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(54) Title: SOYBEAN EVENT 3560.4.3.5 AND COMPOSITIONS AND METHODS FOR THE IDENTIFICATION AND/OR DETECTION THEREOF

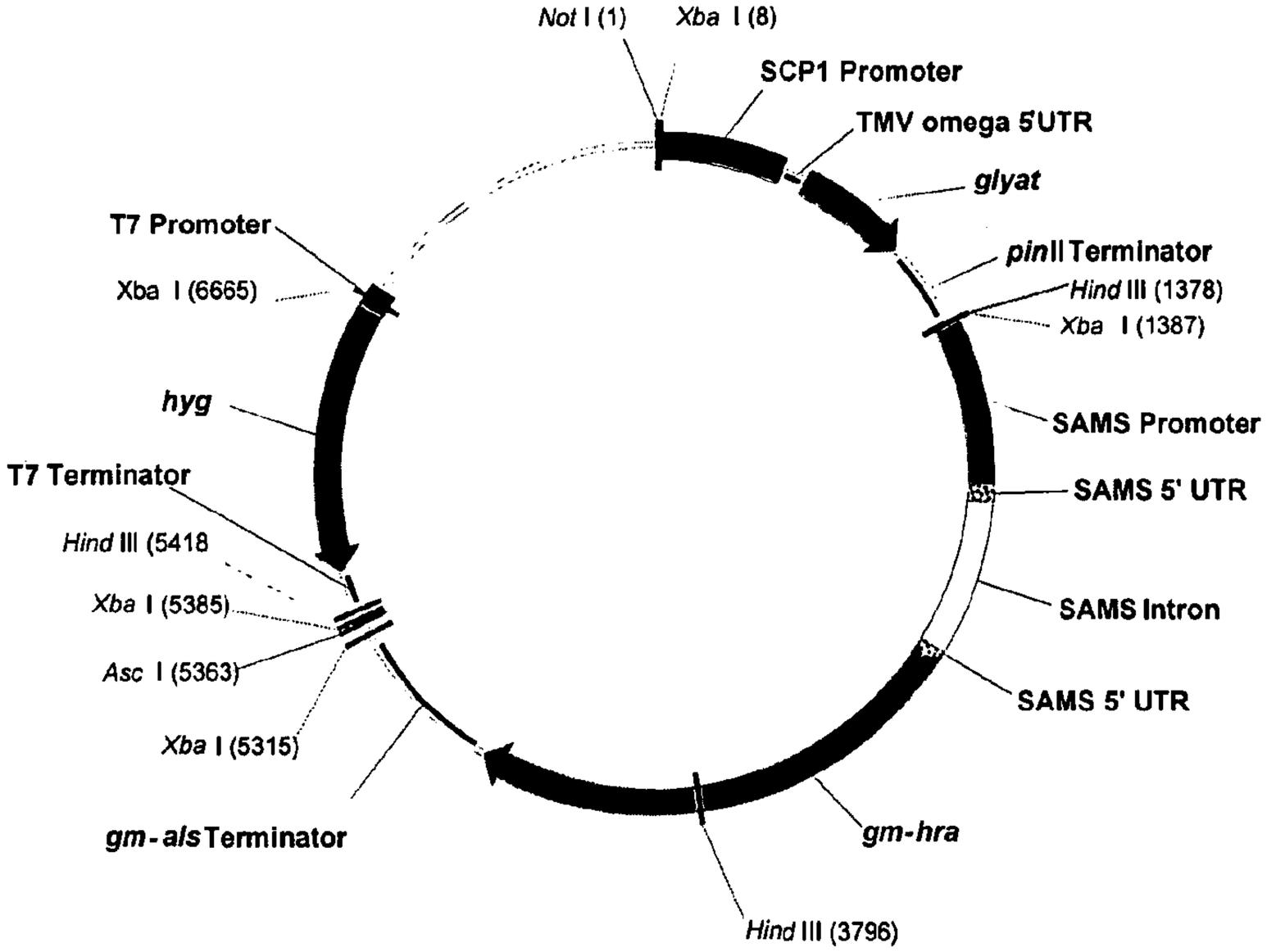


FIGURE 1

(57) Abrégé/Abstract:
Compositions and methods related to transgenic glyphosate/ALS inhibitor-tolerant soybean plants are provided. Specifically, soybean plants having a 3560.4.3.5 event which imparts tolerance to glyphosate and at least one ALS-inhibiting herbicide are

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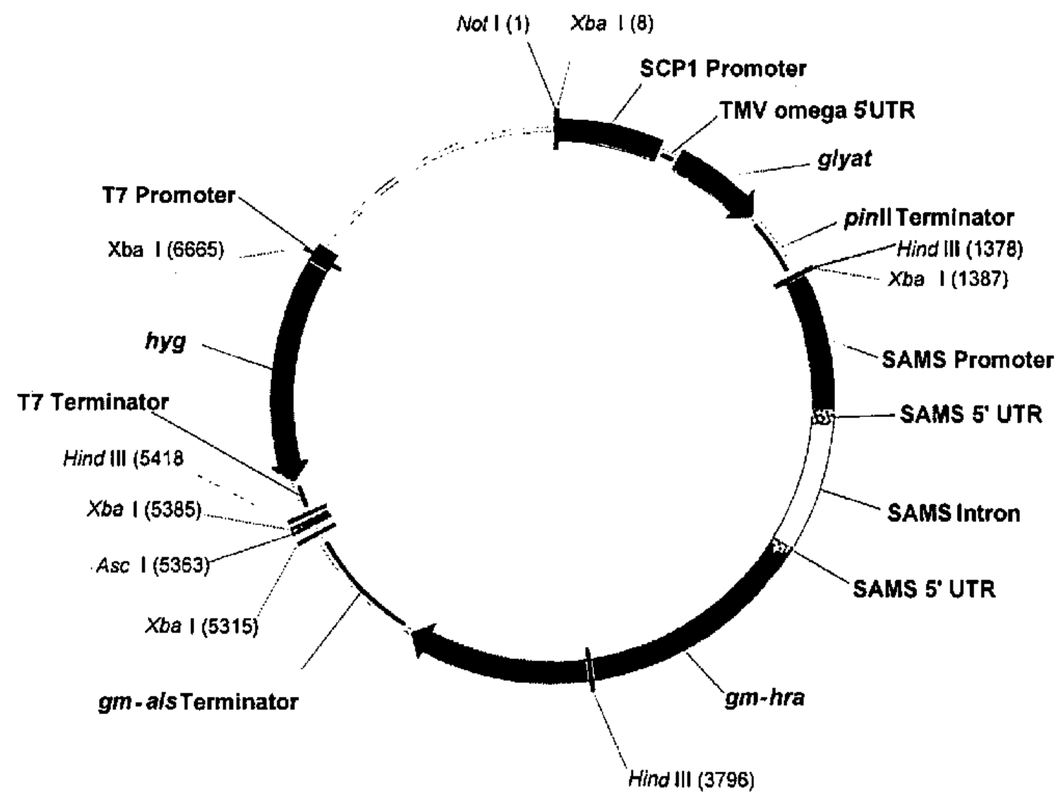


FIGURE 1

(57) Abstract: Compositions and methods related to transgenic glyphosate/ALS inhibitor-tolerant soybean plants are provided. Specifically, soybean plants having a 3560.4.3.5 event which imparts tolerance to glyphosate and at least one ALS-inhibiting herbicide are provided. The soybean plant harboring the 3560.4.3.5 event at the recited chromosomal location comprises genomic/transgene junctions having at least the polynucleotide sequence of SEQ ID NO: 10 and/or 11. The characterization of the genomic insertion site of the 3560.4.3.5 event provides for an enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof. Various methods and compositions for the identification, detection, and use of the soybean 3560.4.3.5 events are provided.

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SOYBEAN EVENT 3560.4.3.5 AND COMPOSITIONS AND METHODS FOR THE IDENTIFICATION AND/OR DETECTION THEREOF

FIELD OF THE INVENTION

This invention is in the field of molecular biology. More specifically, this invention pertains to multiple herbicide tolerances conferred by expression of a sequence that confers tolerance to glyphosate in conjunction with the expression of sequence that confers tolerance to one or more ALS inhibitor chemistries.

BACKGROUND OF THE INVENTION

The expression of foreign genes in plants is known to be influenced by their location in the plant genome, perhaps due to chromatin structure (e.g., heterochromatin) or the proximity of transcriptional regulatory elements (e.g., enhancers) close to the integration site (Weising *et al.* (1988) *Ann. Rev. Genet* 22: 421-477). At the same time the presence of the transgene at different locations in the genome influences the overall phenotype of the plant in different ways. For this reason, it is often necessary to screen a large number of events in order to identify an event characterized by optimal expression of an introduced gene of interest. For example, it has been observed in plants and in other organisms that there may be a wide variation in levels of expression of an introduced gene among events. There may also be differences in spatial or temporal patterns of expression, for example, differences in the relative expression of a transgene in various plant tissues, that may not correspond to the patterns expected from transcriptional regulatory elements present in the introduced gene construct. It is also observed that the transgene insertion can affect the endogenous gene expression. For these reasons, it is common to produce hundreds to thousands of different events and screen those events for a single event that has desired transgene expression levels and patterns for commercial purposes. An event that has desired levels or patterns of transgene expression is

useful for introgressing the transgene into other genetic backgrounds by sexual outcrossing using conventional breeding methods. Progeny of such crosses maintain the transgene expression characteristics of the original transformant. This strategy is used to ensure reliable gene expression in a number of varieties that are well adapted to local growing conditions.

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

In the commercial production of crops, it is desirable to easily and quickly eliminate unwanted plants (*i.e.*, "weeds") from a field of crop plants. An ideal treatment would be one which could be applied to an entire field but which would eliminate only the unwanted plants while leaving the crop plants unharmed. One such treatment system would involve the use of crop plants which are tolerant to a herbicide so that when the herbicide was sprayed on a field of herbicide-tolerant crop plants, the crop plants would continue to thrive while non-herbicide-tolerant weeds were killed or severely damaged. Ideally, such treatment systems would take advantage of varying herbicide properties so that weed control could provide the best possible combination of flexibility and economy. For example, individual herbicides have different longevities in the field, and some herbicides persist and are effective for a relatively long time after they are applied to a field while other herbicides are quickly broken down into other and/or non-active compounds. An ideal treatment system would allow the use of different herbicides so that growers could tailor the choice of herbicides for a particular situation.

Due to local and regional variation in dominant weed species as well as preferred crop species, a continuing need exists for customized systems of crop protection and weed management which can be adapted to the needs of a particular region, geography, and/or locality. Methods and compositions that allow for the rapid identification of events in plants that produce such qualities are needed. For example, a continuing need exists for methods of crop protection and weed management which

can reduce: the number of herbicide applications necessary to control weeds in a field; the amount of herbicide necessary to control weeds in a field; the amount of tilling necessary to produce a crop; and/or programs which delay or prevent the development and/or appearance of herbicide-resistant weeds. A continuing need exists for methods and compositions of crop protection and weed management which allow the targeted use of particular herbicide combinations and for the efficient detection of such an event.

BRIEF SUMMARY OF THE INVENTION

Compositions and methods related to transgenic glyphosate/ALS inhibitor-tolerant soybean plants are provided. Compositions comprise soybean plants containing a 3560.4.3.5 event which imparts tolerance to glyphosate and at least one ALS-inhibiting herbicide. The soybean plant harboring the 3560.4.3.5 event at the recited chromosomal location comprises genomic/transgene junctions having at least the polynucleotide sequence of SEQ ID NO:10 and/or 11. Further provided are the seeds deposited as Patent Deposit No. PTA-8287 and plants, plant cells, plant parts, grain and plant products derived therefrom. The characterization of the genomic insertion site of event 3560.4.3.5 provides for an enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof. Various methods and compositions for the identification, detection, and use of the soybean event 3560.4.3.5 are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a schematic map of PHP20163 indicating plasmid elements and restriction enzyme sites for *Not* I, *Asc* I, *Xba* I and *Hind* III. *Not* I and *Asc* I were used for isolation of fragment PHP20163A (Figure 2).

Figure 2 provides a schematic map of fragment PHP20163A indicating restriction enzyme sites for *Xba* I, *Hind* III and various genetic elements. This fragment was used for microprojectile bombardment to produce the 3560.4.3.5 soybean event. Probes used for Southern hybridization are indicated as boxes beneath the fragment map and are identified as follows: A: *glyat4601* (*glyphosate acetyltransferase)* probe B: *gm-hra* probe (two non-overlapping segments were generated covering the entire region and used together for hybridization.

Figure 3 provides a schematic map of the insertion region in soybean event 3560.4.3.5 and shows the three regions that were sequenced from PCR products generated using genomic DNA as template: inserted DNA from the bombarded PHP20163A DNA fragment, 5' flanking soybean genomic DNA and 3' flanking soybean genomic DNA.

Figure 4A-E provides a complete sequence of DNA insert and flanking genomic border regions in the 3560.4.3.5 soybean event (SEQ ID NO:6). The 5' and 3' flanking genomic border regions, bp 1 to 3317 and bp 8680 to 10849, respectively, are underlined.

Figure 5 provides a breeding diagram for 3560.4.3.5 soybean.

Figure 6 provides a schematic map of fragment PHP20163A indicating location of the genetic elements contained in the two gene expression cassettes and base pair positions for Bgl II and Xba I restriction enzyme sites. The *Not I* and *Asc I* restriction enzyme sites are lost upon excision of this fragment from PHP20163. The total fragment size is 5362 base pairs. Approximate locations of the probes used are shown as numbered boxes below the fragment and are identified below. Additional details on these probes are provided in Table 3.

Figure 7 provides a schematic plasmid map of PHP20163 indicating the location of genetic elements and base pair positions for restriction enzyme sites for *Not I, Bgl II*, *Xba I*, and *Asc I*. The *Not I-Asc I* fragment of this plasmid was isolated (PHP20163A; map, Figure 2) and used for transformation to produce 3560.4.3.5 soybean. The *Xba I* site located at bp 1387 contains a Dam methylase recognition site and is resistant to digestion by *Xba* I if the plasmid is prepared from a Dam^+ strain. The total plasmid size is 7954 base pairs. Backbone probes are indicated schematically as lines within the plasmid diagram and are identified below. Each probe is comprised of two non-overlapping segments that were combined for hybridization.

Figure 8 provides a schematic map of the transgene insertion in 3560.4.3.5 soybean based on Southern blot analysis. The flanking soybean genome is represented by the horizontal dotted line. A single, intact copy of the PHP20163A fragment integrated into the soybean genome. *Bgl* II and *Xba* I restriction enzyme sites are indicated with the sizes of observed fragments on Southern blots shown below the map in base pairs (bp).

Figure 9 provides a map of the 3560.4.3.5 event and depicts where various primers anneal.

Figure 10A-I provides the left genomic border/internal transgene insert/right genomic border of the 3560.4.3.5 event (SEQ ID NO:6). The regions where various primers anneal are illustrated.

