10 nucleotide 1507
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1510
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1512
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1513
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1514
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to  
20 nucleotide 1692
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1694
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1695
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1696
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1703
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to  
30 nucleotide 1704

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1711
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1488
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1506
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1511
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to  
10 nucleotide 1690
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1697
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1709
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1487
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1508
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to  
20 nucleotide 1511
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1512
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1666 to nucleotide 1687
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1688
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1692
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to  
30 nucleotide 1693

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1707
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1490
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1491
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1501
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1509
- 10 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1515
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1691
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1694
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1698
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1489
- 20 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1689
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1708
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1710
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1711
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1492
- 30

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1510
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1666 to nucleotide 1688
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1709
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1712
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to  
10 nucleotide 1488
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1490
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1509
- 15 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1511
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1699
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to  
20 nucleotide 1493
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1494
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1502
- 25 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1692
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1491
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to  
30 nucleotide 1510

- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1712
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1713
- 5 - the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1714
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1489
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to  
10 nucleotide 1700
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1503
- the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1713

15

## c. 3' flanking sequence recognizing primers:

- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 847
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to  
20 nucleotide 849
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 846
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 848
- 25 - the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 848
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 850
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to  
30 nucleotide 845

- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 847
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 849
- 5 - the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 851
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 844
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to  
10 nucleotide 846
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 850
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 852
- 15 - the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 992 to nucleotide 1009
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 731 to nucleotide 752
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to  
20 nucleotide 795
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 731 to nucleotide 753
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 794
- 25 - the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 796
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 793
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to  
30 nucleotide 797

- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 792
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 798
- 5 - the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 752
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 753
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to  
10 nucleotide 754
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 755
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 838 to nucleotide 854
- 15 - the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 246 to nucleotide 263
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 838 to nucleotide 855
- the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 245 to  
20 nucleotide 264

d. foreign DNA sequence recognizing primers for use with 3' flanking sequence recognizing primers:

- the nucleotide sequence of SEQ ID No 3 from nucleotide 173 to nucleotide 192
- 25 - the nucleotide sequence of SEQ ID No 3 from nucleotide 22 to nucleotide 41
- the nucleotide sequence of SEQ ID No 3 from nucleotide 172 to nucleotide 192
- the nucleotide sequence of SEQ ID No 3 from nucleotide 174 to nucleotide 192
- the nucleotide sequence of SEQ ID No 3 from nucleotide 191 to nucleotide 210
- the nucleotide sequence of SEQ ID No 3 from nucleotide 171 to nucleotide 192
- 30 - the nucleotide sequence of SEQ ID No 3 from nucleotide 175 to nucleotide 192
- the nucleotide sequence of SEQ ID No 3 from nucleotide 190 to nucleotide 210

- the nucleotide sequence of SEQ ID No 3 from nucleotide 192 to nucleotide 210
- the nucleotide sequence of SEQ ID No 3 from nucleotide 176 to nucleotide 192
- the nucleotide sequence of SEQ ID No 3 from nucleotide 189 to nucleotide 210
- the nucleotide sequence of SEQ ID No 3 from nucleotide 193 to nucleotide 210
- 5 - the nucleotide sequence of SEQ ID No 3 from nucleotide 188 to nucleotide 210
- the nucleotide sequence of SEQ ID No 3 from nucleotide 194 to nucleotide 210
- the nucleotide sequence of SEQ ID No 3 from nucleotide 199 to nucleotide 218
- the nucleotide sequence of SEQ ID No 3 from nucleotide 200 to nucleotide 218
- the nucleotide sequence of SEQ ID No 3 from nucleotide 197 to nucleotide 218
- 10 - the nucleotide sequence of SEQ ID No 3 from nucleotide 201 to nucleotide 218
- the nucleotide sequence of SEQ ID No 3 from nucleotide 201 to nucleotide 220
- the nucleotide sequence of SEQ ID No 3 from nucleotide 200 to nucleotide 220
- the nucleotide sequence of SEQ ID No 3 from nucleotide 199 to nucleotide 220
- the nucleotide sequence of SEQ ID No 3 from nucleotide 200 to nucleotide 221
- 15 - the nucleotide sequence of SEQ ID No 3 from nucleotide 199 to nucleotide 221
- the nucleotide sequence of SEQ ID No 3 from nucleotide 150 to nucleotide 172

As used herein, “the nucleotide sequence of SEQ ID No. Z from position X to position Y” indicates the nucleotide sequence including both nucleotide endpoints.

20

Preferably, the amplified fragment has a length of between 50 and 500 nucleotides, such as a length between 100 and 350 nucleotides. The specific primers may have a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event and the foreign DNA of the elite event, respectively, provided the mismatches still allow  
25 specific identification of the elite event with these primers under optimized PCR conditions. The range of allowable mismatches however, can easily be determined experimentally and are known to a person skilled in the art.

Detection of integration fragments can occur in various ways, e.g., via size estimation after gel  
30 analysis. The integration fragments may also be directly sequenced. Other sequence specific methods for detection of amplified DNA fragments are also known in the art.

As the sequence of the primers and their relative location in the genome are unique for the elite event, amplification of the integration fragment will occur only in biological samples comprising (the nucleic acid of) the elite event. Preferably when performing a PCR to identify the presence of EE-GM3 in unknown samples, a control is included of a set of primers with which a fragment within a "housekeeping gene" of the plant species of the event can be amplified. Housekeeping genes are genes that are expressed in most cell types and which are concerned with basic metabolic activities common to all cells. Preferably, the fragment amplified from the housekeeping gene is a fragment which is larger than the amplified integration fragment. Depending on the samples to be analyzed, other controls can be included.

Standard PCR protocols are described in the art, such as in "PCR Applications Manual" (Roche Molecular Biochemicals, 2nd Edition, 1999) and other references. The optimal conditions for the PCR, including the sequence of the specific primers, are specified in a "PCR (or Polymerase Chain Reaction) Identification Protocol" for each elite event. It is however understood that a number of parameters in the PCR Identification Protocol may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA may require adjustment of, for instance, the amount of primers, polymerase and annealing conditions used. Similarly, the selection of other primers may dictate other optimal conditions for the PCR Identification Protocol. These adjustments will however be apparent to a person skilled in the art, and are furthermore detailed in current PCR application manuals such as the one cited above.

Alternatively, specific primers can be used to amplify an integration fragment that can be used as a "specific probe" for identifying EE-GM3 in biological samples. Contacting nucleic acid of a biological sample, with the probe, under conditions which allow hybridization of the probe with its corresponding fragment in the nucleic acid, results in the formation of a nucleic acid/probe hybrid. The formation of this hybrid can be detected (e.g., via labeling of the nucleic acid or probe), whereby the formation of this hybrid indicates the presence of EE-GM3. Such identification methods based on hybridization with a specific probe (either on a solid phase carrier or in solution) have been described in the art. The specific probe is preferably a sequence

which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the elite event and preferably also comprising part of the foreign DNA contiguous therewith (hereinafter referred to as "specific region"). Preferably, the specific probe comprises a sequence of between 50 and 500 bp, preferably of 100 to 350 bp which is at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, most preferably between 95% and 100% identical (or complementary) to the nucleotide sequence of a specific region. Preferably, the specific probe will comprise a sequence of about 15 to about 100 contiguous nucleotides identical (or complementary) to a specific region of the elite event.

10

Oligonucleotides suitable as PCR primers for detection of the elite event EE-GM3 can also be used to develop a PCR-based protocol to determine the zygosity status of plants containing the elite event. To this end, two primers recognizing the wild-type locus before integration are designed in such a way that they are directed towards each other and have the insertion site located in between the primers. These primers may be primers specifically recognizing the 5' and 3' flanking sequences contained within SEQ ID NO 2 or 3, respectively. These primers may also be primers specifically recognizing the 5' or 3' flanking sequence. For the current invention, particularly suitable primers recognizing the wild type locus before integration are primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No 4 and SEQ ID 6. This set of primers, together with a third primer complementary to transforming DNA sequences (such as a primer comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No 7) allow simultaneous diagnostic PCR amplification of the EE-GM3 specific locus, as well as of the wild type locus. If the plant is homozygous for the transgenic locus or the corresponding wild type locus, the diagnostic PCR will give rise to a single PCR product typical, preferably typical in length, for either the transgenic or wild type locus. If the plant is hemizygous for the transgenic locus, two locus-specific PCR products will appear, reflecting both the amplification of the transgenic and wild type locus.

25

Furthermore, detection methods specific for elite event EE-GM3 which differ from PCR based amplification methods can also be developed using the elite event specific sequence information provided herein. Such alternative detection methods include linear signal amplification detection

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methods based on invasive cleavage of particular nucleic acid structures, also known as Invader™ technology, (as described e.g. in US patent 5,985,557 “Invasive Cleavage of Nucleic Acids”, 6,001,567 “Detection of Nucleic Acid sequences by Invader Directed Cleavage”, incorporated herein by reference). To this end, the target sequence is hybridized with a labeled  
5 first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1452 to nucleotide 1469 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 223 to nucleotide 240 or its complement and is further hybridized with a second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1434 to nucleotide 1451 or its complement  
10 or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 241 to nucleotide 258 or its complement, wherein the first and second oligonucleotide overlap by at least one nucleotide. The duplex or triplex structure which is produced by this hybridization allows selective probe cleavage with an enzyme (Cleavase®) leaving the target sequence intact. The cleaved labeled probe is subsequently detected,  
15 potentially via an intermediate step resulting in further signal amplification.

A “kit” as used herein refers to a set of reagents for the purpose of performing the method of the invention, more particularly, the identification of the elite event EE-GM3 in biological samples or the determination of the zygosity status of EE-GM3 containing plant material. More  
20 particularly, a preferred embodiment of the kit of the invention comprises at least one or two specific primers, as described above for identification of the elite event, or three specific primers for the determination of the zygosity status. Optionally, the kit can further comprise any other reagent described herein in the PCR Identification Protocol. Alternatively, according to another embodiment of this invention, the kit can comprise a specific probe, as described above, which  
25 specifically hybridizes with nucleic acid of biological samples to identify the presence of EE-GM3 therein. Optionally, the kit can further comprise any other reagent (such as but not limited to hybridizing buffer, label) for identification of EE-GM3 in biological samples, using the specific probe.

30 The kit of the invention can be used, and its components can be specifically adjusted, for purposes of quality control (e.g., purity of seed lots), detection of the presence or absence of the

elite event in plant material or material comprising or derived from plant material, such as but not limited to food or feed products.

As used herein, "sequence identity" with regard to nucleotide sequences (DNA or RNA), refers to the number of positions with identical nucleotides divided by the number of nucleotides in the shorter of the two sequences. The alignment of the two nucleotide sequences is performed by the Wilbur and Lipmann algorithm (Wilbur and Lipmann, 1983, Proc. Nat. Acad. Sci. USA 80:726) using a window-size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4. Computer-assisted analysis and interpretation of sequence data, including sequence alignment as described above, can, e.g., be conveniently performed using the sequence analysis software package of the Genetics Computer Group (GCG, University of Wisconsin Biotechnology Center). Sequences are indicated as "essentially similar" when such sequences have a sequence identity of at least about 75%, particularly at least about 80%, more particularly at least about 85%, quite particularly at least about 90%, especially at least about 95%, more especially at least about 98 %, or at least about 99 %. It is clear that when RNA sequences are said to be essentially similar or have a certain degree of sequence identity with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Also, it is clear that small differences or mutations may appear in DNA sequences over time and that some mismatches can be allowed for the event-specific primers or probes of the invention, so any DNA sequence indicated herein in any embodiment of this invention for any 3' or 5' flanking DNA or for any insert or foreign DNA or any primer or probe of this invention, also includes sequences essentially similar to the sequences provided herein, such as sequences hybridizing to or with at least 90 %, 95 %, 96 %, 97 %, 98 %, or at least 99 % sequence identity to the sequence given for any 3' or 5' flanking DNA, for any primer or probe or for any insert or foreign DNA of this invention.

The term "primer" as used herein encompasses any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process, such as PCR. Typically, primers are oligonucleotides from 10 to 30 nucleotides, but longer sequences can be employed. Primers may be provided in double-stranded form, though the single-stranded form is preferred.

Probes can be used as primers, but are designed to bind to the target DNA or RNA and need not be used in an amplification process.

5 The term “recognizing” as used herein when referring to specific primers, refers to the fact that the specific primers specifically hybridize to a nucleic acid sequence in the elite event under the conditions set forth in the method (such as the conditions of the PCR Identification Protocol), whereby the specificity is determined by the presence of positive and negative controls.

10 The term “hybridizing” as used herein when referring to specific probes, refers to the fact that the probe binds to a specific region in the nucleic acid sequence of the elite event under standard stringency conditions. Standard stringency conditions as used herein refers to the conditions for hybridization described herein or to the conventional hybridizing conditions as described by Sambrook et al., 1989 (Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY) which for instance can comprise the following steps: 1) 15 immobilizing plant genomic DNA fragments on a filter, 2) prehybridizing the filter for 1 to 2 hours at 42°C in 50% formamide, 5 X SSPE, 2 X Denhardt’s reagent and 0.1% SDS, or for 1 to 2 hours at 68°C in 6 X SSC, 2 X Denhardt’s reagent and 0.1% SDS, 3) adding the hybridization probe which has been labeled, 4) incubating for 16 to 24 hours, 5) washing the filter for 20 min. at room temperature in 1X SSC, 0.1 %SDS, 6) washing the filter three times for 20 min. each at 20 68°C in 0.2 X SSC, 0.1 %SDS, and 7) exposing the filter for 24 to 48 hours to X-ray film at -70°C with an intensifying screen.

As used in herein, a biological sample is a sample of a plant, plant material or products comprising plant material. The term “plant” is intended to encompass soybean (*Glycine max*) 25 plant tissues, at any stage of maturity, as well as any cells, tissues, or organs taken from or derived from any such plant, including without limitation, any seeds, leaves, stems, flowers, roots, single cells, gametes, cell cultures, tissue cultures or protoplasts. “Plant material”, as used herein refers to material which is obtained or derived from a plant. Products comprising plant material relate to food, feed or other products which are produced using plant material or can be 30 contaminated by plant material. It is understood that, in the context of the present invention, such biological samples are tested for the presence of nucleic acids specific for EE-GM3, implying the

presence of nucleic acids in the samples. Thus the methods referred to herein for identifying elite event EE-GM3 in biological samples, relate to the identification in biological samples of nucleic acids which comprise the elite event.

5 As used herein “comprising” is to be interpreted as specifying the presence of the stated features, integers, steps, reagents or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid  
10 or protein. A chimeric gene comprising a DNA sequence which is functionally or structurally defined, may comprise additional DNA sequences, such as promoter and transcript termination sequences.

The present invention also relates to the development of an elite event EE-GM3 in soybean  
15 plants comprising this event, the progeny plants and seeds comprising elite event EE-GM3 obtained from these plants and to the plant cells, or plant material derived from plants comprising this event. Plants comprising elite event EE-GM3 can be obtained as described in Example 1. This invention also relates to seed comprising elite event EE-GM3 deposited at the NCIMB under deposit number NCIMB 41659 or derivatives therefrom comprising elite event  
20 EE-GM3. “Derivatives (of seed)” as used herein, refers to plants which can be grown from such seed, progeny resulting from crossing or backcrossing, as well as plant cells, organs, parts, tissue, cell cultures, protoplasts, and plant material of same.

Soybean plants or plant material comprising EE-GM3 can be identified according to the PCR  
25 Identification Protocol described for EE-GM3 in Example 2. Briefly, soybean genomic DNA present in the biological sample is amplified by PCR using a primer which specifically recognizes a sequence within the 5’ or 3’ flanking sequence of EE-GM3 such as the primer with the sequence of SEQ ID NO: 5, and a primer which recognizes a sequence in the foreign DNA, such as the primer with the sequence of SEQ ID NO: 4. DNA primers which amplify part of an  
30 endogenous soybean sequence are used as positive control for the PCR amplification. If upon

PCR amplification, the material yields a fragment of the expected size, the material contains plant material from a soybean plant harboring elite event EE-GM3.

5 Plants harboring EE-GM3 are characterized by their glyphosate tolerance, as well as by their tolerance to HPPD inhibitors such as isoxaflutole. Plants harboring EE-GM3 are also characterized by having agronomical characteristics that are comparable to commercially available varieties of soybean, in the absence of herbicide application. It has been observed that the presence of a foreign DNA in the insertion region of the soybean plant genome described herein, confers particularly interesting phenotypic and molecular characteristics to the plants  
10 comprising this event.

One embodiment of this invention provides an elite event in soybean plants, obtainable by insertion of 2 transgenes at a specific location in the soybean genome, which elite event confers tolerance to glyphosate and an HPPD inhibitor herbicide such as isoxaflutole on such soybean  
15 plants, and wherein such elite event does not cause any effect on the agronomic performance of such soybeans negatively affecting the yield of such soybean plants, compared to isogenic lines (as used herein, "isogenic lines" or "near-isogenic lines" are soybean lines of the same genetic background but lacking the transgenes, such as plants of the same genetic background as the plant used for transformation, or segregating sister lines having lost the transgenes). Particularly,  
20 the current invention provides an elite event in soybean plants, wherein the insertion or presence of said elite event in the genome of such soybean plants does not cause an increased susceptibility to disease, does not cause a yield drag, or does not cause increased lodging, in such soybean plants, as compared to isogenic lines. Hence, the current invention provides an elite event in soybean plants, designated as EE-GM3, which results in soybean plants that can tolerate  
25 the application of glyphosate and an HPPD inhibitor herbicide (either simultaneously or separately) without negatively affecting the yield of said soybean plants compared to isogenic lines, which soybean plants have no statistically significant difference in their disease susceptibility, or lodging, as isogenic soybean plants. These characteristics make the current elite event very interesting to control glyphosate-resistant weeds in soybean fields, and can also  
30 be used in approaches to prevent or delay further glyphosate resistance development in soybean

fields (e.g., by application of glyphosate and isoxaflutole, securing 2 modes of actions applied on a soybean field).

5 Provided herein is also a soybean plant or part thereof comprising event EE-GM3, wherein representative soybean seed comprising event EE-GM3 has been deposited under NCIMB accession number 41659. Further provided herein are seeds of such plants, comprising such event, as well as a soybean product produced from such seeds, wherein said soybean product comprises event EE-GM3. Such soybean product can be or can comprise meal, ground seeds, flour, flakes, etc.. Particularly, such soybean product comprises a nucleic acid that produces an  
10 amplicon diagnostic or specific for event EE-GM3, such amplicon comprising SEQ ID No. 2 or 3. Also provided herein is a method for producing a soybean product, comprising obtaining soybean seed comprising event EE-GM3, and producing such soybean product therefrom.

Also provided herein is a soybean plant, which is progeny of any of the above soybean plants, and which comprises event EE-GM3.

15 Further provided herein is a method for producing a soybean plant tolerant to glyphosate and/or isoxaflutole herbicides, comprising introducing into the genome of such plant event EE-GM3, particularly by crossing a first soybean plant lacking event EE-GM3 with a soybean plant comprising EE-GM3, and selecting a progeny plant tolerant to glyphosate and/or isoxaflutole.

20 Also provided herein is a glyphosate and/or isoxaflutole tolerant plant, particularly without yield drag, and with acceptable agronomical characteristics, comprising a 2mEPSPS and HPPD protein, and capable of producing an amplicon diagnostic for event EE-GM3. Also provided herein are the specific isolated amplicons (DNA sequence fragments) as such, that can be obtained using the specific detection tools described herein, particularly amplicons including in  
25 their sequence a DNA fragment originating from plant DNA and a DNA fragment foreign or heterologous to such plant, such as the DNA inserted in the plant genome by transformation, as defined herein.

Further provided herein is a method for controlling weeds in a field of soybean plants comprising  
30 event EE-GM3, or a field to be planted with such soybean plants, comprising treating the field

with an effective amount of an isoxaflutole-based herbicide, wherein such plants are tolerant to such herbicide.

Further provided herein is a DNA comprising the sequence of SEQ ID No 1 or a sequence  
5 essentially similar thereto, and any plant, cell, tissue or seed, particularly of soybean, comprising such DNA sequence, such as a plant, cell, tissue, or seed comprising EE-GM3, particularly a DNA comprising 2 adjacent regions comprising or consisting (essentially) of SEQ ID No 1, or a DNA comprising 2 adjacent regions comprising or consisting of SEQ ID No 1 with some nucleotides changed, deleted or added, such as a DNA comprising a duplication of SEQ ID No 1  
10 with 4, 6, 8, or 10 nucleotides deleted or replaced, located close (such as 200-500 nt, or less than 2000 or 10.000 nt, separated) to one another. In one embodiment, this includes the DNA of SEQ ID No. 11 from nucleotide position 2257 to nucleotide position 16601 wherein 2, 4, 6, 8 or 10 nucleotides have been replaced by other nucleotides, or wherein 2, 4, 6, 8 or 10 nucleotides have been deleted or added, or the DNA of SEQ ID No. 11 from nucleotide position 2257 to  
15 nucleotide position 16601 of SEQ ID No 11, as well as the DNA of SEQ ID No. 11, and any plant, cell, tissue or seed, particularly of soybean, comprising any of such DNA sequences. Also included herein is any soybean plant, cell, tissue or seed, comprising the DNA sequence (heterologous or foreign to a conventional soybean plant, seed, tissue or cell) of SEQ ID No. 11, or comprising the DNA sequence of SEQ ID No. 11 from nucleotide position 2257 to nucleotide  
20 position 16601, or comprising a DNA sequence with at least 99 % or 99.5 % sequence identity to the sequence of SEQ ID No. 11, or comprising a DNA sequence with at least 99 % or 99.5 % sequence identity to the sequence of SEQ ID No. 11 from nucleotide position 2257 to nucleotide position 16601.