DETAILED DESCRIPTION OF THE INVENTION

The present inventions now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the inventions are shown. Indeed, these inventions may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which these inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

Compositions and methods related to transgenic glyphosate/ALS inhibitor-tolerant soybean plants are provided. Compositions include soybean plants having event 3560.4.3.5. A soybean plant having "event 3560.4.3.5" has been modified by the insertion of the glyphosate acetyltransferase (*glyat*) gene derived from *Bacillus licheniformis* and a modified version of the soybean acetolactate synthase gene (*gm-hra*). The *glyat* gene was functionally improved by a gene shuffling process to optimize the kinetics of glyphosate acetyltransferase (GLYAT) activity for acetylating the herbicide glyphosate. The insertion of the *glyat* gene in the plant confers tolerance to the herbicidal active ingredient glyphosate through the conversion of glyphosate to the non-toxic acetylated form. The insertion of the *gm-hra* gene produces a modified form of the acetolactate synthase (ALS) enzyme. ALS is essential for branched chain amino acid biosynthesis and is inhibited by certain

herbicides. The modification in the *gm-hra* gene overcomes this inhibition and thus provides tolerance to a wide range of ALS-inhibiting herbicides. Thus, a soybean plant having an event 3560.4.3.5 is tolerant to glyphosate and at least one ALS-inhibiting herbicide. The soybean event 3560.4.3.5 is otherwise known as Event DP-356Ø43-5 or 356043 soybean.

The polynucleotides conferring the glyphosate and ALS inhibitor tolerance are linked on the same DNA construct and are inserted at a characterized position in the soybean genome and thereby produce the 3560.4.3.5 soybean event. The soybean plant harboring the 3560.4.3.5 event at the recited chromosomal location comprises genomic/transgene junctions having at least the polynucleotide sequence of SEQ ID NO:10 and/or 11. The characterization of the genomic insertion site of the 3560.4.3.5 event provides for an enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof. Various methods and compositions for the identification, detection, and use of the soybean 3560.4.3.5 events are provided herein. As used herein, the term "event 3560.4.3.5 specific" refers to a polynucleotide sequence which is suitable for discriminatively identifying event 3560.4.3.5 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.

Compositions further include seed deposited as Patent Deposit Nos. PTA-8287 and plants, plant cells, and seed derived therefrom. Applicant(s) have made a deposit of at least 2500 seeds of soybean event 3560.4.3.5 with the American Type Culture Collection (ATCC), Manassas, VA 20110-2209 USA, on March 27, 2007, and the deposits were assigned ATCC Deposit No. PTA-8287. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112.The seeds deposited with the ATCC on March 26, 2007 were taken from the deposit maintained by Pioneer Hi-Bred International, Inc., 7250 NW 62nd Avenue, Johnston, Iowa 50131-1000. Access to this deposit will be available during the pendency of the application to the Commissioner of Patents and Trademarks and persons determined by the Commissioner to be entitled thereto upon request. Upon allowance of any claims in the application, the Applicant(s) will make available to the public, pursuant to 37

C.F.R. § 1.808, sample(s) of the deposit of at least 2500 seeds of soybean event 3560.4.3.5 with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209. This deposit of seed of soybean event 3560.4.3.5 will be maintained in the ATCC depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the enforceable life of the patent, whichever is longer, and will be replaced if it becomes nonviable during that period. Additionally, Applicant(s) have satisfied all the requirements of 37 C.F.R. §§1.801 - 1.809, including providing an indication of the viability of the sample upon deposit. Applicant(s) have no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicant(s) do not waive any infringement of their rights granted under this patent or rights applicable to event 3560.4.3.5 under the Plant Variety Protection Act (7 USC 2321 et seq.). Unauthorized seed multiplication prohibited. The seed may be regulated.

As used herein, the term "soybean" means *Glycine max* and includes all plant varieties that can be bred with soybean. As used herein, the term plant includes plant cells, plant organs, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit, stalks, roots, root tips, anthers, and the like. Grain is intended to mean the mature seed produced by commercial growers for purposes other than growing or reproducing the species. Progeny, variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise a 3650.4.3.5 event.

A transgenic "event" is produced by transformation of plant cells with a heterologous DNA construct(s), including a nucleic acid expression cassette that comprises a transgene of interest, the regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and selection of a particular plant characterized by insertion into a particular genome location. An event is characterized phenotypically by the expression of the transgene(s). At the genetic level, an event is part of the genetic makeup of a plant. The term "event" also refers to progeny produced by a sexual outcross between the transformant and another variety that include the heterologous DNA. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed parent is

present in the progeny of the cross at the same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA and flanking sequence immediately adjacent to the inserted DNA that would be expected to be transferred to a progeny that receives inserted DNA including the transgene of interest as the result of a sexual cross of one parental line that includes the inserted DNA (e.g., the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA.

As used herein, "insert DNA" refers to the heterologous DNA within the expression cassettes used to transform the plant material while "flanking DNA" can comprise either genomic DNA naturally present in an organism such as a plant, or foreign (heterologous) DNA introduced via the transformation process which is extraneous to the original insert DNA molecule, e.g. fragments associated with the transformation event. A "flanking region" or "flanking sequence" as used herein refers to a sequence of at least 20, 50, 100, 200, 300, 400, 1000, 1500, 2000, 2500, or 5000 base pair or greater which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the original foreign insert DNA molecule. Non-limiting examples of the flanking regions of the 3560.4.3.5 event are set forth in SEQ ID NO:4 and 5 and variants and fragments thereof.

Transformation procedures leading to random integration of the foreign DNA will result in transformants containing different flanking regions characteristic of and unique for each transformant. When recombinant DNA is introduced into a plant through traditional crossing, its flanking regions will generally not be changed. Transformants will also contain unique junctions between a piece of heterologous insert DNA and genomic DNA, or two pieces of genomic DNA, or two pieces of heterologous DNA. A "junction" is a point where two specific DNA fragments join. For example, a junction exists where insert DNA joins flanking DNA. A junction point also exists in a transformed organism where two DNA fragments join together in a manner that is modified from that found in the native organism. As used herein, "junction DNA" refers to DNA that comprises a junction point. Non-limiting examples of junction DNA from the 3560.4.3.5 event set are forth in SEQ ID NO:1, 2, 6, 10, 11, 12, 13, 14, 15, 27, 28, 41, 42 or variants and fragments thereof.

A 3560.4.3.5 plant can be bred by first sexually crossing a first parental soybean plant grown from the transgenic 3560.4.3.5 soybean plant (or progeny

thereof derived from transformation with the expression cassettes of the embodiments that confer herbicide tolerance) and a second parental soybean plant that lacks the herbicide tolerance phenotype, thereby producing a plurality of first progeny plants; and then selecting a first progeny plant that displays the desired herbicide tolerance; and selfing the first progeny plant, thereby producing a plurality of second progeny plants; and then selecting from the second progeny plants which display the desired herbicide tolerance. These steps can further include the back-crossing of the first herbicide tolerant progeny plant or the second herbicide tolerant progeny plant to the second parental soybean plant or a third parental soybean plant, thereby producing a soybean plant that displays the desired herbicide tolerance. It is further recognized that assaying progeny for phenotype is not required. Various methods and compositions, as disclosed elsewhere herein, can be used to detect and/or identify the 3560.4.3.5 event.

It is also to be understood that two different transgenic plants can also be sexually crossed to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several references, e.g., Fehr, in *Breeding Methods for Cultivar Development*, Wilcos J. ed., American Society of Agronomy, Madison Wis. (1987).

The term "germplasm" refers to an individual, a group of individuals, or a clone representing a genotype, variety, species or culture, or the genetic material thereof.

A "line" or "strain" is a group of individuals of identical parentage that are generally inbred to some degree and that are generally isogenic or near isogenic.

Inbred soybean lines are typically developed for use in the production of soybean hybrids and for use as germplasm in breeding populations for the creation of new and distinct inbred soybean lines. Inbred soybean lines are often used as targets for the introgression of novel traits through traditional breeding and/or molecular introgression techniques. Inbred soybean lines need to be highly homogeneous, homozygous and reproducible to be useful as parents of commercial hybrids. Many analytical methods are available to determine the homozygosity and phenotypic stability of inbred lines.

The phrase "hybrid plants" refers to plants which result from a cross between genetically different individuals.

The term "crossed" or "cross" in the context of this invention means the fusion of gametes, e.g., via pollination to produce progeny (i.e., cells, seeds, or plants) in the case of plants. The term encompasses both sexual crosses (the pollination of one plant by another) and, in the case of plants, selfing (self-pollination, i.e., when the pollen and, ovule are from the same plant).

The term "introgression" refers to the transmission of a desired allele of a genetic locus from one genetic background to another. In one method, the desired alleles can be introgressed through a sexual cross between two parents, wherein at least one of one of the parents has the desired allele in its genome.

In some embodiments, the polynucleotide conferring the soybean 3560.4.3.5 event of the invention are engineered into a molecular stack. In other embodiments, the molecular stack further comprises at least one additional polynucleotide that confers tolerance to a 3rd herbicide. In one embodiment, the sequence confers tolerance to glufosinate, and in a specific embodiment, the sequence comprises pat.

In other embodiments, the soybean 3560.4.3.5 event of the invention comprise one or more trait of interest, and in more specific embodiments, the plant is stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired combination of traits. A trait, as used herein, refers to the phenotype derived from a particular sequence or groups of sequences. For example, herbicide-tolerance polynucleotides may be stacked with any other polynucleotides encoding polypeptides having pesticidal and/or insecticidal activity, such as *Bacillus thuringiensis* toxic proteins (described in U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; Geiser *et al.* (1986) *Gene* 48: 109; Lee *et al.* (2003) *Appl. Environ. Microbiol.* 69: 4648-4657 (*Vip3A*); Galitzky *et al.* (2001) *Acta Crystallogr. D. Biol. Crystallogr.* 57: 1101-1109 (*Cry3Bb1*); and Herman *et al.* (2004) *J. Agric. Food Chem.* 52: 2726-2734 (*Cry1F*)), lectins (Van Damme *et al.* (1994) *Plant Mol. Biol.* 24: 825, pentin (described in U.S. Patent No. 5,981,722), and the like. The combinations generated can also include multiple copies of any one of the polynucleotides of interest.

In some embodiments, soybean 3560.4.3.5 event may be stacked with other herbicide-tolerance traits to create a transgenic plant of the invention with further improved properties. Other herbicide-tolerance polynucleotides that could be used in

such embodiments include those conferring tolerance to glyphosate or to ALS inhibitors by other modes of action, such as, for example, a gene that encodes a glyphosate oxido-reductase enzyme as described more fully in U.S. Patent Nos. 5,776,760 and 5,463,175. Other traits that could be combined with the soybean 3560.4.3.5 events include those derived from polynucleotides that confer on the plant the capacity to produce a higher level of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), for example, as more fully described in U.S. Patent Nos. 6,248,876 B1; 5,627,061; 5,804,425; 5,633,435; 5,145,783; 4,971,908; 5,312,910; 5,188,642; 4,940,835; 5,866,775; 6,225,114 B1; 6,130,366; 5,310,667; 4,535,060; 4,769,061; 5,633,448; 5,510,471; Re. 36,449; RE 37,287 E; and 5,491,288; and international publications WO 97/04103; WO 00/66746; WO 01/66704; and WO 00/66747. Other traits that could be combined with the soybean 3560.4.3.5 event include those conferring tolerance to sulfonylurea and/or imidazolinone, for example, as described more fully in U.S. Patent Nos. 5,605,011; 5,013,659; 5,141,870; 5,767,361; 5,731,180; 5,304,732; 4,761,373; 5,331,107; 5,928,937; and 5,378,824; and international publication WO 96/33270.

In some embodiments, the soybean 3560.4.3.5 event may be stacked with, for example, hydroxyphenylpyruvatedioxygenases which are enzymes that catalyze the reaction in which para-hydroxyphenylpyruvate (HPP) is transformed into homogentisate. Molecules which inhibit this enzyme and which bind to the enzyme in order to inhibit transformation of the HPP into homogentisate are useful as herbicides. Traits conferring tolerance to such herbicides in plants are described in U.S. Patent Nos. 6,245,968 B1; 6,268,549; and 6,069,115; and international publication WO 99/23886. Other examples of suitable herbicide-tolerance traits that could be stacked with the soybean 3560.4.3.5 event include aryloxyalkanoate dioxygenase polynucleotides (which reportedly confer tolerance to 2,4-D and other phenoxy auxin herbicides as well as to aryloxyphenoxypropionate herbicides as described, for example, in WO2005/107437) and dicamba-tolerance polynucleotides as described, for example, in Herman *et al.* (2005) *J. Biol. Chem.* 280: 24759-24767.

Other examples of herbicide-tolerance traits that could be combined with the soybean 3560.4.3.5 event include those conferred by polynucleotides encoding an exogenous phosphinothricin acetyltransferase, as described in U.S. Patent Nos. 5,969,213; 5,489,520; 5,550,318; 5,874,265; 5,919,675; 5,561,236; 5,648,477; 5,646,024; 6,177,616; and 5,879,903. Plants containing an exogenous

phosphinothricin acetyltransferase can exhibit improved tolerance to glufosinate herbicides, which inhibit the enzyme glutamine synthase. Other examples of herbicide-tolerance traits that could be combined with the soybean 3560.4.3.5 event include those conferred by polynucleotides conferring altered protoporphyrinogen oxidase (protox) activity, as described in U.S. Patent Nos. 6,288,306 B1; 6,282,837 B1; and 5,767,373; and international publication WO 01/12825. Plants containing such polynucleotides can exhibit improved tolerance to any of a variety of herbicides which target the protox enzyme (also referred to as "protox inhibitors").

Other examples of herbicide-tolerance traits that could be combined with the soybean 3560.4.3.5 event include those conferring tolerance to at least one herbicide in a plant such as, for example, a soybean plant or horseweed. Herbicide-tolerant weeds are known in the art, as are plants that vary in their tolerance to particular herbicides. See, *e.g.*, Green and Williams (2004) "Correlation of Corn (*Zea mays*) Inbred Response to Nicosulfuron and Mesotrione," poster presented at the WSSA Annual Meeting in Kansas City, Missouri, February 9-12, 2004; Green (1998) *Weed Technology* 12: 474-477; Green and Ulrich (1993) *Weed Science* 41: 508-516. The trait(s) responsible for these tolerances can be combined by breeding or via other methods with the soybean 3560.4.3.5 event to provide a plant of the invention as well as methods of use thereof.

The soybean 3560.4.3.5 event can also be combined with at least one other trait to produce plants of the present invention that further comprise a variety of desired trait combinations including, but not limited to, traits desirable for animal feed such as high oil content (*e.g.*, U.S. Patent No. 6,232,529); balanced amino acid content (*e.g.*, hordothionins (U.S. Patent Nos. 5,990,389; 5,885,801; 5,885,802; and 5,703,409; U.S. Patent No. 5,850,016); barley high lysine (Williamson *et al*. (1987) *Eur. J. Biochem*. 165: 99-106; and WO 98/20122) and high methionine proteins (Pedersen *et al*. (1986) *J. Biol. Chem*. 261: 6279; Kirihara *et al*. (1988) *Gene* 71: 359; and Musumura *et al*. (1989) *Plant Mol. Biol*. 12:123)); increased digestibility (*e.g.*, modified storage proteins (U.S. Application Serial No. 10/053,410, filed November 7, 2001); and thioredoxins (U.S. Application Serial No. 10/005,429, filed December 3, 2001)); the disclosures of which are herein incorporated by reference. Desired trait combinations also include LLNC (low linolenic acid content; see, *e.g.*, Dyer *et al.* (2002) *Appl. Microbiol. Biotechnol.* 59: 224-230) and OLCH (high oleic acid content; see, *e.g.*, Fernandez-Moya *et al.* (2005) *J. Agric. Food Chem.* 53: 5326-5330).