25 Also provided herein is a transgenic soybean plant, plant cell, tissue, or seed, comprising in their genome event EE-GM3 characterized by a nucleic acid molecule comprising a nucleotide sequence essentially similar to SEQ ID No. 2 from nucleotide 1441 to nucleotide 1462 and a nucleic acid molecule comprising a nucleotide sequence essentially similar to SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences, as well as a soybean plant,  
30 plant cell, tissue, or seed, comprising in their genome event EE-GM3 characterized by a nucleic acid molecule comprising a nucleotide sequence essentially similar to SEQ ID No. 2 and a

nucleic acid molecule comprising a nucleotide sequence essentially similar to SEQ ID No. 3, or the complement of said sequences.

Even further provided herein is a soybean plant, cell, tissue or seed, comprising EE-GM3, characterized by comprising in the genome of its cells a nucleic acid sequence with at least 80%, 90%, 95 % or 100 % sequence identity to SEQ ID No. 2 from nucleotide 1431 to 1472 and a nucleic acid sequence with at least 80%, 90%, 95 % or 100 % sequence identity to SEQ ID No. 3 from nucleotide 220 to 261, or the complement of said sequences.

10 The term "isoxaflutole", as used herein, refers to the herbicide isoxaflutole [i.e.(5-cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone], the active metabolite thereof, diketonitrile, and any mixtures or solutions comprising said compounds. HPPD inhibiting herbicides useful for application on the event of this invention are the diketonitriles, e.g. 2-cyano-3-cyclopropyl-1-(2-methylsulphonyl-4-trifluoromethylphenyl)-propane-1,3-dione and 2-cyano-1-[4-(methylsulphonyl)-2-trifluoromethylphenyl]-3-(1-methylcyclopropyl)propane-1,3-dione; other isoxazoles; and the pyrazolines, e.g. topramezone [i.e.[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone], and pyrasulfotole [(5-hydroxy-1,3-dimethylpyrazol-4-yl)(2-methyl-4-trifluoromethylphenyl) methanone]; or pyrazofen [2-[4-(2,4-dichlorobenzoyl)-1,3-dimethylpyrazol-5-yl]oxy]acetophenone].

In one embodiment of this invention, a field to be planted with soybean plants containing the EE-GM3 event, can be treated with an HPPD inhibitor herbicide, such as isoxaflutole ("IFT"), before the soybean plants are planted or the seeds are sown, which cleans the field of weeds that are killed by the HPPD inhibitor, allowing for no-till practices, followed by planting or sowing of the soybeans in that same pre-treated field later on (burndown application using an HPPD inhibitor herbicide). The residual activity of IFT will also protect the emerging and growing soybean plants from competition by weeds in the early growth stages. Once the soybean plants have a certain size, and weeds tend to re-appear, glyphosate, or an HPPD inhibitor-glyphosate mixture, can be applied as post-emergent herbicide over the top of the plants.

In another embodiment of this invention, a field in which seeds containing the EE-GM3 event were sown, can be treated with an HPPD inhibitor herbicide, such as IFT, before the soybean plants emerge but after the seeds are sown (the field can be made weed-free before sowing using other means, typically conventional tillage practices such as ploughing, chissel ploughing, or seed bed preparation), where residual activity will keep the field free of weeds killed by the herbicide so that the emerging and growing soybean plants have no competition by weeds (pre-emergence application of an HPPD inhibitor herbicide). Once the soybean plants have a certain size, and weeds tend to re-appear, glyphosate - or an HPPD inhibitor-glyphosate mixture - can be applied as post-emergent herbicide over the top of the plants.

10

In another embodiment of this invention, plants containing the EE-GM3 event, can be treated with an HPPD inhibitor herbicide, such as IFT, over the top of the soybean plants that have emerged from the seeds that were sown, which cleans the field of weeds killed by the HPPD inhibitor, which application can be together with (e.g., in a spray tank mix), followed by or preceded by a treatment with glyphosate as post-emergent herbicide over the top of the plants (post-emergence application of an HPPD inhibitor herbicide (with or without glyphosate)).

15

Also, in accordance with the current invention, soybean plants harboring EE-GM3 may be treated with the following insecticides, herbicides or fungicides or soybean seeds harboring EE-GM3 may be coated with a coat comprising the following insecticides, herbicides or fungicides:

20

Soybean Herbicides:

Alachlor, Bentazone, Trifluralin, Chlorimuron-Ethyl, Cloransulam-Methyl, Fenoxaprop, Fomesafen, Fluazifop, Glyphosate, Imazamox, Imazaquin, Imazethapyr, (S-)Metolachlor, Metribuzin, Pendimethalin, Tepraloxym, Isoxaflutole.

25

Soybean Insecticides:

Lambda-cyhalothrin, Methomyl, Parathion, Thiocarb, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinotofuran, Flubendiamide, Rynaxypyr, Cyazypyr, Spinosad, Spinetoram, Emamectin-Benzoate, Fipronil, Ethiprole, Deltamethrin,  $\beta$ -Cyfluthrin, gamma and lambda Cyhalothrin, 4-[[[(6-Chloropyridin-3-yl)methyl](2,2-difluoroethyl)amino]furan-2(5H)-on, Spirotetramat, Spinodiclofen, Triflumuron, Flonicamid, Thiodicarb, beta-Cyfluthrin.

30

Soybean Fungicides:

Azoxystrobin, Cyproconazole, Epoxiconazole, Flutriafol, Pyraclostrobin, Tebuconazole, Trifloxystrobin, Prothioconazole, Tetraconazole.

- 5 The following examples describe the development and identification of elite event EE-GM3, the development of different soybean lines comprising this event, and the development of tools for the specific identification of elite event EE-GM3 in biological samples.

Unless stated otherwise in the Examples, all recombinant techniques are carried out according to  
10 standard protocols as described in “Sambrook J and Russell DW (eds.) (2001) *Molecular Cloning: A Laboratory Manual*, 3rd Edition, Cold Spring Harbor Laboratory Press, New York” and in “Ausubel FA, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA and Struhl K (eds.) (2006) *Current Protocols in Molecular Biology*. John Wiley & Sons, New York”.

15 Standard materials and references are described in “Croy RDD (ed.) (1993) *Plant Molecular Biology LabFax*, BIOS Scientific Publishers Ltd., Oxford and Blackwell Scientific Publications, Oxford” and in “Brown TA, (1998) *Molecular Biology LabFax*, 2nd Edition, Academic Press, San Diego”. Standard materials and methods for polymerase chain reactions (PCR) can be found in “McPherson MJ and Møller SG (2000) *PCR (The Basics)*, BIOS Scientific Publishers Ltd.,  
20 Oxford” and in “*PCR Applications Manual*, 3rd Edition (2006), Roche Diagnostics GmbH, Mannheim or [www.roche-applied-science.com](http://www.roche-applied-science.com) “

It should be understood that a number of parameters in any lab protocol such as the PCR  
25 protocols in the below Examples may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA or the selection of other primers in a PCR method may dictate other optimal conditions for the PCR protocol. These adjustments will however be apparent to a person skilled in the art, and are furthermore detailed in current PCR application manuals.

30 In the description and examples, reference is made to the following sequences:

SEQ ID No. 1:	Sall fragment nucleotide sequence of vector pSF10.
SEQ ID No. 2:	nucleotide sequence comprising the 5' region flanking the foreign DNA comprising the herbicide tolerance genes in EE-GM3.
SEQ ID No. 3:	nucleotide sequence comprising the 3' region flanking the foreign DNA comprising the herbicide tolerance genes in EE-GM3.
5	
SEQ ID No. 4:	primer SOY028
SEQ ID No. 5:	primer SOY029
SEQ ID No. 6:	primer SMP187
SEQ ID No. 7:	primer STV019
10	
SEQ ID No. 8:	nucleotide sequence of the amplicon
SEQ ID No. 9:	primer 1 for amplification of control fragment (SOY01)
SEQ ID No. 10:	primer 2 for amplification of control fragment (SOY02)
SEQ ID No. 11:	nucleotide sequence of foreign DNA and plant flanking sequences in EE-GM3
15	
SEQ ID No. 12:	primer SHA130
SEQ ID No. 13:	primer SHA178

### Examples

#### **1. Transformation of *Glycine max* with herbicide tolerance genes.**

20

##### **1.1. Description of the foreign DNA comprising the 2mEPSPS and HPPD-Pf-W336 chimeric genes**

Plasmid pSF10 is a pUC19 derived cloning vector which contains a chimeric *2mepsps* gene and a chimeric *hppd-Pf-W336* gene located on a Sall fragment of about 7.3 kb. A full description of the DNA comprised between the two Sall restriction sites is given in Table 1 below. The nucleotide sequence is represented in SEQ ID No. 1.