The soybean 3560.4.3.5 event can also be combined with other desirable traits such as, for example, fumonisim detoxification genes (U.S. Patent No. 5,792,931), avirulence and disease resistance genes (Jones *et al*. (1994) *Science* 266: 789; Martin *et al*. (1993) *Science* 262: 1432; Mindrinos *et al*. (1994) *Cell* 78: 1089), and traits desirable for processing or process products such as modified oils (*e.g.*, fatty acid desaturase genes (U.S. Patent No. 5,952,544; WO 94/11516)); modified starches (*e.g.*, ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE), and starch debranching enzymes (SDBE)); and polymers or bioplastics (*e.g.*, U.S. Patent No. 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase, and acetoacetyl-CoA reductase (Schubert *et al.* (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)); the disclosures of which are herein incorporated by reference. One could also combine herbicide-tolerant polynucleotides with polynucleotides providing agronomic traits such as male sterility (*e.g.*, see U.S. Patent No. 5.583,210), stalk strength, flowering time, or transformation technology traits such as cell cycle regulation or gene targeting (*e.g.*, WO 99/61619, WO 00/17364, and WO 99/25821); the disclosures of which are herein incorporated by reference.

In another embodiment, the soybean 3560.4.3.5 event can also be combined with the Rcg1 sequence or biologically active variant or fragment thereof. The Rcg1 sequence is an anthracnose stalk rot resistance gene in corn. See, for example, U.S. Patent Application No. 11/397,153, 11/397,275, and 11/397,247, each of which is herein incorporated by reference.

These stacked combinations can be created by any method including, but not limited to, breeding plants by any conventional or TopCross methodology, or genetic transformation. If the sequences are stacked by genetically transforming the plants, the polynucleotide sequences of interest can be combined at any time and in any order. The traits can be introduced simultaneously in a co-transformation protocol with the polynucleotides of interest provided by any combination of transformation cassettes. For example, if two sequences will be introduced, the two sequences can be contained in separate transformation cassettes (trans) or contained on the same transformation cassette (cis). Expression of the sequences can be driven by the same promoter or by different promoters. In certain cases, it may be desirable to introduce a transformation cassette that will suppress the expression of the polynucleotide of interest. This may be combined with any combination of other suppression cassettes

or overexpression cassettes to generate the desired combination of traits in the plant. It is further recognized that polynucleotide sequences can be stacked at a desired genomic location using a site-specific recombination system. See, for example, WO99/25821, WO99/25854, WO99/25840, WO99/25855, and WO99/25853, all of which are herein incorporated by reference.

As used herein, the use of the term "polynucleotide" is not intended to limit a polynucleotide to comprise DNA. Those of ordinary skill in the art will recognize that polynucleotides, can comprise ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues. The polynucleotides also encompass all forms of sequences including, but not limited to, single-stranded forms, double-stranded forms, hairpins, stem-and-loop structures, and the like.

A 3560.4.3.5 plant comprises an expression cassette having a glyphosate acetyltransferase polynucleotide and a genetically modified acetolactate synthase polynucleotide (*gm-hra*). The cassette can include 5' and 3' regulatory sequences operably linked to the *glyat* and the *gm-hra* polynucleotides. "Operably linked" is intended to mean a functional linkage between two or more elements. For example, an operable linkage between a polynucleotide of interest and a regulatory sequence (i.e., a promoter) is functional link that allows for the expression of the polynucleotide of interest. Operably linked elements may be contiguous or non-contiguous. When used to refer to the joining of two protein coding regions, by operably linked it is intended that the coding regions are in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes. Such an expression cassette is provided with a plurality of restriction sites and/or recombination sites for insertion of the polynucleotide to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a coding region, and a transcriptional and translational termination region functional in plants. "Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a

promoter sequence. The promoter sequence can comprise proximal and more distal upstream elements, the latter elements are often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters that cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15: 1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The expression cassettes may also contain 5' leader sequences. Such leader sequences can act to enhance translation. The regulatory regions (i.e., promoters, transcriptional regulatory regions, RNA processing or stability regions, introns, polyadenylation signals, and translational termination regions) and/or the coding region may be native/analogous or heterologous to the host cell or to each other.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect numerous parameters including, processing of the primary transcript to mRNA, mRNA stability and/or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3: 225-236). The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3'

non-coding sequences is exemplified by Ingelbrecht *et al.* (1989) *Plant Cell 1*: 671-680.

As used herein, "heterologous" in reference to a sequence is a sequence that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous polynucleotide is from a species different from the species from which the polynucleotide was derived, or, if from the same/analogous species, one or both are substantially modified from their original form and/or genomic locus, or the promoter is not the native promoter for the operably linked polynucleotide.

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved. The expression cassette can also comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues.

Isolated polynucleotides are provided that can be used in various methods for the detection and/or identification of the soybean 3560.4.3.5 event. An "isolated" or "purified" polynucleotide, or biologically active portion thereof, is substantially or essentially free from components that normally accompany or interact with the polynucleotide as found in its naturally occurring environment. Thus, an isolated or purified polynucleotide is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. Optimally, an "isolated" polynucleotide is free of sequences (optimally protein encoding sequences) that naturally flank the polynucleotide (i.e., sequences located at the 5' and 3' ends of the polynucleotide) in the genomic DNA of the organism from which the polynucleotide is derived. For example, in various embodiments, the isolated polynucleotide can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide

sequence that naturally flank the polynucleotide in genomic DNA of the cell from which the polynucleotide is derived.

In specific embodiments, the polynucleotides comprise the junction DNA sequence set forth in SEQ ID NO:10 or 11. In other embodiments, the polynucleotides comprise the junction DNA sequences set forth in SEQ ID NO:1, 2, 6, 12, 13, 14, 15, 27, 28, 41 or 42 or variants and fragments thereof. Fragments and variants of junction DNA sequences are suitable for discriminatively identifying event 3560.4.3.5. As discussed elsewhere herein, such sequences find use as primers and/or probes.

In other embodiments, the polynucleotides are provided that can detect a 3560.4.3.5 event or a 3560.4.3.5 specific region. Such sequences include any polynucleotide set forth in SEQ ID NOS:1-56 or variants and fragments thereof. In specific embodiments, the polynucleotide used to detect a 3560.4.3.5 event comprise the sequence set forth in SEQ ID NO: 43 or a fragment of SEQ ID NO:43 having at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, or 180 nucleotides. Fragments and variants of polynucleotides that detect a 3560.4.3.5 event or a 3560.4.3.5 specific region are suitable for discriminatively identifying event 3560.4.3.5. As discussed elsewhere herein, such sequences find use as primers and/or probes. Further provided are isolated DNA nucleotide primer sequences comprising or consisting of a sequence set forth in SEQ ID NO:7, 8, 9, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 37, 38, 39, 40, 43, 44, 45, 46, 51, 52, 53, 54, or 55 or a complement thereof or variants and fragments of SEQ ID NO:1, 2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 15, 27, 28, 41, 42, or 43.

"Variants" is intended to mean substantially similar sequences. For polynucleotides, a variant comprises a polynucleotide having deletions (i.e., truncations) at the 5' and/or 3' end; deletion and/or addition of one or more nucleotides at one or more internal sites in the native polynucleotide; and/or substitution of one or more nucleotides at one or more sites in the native polynucleotide.

As used herein, a "probe" is an isolated polynucleotide to which is attached a conventional detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, enzyme, etc. Such a probe is complementary to a strand of a target polynucleotide, in the instant case, to a strand of isolated DNA from soybean event 3560.4.3.5 whether from a soybean plant or from a sample that includes DNA

from the event. Probes include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that can specifically detect the presence of the target DNA sequence.

As used herein, "primers" are isolated polynucleotides that are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase. Primer pairs refer to their use for amplification of a target polynucleotide, e.g., by the polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods. "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (see, U.S. Pat. Nos. 4,683,195 and 4,800,159; herein incorporated by reference). Any combination of primers disclosed herein can be used such that the pair allows for the detection a 3560.4.3.5 event or specific region (i.e., SEQ ID NOS: 7-9, 16-26, 37, 38, 39, 40, 44-46, or 51-55). Non-limiting examples of primer pairs include SEQ ID NOS:16 and 17; SEQ ID NOS:23 and 20; SEQ ID NOS:23 and 19; SEQ ID NOS:18 AND 22; SEQ ID NOS:21 and 22;; SEQ ID NO: 7 and 9; SEQ ID NO:8 and 9; SEQ ID NO:7 and 8; SEQ ID NO:37 and 39; and SEQ ID NO:38 and 39; and SEQ ID NO: 44 and 45 and SEQ ID NOS: 37 and 38.

Probes and primers are of sufficient nucleotide length to bind to the target DNA sequence and specifically detect and/or identify a polynucleotide having a 3560.4.3.5 event. It is recognized that the hybridization conditions or reaction conditions can be determined by the operator to achieve this result. This length may be of any length that is of sufficient length to be useful in a detection method of choice. Generally, 8, 11, 14, 16, 18, 20, 22, 24, 26, 28, 30, 40, 50, 75, 100, 200, 300, 400, 500, 600, 700 nucleotides or more, or between about 11-20, 20-30, 30-40, 40-50, 50-100, 100-200, 200-300, 300-400, 400-500, 500-600, 600-700, 700-800, or more nucleotides in length are used. Such probes and primers can hybridize specifically to a target sequence under high stringency hybridization conditions. Probes and primers according to embodiments may have complete DNA sequence identity of contiguous nucleotides with the target sequence, although probes differing from the target DNA sequence and that retain the ability to specifically detect and/or identify a target DNA sequence may be designed by conventional methods. Accordingly, probes and primers can share about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater sequence identity or complementarity to the target

polynucleotide (i.e., SEQ ID NO:1-46 and 47-55), or can differ from the target sequence (i.e., SEQ ID NO: 1-46 and 47-55) by 1, 2, 3, 4, 5, 6 or more nucleotides. Probes can be used as primers, but are generally designed to bind to the target DNA or RNA and are not used in an amplification process.

Specific primers can be used to amplify an integration fragment to produce an amplicon that can be used as a "specific probe" or can itself be detected for identifying event 3560.4.3.5 in biological samples. Alternatively, a probe can be used during the PCR reaction to allow for the detection of the amplification event (i.e., a Taqman probe or a MGB probe) (so called real time PCR). When the probe is hybridized with the polynucleotides of a biological sample under conditions which allow for the binding of the probe to the sample, this binding can be detected and thus allow for an indication of the presence of event 3560.4.3.5 in the biological sample. Such identification of a bound probe has been described in the art. In an embodiment, the specific probe is a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the event and also comprises a part of the foreign DNA contiguous therewith. The specific probe may comprise a sequence of at least 80%, between 80 and 85%, between 85 and 90%, between 90 and 95%, and between 95 and 100% identical (or complementary) to a specific region of the 3560.4.3.5 event.

As used herein, "amplified DNA" or "amplicon" refers to the product of polynucleotide amplification of a target polynucleotide that is part of a nucleic acid template. For example, to determine whether a soybean plant resulting from a sexual cross contains the 3560.4.3.5 event, DNA extracted from the soybean plant tissue sample may be subjected to a polynucleotide amplification method using a DNA primer pair that includes a first primer derived from flanking sequence adjacent to the insertion site of inserted heterologous DNA, and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the 3560.4.3.5 event DNA. By "diagnostic" for a 3650.4.3.5 event the use of any method or assay which discriminates between the presence or the absence of a 3560.4.3.5 event in a biological sample is intended. Alternatively, the second primer may be derived from the flanking sequence. In still other embodiments, primer pairs can be derived from flanking sequence on both sides of the inserted DNA so as to produce an amplicon that includes the entire insert polynucleotide of the expression construct as well as the sequence flanking the transgenic insert. See, Figure 1. The

amplicon is of a length and has a sequence that is also diagnostic for the event (i.e., has a junction DNA from a 3560.4.3.5 event). The amplicon may range in length from the combined length of the primer pairs plus one nucleotide base pair to any length of amplicon producible by a DNA amplification protocol. A member of a primer pair derived from the flanking sequence may be located a distance from the inserted DNA sequence, this distance can range from one nucleotide base pair up to the limits of the amplification reaction, or about twenty thousand nucleotide base pairs. The use of the term "amplicon" specifically excludes primer dimers that may be formed in the DNA thermal amplification reaction.

Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual,* 2.sup.nd ed, vol. 1-3, ed. Sambrook *et al*., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989 (hereinafter, "Sambrook *et al*., 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al*., Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al*., 1992"); and Innis *et al.,* PCR Protocols: A Guide to Methods and Applications, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as the PCR primer analysis tool in Vector NTI version 6 (Informax Inc., Bethesda Md.); PrimerSelect (DNASTAR Inc., Madison, Wis.); and Primer (Version 0.5.COPYRGT., 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Additionally, the sequence can be visually scanned and primers manually identified using guidelines known to one of skill in the art.

It is to be understood that as used herein the term "transgenic" includes any cell, cell line, callus, tissue, plant part, or plant, the genotype of which has been altered by the presence of a heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host

organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include Agrobacterium-mediated transformation (De Blaere *et al.* (1987) *Meth. Enzymol.* 143: 277) and particle-accelerated or "gene gun" transformation technology (Klein *et al.* (1987) *Nature* (London) 327: 70-73; U.S. Pat. No. 4,945,050, incorporated herein by reference). Additional transformation methods are disclosed below.

Thus, isolated polynucleotides can be incorporated into recombinant constructs, typically DNA constructs, which are capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels *et al.* (1985; Supp. 1987) *Cloning Vectors: A Laboratory Manual*, Weissbach and Weissbach (1989) *Methods for Plant Molecular Biology* (Academic Press, New York); and Flevin *et al.* (1990) *Plant Molecular Biology Manual* (Kluwer Academic Publishers). Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

Various methods and compositions for identifying event 3560.4.3.5 are provided. Such methods find use in identifying and/or detecting a 3560.4.3.5 event in any biological material. Such methods include, for example, methods to confirm seed purity and methods for screening seeds in a seed lot for a 3560.4.3.5 event. In one embodiment, a method for identifying event 3560.4.3.5 in a biological sample is provided and comprises contacting the sample with a first and a second primer; and, amplifying a polynucleotide comprising a 3560.4.3.5 specific region.

A biological sample can comprise any sample in which one desires to determine if DNA having event 3560.4.3.5 is present. For example, a biological sample can comprise any plant material or material comprising or derived from a plant material such as, but not limited to, food or feed products. As used herein,

"plant material" refers to material which is obtained or derived from a plant or plant part. In specific embodiments, the biological sample comprises a soybean tissue.