30

**Table 1. Nucleotide positions of the DNA comprised between the Sall restriction sites of pSF10 (SEQ ID No 1)**

Nucleotide positions	Orientation	Description and references
188-479	complement	3'nos: sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 of <i>Agrobacterium tumefaciens</i> (Depicker et al., 1982, <i>Journal of Molecular and Applied Genetics</i> , 1, 561-573)
480-1556	complement	hppdPf W336: the coding sequence of the 4-hydroxyphenylpyruvate dioxygenase of <i>Pseudomonas fluorescens</i> strain A32 modified by the replacement of the amino acid Glycine 336 with a Tryptophane, as described by Boudec et al. (2001) US Patent US6245968B1
1557-1928	complement	TPotp Y: coding sequence of an optimized transit peptide derivative (position 55 changed into Tyrosine), containing sequence of the RuBisCO small subunit genes of <i>Zea mays</i> (corn) and <i>Helianthus annuus</i> (sunflower), as described by Lebrun et al. (1996) US5510471
1929-2069	complement	5'tev: sequence including the leader sequence of the tobacco etch virus as described by Carrington and Freed (1990) <i>Journal of Virology</i> , 64, 1590-1597
2070-3359	complement	Ph4a748 ABBC: sequence including the promoter region of the histone H4 gene of <i>Arabidopsis thaliana</i> , containing an internal duplication (Chabouté et al., 1987) <i>Plant Molecular Biology</i> , 8, 179-191.
3360-4374		Ph4a748: sequence including the promoter region of the histone H4 gene of <i>Arabidopsis thaliana</i> (Chabouté et al., 1987) <i>Plant Molecular Biology</i> , 8, 179-191.
4375-4855		intron1 h3At: first intron of gene II of the histone H3.III variant of <i>Arabidopsis thaliana</i> (Chaubet et al., 1992) <i>Journal of Molecular Biology</i> , 225, 569-574.
4856-5227		TPotp C: coding sequence of the optimized transit peptide, containing sequence of the RuBisCO small subunit genes of <i>Zea mays</i> (corn) and <i>Helianthus annuus</i> (sunflower), as described by Lebrun et al. (1996) US5510471
5228-6565		2mepsps: the coding sequence of the double-mutant 5-enol-pyruvylshikimate-3-phosphate synthase gene of <i>Zea mays</i> (corn) (Lebrun et al., 1997) WO9704103-A 1

Nucleotide positions	Orientation	Description and references
6566-7252		3'histonAt: sequence including the 3' untranslated region of the histone H4 gene of <i>Arabidopsis thaliana</i> (Chabouté et al., 1987) <i>Plant Molecular Biology</i> , 8, 179-191.

## 1.2. Event EE-GM3

5 The HPLC purified Sall-linearized pSF10 fragment of about 7.3 kb (containing the *2mEPSPS* glyphosate-tolerance gene and the HPPD inhibitor tolerance gene *HPPD-Pf- W336*) was used to obtain transformed soybean plants by means of direct gene transfer into cells of soybean type Jack (Nickell, C. D., G. R. Noel, D. J. Thomas, and R. Waller. Registration of 'Jack' soybean. *Crop Sci* 1365. 30.1990), followed by regeneration of transformed plant cells into transgenic  
 10 fertile soybean plants.

### 1.2.1 Identification of elite event EE-GM3

Elite event EE-GM3 was selected based on an extensive selection procedure based on good  
 15 expression and stability of the herbicide tolerance genes, and its compatibility with optimal agronomic characteristics such as plant height, height to node, stand, vigor, seed yield, were evaluated. Soybean plants containing this event were selected from a wide range of different transformation events obtained using the same chimeric genes. Parameters used in the selection of this event were: a) acceptable tolerance to isoxaflutole herbicide application in field trials, b)  
 20 acceptable tolerance to glyphosate herbicide application in field trials, c) acceptable tolerance to combined application of isoxaflutole and glyphosate herbicides in field trials, d) an insertion of the herbicide tolerance transgenes at a single locus in the soybean plant genome, with absence of vector backbone, e) overall agronomy similar to the parent plants used for transformation (maturity, lodging, disease susceptibility, etc.), and f) no significant yield drag caused by the  
 25 insertion of the transforming DNA (as compared to an isogenic line without the event, such as the plant line used for transformation, grown under the same conditions).

At the T3 generation, a homozygous line of the soybean transformation event EE-GM3 was selected for seed production. Multi-location replicated agronomic field studies were conducted in the regions of adaptation of the parent variety, Jack. Field evaluations included herbicide tolerance and agronomic performance. The agronomic performance of plants containing EE-GM3 was found comparable to Jack (when no herbicides were applied).

The field evaluations also showed that plants carrying the EE-GM3 event have :

- similar plant morphology and seed characteristics compared to Jack,
- no change in response to soybean diseases compared to Jack, and
- no changes in seed germination or dormancy compared to Jack.

Seed (T1 or S1 generation) harvested from the initial transformant (T0) plant (the plant transformed with the construct so as to produce event EE-GM3) in the greenhouse were planted in the field. Three blocks were planted and sprayed with 0, 2, or 4 kg/ha glyphosate. Seed was harvested from plants demonstrating the desired level of tolerance to the herbicide, glyphosate.

Seeds (T2 generation) harvested from self-pollinated T1 plants grown in the field were sown "plant to row". Chi square analysis of segregation data for rows (fully or partially tolerant) and of individual plants within rows (tolerant or sensitive) demonstrates the expected Mendelian inheritance of a single insertion for EE-GM3.

Selection and seed increase continued until a line was determined to be homozygous for transformation event EE-GM3 and selected for core seed production in the fourth generation. Then, T5 generation seed served as a candidate for the development of different varieties. Plants in the sixth generation (T6 generation) were crossed with conventional soybean breeding lines in an introgression program designed to move the event into a broader base of commercial soybean germplasm. F1 hybrid plants (EE-GM3 lines x conventional lines) were grown to maturity and the F2 seed was planted. Leaf samples of 901 F2 plants were analyzed by PCR primers designed to identify the zygosity of the EE-GM3 insert. The expected ratio of 1:2:1 for a single-insertion segregation by the rules of Mendel was observed.

The selected event EE-GM3 was introduced in different commercial genetic backgrounds, and results of field trials on different locations were compared. Plants were challenged with glyphosate herbicide and/or isoxaflutole herbicide using different treatments. The plants exhibited good herbicide tolerance. Hundreds of different soybean cultivars containing event  
5 EE-GM3 were used in an inheritance study, and herbicides were applied. Selected lines from this trial were later increased in the field and also treated with herbicide. From that trial, 50 selected lines were increased, and these were also herbicide treated. The phytotoxicity scores for the latter lines sprayed with isoxaflutole and glyphosate showed some variability in response, but the range of responses among the lines reflected similar variability as was observed across 4  
10 replications of the EE-GM3 event in the original Jack background, grown under the same treatment and environmental conditions. Hence, tolerance to the relevant herbicides across a broad range of germplasm was observed for plants comprising EE-GM3.

Furthermore, plants containing the event EE-GM3 had normal leaf, flower and pod morphology,  
15 excellent fertility, and showed no disease or abnormal insect susceptibility in multiple genetic backgrounds. During introgression into multiple genetic backgrounds no aberrant problems or abnormalities were observed.

In one season, a 10-location study was designed to compare the agronomic performance of  
20 double herbicide tolerant soybean comprising transformation event EE-GM3 to the transformation parent variety, Jack and some non-transgenic soybean varieties. Using a randomized complete block design, EE-GM3 plants were grown in replicated plots with either conventional weed control or with the intended herbicides, glyphosate and isoxaflutole. Plots with soybean plants containing transformation event EE-GM3 were sprayed with isoxaflutole  
25 herbicide at a target rate of 70 grams ai/Ha and with glyphosate herbicide at a target rate of 1060 grams ai/Ha. Herbicide application was made to these plants as a foliar spray at about the V4-V5 plant growth stage. Agronomic observations were made in the early, mid and late season. The plant density (parameter; stand count) was higher for the Jack and the non-transgenic variety plots than in the event EE-GM3 plots by one standard deviation. The early stand count  
30 difference may have been the result of seed lot quality, as the EE-GM3 planting seed was produced in counter season nursery, while the seed of the non-transgenic varieties was produced

in the contiguous US, normal production season. However, the number of days to achieve 50% emergence and the plant vigor ratings were the same, indicating that the seed lots were comparable for these performance parameters. In the late season stand counts, Jack and the non-transgenic varieties remained different by one standard deviation from EE-GM3 plants. Plot yields of EE-GM3 event plants were also lower than those of Jack by one standard deviation, perhaps a result of the lower plant density of the EE-GM3 event plots. The yield of the non-transgenic varieties was more than Jack as could be expected because of the advancement in yield potential found in more recent varieties.

In one trial, plant health ratings were made at three growth stages: V4-5, R1 and full maturity. The first evaluation was shortly after the intended herbicide application. At the time of the final plant health evaluation, the EE-GM3-containing plants sprayed with both herbicides had the same score as the unsprayed Jack plants, or the unsprayed plants comprising EE-GM3. In ratings by the agronomic staff, the herbicide-sprayed plants received a health rating of 3-4 (moderate injury) at the V4-5 and R1 plant growth stages. The unsprayed plants (untransformed Jack or soybean plants containing EE-GM3) were rated as 4.6-4.8 (rating of 5 indicates no injury). At the final plant health rating, all the plots received the same rating of 5 (no injury).

One trial season was one of exceptional rainfall, and crop injury in the EE-GM3 plants following the intended herbicide application was more obvious than observed in other seasons. The field evaluations also included monitoring of the fitness characters (reproduction, disease resistance, fecundity, seed dispersal, dormancy, persistence). For the reproductive characteristics; days to emergence, days to 50% flowering and days to 90% pods maturing, the EE-GM3 and Jack plants were not different. No difference was noted in the reaction to natural infestations of plant diseases and insect pests. Although EE-GM3 produced less ultimate yield than Jack, no difference in fecundity (100-seed weight) was found. The assessment of seed dispersal parameters (pod shattering and plant lodging) found EE-GM3 and Jack to have the same pod shattering score, but found EE-GM3 plants to be less prone to lodging. Evaluation of seed harvested from the 10 locations found no concerns raised by germination or dormancy testing.

In these trials during the season with exceptional rainfall, the final yield of EE-GM3 plants, regardless of the weed control treatment, was less than the yield of Jack by one standard deviation (perhaps a result of the lower plant density of the EE-GM3 event plots). In the exceptionally wet season, crop injury (bleaching in 10-30% of the crop area) was reported for EE-GM3 plots up to six weeks following foliar application of the glyphosate and isoxaflutole herbicides. However, by maturity, "no injury" plant health ratings were assigned to all the plots. Replicated multi-location field trials with EE-GM3 introgressed in elite soybean cultivar background, when compared to near-isogenic sister lines not containing the transgene, are expected to show no yield difference between plants containing event EE-GM3 and the near-isogenic lines (in the absence of herbicide treatment).

Further, in a replicated field trial significant crop tolerance (bleaching of less than 10 %) was found in soybean plants comprising EE-GM3 when treated either pre- or post-emergence with IFT (70 gr ai/ha with 0.5 % NIS, Agridex), but also significant crop tolerance (bleaching of less than 10 %) was found in soybean plants comprising EE-GM3 when treated with a post-emergence application of pyrasulfotole (35 gr ai/ha with 0.5 % NIS, Agridex), another HPPD inhibitor herbicide.