Primers and probes based on the flanking DNA and insert sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed sequences by conventional methods, e.g., by re-cloning and sequencing such sequences. The polynucleotide probes and primers specifically detect a target DNA sequence. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of DNA from a transgenic event in a sample. By "specifically detect" it is intended that the polynucleotide can be used either as a primer to amplify a 3560.4.3.5 specific region or the polynucleotide can be used as a probe that hybridizes under stringent conditions to a polynucleotide having a 3560.4.3.5 event or a 3560.4.3.5 specific region. The level or degree of hybridization which allows for the specific detection of a 3560.4.3.5 event or a specific region of a 3560.4.3.5 event is sufficient to distinguish the polynucleotide with the 3560.4.3.5 specific region from a polynucleotide lacking this region and thereby allow for discriminately identifying a 3560.4.3.5 event. By "shares sufficient sequence identity or complementarity to allow for the amplification of a 3560.4.3.5 specific event" is intended the sequence shares at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity or complementarity to a fragment or across the full length of the polynucleotide having the 3560.4.3.5 specific region.

Regarding the amplification of a target polynucleotide (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize to the target polynucleotide to which a primer having the corresponding wild-type sequence (or its complement) would bind and preferably to produce an identifiable amplification product (the amplicon) having a 3560.4.3.5 specific region in a DNA thermal amplification reaction. In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify a 3560.4.3.5 specific region. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook *et al*. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis *et al*., eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Methods of

amplification are further described in US Patent No. 4,683,195, 4,683,202 and Chen *et al.* (1994) *PNAS* 91:5695-5699. These methods as well as other methods known in the art of DNA amplification may be used in the practice of the other embodiments. It is understood that a number of parameters in a specific PCR protocol may need to be adjusted to specific laboratory conditions and may be slightly modified and yet allow for the collection of similar results. These adjustments will be apparent to a person skilled in the art.

The amplified polynucleotide (amplicon) can be of any length that allows for the detection of the 3560.4.3.5 event or a 3560.4.3.5 specific region. For example, the amplicon can be about 10, 50, 100, 200, 300, 500, 700, 100, 2000, 3000, 4000, 5000 nucleotides in length or longer.

In specific embodiments, the specific region of the 3560.4.3.5 event is detected.

Any primer that allows a 3560.4.3.5 specific region to be amplified and/or detected can be employed in the methods. For example, in specific embodiments, the first primer comprises a fragment of a polynucleotide of SEQ ID NO: 4 or 5, wherein the first or the second primer shares sufficient sequence identity or complementarity to the polynucleotide to amplify the 3560.4.3.5 specific region. The primer pair can comprise a fragment of SEQ ID NO:4 and a fragment of SEQ ID NO:5 or 3, or alternatively, the primer pair can comprise a fragment of SEQ ID NO:5 and a fragment of SEQ ID NO: 3 or 4. In still further embodiments, the first and the second primer can comprise any one or any combination of the sequences set forth in SEQ ID NO:7, 8, 9, 16-26, 37, 38, 39, 40, 44-46, or 51-55. The primers can be of any length sufficient to amplify a 3560.4.3.5 region including, for example, at least 6, 7, 8, 9, 10, 15, 20, 15, or 30 or about 7-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45 nucleotides or longer.

As discussed elsewhere herein, any method to PCR amplify the 3560.4.3.5 event or specific region can be employed, including for example, real time PCR. See, for example, Livak *et al.* (1995) *PCR Methods and Applications* 4:357-362; US Patent 5,538,848; US Patent 5,723,591; Applied Biosystems User Bulletin No. 2, "Relative Quantitation of Gene Expression," P/N 4303859; and, Applied Biosystems User Bulletin No. 5, "Multiplex PCR with Taqman VIC probes," P/N 4306236; each of which is herein incorporated by reference.

Thus, in specific embodiments, a method of detecting the presence of soybean event 3560.4.3.5 or progeny thereof in a biological sample is provided. The method comprises (a) extracting a DNA sample from the biological sample; (b) providing a pair of DNA primer molecules (i.e, any combination of SEQ ID NOS: 7-9, 16-26, 37, 38, 39, 40, 44-46, or 51-55, wherein said combination amplifies a 3560.4.3.5 event), including, but not limited to, i) the sequences of SEQ ID NO:16 and SEQ ID NO:17, ii) the sequences of SEQ ID NO:23 and SEQ ID NO:20; iii) the sequences of SEQ ID NO:23 and SEQ ID NO:19; iv) the sequences of SEQ ID NO:18 and SEQ ID NO:22; v) SEQ ID NO:21 and SEQ ID NO:22; vi) SEQ ID NO: 7 and 9; vii) SEQ ID NO: 8 and 9; iix) SEQ ID NO: 7 and 8; ix) SEQ ID NO: 37 and 39; x) SEQ ID NO: 38 and 39; xi) SEQ ID NO: 44 and 45; xii) SEQ ID NO: 25 and 26; xiii) SEQ ID NO:25 and SEQ ID NO:24; (c) providing DNA amplification reaction conditions; (d) performing the DNA amplification reaction, thereby producing a DNA amplicon molecule; and (e) detecting the DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in the DNA amplification reaction indicates the presence of soybean event 3560.4.3.5. In order for a nucleic acid molecule to serve as a primer or probe it needs only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

In hybridization techniques, all or part of a polynucleotide that selectively hybridizes to a target polynucleotide having a 3560.4.3.5 specific event is employed. By "stringent conditions" or "stringent hybridization conditions" when referring to a polynucleotide probe conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background) are intended. Regarding the amplification of a target polynucleotide (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize to the target polynucleotide to which a primer having the corresponding wild-type. Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of identity are detected (heterologous

probing). Generally, a probe is less than about 1000 nucleotides in length or less than 500 nucleotides in length.

As used herein, a substantially identical or complementary sequence is a polynucleotide that will specifically hybridize to the complement of the nucleic acid molecule to which it is being compared under high stringency conditions. Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45° C., followed by a wash of 2XSSC at 50° C., are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions for hybridization and detection will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Optionally, wash buffers may comprise about 0.1% to about 1% SDS. Duration of hybridization is generally less than about 24 hours, usually about 4 to about 12 hours. The duration of the wash time will be at least a length of time sufficient to reach equilibrium.

In hybridization reactions, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: T_m = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature

(under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with ≥90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is optimal to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes,* Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al*. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York) and Haymes *et al.* (1985) In: *Nucleic Acid Hybridization, a Practical Approach*, IRL Press, Washington, D.C.

A polynucleotide is said to be the "complement" of another polynucleotide if they exhibit complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the polynucleotide molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to

permit them to remain annealed to one another under conventional "high-stringency" conditions.

Further provided are methods of detecting the presence of DNA corresponding to the 3560.4.3.5 event in a sample. In one embodiment, the method comprises (a) contacting the biological sample with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from soybean event 3560.4.3.5 and specifically detects the 3560.4.3.5 event; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the 3560.4.3.5 event. In one embodiment, the DNA is digested with appropriate enzymes are preformed prior to the hybridization event.

Various method can be used to detect the 3560.4.3.5 specific region or amplicon thereof, including, but not limited to, Genetic Bit Analysis (Nikiforov *et al.* (1994) *Nucleic Acid Res.* 22: 4167-4175). In one method, a DNA oligonucleotide is designed which overlaps both the adjacent flanking DNA sequence and the inserted DNA sequence. In other embodiments, DNA oligos are designed to allow for a 3560.4.3.5 specific amplicon. The oligonucleotide is immobilized in wells of a microwell plate. Following PCR of the region of interest a single-stranded PCR product can be hybridized to the immobilized oligonucleotide and serve as a template for a single base extension reaction using a DNA polymerase and labeled ddNTPs specific for the expected next base. Readout may be fluorescent or ELISA-based. A signal indicates presence of the insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Another detection method is the Pyrosequencing technique as described by Winge ((2000) *Innov. Pharma. Tech.* 00: 18-24). In this method, an oligonucleotide is designed that overlaps the adjacent DNA and insert DNA junction or a pair of oligos are employed that can amplify a 3560.4.3.5 specific region. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted sequence and one in the flanking sequence) and incubated in the presence of a DNA polymerase, ATP, sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. dNTPs are added individually and the incorporation results in a light signal which is measured. A light signal indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single or multi-base extension.

Fluorescence Polarization as described by Chen *et al.* ((1999) *Genome Res. 9*: 492-498, 1999) is also a method that can be used to detect an amplicon of the invention. Using this method, an oligonucleotide is designed which overlaps the flanking and inserted DNA junction or a pair of oligos are employed that can amplify a 3560.4.3.5 specific region. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted DNA and one in the flanking DNA sequence) and incubated in the presence of a DNA polymerase and a fluorescent-labeled ddNTP. Single base extension results in incorporation of the ddNTP. Incorporation can be measured as a change in polarization using a fluorometer. A change in polarization indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Taqman® (PE Applied Biosystems, Foster City, Calif.) is described as a method of detecting and quantifying the presence of a DNA sequence and is fully understood in the instructions provided by the manufacturer. Briefly, a FRET oligonucleotide probe is designed which overlaps the flanking and insert DNA junction or a pair of oligos are employed that can amplify a 3560.4.3.5 specific region. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Hybridization of the FRET probe results in cleavage and release of the fluorescent moiety away from the quenching moiety on the FRET probe. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

Molecular Beacons have been described for use in sequence detection as described in Tyangi *et al.* ((1996) *Nature Biotech. 14*: 303-308). Briefly, a FRET oligonucleotide probe is designed that overlaps the flanking and insert DNA junction or a pair of oligos are employed that can amplify a 3560.4.3.5 specific region. . The unique structure of the FRET probe results in it containing secondary structure that keeps the fluorescent and quenching moieties in close proximity. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Following successful PCR amplification, hybridization of the FRET probe to the target sequence results in the removal of the probe secondary structure and spatial separation of the fluorescent and quenching moieties. A fluorescent signal results. A

fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

A hybridization reaction using a probe specific to a sequence found within the amplicon is yet another method used to detect the amplicon produced by a PCR reaction.

As used herein, "kit" refers to a set of reagents for the purpose of performing the method embodiments, more particularly, the identification and/or the detection of the 3560.4.3.5 event in biological samples. The kit can be used, and its components can be specifically adjusted, for purposes of quality control (e.g. purity of seed lots), detection of event 3560.4.3.5 in plant material, or material comprising or derived from plant material, such as but not limited to food or feed products.

In specific embodiments, a kit for identifying event 3560.4.3.5 in a biological sample is provided. The kit comprises a first and a second primer, wherein the first and second primer amplify a polynucleotide comprising a 3560.4.3.5 specific region. In further embodiments, the kit also comprises a polynucleotide for the detection of the 3560.4.3.5 specific region. The kit can comprise, for example, a first primer comprising a fragment of a polynucleotide of SEQ ID NO:4 or 5, wherein the first or the second primer shares sufficient sequence homology or complementarity to the polynucleotide to amplify said 3560.4.3.5 specific region. For example, in specific embodiments, the first primer comprises a fragment of a polynucleotide of SEQ ID NO:4 or 5, wherein the first or the second primer shares sufficient sequence homology or complementarity to the polynucleotide to amplify said 3560.4.3.5 specific region. The primer pair can comprises a fragment of SEQ ID NO:4 and a fragment of SEQ ID NO:5 or 3, or alternatively, the primer pair can comprises a fragment of SEQ ID NO:5 and a fragment of SEQ ID NO:3 or 4. In still further embodiments, the first and the second primer can comprise any one or any combination of the sequences set forth in SEQ ID NO:7, 8, 9, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 37, 38, 39, 40, 43, 44, 45, 46, or 51-55. The primers can be of any length sufficient to amplify the 3560.4.3.5 region including, for example, at least 6, 7, 8, 9, 10, 15, 20, 15, or 30 or about 7-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40, 40-45 nucleotides or longer.

Further provided are DNA detection kits comprising at least one polynucleotide that can specifically detect a 3560.4.3.5 specific region, wherein said polynucleotide comprises at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO: 3, 4 or 5. In

specific embodiments, the DNA detection kit comprises a polynucleotide having SEQ ID NO:10 or 11 or comprises a sequence which hybridizes with sequences selected from the group consisting of: a) the sequences of SEQ ID NO: 4 and SEQ ID NO:3; and, b) the sequences of SEQ ID NO: 5 and SEQ ID NO: 3, and a sequence of SEQ ID NO:4 and 43.

Any of the polynucleotides and fragments and variants thereof employed in the methods and compositions can share sequence identity to a region of the transgene insert of the 3560.4.3.5 event, a junction sequence of the 3560.4.3.5 event or a flanking sequence of the 3560.4.3.5 event. Methods to determine the relationship of various sequences are known. As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two polynucleotides. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent sequence identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local alignment algorithm of Smith *et al*. (1981) *Adv. Appl. Math.* 2:482; the global alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-local alignment method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 872264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations

include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the GCG Wisconsin Genetics Software Package, Version 10 (available from Accelrys Inc., 9685 Scranton Road, San Diego, California, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al*. (1988) *Gene* 73:237-244 (1988); Higgins *et al*. (1989) *CABIOS* 5:151-153; Corpet *et al*. (1988) *Nucleic Acids Res*. 16:10881-90; Huang *et al*. (1992) *CABIOS* 8:155-65; and Pearson *et al*. (1994) *Meth*. *Mol*. *Biol*. 24:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al*. (1997) *Nucleic Acids Res*. 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al*. (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.nlm.nih.gov. Alignment may also be performed manually by inspection.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. By "equivalent program" any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue

matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10 is intended.

GAP uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the GCG Wisconsin Genetics Software Package for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater.

GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the Quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the GCG Wisconsin Genetics Software Package is BLOSUM62 (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

As used herein, "sequence identity" or "identity" in the context of two polynucleotides or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical

often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Methods are provided for controlling weeds in an area of cultivation, preventing the development or the appearance of herbicide resistant weeds in an area of cultivation, producing a crop, and increasing crop safety. The term "controlling," and derivations thereof, for example, as in "controlling weeds" refers to one or more of inhibiting the growth, germination, reproduction, and/or proliferation of; and/or killing, removing, destroying, or otherwise diminishing the occurrence and/or activity of a weed.

As used herein, an "area of cultivation" comprises any region in which one desires to grow a plant. Such areas of cultivations include, but are not limited to, a field in which a plant is cultivated (such as a crop field, a sod field, a tree field, a

managed forest, a field for culturing fruits and vegetables, etc), a greenhouse, a growth chamber, etc.

The methods comprise planting the area of cultivation with the soybean 3560.4.3.5 seeds or plants, and in specific embodiments, applying to the crop, seed, weed or area of cultivation thereof an effective amount of a herbicide of interest. It is recognized that the herbicide can be applied before or after the crop is planted in the area of cultivation. Such herbicide applications can include an application of glyphosate, an ALS inhibitor chemistry, or any combination thereof. In specific embodiments, a mixture of an ALS inhibitor chemistry in combination with glyphosate is applied to the soybean 3560.4.3.5, wherein the effective concentration of at least the ALS inhibitor chemistry would significantly damage an appropriate control plant. In one non-limiting embodiment, the herbicide comprises at least one of a sulfonylaminocarbonyltriazolinone; a triazolopyrimidine; a pyrimidinyl(thio)benzoate; an imidazolinone; a triazine; and/or a phosphinic acid.

In another non-limiting embodiment, the combination of herbicides comprises glyphosate, imazapyr, chlorimuron-ethyl, quizalofop, and fomesafen, wherein an effective amount is tolerated by the crop and controls weeds. As disclosed elsewhere herein, any effective amount of these herbicides can be applied. In specific embodiments, this combination of herbicides comprises an effective amount of glyphosate comprising about 1110 to about 1130 g ai/hectare; an effective amount of imazapyr comprising about 7.5 to about 27.5 g ai/hectare; an effective amount of chlorimuron-ethyl comprising about 7.5 to about 27.5 g ai/hectare; an effective amount of quizalofop comprising about 50 to about 70 g ai/hectare; and, an effective amount of fomesafen comprising about 240 to about 260 g ai/hectare.

In other embodiments, a combination of at least two herbicides is applied, wherein the combination does not include glyphosate. In other embodiments, at least one ALS inhibitor and glyphosate is applied to the plant. More details regarding the various herbicide combinations that can be employed in the methods are discussed elsewhere herein.

In one embodiment, the method of controlling weeds comprises planting the area with the 3560.4.3.5 soybean seeds or plants and applying to the crop, crop part, seed of said crop or the area under cultivation, an effective amount of a herbicide, wherein said effective amount comprises

i) an amount that is not tolerated by a first control crop when applied to the first control crop, crop part, seed or the area of cultivation, wherein said first control crop expresses a first polynucleotide encoding GLYAT polypeptide that confers tolerance to glyphosate and does not express a second polynucleotide that encodes the gm-hra polypeptide;

ii) an amount that is not tolerated by a second control crop when applied to the second crop, crop part, seed or the area of cultivation, wherein said second control crop expresses the *gm-hra* polynucleotide and does not express the *glyat* polynucleotide; and,

iii) an amount that is tolerated when applied to the 3560.4.3.5 soybean crop, crop part, seed, or the area of cultivation thereof. The herbicide can comprise a combination of herbicides that either includes or does not include glyphosate. In specific embodiments, the combination of herbicides comprises ALS inhibitor chemistries as discussed in further detail below.

In another embodiment, the method of controlling weeds comprises planting the area with a 3560.4.3.5 soybean crop seed or plant and applying to the crop, crop part, seed of said crop or the area under cultivation, an effective amount of a herbicide, wherein said effective amount comprises a level that is above the recommended label use rate for the crop, wherein said effective amount is tolerated when applied to the 3560.4.3.5 soybean crop, crop part, seed, or the area of cultivation thereof. The herbicide applied can comprise a combination of herbicides that either includes or does not include glyphosate. In specific embodiments, the combination of herbicides comprises at least one ALS inhibitor chemistry as discussed in further detail below. Further herbicides and combinations thereof that can be employed in the various methods are discussed in further detail below.

A "control" or "control plant" or "control plant cell" provides a reference point for measuring changes in phenotype of the subject plant or plant cell, and may be any suitable plant or plant cell. A control plant or plant cell may comprise, for example: (a) a wild-type plant or cell, *i.e.*, of the same genotype as the starting material for the genetic alteration which resulted in the subject plant or cell; (b) a plant or plant cell of the same genotype as the starting material but which has been transformed with a null construct (*i.e.*, with a construct which has no known effect on the trait of interest, such as a construct comprising a marker gene); (c) a plant or plant cell which is a non-transformed segregant among progeny of a subject plant or plant

cell; (d) a plant or plant cell which is genetically identical to the subject plant or plant cell but which is not exposed to the same treatment (*e.g.*, herbicide treatment) as the subject plant or plant cell; (e) the subject plant or plant cell itself, under conditions in which the gene of interest is not expressed; or (f) the subject plant or plant cell itself, under conditions in which it has not been exposed to a particular treatment such as, for example, a herbicide or combination of herbicides and/or other chemicals. In some instances, an appropriate control plant or control plant cell may have a different genotype from the subject plant or plant cell but may share the herbicide-sensitive characteristics of the starting material for the genetic alteration(s) which resulted in the subject plant or cell (see, *e.g.*, Green (1998) *Weed Technology* 12: 474-477; Green and Ulrich (1993) *Weed Science* 41: 508-516. In some instances, an appropriate control soybean plant is a "Jack" soybean plant (Illinois Foundation Seed, Champaign, Illinois). In other embodiments, the null segregant can be used as a control, as they are near isogenic to 3560.4.3.5 with the exception of the transgenic insert DNA.

Any herbicide can be applied to the 3560.4.3.5 soybean crop, crop part, or the area of cultivation containing the crop plant. Classification of herbicides (*i.e.*, the grouping of herbicides into classes and subclasses) is well-known in the art and includes classifications by HRAC (Herbicide Resistance Action Committee) and WSSA (the Weed Science Society of America) (see also, Retzinger and Mallory-Smith (1997) *Weed Technology* 11: 384-393). An abbreviated version of the HRAC classification (with notes regarding the corresponding WSSA group) is set forth below in Table 1.

Herbicides can be classified by their mode of action and/or site of action and can also be classified by the time at which they are applied (*e.g.*, preemergent or postemergent), by the method of application (*e.g.*, foliar application or soil application), or by how they are taken up by or affect the plant. For example, thifensulfuron-methyl and tribenuron-methyl are applied to the foliage of a crop and are generally metabolized there, while rimsulfuron and chlorimuron-ethyl are generally taken up through both the roots and foliage of a plant. "Mode of action" generally refers to the metabolic or physiological process within the plant that the herbicide inhibits or otherwise impairs, whereas "site of action" generally refers to the physical location or biochemical site within the plant where the herbicide acts or

directly interacts. Herbicides can be classified in various ways, including by mode of action and/or site of action (see, *e.g.*, Table 1).

Often, an herbicide-tolerance gene that confers tolerance to a particular herbicide or other chemical on a plant expressing it will also confer tolerance to other herbicides or chemicals in the same class or subclass, for example, a class or subclass set forth in Table 1. Thus, in some embodiments, a transgenic plant is tolerant to more than one herbicide or chemical in the same class or subclass, such as, for example, an inhibitor of PPO, a sulfonylurea, or a synthetic auxin.

Typically, the plants can tolerate treatment with different types of herbicides (*i.e.*, herbicides having different modes of action and/or different sites of action) as well as with higher amounts of herbicides than previously known plants, thereby permitting improved weed management strategies that are recommended in order to reduce the incidence and prevalence of herbicide-tolerant weeds. Specific herbicide combinations can be employed to effectively control weeds.

Transgenic soybean plants are provided which can be selected for use in crop production based on the prevalence of herbicide-tolerant weed species in the area where the transgenic crop is to be grown. Weed management techniques, such as for example, crop rotation using a crop that is tolerant to a herbicide to which the local weed species are not tolerant can be used. See, for example, the Herbicide Resistance Action Committee (HRAC), the Weed Science Society of America, and various state agencies and the herbicide tolerance scores for various broadleaf weeds from the 2004 Illinois Agricultural Pest Management Handbook). See also, Owen and Hartzler (2004), *2005 Herbicide Manual for Agricultural Professionals*, Pub. WC 92 Revised (Iowa State University Extension, Iowa State University of Science and Technology, Ames, Iowa); *Weed Control for Corn, Soybeans, and Sorghum*, Chapter 2 of "2004 Illinois Agricultural Pest Management Handbook" (University of Illinois Extension, University of Illinois at Urbana-Champaign, Illinois); *Weed Control Guide for Field Crops*, MSU Extension Bulletin E434 (Michigan State University, East Lansing, Michigan)).

Table 1: Abbreviated version of HRAC Herbicide Classification

I. ALS Inhibitors (WSSA Group 2)
A. Sulfonylureas
1. Azimsulfuron
2. Chlorimuron-ethyl

3. Metsulfuron-methyl
4. Nicosulfuron
5. Rimsulfuron
6. Sulfometuron-methyl
7. Thifensulfuron-methyl
8. Tribenuron-methyl
9. Amidosulfuron
10. Bensulfuron-methyl
11. Chlorsulfuron
12. Cinosulfuron
13. Cyclosulfamuron
14. Ethametsulfuron-methyl
15. Ethoxysulfuron
16. Flazasulfuron
17. Flupyrsulfuron- methyl
18. Foramsulfuron
19. Imazosulfuron
20. Iodosulfuron-methyl
21. Mesosulfuron-methyl
22. Oxasulfuron
23. Primisulfuron-methyl
24. Prosulfuron
25. Pyrazosulfuron-ethyl
26. Sulfosulfuron
27. Triasulfuron
28. Trifloxysulfuron
29. Triflusulfuron-methyl
30. Tritosulfuron
31. Halosulfuron-methyl
32. Flucetosulfuron

B. Sulfonylaminocarbonyltriazolinones
   1. Flucarbazone
   2. Procarbazone

C. Triazolopyrimidines
   1. Cloransulam-methyl
   2. Flumetsulam
   3. Diclosulam
   4. Florasulam
   5. Metosulam
   6. Penoxsulam
   7. Pyroxsulam

D. Pyrimidinyloxy(thio)benzoates
   1. Bispyribac
   2. Pyriftalid
   3. Pyribenzoxim
   4. Pyrithiobac
   5. Pyriminobac-methyl

E. Imidazolinones
   1. Imazapyr

2. Imazethapyr
3. Imazaquin
4. Imazapic
5. Imazamethabenz-methyl
6. Imazamox

II. Other Herbicides--Active Ingredients/ Additional Modes of Action

A. Inhibitors of Acetyl CoA carboxylase (ACCase) (WSSA Group 1)

1. Aryloxyphenoxypropionates ('FOPs')
   a. Quizalofop-P-ethyl
   b. Diclofop-methyl
   c. Clodinafop-propargyl
   d. Fenoxaprop-P-ethyl
   e. Fluazifop-P-butyl
   f. Propaquizafop
   g. Haloxyfop-P-methyl
   h. Cyhalofop-butyl
   i. Quizalofop-P-ethyl

2. Cyclohexanediones ('DIMs')
   a. Alloxydim
   b. Butroxydim
   c. Clethodim
   d. Cycloxydim
   e. Sethoxydim
   f. Tepraloxydim
   g. Tralkoxydim

B. Inhibitors of Photosystem II—HRAC Group C1/ WSSA Group 5

1. Triazines
   a. Ametryne
   b. Atrazine
   c. Cyanazine
   d. Desmetryne
   e. Dimethametryne
   f. Prometon
   g. Prometryne
   h. Propazine
   i. Simazine
   j. Simetryne
   k. Terbumeton
   l. Terbuthylazine
   m. Terbutryne
   n. Trietazine

2. Triazinones
   a. Hexazinone
   b. Metribuzin
   c. Metamitron

3. Triazolinone
   a. Amicarbazone

4. Uracils
   a. Bromacil
   b. Lenacil
   c. Terbacil

5. Pyridazinones
   a. Pyrazon

6. Phenyl carbamates
   a. Desmedipham
   b. Phenmedipham

C. Inhibitors of Photosystem II--HRAC Group C2/WSSA Group 7

1. Ureas
   a. Fluometuron
   b. Linuron
   c. Chlorobromuron
   d. Chlorotoluron
   e. Chloroxuron
   f. Dimefuron
   g. Diuron
   h. Ethidimuron
   i. Fenuron
   j. Isoproturon
   k. Isouron
   l. Methabenzthiazuron

m. Metobromuron
n. Metoxuron
o. Monolinuron
p. Neburon
q. Siduron
r. Tebuthiuron

2. Amides
a. Propanil
b. Pentanochlor

D. Inhibitors of Photosystem II--HRAC Group C3/ WSSA Group 6
1. Nitriles
a. Bromofenoxim
b. Bromoxynil
c. Ioxynil

2. Benzothiadiazinone (Bentazon)
a. Bentazon

3. Phenylpyridazines
a. Pyridate
b. Pyridafol

E. Photosystem-I-electron diversion (Bipyridyliums) (WSSA Group 22)
1. Diquat
2. Paraquat

F. Inhibitors of PPO (protoporphyrinogen oxidase) (WSSA Group 14)
1. Diphenylethers
a. Acifluorfen-Na
b. Bifenox
c. Chlomethoxyfen
d. Fluoroglycofen-ethyl
e. Fomesafen
f. Halosafen
g. Lactofen
h. Oxyfluorfen

2. Phenylpyrazoles
a. Fluazolate
b. Pyraflufen-ethyl

3. N-phenylphthalimides
a. Cinidon-ethyl
b. Flumioxazin
c. Flumiclorac-pentyl

4. Thiadiazoles
a. Fluthiacet-methyl
b. Thidiazimin

5. Oxadiazoles
a. Oxadiazon

b. Oxadiargyl

6. Triazolinones
   a. Carfentrazone-ethyl
   b. Sulfentrazone

7. Oxazolidinediones
   a. Pentoxazone

8. Pyrimidindiones
   a. Benzfendizone
   b. Butafenicil

9. Others
   a. Pyrazogyl
   b. Profluazol

G. Bleaching: Inhibition of carotenoid biosynthesis at the phytoene desaturase step (PDS) (WSSA Group 12)

1. Pyridazinones
   a. Norflurazon

2. Pyridinecarboxamides
   a. Diflufenican
   b. Picolinafen

3. Others
   a. Beflubutamid
   b. Fluridone
   c. Flurochloridone
   d. Flurtamone

H. Bleaching: Inhibition of 4-hydroxyphenyl-pyruvate-dioxygenase (4-HPPD) (WSSA Group 28)

1. Triketones
   a. Mesotrione
   b. Sulcotrione

2. Isoxazoles
   a. Isoxachlortole
   b. Isoxaflutole

3. Pyrazoles
   a. Benzofenap
   b. Pyrazoxyfen
   c. Pyrazolynate

4. Others
   a. Benzobicyclon

I. Bleaching: Inhibition of carotenoid biosynthesis (unknown target) (WSSA Group 11 and 13)

1. Triazoles (WSSA Group 11)
   a. Amitrole

2. Isoxazolidinones (WSSA Group 13)
   a. Clomazone

3. Ureas
   a. Fluometuron

3. Diphenylether
   a. Aclonifen

J. Inhibition of EPSP Synthase
   1. Glycines (WSSA Group 9)
      a. Glyphosate
      b. Sulfosate

K. Inhibition of glutamine synthetase
   1. Phosphinic Acids
      a. Glufosinate-ammonium
      b. Bialaphos

L. Inhibition of DHP (dihydropteroate) synthase (WSSA Group 18)
   1 Carbamates
      a. Asulam

M. Microtubule Assembly Inhibition (WSSA Group 3)
   1. Dinitroanilines
      a. Benfluralin
      b. Butralin
      c. Dinitramine
      d. Ethalfluralin
      e. Oryzalin
      f. Pendimethalin
      g. Trifluralin