### **1.2.2. Identification of the flanking regions and foreign DNA of elite event EE-GM3**

The sequence of the regions flanking the foreign DNA comprising the herbicide tolerance genes in the EE-GM3 elite event was determined to be as follows:

#### **1.2.2.1. Right (5') flanking region**

The fragment identified as comprising the 5' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 2. The sequence between nucleotide 1 and 1451 corresponds to plant DNA, while the sequence between nucleotide 1452 and 1843 corresponds to foreign DNA.

#### **1.2.2.2. Left (3') flanking region**

The fragment identified as comprising the 3' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 3. The sequence between nucleotide 1 and 240 corresponds to foreign DNA, while the sequence between nucleotide 241 and 1408 corresponds to plant DNA.

#### 1.2.2.3. Foreign DNA comprising the herbicide tolerance genes of EE-GM3

Using different molecular techniques, it has been determined that the foreign DNA of elite event EE-GM3 comprising the herbicide tolerance genes contains two partial 3' histonAt sequences in a head-to-head orientation, followed by 2 almost complete copies of the Sall fragment of pSF10 arranged in head-to-tail orientation (see Figure 1).

The foreign DNA comprising the herbicide tolerance genes of EE-GM3 thus contains in order the following sequences:

- from nucleotide 1 to nucleotide 199: the nucleotide sequence corresponding to complement of the nucleotide sequence of SEQ ID 1 from nt 6760 to nt 6958;
- from nucleotide 200 to nucleotide 624: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 1 from nt 6874 to nt 7298;
- from nucleotide 625 to nucleotide 7909: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 1 from nt 7 to nt 7291;
- from nucleotide 7910 to nucleotide 15163: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 1 from nt 12 to nt 7265; and
- from nucleotide 15164 to nucleotide 15187: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 3 from nt 217 to nt 240 (this sequence does not correspond to either pSF10 plasmid DNA or wt plant DNA and therefore is designated filler DNA).

This foreign DNA is preceded immediately upstream and contiguous with the foreign DNA by the 5' flanking sequence of SEQ ID No 2 from nucleotide 1 to 1451 and is followed immediately downstream and contiguous with the foreign DNA by the 3' flanking sequence of SEQ ID No 3 from nucleotide 241 to nucleotide 1408.

Confirmed full DNA sequencing of the foreign DNA and flanking DNA sequences in EE-GM3 resulted in the sequence reported in SEQ ID No. 11. In this sequence, the inserted DNA is from nucleotide position 1452 to nucleotide position 16638, and the 2 almost complete copies from pSF10 arranged in head-to-tail orientation are from nucleotide position 2257 to nucleotide position 16601. The 5' flanking DNA sequence in SEQ ID No. 11 is the sequence from nucleotide position 1 to nucleotide position 1451 in SEQ ID No. 11, and the 3' flanking DNA sequence in SEQ ID No. 11 is the sequence from nucleotide position 16639 to nucleotide position 17806 in SEQ ID No. 11.

## 2. Development of Polymerase Chain Reaction Identification Protocols for EE-GM3

### 2.1. Primers

Specific primers were developed which recognize sequences within the elite event.

A primer was developed which recognizes a sequence within the 3' flanking region of EE-GM3. A second primer was then selected within the sequence of the foreign DNA so that the primers span a sequence of about 263 nucleotides. The following primers were found to give particularly clear and reproducible results in a PCR reaction on EE-GM3 DNA:

SOY028: 5'-ATC.gCT.TTA.ACg.TCC.CTC.Ag -3 (SEQ ID No.: 4)  
(target: insert DNA)

SOY029: 5'-CAA.ggC.CTC.gAg.ATT.ATC -3' (SEQ ID No.: 5)  
(target: plant DNA)

Primers targeting an endogenous sequence are preferably included in the PCR cocktail. These primers serve as an internal control in unknown samples and in the DNA positive control. A positive result with the endogenous primer-pair (presence of an PCR amplified fragment of 413 bp) demonstrates that there is ample DNA of adequate quality in the genomic DNA preparation

for a PCR product to be generated. The endogenous primers were selected to recognize the endogenous actin soybean gene:

SOY01        5'-gTC.AgC.CAC.ACA.gTg.CCT.AT -3'    (SEQ ID No.: 9)

5

SOY02        5'-gTT.ACC.gTA.CAg.gTC.TTT.CC -3'    (SEQ ID No.: 10)

## 2.2. Amplified fragments

10    The expected amplified fragments in the PCR reaction are:

For primer pair SOY01-SOY02:            413bp (endogenous control)

For primer pair SOY028-SOY029:        263bp (EE-GM3 elite event)

## 15    2.3. Template DNA

Template DNA was prepared from a leaf punch according to Edwards *et al.* (Nucleic Acid Research, 19, p1349, 1991). When using DNA prepared with other methods, a test run utilizing different amounts of template should be done. Usually 50 ng of genomic template DNA yields  
20    the best results.

## 2.4. Assigned positive and negative controls

To avoid false positives or negatives, it was determined that the following positive and negative  
25    controls should be included in a PCR run:

- Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result, no PCR products, is observed this indicates that the PCR cocktail was not contaminated with target DNA.

30

- A DNA positive control (genomic DNA sample known to contain the transgenic sequences). Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.
- 5 - A wild-type DNA control. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

10

### 2.5. PCR conditions

Optimal results were obtained under the following conditions (In describing the various conditions for optimal results is meant to provide examples of such conditions. Clearly one skilled in the art could vary conditions, reagents and parameters such as using other Taq polymerases, and achieve desirable results):

15

- the PCR mix for 25 $\mu$ l reactions contains:

20

20 ng template DNA

2.5  $\mu$ l 10x Amplification Buffer (supplied by the manufacturer with the Taq polymerase)

0.5  $\mu$ l 10 mM dNTP's

0.4  $\mu$ l SOY01 (10pmoles/ $\mu$ l)

25

0.4  $\mu$ l SOY02 (10pmoles/ $\mu$ l)

0.7  $\mu$ l SOY028 (10pmoles/ $\mu$ l)

0.7  $\mu$ l SOY029 (10pmoles/ $\mu$ l)

0.1  $\mu$ l Taq DNA polymerase (5 units/ $\mu$ l)

water up to 25  $\mu$ l

30

- the thermocycling profile to be followed for optimal results is the following:

4 min. at 95°C

Followed by: 1 min. at 95°C  
1 min. at 57°C  
5 2 min. at 72°C  
For 5 cycles

Followed by: 30 sec. at 92°C  
30 sec. at 57°C  
10 1 min. at 72°C  
For 25 cycles

Followed by: 10 minutes at 72°C

## 15 **2.6. Agarose gel analysis**

To optimally visualize the results of the PCR it was determined that between 10 and 20µl of the PCR samples should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate molecular weight marker (e.g. 100bp ladder Pharmacia).

20

## **2.7. Validation of the results**

It was determined that data from transgenic plant DNA samples within a single PCR run and a single PCR cocktail should not be acceptable unless 1) the DNA positive control shows the expected PCR products (transgenic and endogenous fragments), 2) the DNA negative control is negative for PCR amplification (no fragments) and 3) the wild-type DNA control shows the expected result (endogenous fragment amplification).

When following the PCR Identification Protocol for EE-GM3 as described above, lanes showing visible amounts of the transgenic and endogenous PCR products of the expected sizes, indicate that the corresponding plant from which the genomic template DNA was prepared, has inherited

30

the EE-GM3 elite event. Lanes not showing visible amounts of either of the transgenic PCR products and showing visible amounts of the endogenous PCR product, indicate that the corresponding plant from which the genomic template DNA was prepared, does not comprise the elite event. Lanes not showing visible amounts of the endogenous and transgenic PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated. These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

### 2.8. Use of discriminating PCR protocol to identify EE-GM3

10

Before attempting to screen unknowns, a test run, with all appropriate controls, is performed. The developed protocol might require optimization for components that may differ between labs (template DNA preparation, Taq DNA polymerase, quality of the primers, dNTP's, thermocycler, etc.).

15

Amplification of the endogenous sequence plays a key role in the protocol. One has to attain PCR and thermocycling conditions that amplify equimolar quantities of both the endogenous and the transgenic sequence in a known transgenic genomic DNA template. Whenever the targeted endogenous fragment is not amplified or whenever the targeted sequences are not amplified with the same ethidium bromide staining intensities, as judged by agarose gel electrophoresis, optimization of the PCR conditions may be required.

Leaf material from a number of soybean plants, some of which comprising EE-GM3 were tested according to the above-described protocol. Samples from elite event EE-GM3 and from soybean wild-type were taken as positive and negative controls, respectively.

Figure 2 illustrates the result obtained with the elite event PCR Identification Protocol for EE-GM3 on a number of soybean plant samples. The samples in lanes 2 and 3 were found to contain elite event EE-GM3 as the 263 bp band is detected, while the samples in lanes 4 to 8 do not comprise EE-GM3. Lanes 6 and 7 comprise samples from other soybean transformation events obtained using the same herbicide tolerance chimeric genes; lane 8 contains DNA from wild type

soybean plants and lane 9 represents the negative control (water) sample, lanes 1 and 10 represent the Molecular Weight Marker (100 bp ladder).

5

### 2.9. dPCR assay for EE-GM3 detection in bulked seed

A discriminating PCR (dPCR) assay is set up to detect low level presence of EE-GM3 in bulked seeds. A minimum level of 0.4% (w/w) of transgenic seeds in a bulk of non transgenic seeds was successfully detected under repeatable conditions. Therefore the Limit of Detection is determined to be 0.4% (w/w).

The following primers are applied in this target PCR reaction:

15 Forward primer targeted to the T-DNA sequence:

SHA130 5' – CTA.TAT.TCT.ggT.TCC.AAT.TTA.TC -3' (SED ID No.12)

Reverse primer targeted to the 3' flanking sequence:

SMP178 5' – TgA.ggC.ACg.TAT.TgA.TgA.CC – 3' (SEQ ID No. 13)

The expected amplified fragment in the PCR reaction from these primers is 115 bp.

20

The target PCR reaction is performed on approximately 200ng of template DNA prepared from ground bulked seed according to a modified Gentra Puregene DNA purification extraction kit (Qiagen). When using DNA prepared with other methods, a test run using samples with known relative levels of EE-GM3 should be performed.

25 A validated reference system PCR reaction, targeting an endogenous sequence, should ideally be performed in a separate PCR run to verify the suitability of the DNA sample for PCR analysis to avoid false negative results.

For unknown test samples the PCR experiment should ideally include the appropriate positive and negative control samples, i.e.:

30 - Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result (no PCR product) is observed for both the target and the

reference system reaction this indicates that the PCR cocktail was not contaminated with target DNA.

- A DNA positive control (genomic DNA sample known to contain the transgenic sequences). Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.
- Also a wild-type DNA control can be added in this PCR. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

Optimal results are obtained under the following conditions:

Obviously, other Taq polymerases can be used, and then the conditions can differ to follow supplier recommendations.

15

- the PCR mix for 25µl reactions contains:

200 ng template DNA

5 µl 5x Reaction Buffer

20 0.25 µl 20 mM dNTP's

0.7 µl SHA130 (10pmoles/l)

0.4 µl SMP178 (10pmoles/l)

0.1 µl GO-Taq DNA polymerase (5 units/l)

Add water up to 25 µl

25

- the thermocycling profile to be followed for optimal results is the following:

4 min. at 95°C

Followed by: 1 min. at 95°C

30 1 min. at 57°C

2 min. at 72°C

For 5 cycles

Followed by: 30 sec. at 92°C

30 sec. at 57°C

5 1 min. at 72°C

For 30 cycles

Followed by: 10 minutes at 72°C

10 To optimally visualize the results of the PCR it was determined that 25µl of the PCR product should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate molecular weight marker (e.g. 50bp ladder).

15 When following the PCR method as described above, lanes showing visible amounts of the target and reference system PCR products of the expected sizes, indicate that the test sample from which the genomic template DNA was prepared, contained levels of EE-GM3 elite event above the detection limit of the target reaction.

20 Lanes not showing visible amounts of the target PCR products but showing visible amounts of the reference system PCR product, indicate that the test sample from which the genomic template DNA was prepared, contained levels of EE-GM3 elite event below the detection limit of the target reaction .

25 Lanes not showing visible amounts of the endogenous and transgenic PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated. These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

### **3. Use of a specific integration fragment as a probe for detection of material comprising EE-GM3**

30 A specific integration fragment of EE-GM3 is obtained by PCR amplification using specific primers SOY028 (SEQ ID No. 4) and SOY029 (SEQ ID No. 5) yielding an amplicon with the



(target: plant DNA of the 3' flanking sequence)

STV019 5' - ggC.ATT.AAA.TTg.gTg.AAA.ATT.gC-3' (SEQ ID No.: 7)  
(target: insert DNA)

5

#### 4.2. Amplified fragments

The expected amplified fragments in the PCR reaction are:

- 10 For primer pair SMP187 – SOY029: 319 bp (wild-type locus)  
For primer pair STV019 – SOY029: 706 bp (EE-GM3 locus)

#### 4.3. Template DNA

- 15 Template DNA was prepared from a leaf punch according to Edwards *et al.* (Nucleic Acid Research, 19, p1349, 1991). When using DNA prepared with other methods, a test run utilizing different amounts of template should be done. Usually 25 or 50 ng of genomic template DNA yields the best results.

#### 20 4.4. Assigned positive and negative controls

To avoid false positives or negatives, it is advisable that the following positive and negative controls should be included in a PCR run:

- 25 - Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result, no PCR products, is observed this indicates that the PCR cocktail was not contaminated with target DNA.
- A DNA positive control (genomic DNA sample known to contain the transgenic sequences).
- 30 Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.

- A wild-type DNA control. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

#### 4.5. PCR conditions

Optimal results were obtained under the following conditions. Obviously, other Taq polymerases, such as GO-Taq, can be used, and then the conditions can differ to follow supplier recommendations.

- the PCR mix for 25µl reactions contains:

15

x µl template DNA (25 ng)  
2.5 µl 10x Amplification Buffer (supplied by the manufacturer with the Taq polymerase)  
0.5 µl 10 mM dNTP's  
0.5 µl SMP187(10pmoles/µl)  
0.5 µl STV019(10pmoles/µl)  
1 µl SOY029 (10pmoles/µl)  
0.1 µl Taq DNA polymerase (5 units/µl)  
water up to 25 µl

25

- the thermocycling profile to be followed for optimal results is the following:

4 min. at 95°C

Followed by: 1 min. at 95°C

30

1 min. at 57°C

2 min. at 72°C

For 5 cycles

Followed by: 30 sec. at 92°C

30 sec. at 57°C

5 1 min. at 72°C

For 25 cycles

Followed by: 10 minutes at 72°C

10

#### 4.6. Agarose gel analysis

To optimally visualize the results of the PCR it was determined that between 10 and 20µl of the PCR samples should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate  
15 molecular weight marker (e.g. 100bp ladder Pharmacia).

#### 4.7. Validation of the results

Data from transgenic plant DNA samples within a single PCR run and a single PCR

20 Master Mix will not be acceptable unless:

- the positive control shows the expected PCR products (transgenic target amplification)
- the wild-type-positive DNA control shows the expected result (wild-type target amplification).
- the negative control is negative for PCR amplification (no fragments).

25

Lanes showing visible amounts of the transgenic PCR product of the expected size and not showing visible amounts of the wild type PCR product, indicate that the corresponding plant from which the genomic DNA template was prepared, is homozygous for the transgenic gene cassette.

30

Lanes showing visible amounts of the transgenic and wild type PCR products of the expected sizes, indicate that the corresponding plant from which the genomic template DNA was prepared, is hemizygous for the transgenic gene cassette. Lanes not showing visible amounts of the transgenic PCR product and showing visible amounts of the wild type PCR product, indicate  
5 that the corresponding plant from which the genomic template DNA was prepared, has not inherited the transgenic sequence assayed for and is thus homozygous for the wild type locus.

Lanes not showing visible amounts of transgenic and wild type PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated.  
10 These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

#### **4.8. Use of the zygosity scoring protocol for identification of zygosity status in EE-GM3 containing plants**

15 Figure 3 illustrates the result obtained with the zygosity scoring PCR for EE-GM3 on a number of soybean plant samples. The samples in lanes 2 and 5 were found to contain only the PCR fragment (706 bp) characteristic for elite event EE-GM3, while the samples in lanes 4, 6 and 7 contained only the fragment characteristic for the presence of the wt locus. Lanes 3, 8 and 9  
20 contained both fragments. Lanes 2 and 5 therefore contain EE-GM3 in homozygous form, lanes 3, 8 and 9 contain EE-GM3 in hemizygous form and lanes 4, 6 and 7 contain the wt locus in homozygous form (azygous for EE-GM3). Lane 10 represents the negative control (water) sample, and lanes 1 and 11 represent the Molecular Weight Marker (100 bp ladder).

#### **5. Introgression of EE-GM3 into preferred cultivars**

Elite event EE-GM3 is introduced by repeated back-crossing into commercial soybean cultivars such as but not limited to Soybean Cultivar 7631014 (US2009252860); Soybean Cultivar 7431014 (US2009252859); Soybean Cultivar 7925084 (US2009252858); Soybean Cultivar  
30 7311153 (US2009252857); Soybean Cultivar S070159 (US2009252856); Soybean Cultivar 7535357 (US2009246353); Soybean Cultivar S070160 (US2009246352); Soybean Cultivar

26074414 (US2009249508); Soybean Cultivar 7509171 (US2009249507); Soybean Cultivar  
S070158 (US2009246351); Soybean Cultivar 7511119 (US2009249506); Soybean Cultivar  
7113111 (US2009238945); Soybean cultivar S06-02RM018047 (US7592518); Soybean Cultivar  
7013345 (US2009232957); Soybean Cultivar 7041461 (US2009235376); Soybean Cultivar  
5 7549450 (US2009232956); Soybean Cultivar 7317090 (US2009232955); Soybean Cultivar  
2N2V58015 (US2009226597); Soybean Cultivar 7243182 (US2009226596); Soybean Cultivar  
7143182 (US2009226595); Soybean Cultivar 7043182 (US2009220673); Soybean Cultivar  
S070157 (US2009222950); Soybean Cultivar 306924721 (US2009220672); Soybean Cultivar  
7614385 (US2009220671); Soybean Cultivar 7925118 (US2009214750); Soybean Cultivar  
10 7821295 (US2009214749); Soybean Cultivar 7811336 (US2009214748); Soybean Cultivar  
S070150 (US2009214747); Soybean Cultivar 6214260 (US2009214746); Soybean Cultivar  
S070152 (US2009214745); Soybean Cultivar 7429331 (US2009214751); Soybean Cultivar  
26034631 (US2009208634); Soybean cultivar S07-03JR108674 (US7560621); Soybean cultivar  
S07-03KL016279 (US7560620); Soybean cultivar S06-CL959411 (US7554017); SOYBEAN  
15 CULTIVAR SG3870NRR (US2009158453); SOYBEAN CULTIVAR HFPR-G (CA2645702);  
Soybean cultivar S06-02JR423016 (US7521606); Soybean cultivar S06-01JR119814  
(US7518039); Soybean cultivar S06-01JR119448 (US7518038); Soybean Cultivar 6540220  
(US2009055960); Soybean Cultivar S060292 (US2009055959); Soybean Cultivar S050228  
(US2009055958); Soybean cultivar S06-02JR423003 (US7491873); Soybean cultivar S06-  
20 02JR423005 (US7491872); Soybean cultivar S06-02JR409114 (US7485782); Soybean cultivar  
S06-SJ144056 (US7473823); Soybean cultivar (US7470835); Soybean cultivar 6910450  
(US2008282369); SOYBEAN CULTIVAR 6223012 (US7446246); SOYBEAN CULTIVAR  
6549250 (US7446245); Soybean Cultivar 17731225 (US2008271204); Soybean Cultivar  
6928285 (US2008271203); Soybean Cultivar 6736054 (US2008271169); Soybean Cultivar  
25 S060299 (US2008271199); Soybean Cultivar S060294 (US2008271202); Soybean Cultivar  
6943322 (US2008271201); Soybean cultivar 5343260 (US2008263719); Soybean cultivar  
6439359 (US2008263704); Soybean cultivar 6238359 (US2008263703); Soybean cultivar  
6547272 (US2008263702); Soybean cultivar 6929431 (US2008263701); Soybean cultivar  
6703392 (US2008263700); Soybean cultivar 6044483 (US2008263699); Soybean cultivar  
30 S050224 (US2008263698); Soybean cultivar 6719022 (US2008263697); Soybean cultivar  
5826056 (US2008263696); Soybean cultivar 6265047 (US2008263724); Soybean cultivar