   2. Phosphoroamidates
      a. Amiprophos-methyl
      b. Butamiphos

   3. Pyridines
      a. Dithiopyr
      b. Thiazopyr

   4. Benzamides
      a. Pronamide
      b. Tebutam

   5. Benzenedicarboxylic acids
      a. Chlorthal-dimethyl

N. Inhibition of mitosis/microtubule organization WSSA Group 23)
   1. Carbamates

a. Chlorpropham
b. Propham
c. Carbetamide

O. Inhibition of cell division (Inhibition of very long chain fatty acids as proposed mechanism; WSSA Group 15)
1. Chloroacetamides
a. Acetochlor
b. Alachlor
c. Butachlor
d. Dimethachlor
e. Dimethanamid
f. Metazachlor
g. Metolachlor
h. Pethoxamid
i. Pretilachlor
j. Propachlor
k. Propisochlor
l. Thenylchlor

2. Acetamides
a. Diphenamid
b. Napropamide
c. Naproanilide

3. Oxyacetamides
a. Flufenacet
b. Mefenacet

4. Tetrazolinones
a. Fentrazamide

5. Others
a. Anilofos
b. Cafenstrole
c. Indanofan
d. Piperophos

P. Inhibition of cell wall (cellulose) synthesis
1. Nitriles (WSSA Group 20)
a. Dichlobenil
b. Chlorthiamid

2. Benzamides (isoxaben (WSSA Group 21))
a. Isoxaben

3. Triazolocarboxamides (flupoxam)
a. Flupoxam

Q. Uncoupling (membrane disruption): (WSSA Group 24)
1. Dinitrophenols
a. DNOC
b. Dinoseb

c. Dinoterb

R. Inhibition of Lipid Synthesis by other than ACC inhibition

1. Thiocarbamates (WSSA Group 8)
   a. Butylate
   b. Cycloate
   c. Dimepiperate
   d. EPTC
   e. Esprocarb
   f. Molinate
   g. Orbencarb
   h. Pebulate
   i. Prosulfocarb
   j. Benthiocarb
   k. Tiocarbazil
   l. Triallate
   m. Vernolate

2. Phosphorodithioates
   a. Bensulide

3. Benzofurans
   a. Benfuresate
   b. Ethofumesate

4. Halogenated alkanoic acids (WSSA Group 26)
   a. TCA
   b. Dalapon
   c. Flupropanate

S. Synthetic auxins (IAA-like) (WSSA Group 4)
  1. Phenoxycarboxylic acids
    a. Clomeprop
    b. 2,4-D
    c. Mecoprop

  2. Benzoic acids
    a. Dicamba
    b. Chloramben
    c. TBA

  3. Pyridine carboxylic acids
    a. Clopyralid
    b. Fluroxypyr
    c. Picloram
    d. Tricyclopyr
  4. Quinoline carboxylic acids
    a. Quinclorac
    b. Quinmerac

  5. Others (benazolin-ethyl)
    a. Benazolin-ethyl

T. Inhibition of Auxin Transport
  1. Phthalamates; semicarbazones (WSSA Group 19)
    a. Naptalam
    b. Diflufenzopyr-Na

U. Other Mechanism of Action
  1. Arylaminopropionic acids
    a. Flamprop-M-methyl /-isopropyl

  2. Pyrazolium
    a. Difenzoquat

  3. Organoarsenicals
    a. DSMA
    b. MSMA

  4. Others
    a. Bromobutide
    b. Cinmethylin
    c. Cumyluron
    d. Dazomet
    e. Daimuron-methyl
    f. Dimuron
    g. Etobenzanid
    h. Fosamine
    i. Metam
    j. Oxaziclomefone
    k. Oleic acid

l. Pelargonic acid
m. Pyributicarb

In one embodiment, one ALS inhibitor or at least two ALS inhibitors are applied to the 3560.4.3.5 soybean crop or area of cultivation. In non-limiting embodiments, the combination of ALS inhibitor herbicides can include or does not include glyphosate. The ALS inhibitor can be applied at any effective rate that selectively controls weeds and does not significantly damage the crop. In specific embodiments, at least one ALS inhibitor is applied at a level that would significantly damage an appropriate control plant. In other embodiments, at least one ALS inhibitor is applied above the recommended label use rate for the crop. In still other embodiments, a mixture of ALS inhibitors is applied at a lower rate than the recommended use rate and weeds continue to be selectively controlled. Herbicides that inhibit acetolactate synthase (also known as acetohydroxy acid synthase) and are therefore useful in the methods include sulfonylureas as listed in Table 1, including agriculturally suitable salts (*e.g.*, sodium salts) thereof; sulfonylaminocarbonyltriazolinones as listed in Table 1, including agriculturally suitable salts (*e.g.*, sodium salts) thereof; triazolopyrimidines as listed in Table 1, including agriculturally suitable salts (*e.g.*, sodium salts) thereof; pyrimidinyloxy(thio)benzoates as listed in Table 1, including agriculturally suitable salts (*e.g.*, sodium salts) thereof; and imidazolinones as listed in Table 1, including agriculturally suitable salts (*e.g.*, sodium salts) thereof. In some embodiments, methods comprise the use of a sulfonylurea which is not chlorimuron-ethyl, chlorsulfuron, rimsulfuron, thifensulfuron-methyl, or tribenuron-methyl.

In still further methods, glyphosate, alone or in combination with another herbicide of interest, can be applied to the 3560.4.3.5 soybean plants or their area of cultivation. Non-limiting examples of glyphosate formations are set forth in Table 2. In specific embodiments, the glyphosate is in the form of a salt, such as, ammonium, isopropylammonium, potassium, sodium (including sesquisodium) or trimesium (alternatively named sulfosate). In still further embodiments, a mixture of a synergistically effective amount of a combination of glyphosate and an ALS inhibitor (such as a sulfonylurea) is applied to the 3560.4.3.5 soybean plants or their area of cultivation.

Table 2. Glyphosate formulations comparisons.

Herbicide by Registered Trademark	*Manufacturer*	*Salt*	*Active ingredient per gallon*	*Acid equivalent per gallon*	*Apply fl oz/ acre*	*Acid equivalent per acre*
Roundup Original	Monsanto	Isopropylamine	4	3	32	0.750
Roundup Original II	Monsanto	Isopropylamine	4	3	32	0.750
Roundup Original MAX	Monsanto	Potassium	5.5	4.5	22	0.773
RoundUp UltraMax	Monsanto	Isopropylamine	5	3.68	26	0.748
Roundup UltraMax II	Monsanto	Potassium	5.5	4.5	22	0.773
Roundup Weathermax	Monsanto	Potassium	5.5	4.5	22	0.773
Touchdown	Syngenta	Diammonium	3.7	3	32	0.750
Touchdown HiTech	Syngenta	Potassium	6.16	5	20	0.781
Touchdown Total	Syngenta	Potassium	5.14	4.17	24	0.782
Durango	Dow AgroSciences	Isopropylamine	5.4	4	24	0.750
Glyphomax	Dow AgroSciences	Isopropylamine	4	3	32	0.750
Glyphomax Plus	Dow AgroSciences	Isopropylamine	4	3	32	0.750
Glyphomax XRT	Dow AgroSciences	Isopropylamine	4	3	32	0.750
Gly Star Plus	AlbaughAgri Star	Isopropylamine	4	3	32	0.750
Gly Star 5	AlbaughAgri Star	Isopropylamine	5.4	4	24	0.750
Gly Star Original	AlbaughAgri Star	Isopropylamine	4	3	32	0.750
Gly Flo	Micro Flo	Isopropylamine	4	3	32	0.750
Credit	Nufarm	Isopropylamine	4	3	32	0.750
Credit Extra	Nufarm	Isopropylamine	4	3	32	0.750
Credit Duo	Nufarm	Isopro.+ monoamm.	4	3	32	0.750
Credit Duo Extra	Nufarm	Isopro.+ monoamm.	4	3	32	0.750
Extra Credit 5	Nufarm	Isopropylamine	5	3.68	26	0.748
Cornerstone	Agriliance	Isopropylamine	4	3	32	0.750
Cornerstone Plus	Agriliance	Isopropylamine	4	3	32	0.750
Glyfos	Chemnova	Isopropylamine	4	3	32	0.750
Glyfos X-TRA	Chemnova	Isopropylamine	4	3	32	0.750
Rattler	Helena	Isopropylamine	4	3	32	0.750
Rattler Plus	Helena	Isopropylamine	4	3	32	0.750
Mirage	UAP	Isopropylamine	4	3	32	0.750
Mirage Plus	UAP	Isopropylamine	4	3	32	0.750
Glyphosate 4 Plus	Helm Agro USA	Isopropylamine	4	3	32	0.750
Buccaneer	Tenkoz	Isopropylamine	4	3	32	0.750
Buccaneer Plus	Tenkoz	Isopropylamine	4	3	32	0.750
Honcho	Monsanto	Isopropylamine	4	3	32	0.750
Honcho Plus	Monsanto	Isopropylamine	4	3	32	0.750
Gly-4	Univ. Crop Prot. Alli.	Isopropylamine	4	3	32	0.750
Gly-4 Plus	Univ. Crop Prot. Alli.	Isopropylamine	4	3	32	0.750
ClearOut 41	Chemical Products Tech	Isopropylamine	4	3	32	0.750
ClearOut 41 Plus	Chemical Products Tech	Isopropylamine	4	3	32	0.750
Spitfire	Control Solutions	Isopropylamine	4	3	32	0.750
Spitfire Plus	Control Solutions	Isopropylamine	4	3	32	0.750
Glyphosate 4	FarmerSaver.com	Isopropylamine	4	3	32	0.750
FS Glyphosate Plus	Growmark	Isopropylamine	4	3	32	0.750
Glyphosate Original	Griffin, LLC.	Isopropylamine	4	3	32	0.750

Thus, in some embodiments, a transgenic plant is used in a method of growing a 3560.4.3.5 soybean crop by the application of herbicides to which the plant is

tolerant. In this manner, treatment with a combination of one of more herbicides which include, but are not limited to: acetochlor, acifluorfen and its sodium salt, aclonifen, acrolein (2-propenal), alachlor, alloxydim, ametryn, amicarbazone, amidosulfuron, aminopyralid, amitrole, ammonium sulfamate, anilofos, asulam, atrazine, azimsulfuron, beflubutamid, benazolin, benazolin-ethyl, bencarbazone, benfluralin, benfuresate, bensulfuron-methyl, bensulide, bentazone, benzobicyclon, benzofenap, bifenox, bilanafos, bispyribac and its sodium salt, bromacil, bromobutide, bromofenoxim, bromoxynil, bromoxynil octanoate, butachlor, butafenacil, butamifos, butralin, butroxydim, butylate, cafenstrole, carbetamide, carfentrazone-ethyl, catechin, chlomethoxyfen, chloramben, chlorbromuron, chlorflurenol-methyl, chloridazon, chlorimuron-ethyl, chlorotoluron, chlorpropham, chlorsulfuron, chlorthal-dimethyl, chlorthiamid, cinidon-ethyl, cinmethylin, cinosulfuron, clethodim, clodinafop-propargyl, clomazone, clomeprop, clopyralid, clopyralid-olamine, cloransulam-methyl, CUH-35 (2-methoxyethyl 2-[[[4-chloro-2-fluoro-5-[(1-methyl-2-propynyl)oxy]phenyl](3-fluorobenzoyl)amino]carbonyl]-1-cyclohexene-1-carboxylate), cumyluron, cyanazine, cycloate, cyclosulfamuron, cycloxydim, cyhalofop-butyl, 2,4-D and its butotyl, butyl, isoctyl and isopropyl esters and its dimethylammonium, diolamine and trolamine salts, daimuron, dalapon, dalapon-sodium, dazomet, 2,4-DB and its dimethylammonium, potassium and sodium salts, desmedipham, desmetryn, dicamba and its diglycolammonium, dimethylammonium, potassium and sodium salts, dichlobenil, dichlorprop, diclofop-methyl, diclosulam, difenzoquat metilsulfate, diflufenican, diflufenzopyr, dimefuron, dimepiperate, dimethachlor, dimethametryn, dimethenamid, dimethenamid-P, dimethipin, dimethylarsinic acid and its sodium salt, dinitramine, dinoterb, diphenamid, diquat dibromide, dithiopyr, diuron, DNOC, endothal, EPTC, esprocarb, ethalfluralin, ethametsulfuron-methyl, ethofumesate, ethoxyfen, ethoxysulfuron, etobenzanid, fenoxaprop-ethyl, fenoxaprop-P-ethyl, fentrazamide, fenuron, fenuron-TCA, flamprop-methyl, flamprop-M-isopropyl, flamprop-M-methyl, flazasulfuron, florasulam, fluazifop-butyl, fluazifop-P-butyl, flucarbazone, flucetosulfuron, fluchloralin, flufenacet, flufenpyr, flufenpyr-ethyl, flumetsulam, flumiclorac-pentyl, flumioxazin, fluometuron, fluoroglycofen-ethyl, flupyrsulfuron-methyl and its sodium salt, flurenol, flurenol-butyl, fluridone, flurochloridone, fluroxypyr, flurtamone, fluthiacet-methyl, fomesafen, foramsulfuron, fosamine-ammonium, glufosinate, glufosinate-ammonium, glyphosate and its salts

such as ammonium, isopropylammonium, potassium, sodium (including sesquisodium) and trimesium (alternatively named sulfosate), halosulfuron-methyl, haloxyfop-etotyl, haloxyfop-methyl, hexazinone, HOK-201 (*N*-(2,4-difluorophenyl)-1,5-dihydro-*N*-(1-methylethyl)-5-oxo-1-[(tetrahydro-2*H*-pyran-2-yl)methyl]-4*H*-1,2,4-triazole-4-carboxamide), imazamethabenz-methyl, imazamox, imazapic, imazapyr, imazaquin, imazaquin-ammonium, imazethapyr, imazethapyr-ammonium, imazosulfuron, indanofan, iodosulfuron-methyl, ioxynil, ioxynil octanoate, ioxynil-sodium, isoproturon, isouron, isoxaben, isoxaflutole, isoxachlortole, lactofen, lenacil, linuron, maleic hydrazide, MCPA and its salts (e.g., MCPA-dimethylammonium, MCPA-potassium and MCPA-sodium, esters (e.g., MCPA-2-ethylhexyl, MCPA-butotyl) and thioesters (e.g., MCPA-thioethyl), MCPB and its salts (e.g., MCPB-sodium) and esters (e.g., MCPB-ethyl), mecoprop, mecoprop-P, mefenacet, mefluidide, mesosulfuron-methyl, mesotrione, metam-sodium, metamifop, metamitron, metazachlor, methabenzthiazuron, methylarsonic acid and its calcium, monoammonium, monosodium and disodium salts, methyldymron, metobenzuron, metobromuron, metolachlor, S-metholachlor, metosulam, metoxuron, metribuzin, metsulfuron-methyl, molinate, monolinuron, naproanilide, napropamide, naptalam, neburon, nicosulfuron, norflurazon, orbencarb, oryzalin, oxadiargyl, oxadiazon, oxasulfuron, oxaziclomefone, oxyfluorfen, paraquat dichloride, pebulate, pelargonic acid, pendimethalin, penoxsulam, pentanochlor, pentoxazone, perfluidone, pethoxyamid, phenmedipham, picloram, picloram-potassium, picolinafen, pinoxaden, piperofos, pretilachlor, primisulfuron-methyl, prodiamine, profoxydim, prometon, prometryn, propachlor, propanil, propaquizafop, propazine, propham, propisochlor, propoxycarbazone, propyzamide, prosulfocarb, prosulfuron, pyraclonil, pyraflufen-ethyl, pyrasulfotole, pyrazogyl, pyrazolynate, pyrazoxyfen, pyrazosulfuron-ethyl, pyribenzoxim, pyributicarb, pyridate, pyriftalid, pyriminobac-methyl, pyrimisulfan, pyrithiobac, pyrithiobac-sodium, pyroxsulam, quinclorac, quinmerac, quinoclamine, quizalofop-ethyl, quizalofop-P-ethyl, quizalofop-P-tefuryl, rimsulfuron, sethoxydim, siduron, simazine, simetryn, sulcotrione, sulfentrazone, sulfometuron-methyl, sulfosulfuron, 2,3,6-TBA, TCA, TCA-sodium, tebutam, tebuthiuron, tefuryltrione, tembotrione, tepraloxydim, terbacil, terbumeton, terbuthylazine, terbutryn, thenylchlor, thiazopyr, thiencarbazone, thifensulfuron-methyl, thiobencarb, tiocarbazil, topramezone, tralkoxydim, tri-allate, triasulfuron, triaziflam, tribenuron-methyl, triclopyr, triclopyr-butotyl, triclopyr-triethylammonium,

tridiphane, trietazine, trifloxysulfuron, trifluralin, triflusulfuron-methyl, tritosulfuron and vernolate is disclosed.