6928331 (US2008263695); Soybean cultivar 6719331 (US2008263694); Soybean cultivar  
6636454 (US2008263693); Soybean cultivar 6226454 (US2008263718); Soybean cultivar  
Q0073801 (US2008256657); SOYBEAN CULTIVAR 6326393 (US2008256652); SOYBEAN  
CULTIVAR 6408448 (US2008256651); Soybean cultivar 6449315 (US2008250524); Soybean  
5 cultivar S060296 (US2008250523); Soybean cultivar 6012078 (US2008250522); Soybean  
cultivar 6342078 (US2008250521); Soybean cultivar 6419156 (US2008250520); Soybean  
cultivar 5723264 (US2008250519); Soybean cultivar S050225 (US2008250518); Soybean  
cultivar S060298 (US2008244783); Soybean cultivar 6935331 (US2008244782); Soybean  
cultivar 6819456 (US2008244787); Soybean cultivar S060297 (US2008244781); Soybean  
10 cultivar 6135319 (US2008244786); Soybean cultivar 6819331 (US2008244780); Soybean  
cultivar 6137445 (US2008244779); Soybean cultivar 6917445 (US2008244778); Soybean  
cultivar 6111333 (US2008244777); Soybean Cultivar S050229 (US2008244776); Soybean  
Cultivar 6114011 (US2008244775); Soybean Cultivar 6900358 (US2008244784); Soybean  
Cultivar 6345184 (US2008244774); Soybean Cultivar 6836085 (US2008244773); Soybean  
15 Cultivar 6635047 (US2008244772); Soybean Cultivar 6139105 (US2008244771); SOYBEAN  
CULTIVAR 6434385 (US2008244770); SOYBEAN CULTIVAR S060295 (US2008244769);  
Soybean Cultivar 6035184 (US2008244768); Soybean Cultivar S060293 (US2008209590);  
Soybean Cultivar 6733322 (US2008209594); SOYBEAN CULTIVAR 6421326  
(US2008209593); Soybean Cultivar S060077 (US2008209589); SOYBEAN CULTIVAR  
20 6600375 (US2008209592); Soybean cultivar S06-CL821457 (US7420104); Soybean cultivar  
S07-02KG294306 (US7414178); Soybean cultivar S06-BA046119 (US7414175); Soybean  
cultivar S07-02KG294307 (US7411114); Soybean Cultivar SG3865N (US2008189802);  
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5 (US6143954); Soybean variety 93B53 (US6335197).

It is observed that the introgression of the elite event into these cultivars does not significantly influence any of the desirable phenotypic or agronomic characteristics of these cultivars (no yield drag) while expression of the transgene, as determined by glyphosate and/or isoxaflutole  
10 tolerance, meets commercially acceptable levels. This confirms the status of event EE-GM3 as an elite event.

Elite event EE-GM3 may be advantageously combined with one or more of other soybean events available in the market, including but not limited to other herbicide tolerance events, such as  
15 events described in USDA-APHIS petitions: 09-349-01p, 09-201-01p, 09-183-01p, 09-082-01p, 09-015-01p, 06-354-01p, 06-271-01p, 06-178-01p, 98-238-01p, 98-014-01p, 97-008-01p, 96-068-01p, 95-335-01p, 93-258-01p (see, e.g., [www.aphis.usda.gov/brs/not\\_reg.html](http://www.aphis.usda.gov/brs/not_reg.html)), or event MON89788 (Glyphosate tolerance) described in US 2006-282915, event DP-305423-1 (High oleic acid / ALS inhibitor tolerance) described in WO 2008/054747, events A2704-12 and  
20 A5547-127 (Glufosinate tolerance) described respectively in WO 2006/108674 or WO 2006/108675, MON87701 described in US2009130071, event 3560.4.3.5 described in US2009036308, event DP-305423-1 described in US2008312082, or event BPS-CV127-9 (Event 127) of WO 2010/080829.

25 As used in the claims below, unless otherwise clearly indicated, the term "plant" is intended to encompass plant tissues, at any stage of maturity, as well as any cells, tissues, or organs taken from or derived from any such plant, including without limitation, any seeds, leaves, stems, flowers, roots, single cells, gametes, cell cultures, tissue cultures or protoplasts.

30 Reference seed comprising elite event EE-GM3 was deposited as 32-RRMM-0531 at the NCIMB (Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB9YA, Scotland) on

October 12, 2009, under NCIMB accession number NCIMB 41659, and the viability thereof was confirmed. Alternative names for EE-GM3 are event FG-072, or event MST-FGØ72-3.

The above description of the invention is intended to be illustrative and not limiting.

5

Various changes or modifications in the embodiments described may occur to those skilled in the art. These can be made without departing from the spirit or scope of the invention.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

**Claims:**

1. A method for identifying elite event EE-GM3 in biological samples, which method comprises detection of an EE-GM3 specific region with a specific primer pair or probe which specifically recognizes the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3 and the inserted foreign DNA contiguous therewith, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451, said 3' flanking region comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, said inserted foreign DNA comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843, the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, the nucleotide sequence of SEQ ID No. 1, or comprising the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or its complement.
  
2. A primer pair suitable for use in an EE-GM3 specific detection, comprising a first primer having a sequence which, under optimized detection conditions specifically recognizes a sequence within the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451 and said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, and a second primer having a sequence which, under optimized detection conditions specifically recognizes a sequence within the inserted foreign DNA contiguous with the 5' or 3' flanking region of said foreign DNA, said inserted foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or its complement, wherein
  - a) at least one of said primers is labeled,

- b) the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence,
- c) at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides 240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos 2 or 3, or
- d) at least one of said primers comprises a sequence which is at least 80 and less than 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, provided the mismatches still allow specific identification of the elite event with these primers under optimized PCR conditions.
3. The primer pair of claim 2, wherein said first primer comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451 or a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, and said second primer comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or its complement, wherein
- a) at least one of said primers is labeled, or
- b) the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.

4. The primer pair of claim 2, wherein said first primer comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451 or a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, and said second primer comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240 or the nucleotide sequence of SEQ ID No 1 or its complement, or the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or its complement, wherein
  - a) at least one of said primers is labeled,
  - b) the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.
  
5. A primer pair comprising a first primer comprising at its extreme 3' end the sequence of SEQ ID No. 5 and a second primer comprising at its extreme 3' end the sequence of SEQ ID No. 4 or 7, wherein
  - a) at least one of said primers is labeled, or
  - b) the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.
  
6. The method of claim 1, said method comprising amplifying a DNA fragment of between 50 and 1000 bp from a nucleic acid present in said biological samples using a polymerase chain reaction with the primers of any one of claims 2 to 5.

7. A kit for identifying elite event EE-GM3 in biological samples, said kit comprising the primers of any one of claims 2-5.
8. A specific probe for the identification of elite event EE-GM3 in biological samples,, which comprises a nucleotide sequence having at least 80% sequence identity with a sequence comprising part of the 5' or 3' flanking sequence of EE-GM3 and part of the sequence of the foreign DNA contiguous therewith, or the complement thereof, wherein said 5' flanking sequence comprises the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451, and the foreign DNA contiguous therewith comprises the nucleotide sequence of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843, and wherein said 3' flanking sequence comprises the nucleotide sequence of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and the foreign DNA contiguous therewith comprises the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240.
9. The probe of claim 8 which comprises the nucleotide sequence of SEQ ID No. 2 from nucleotide 1441 to 1462 or SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences.
10. A specific probe for the identification of elite event EE-GM3 in biological samples comprising a nucleotide sequence with at least 90 % sequence identity to SEQ ID No. 2 from nucleotide 1441 to 1462 or SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences.
11. The method of claim 1, which method comprises hybridizing a nucleic acid of biological samples with the probe of any one of claims 8 to 10.
12. A kit comprising the probe of any one of claims 8 to 10.
13. The method of any one of claims 1, 6, or 11, for confirming seed purity, or for screening seeds for the presence of EE-GM3.

14. A method of detecting the presence of elite event EE-GM3 in biological samples through hybridization with a substantially complementary labeled nucleic acid probe in which the probe:target nucleic acid ratio is amplified through recycling of the target nucleic acid sequence, said method comprising:
  - a) hybridizing said target nucleic acid sequence to a first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1452 to nucleotide 1469 or its complement or said first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 223 to nucleotide 240 or its complement;
  - b) hybridizing said target nucleic acid sequence to a second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1434 to nucleotide 1451 or its complement or said second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 241 to nucleotide 258 or its complement, wherein said first and second oligonucleotide overlap by at least one nucleotide and wherein either said first or said second oligonucleotide is labeled to be said labeled nucleic acid probe;
  - c) cleaving only the labeled probe within the probe:target nucleic acid sequence duplex with an enzyme which causes selective probe cleavage resulting in duplex disassociation, leaving the target sequence intact;
  - d) recycling of the target nucleic acid sequence by repeating steps (a) to (c); and
  - e) detecting cleaved labeled probe, thereby determining the presence of said target nucleic acid sequence.
  
15. A transgenic soybean plant, or cells, parts, seed or progeny thereof, each comprising elite event EE-GM3 in its genome, reference seed comprising said event having being deposited at the NCIMB under deposit number NCIMB 41659.
  
16. The transgenic soybean plant, seed, cells, parts or progeny of claim 15, the genomic DNA of which, when analyzed using the Elite event identification protocol for

EE-GM3 with two primers comprising the nucleotide sequence of SEQ ID 4 and SEQ ID 5 respectively, yields a DNA fragment of about 263 bp.

17. A soybean plant, plant part, cell or tissue, or seed comprising elite event EE-GM3 obtainable from the seed deposited at the NCIMB under deposit number NCIMB 41659 or derivatives therefrom.
18. A soybean plant, or seed, cells or tissues thereof, each comprising elite event EE-GM3 in its genome, obtainable by propagation of and/or breeding with a soybean plant grown from the seed deposited at the NCIMB under deposit number NCIMB 41659.
19. A method for producing a soybean plant or seed comprising elite event EE-GM3 comprising crossing a plant according to any one of claims 15 to 18 with another soybean plant, and planting the seed obtained from said cross.
20. Soybean genomic DNA comprising elite event EE-GM3.
21. A nucleic acid molecule comprising a nucleotide sequence with at least 90 % sequence identity to the sequence of SEQ ID No. 2 from nucleotide 1441 to nucleotide 1462 or SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences.
22. The nucleic acid molecule of claim 21 comprising a nucleotide sequence with at least 90 % sequence identity to the sequence of SEQ ID No. 2 from nucleotide 1431 to nucleotide 1472 or SEQ ID No. 3 from nucleotide 220 to 261, or the complement of said sequences.
23. A transgenic soybean plant, plant cell, tissue, or seed, comprising in its genome event EE-GM3 comprising a nucleic acid molecule comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1441 to nucleotide 1462 and the

- nucleotide sequence of SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences.
24. A soybean plant, plant cell, tissue, or seed, comprising in its genome the nucleotide sequence of SEQ ID No. 11 or the complement thereof.
  25. A nucleic acid molecule comprising a nucleotide sequence with at least 99% sequence identity to the nucleotide sequence of SEQ ID No. 11 from nucleotide position 1452 to nucleotide position 16638 or the complement thereof, or a nucleotide sequence with at least 99% sequence identity to SEQ ID No. 11 or the complement thereof.
  26. A nucleic acid molecule comprising the nucleotide sequence of SEQ ID No. 11 or the complement thereof.
  27. Use of the seed of any one of claims 15 to 18, 23 or 24 to obtain a soybean product.
  28. The use of claim 27, wherein said soybean product is soybean meal, ground soybean seeds, soybean flour or soybean flakes.
  29. A soybean product produced from the seed of any one of claims 15 to 18, 23 or 24, wherein said soybean product is soybean meal, ground soybean seeds, soybean flour or soybean flakes, and comprises nucleic acids specific for elite event EE-GM3.
  30. A process for protecting emerging soybean plants from competition by weeds, comprising treating a field in which seeds of any one of claims 15 to 18, 23 or 24 were sown with an HPPD inhibitor herbicide, such as isoxaflutole, before the soybean plants emerge but after the seeds are sown, optionally followed by application of glyphosate or an HPPD inhibitor-glyphosate mixture as post-emergent herbicide over the top of the plants.