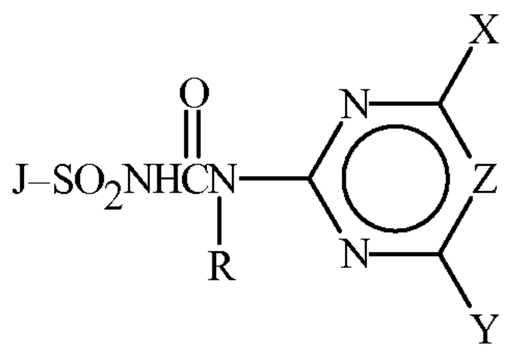
Other suitable herbicides and agricultural chemicals are known in the art, such as, for example, those described in WO 2005/041654. Other herbicides also include bioherbicides such as *Alternaria destruens* Simmons, *Colletotrichum gloeosporiodes* (Penz.) Penz. & Sacc., *Drechsiera monoceras* (MTB-951), *Myrothecium verrucaria* (Albertini & Schweinitz) Ditmar: Fries, *Phytophthora palmivora* (Butl.) Butl. and *Puccinia thlaspeos* Schub. Combinations of various herbicides can result in a greater-than-additive (*i.e.*, synergistic) effect on weeds and/or a less-than-additive effect (*i.e.* safening) on crops or other desirable plants. In certain instances, combinations of glyphosate with other herbicides having a similar spectrum of control but a different mode of action will be particularly advantageous for preventing the development of resistant weeds. Herbicidally effective amounts of any particular herbicide can be easily determined by one skilled in the art through simple experimentation.

Herbicides may be classified into groups and/or subgroups as described herein above with reference to their mode of action, or they may be classified into groups and/or subgroups in accordance with their chemical structure.

Sulfonamide herbicides have as an essential molecular structure feature a sulfonamide moiety (–$S(O)_2NH$–). As referred to herein, sulfonamide herbicides particularly comprise sulfonylurea herbicides, sulfonylaminocarbonyltriazolinone herbicides and triazolopyrimidine herbicides. In sulfonylurea herbicides the sulfonamide moiety is a component in a sulfonylurea bridge (–$S(O)_2NHC(O)NH(R)$–). In sulfonylurea herbicides the sulfonyl end of the sulfonylurea bridge is connected either directly or by way of an oxygen atom or an optionally substituted amino or methylene group to a typically substituted cyclic or acyclic group. At the opposite end of the sulfonylurea bridge, the amino group, which may have a substituent such as methyl (R being CH_3) instead of hydrogen, is connected to a heterocyclic group, typically a symmetric pyrimidine or triazine ring, having one or two substituents such as methyl, ethyl, trifluoromethyl, methoxy, ethoxy, methylamino, dimethylamino, ethylamino and the halogens. In sulfonylaminocarbonyltriazolinone herbicides, the sulfonamide moiety is a component of a sulfonylaminocarbonyl bridge (–$S(O)_2NHC(O)$–). In sulfonylaminocarbonyltriazolinone herbicides the sulfonyl end of the sulfonylaminocarbonyl bridge is typically connected to substituted phenyl ring.

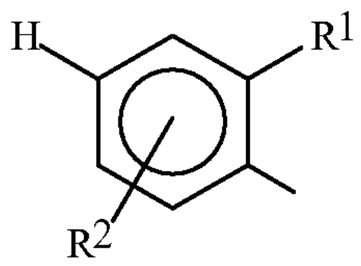
At the opposite end of the sulfonylaminocarbonyl bridge, the carbonyl is connected to the 1-position of a triazolinone ring, which is typically substituted with groups such as alkyl and alkoxy. In triazolopyrimidine herbicides the sulfonyl end of the sulfonamide moiety is connected to the 2-position of a substituted [1,2,4]triazolopyrimidine ring system and the amino end of the sulfonamide moiety is connected to a substituted aryl, typically phenyl, group or alternatively the amino end of the sulfonamide moiety is connected to the 2-position of a substituted [1,2,4]triazolopyrimidine ring system and the sulfonyl end of the sulfonamide moiety is connected to a substituted aryl, typically pyridinyl, group.

Representative of the sulfonylurea herbicides useful in the embodiments are those of the formula:

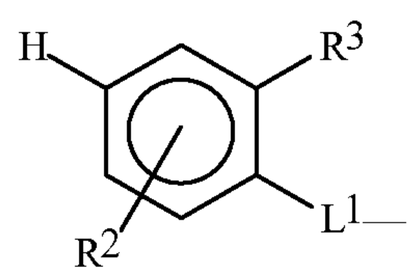


wherein:

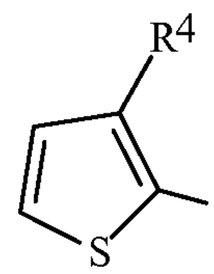
J is selected from the group consisting of



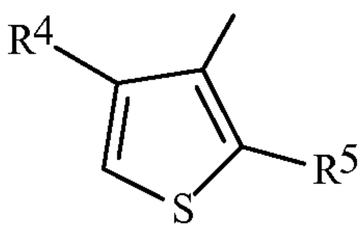
J-1



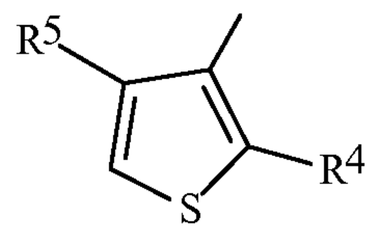
J-2



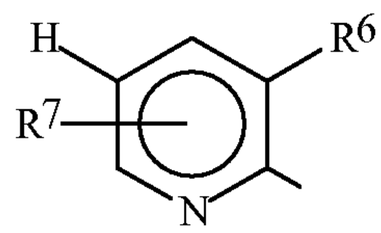
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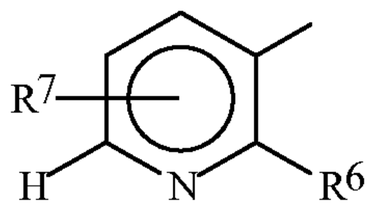
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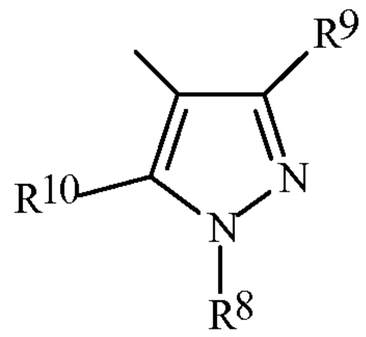
J-5



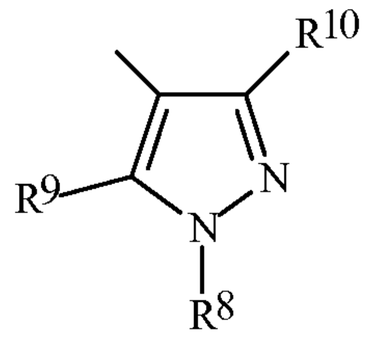
J-6



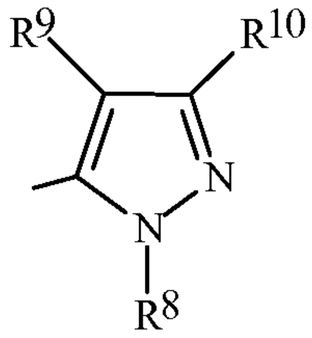
J-7



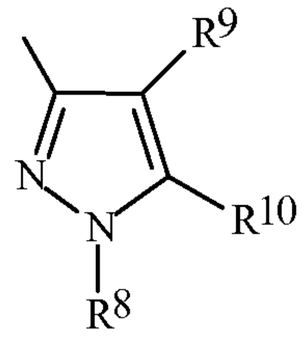
J-8



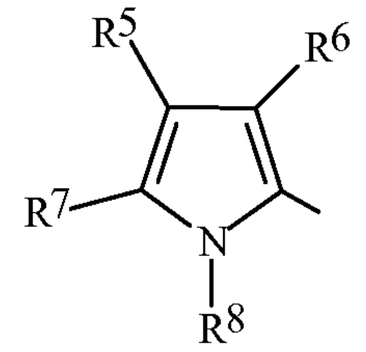
J-9



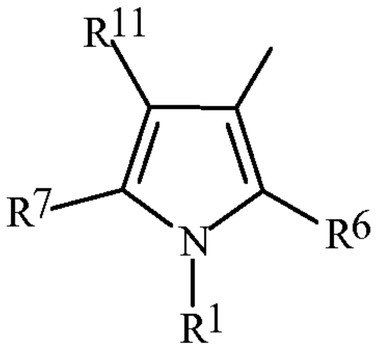
J-10



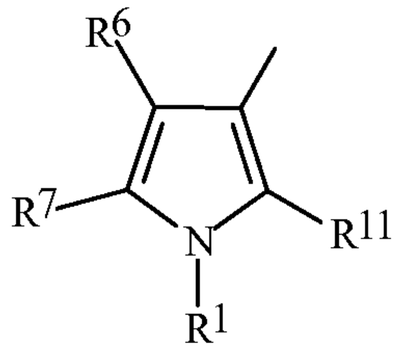
J-11



J-12

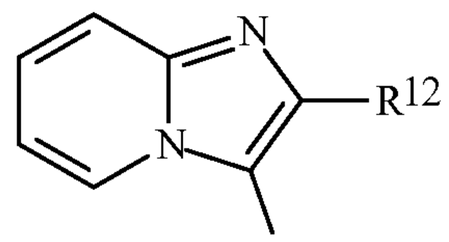


J-13



J-14

and



J-15

; or

J is $R^{13}SO_2N(CH_3)$-;

R is H or CH_3;

R^1 is F, Cl, Br, NO_2, C_1–C_4 alkyl, C_1–C_4 haloalkyl, C_3–C_4 cycloalkyl, C_2–C_4 haloalkenyl, C_1–C_4 alkoxy, C_1–C_4 haloalkoxy, C_2–C_4 alkoxyalkoxy, CO_2R^{14}, $C(O)NR^{15}R^{16}$, $SO_2NR^{17}R^{18}$, $S(O)_nR^{19}$, $C(O)R^{20}$, CH_2CN or L;

R^2 is H, F, Cl, Br, I, CN, CH_3, OCH_3, SCH_3, CF_3 or OCF_2H;

R^3 is Cl, NO_2, CO_2CH_3, $CO_2CH_2CH_3$, $C(O)CH_3$, $C(O)CH_2CH_3$, C(O)-cyclopropyl, $SO_2N(CH_3)_2$, SO_2CH_3, $SO_2CH_2CH_3$, OCH_3 or OCH_2CH_3;

R^4 is C_1–C_3 alkyl, C_1–C_2 haloalkyl, C_1–C_2 alkoxy, C_2–C_4 haloalkenyl, F, Cl, Br, NO_2, CO_2R^{14}, $C(O)NR^{15}R^{16}$, $SO_2NR^{17}R^{18}$, $S(O)_nR^{19}$, $C(O)R^{20}$ or L;

R^5 is H, F, Cl, Br or CH_3;

R^6 is C_1–C_3 alkyl optionally substituted with 0–3 F, 0–1 Cl and 0–1 C_3–C_4 alkoxyacetyloxy, or R^6 is C_1–C_2 alkoxy, C_2–C_4 haloalkenyl, F, Cl, Br, CO_2R^{14}, $C(O)NR^{15}R^{16}$, $SO_2NR^{17}R^{18}$, $S(O)_nR^{19}$, $C(O)R^{20}$ or L;

R^7 is H, F, Cl, CH_3 or CF_3;

R^8 is H, C_1–C_3 alkyl or pyridinyl;

R^9 is C_1–C_3 alkyl, C_1–C_2 alkoxy, F, Cl, Br, NO_2, CO_2R^{14}, $SO_2NR^{17}R^{18}$, $S(O)_nR^{19}$, OCF_2H, $C(O)R^{20}$, C_2–C_4 haloalkenyl or L;

R^{10} is H, Cl, F, Br, C_1–C_3 alkyl or C_1–C_2 alkoxy;

R^{11} is H, C_1–C_3 alkyl, C_1–C_2 alkoxy, C_2–C_4 haloalkenyl, F, Cl, Br, CO_2R^{14}, $C(O)NR^{15}R^{16}$, $SO_2NR^{17}R^{18}$, $S(O)_nR^{19}$, $C(O)R^{20}$ or L;

R^{12} is halogen, C_1–C_4 alkyl or C_1–C_3 alkylsulfonyl;

R^{13} is C_1–C_4 alkyl;

R^{14} is allyl, propargyl or oxetan-3-yl; or R^{14} is C_1–C_3 alkyl optionally substituted by at least one member independently selected from halogen, C_1–C_2 alkoxy and CN;

R^{15} is H, C_1–C_3 alkyl or C_1–C_2 alkoxy;

R^{16} is C_1–C_2 alkyl;

R^{17} is H, C_1–C_3 alkyl, C_1–C_2 alkoxy, allyl or cyclopropyl;

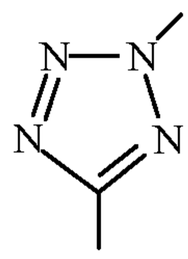
R^{18} is H or C_1–C_3 alkyl;

R^{19} is C_1–C_3 alkyl, C_1–C_3 haloalkyl, allyl or propargyl;