31. A method for protecting emerging soybean plants from competition by weeds, comprising treating a field to be planted or sown with soybean plants or seeds of any one of claims 15 to 18, 23 or 24 with an HPPD inhibitor herbicide, before the soybean plants are planted or the seeds are sown, followed by planting or sowing of said soybean plants or seeds in said pre-treated field, optionally wherein glyphosate, or an HPPD inhibitor-glyphosate mixture, is applied as post-emergent herbicide over the top of the plants.
32. A method for controlling weeds in a field of soybean plants of any one of claims 15 to 18, 23 or 24, comprising treating said field with an effective amount of an HPPD inhibitor herbicide, wherein such plants are tolerant to such herbicide.
33. The method of claim 32, wherein the treatment with an HPPD inhibitor herbicide is applied together with, followed by or preceded by a treatment with glyphosate as post-emergent herbicide.
34. A method for producing a soybean product, comprising obtaining soybean seed of any one of claims 15 to 18, 23 or 24, and producing such soybean product therefrom.
35. A method for treating soybean plants so as to control weeds in a field of soybean plants comprising treating soybean plants of any one of claims 15 to 18, 23 or 24, with an effective amount of an isoxaflutole-based and glyphosate-based herbicide, wherein such plants are tolerant to such herbicides.

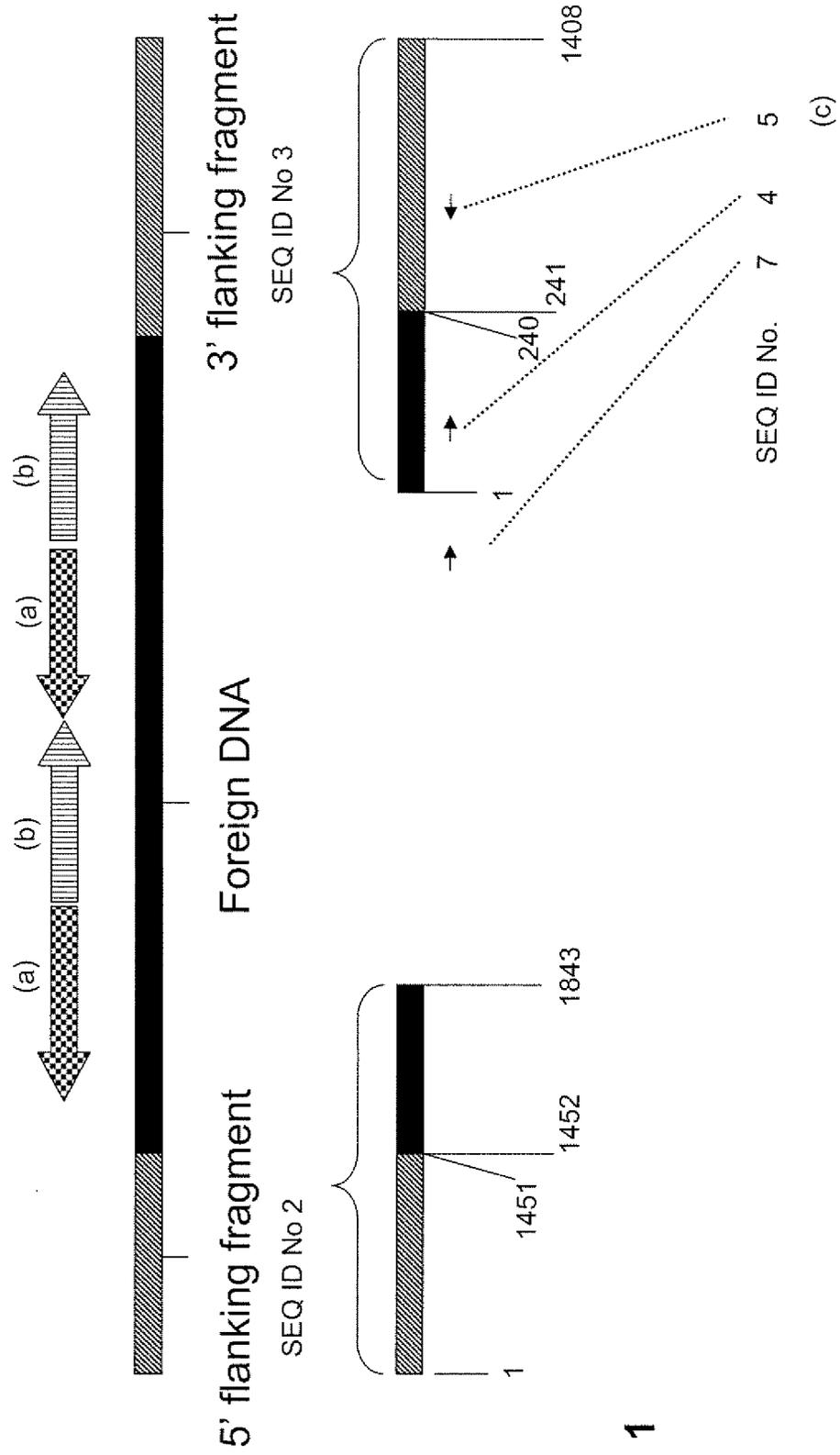


Fig. 1

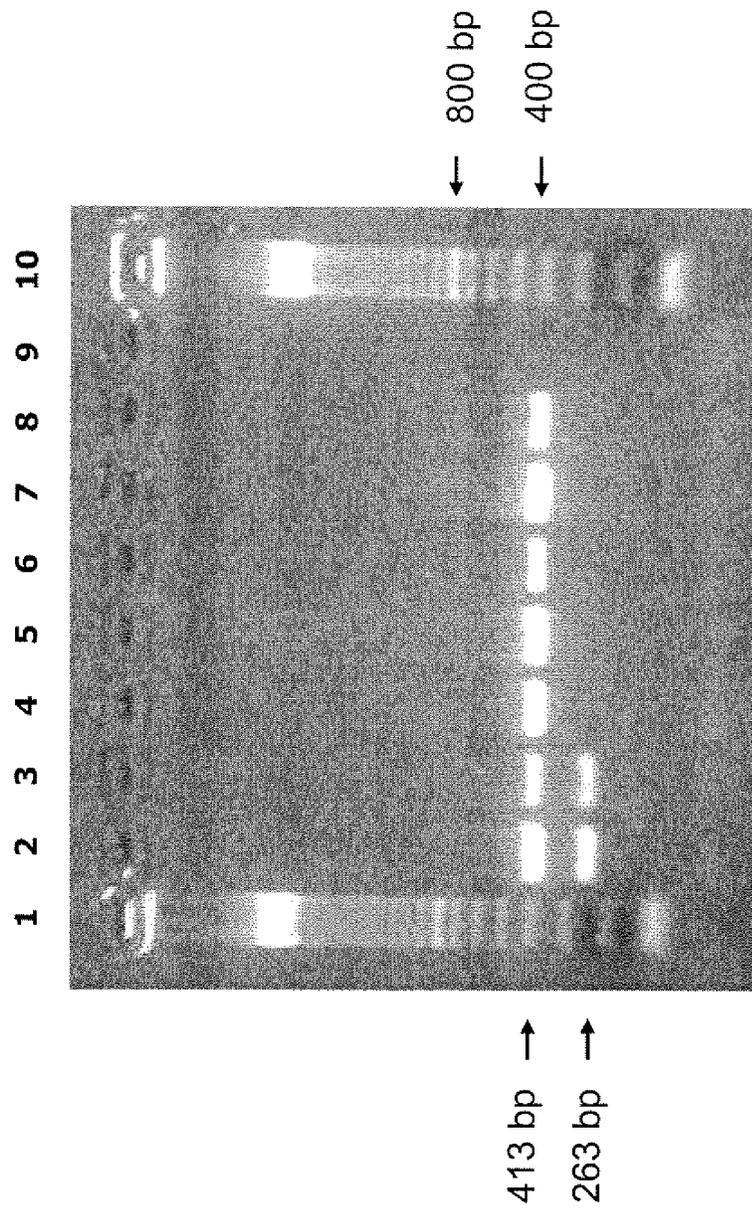


Fig. 2

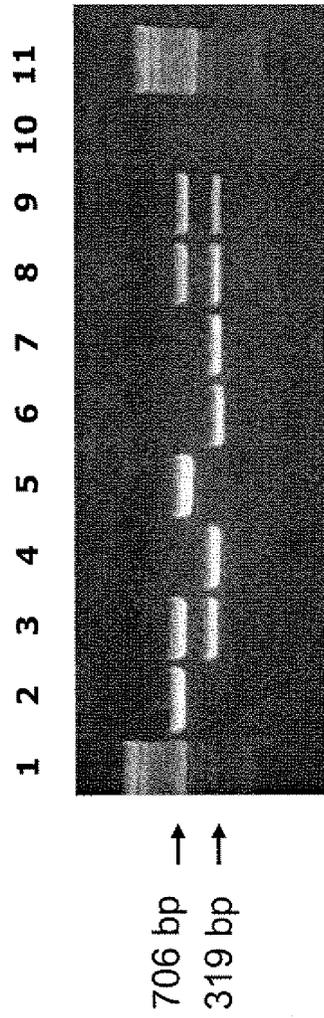


Fig. 3

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WELZ Gunter  
VERHAEGHE Steven  
DE BEUCKELEER Marc  
HABEX Veerle  
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 <223> plant DNA

<220>  
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 in event EE-GM3

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