R^{20} is C_1–C_4 alkyl, C_1–C_4 haloalkyl or C_3–C_5 cycloalkyl optionally substituted by halogen;

n is 0, 1 or 2;

L is



;

L^1 is CH_2, NH or O;

R^{21} is H or C_1–C_3 alkyl;

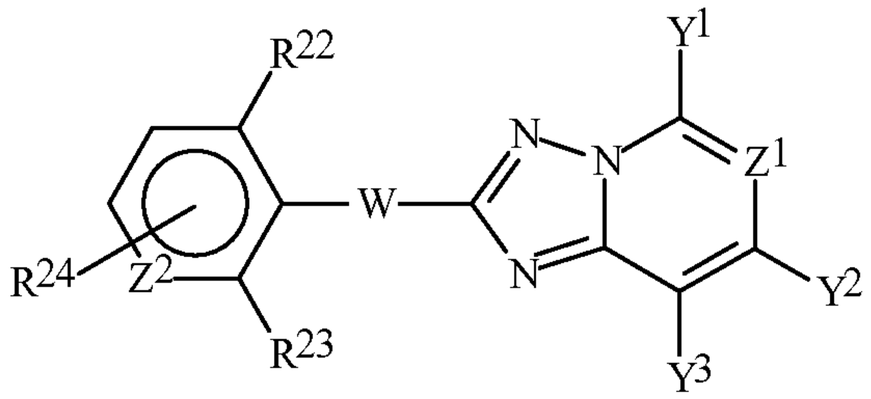
X is H, C_1–C_4 alkyl, C_1–C_4 alkoxy, C_1–C_4 haloalkoxy, C_1–C_4 haloalkyl, C_1–C_4 haloalkylthio, C_1–C_4 alkylthio, halogen, C_2–C_5 alkoxyalkyl, C_2–C_5 alkoxyalkoxy, amino, C_1–C_3 alkylamino or di(C_1–C_3 alkyl)amino;

Y is H, C_1–C_4 alkyl, C_1–C_4 alkoxy, C_1–C_4 haloalkoxy, C_1–C_4 alkylthio, C_1–C_4 haloalkylthio, C_2–C_5 alkoxyalkyl, C_2–C_5 alkoxyalkoxy, amino, C_1–C_3 alkylamino, di(C_1–C_3 alkyl)amino, C_3–C_4 alkenyloxy, C_3–C_4 alkynyloxy, C_2–C_5 alkylthioalkyl, C_2–C_5 alkylsulfinylalkyl, C_2–C_5 alkylsulfonylalkyl, C_1–C_4 haloalkyl, C_2–C_4 alkynyl, C_3–C_5 cycloalkyl, azido or cyano; and

Z is CH or N;

provided that (i) when one or both of X and Y is C_1 haloalkoxy, then Z is CH; and (ii) when X is halogen, then Z is CH and Y is OCH_3, OCH_2CH_3, $N(OCH_3)CH_3$, $NHCH_3$, $N(CH_3)_2$ or OCF_2H. Of note is the present single liquid herbicide composition comprising one or more sulfonylureas of Formula I wherein when R^6 is alkyl, said alkyl is unsubstituted.

Representative of the triazolopyrimidine herbicides contemplated for use in the embodiments are those of the formula:



wherein:

R^{22} and R^{23} each independently halogen, nitro, C_1–C_4 alkyl, C_1–C_4 haloalkyl, C_1–C_4 alkoxy, C_1–C_4 haloalkoxy or C_2–C_3 alkoxycarbonyl;

R^{24} is H, halogen, C_1–C_2 alkyl or C_1–C_2 alkoxy;

W is $-NHS(O)_2-$ or $-S(O)_2NH-$;

Y^1 is H, C_1–C_2 alkyl or C_1–C_2 alkoxy;

Y^2 is H, F, Cl, Br, C_1–C_2 alkyl or C_1–C_2 alkoxy;

Y^3 is H, F or methoxy;

Z^1 is CH or N; and

Z^2 is CH or N;

provided that at least one of Y^1 and Y^2 is other than H.

In the above Markush description of representative triazolopyrimidine herbicides, when W is $-NHS(O)_2-$ the sulfonyl end of the sulfonamide moiety is connected to the [1,2,4]triazolopyrimidine ring system, and when W is $-S(O)_2NH-$ the amino end of the sulfonamide moiety is connected to the [1,2,4]triazolopyrimidine ring system.

In the above recitations, the term "alkyl", used either alone or in compound words such as "alkylthio" or "haloalkyl" includes straight-chain or branched alkyl, such as, methyl, ethyl, *n*-propyl, *i*-propyl, or the different butyl isomers. "Cycloalkyl" includes, for example, cyclopropyl, cyclobutyl and cyclopentyl. "Alkenyl" includes straight-chain or branched alkenes such as ethenyl, 1-propenyl, 2-propenyl, and the different butenyl isomers. "Alkenyl" also includes polyenes such as 1,2-propadienyl and 2,4-butadienyl. "Alkynyl" includes straight-chain or branched alkynes such as ethynyl, 1-propynyl, 2-propynyl and the different butynyl isomers. "Alkynyl" can also include moieties comprised of multiple triple bonds such as 2,5-hexadiynyl. "Alkoxy" includes, for example, methoxy, ethoxy, *n*-propyloxy, isopropyloxy and the different butoxy isomers. "Alkoxyalkyl" denotes alkoxy substitution on alkyl. Examples of "alkoxyalkyl" include CH_3OCH_2, $CH_3OCH_2CH_2$, $CH_3CH_2OCH_2$,

$CH_3CH_2CH_2CH_2OCH_2$ and $CH_3CH_2OCH_2CH_2$. "Alkoxyalkoxy" denotes alkoxy substitution on alkoxy. "Alkenyloxy" includes straight-chain or branched alkenyloxy moieties. Examples of "alkenyloxy" include $H_2C=CHCH_2O$, $(CH_3)CH=CHCH_2O$ and $CH_2=CHCH_2CH_2O$. "Alkynyloxy" includes straight-chain or branched alkynyloxy moieties. Examples of "alkynyloxy" include $HC\equiv CCH_2O$ and $CH_3C\equiv CCH_2O$. "Alkylthio" includes branched or straight-chain alkylthio moieties such as methylthio, ethylthio, and the different propylthio isomers. "Alkylthioalkyl" denotes alkylthio substitution on alkyl. Examples of "alkylthioalkyl" include CH_3SCH_2, $CH_3SCH_2CH_2$, $CH_3CH_2SCH_2$, $CH_3CH_2CH_2CH_2SCH_2$ and $CH_3CH_2SCH_2CH_2$; "alkylsulfinylalkyl" and "alkylsulfonylalkyl" include the corresponding sulfoxides and sulfones, respectively. Other substituents such as "alkylamino", "dialkylamino" are defined analogously.

The total number of carbon atoms in a substituent group is indicated by the "C_i–C_j" prefix where i and j are numbers from 1 to 5. For example, C_1–C_4 alkyl designates methyl through butyl, including the various isomers. As further examples, C_2 alkoxyalkyl designates CH_3OCH_2; C_3 alkoxyalkyl designates, for example, $CH_3CH(OCH_3)$, $CH_3OCH_2CH_2$ or $CH_3CH_2OCH_2$; and C_4 alkoxyalkyl designates the various isomers of an alkyl group substituted with an alkoxy group containing a total of four carbon atoms, examples including $CH_3CH_2CH_2OCH_2$ and $CH_3CH_2OCH_2CH_2$.

The term "halogen", either alone or in compound words such as "haloalkyl", includes fluorine, chlorine, bromine or iodine. Further, when used in compound words such as "haloalkyl", said alkyl may be partially or fully substituted with halogen atoms which may be the same or different. Examples of "haloalkyl" include F_3C, $ClCH_2$, CF_3CH_2 and CF_3CCl_2. The terms "haloalkoxy", "haloalkylthio", and the like, are defined analogously to the term "haloalkyl". Examples of "haloalkoxy" include CF_3O, CCl_3CH_2O, $HCF_2CH_2CH_2O$ and CF_3CH_2O. Examples of "haloalkylthio" include CCl_3S, CF_3S, CCl_3CH_2S and $ClCH_2CH_2CH_2S$.

The following sulfonylurea herbicides illustrate the sulfonylureas useful for this invention: amidosulfuron (*N*-[[[[(4,6-dimethoxy-2-pyrimdinyl)amino]carbonyl]amino]sulfonyl]-*N*-methylmethanesulfonamide),

azimsulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-1-methyl-4-(2-methyl-2*H*-tetrazol-5-yl)-1*H*-pyrazole-5-sulfonamide), bensulfuron-methyl (methyl 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]-sulfonyl]methyl]benzoate), chlorimuron-ethyl (ethyl 2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoate), chlorsulfuron (2-chloro-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide), cinosulfuron (*N*-[[(4,6-dimethoxy-1,3,5-triazin-2-yl)amino]carbonyl]-2-(2-methoxy-ethoxy)benzenesulfonamide), cyclosulfamuron (*N*-[[[2-(cyclopropylcarbonyl)phenyl]-amino]sulfonyl]-N^1-(4,6-dimethoxypyrimidin-2-yl)urea), ethametsulfuron-methyl (methyl 2-[[[[[4-ethoxy-6-(methylamino)-1,3,5-triazin-2-yl]amino]carbonyl]amino]-sulfonyl]benzoate), ethoxysulfuron (2-ethoxyphenyl [[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]sulfamate), flazasulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(trifluoromethyl)-2-pyridinesulfonamide), flucetosulfuron (1-[3-[[[[(4,6-dimethoxy-2-pyrimidinyl)-amino]carbonyl]amino]sulfonyl]-2-pyridinyl]-2-fluoropropyl methoxyacetate), flupyrsulfuron-methyl (methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-amino]sulfonyl]-6-(trifluoromethyl)-3-pyridinecarboxylate), foramsulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-(formylamino)-*N,N*-dimethylbenzamide), halosulfuron-methyl (methyl 3-chloro-5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1*H*-pyrazole-4-carboxylate), imazosulfuron (2-chloro-*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]-carbonyl]imidazo[1,2-*a*]pyridine-3-sulfonamide), iodosulfuron-methyl (methyl 4-iodo-2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate), mesosulfuron-methyl (methyl 2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-[[(methylsulfonyl)amino]methyl]benzoate), metsulfuron-methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate), nicosulfuron (2-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-*N,N*-dimethyl-3-pyridinecarboxamide), oxasulfuron (3-oxetanyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoate), primisulfuron-methyl (methyl 2-[[[[[4,6-bis(trifluoromethoxy)-2-pyrimidinyl]amino]carbonyl]amino]sulfonyl]benzoate), prosulfuron (*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]-2-(3,3,3-trifluoro-

propyl)benzenesulfonamide), pyrazosulfuron-ethyl (ethyl 5-[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-1-methyl-1*H*-pyrazole-4-carboxylate), rimsulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide), sulfometuron-methyl (methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoate), sulfosulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-2-(ethylsulfonyl)imidazo[1,2-*a*]pyridine-3-sulfonamide), thifensulfuron-methyl (methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-thiophenecarboxylate), triasulfuron (2-(2-chloroethoxy)-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]benzenesulfonamide), tribenuron-methyl (methyl 2-[[[[*N*-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-*N*-methylamino]carbonyl]amino]-sulfonyl]benzoate), trifloxysulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]-carbonyl]-3-(2,2,2-trifluoroethoxy)-2-pyridinesulfonamide), triflusulfuron-methyl (methyl 2-[[[[[4-dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]amino]-carbonyl]amino]sulfonyl]-3-methylbenzoate) and tritosulfuron (*N*-[[[4-methoxy-6-(trifluoromethyl)-1,3,5-triazin-2-yl]amino]carbonyl]-2-(trifluoromethyl)benzene-sulfonamide).

The following triazolopyrimidine herbicides illustrate the triazolopyrimidines useful for this invention: cloransulam-methyl (methyl 3-chloro-2-[[(5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)sulfonyl]amino]benzoate, diclosulam (*N*-(2,6-dichlorophenyl)-5-ethoxy-7-fluoro[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide, florasulam (*N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo-[1,5-*c*]pyrimidine-2-sulfonamide), flumetsulam (*N*-(2,6-difluorophenyl)-5-methyl[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide), metosulam (*N*-(2,6-dichloro-3-methylphenyl)-5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidine-2-sulfonamide), penoxsulam (2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6-(trifluoromethyl)benzenesulfonamide) and pyroxsulam (*N*-(5,7-dimethoxy[1,2,4]triazolo[1,5-*a*]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide).

The following sulfonylaminocarbonyltriazolinone herbicides illustrate the sulfonylaminocarbonyltriazolinones useful for this invention: flucarbazone (4,5-dihydro-3-methoxy-4-methyl-5-oxo-*N*-[[2-(trifluoromethoxy)phenyl]sulfonyl]-1*H*-1,2,4-triazole-1-carboxamide) and procarbazone (methyl 2-[[[(4,5-dihydro-4-methyl-5-oxo-3-propoxy-1*H*-1,2,4-triazol-1-yl)carbonyl]amino]sulfonyl]benzoate).

Additional herbicides include phenmedipham, triazolinones, and the herbicides disclosed in WO2006/012981, herein incorporated by reference in its entirety.

The methods further comprise applying to the crop and the weeds in a field a sufficient amount of at least one herbicide to which the crop seeds or plants is tolerant, such as, for example, glyphosate, a hydroxyphenylpyruvatedioxygenase inhibitor (*e.g.*, mesotrione or sulcotrione), a phytoene desaturase inhibitor (*e.g.*, diflufenican), a pigment synthesis inhibitor, sulfonamide, imidazolinone, bialaphos, phosphinothricin, azafenidin, butafenacil, sulfosate, glufosinate, triazolopyrimidine, pyrimidinyloxy(thio)benzoate, or sulonylaminocarbonyltriazolinone, an acetyl Co-A carboxylase inhibitor such as quizalofop-P-ethyl, a synthetic auxin such as quinclorac, or a protox inhibitor to control the weeds without significantly damaging the crop plants.

Generally, the effective amount of herbicide applied to the field is sufficient to selectively control the weeds without significantly affecting the crop. "Weed" as used herein refers to a plant which is not desirable in a particular area. Conversely, a "crop plant" as used herein refers to a plant which is desired in a particular area, such as, for example, a soybean plant. Thus, in some embodiments, a weed is a non-crop plant or a non-crop species, while in some embodiments, a weed is a crop species which is sought to be eliminated from a particular area, such as, for example, an inferior and/or non-transgenic soybean plant in a field planted with soybean event 3560.4.3.5, or a soybean plant in a field planted with 3560.4.3.5. Weeds can be either classified into two major groups: monocots and dicots.

Many plant species can be controlled (*i.e.*, killed or damaged) by the herbicides described herein. Accordingly, the methods are useful in controlling these plant species where they are undesirable (*i.e.*, where they are weeds). These plant species include crop plants as well as species commonly considered weeds, including but not limited to species such as: blackgrass (*Alopecurus myosuroides*), giant foxtail (*Setaria faberi*), large crabgrass (*Digitaria sanguinalis*), Surinam grass (*Brachiaria decumbens*), wild oat (*Avena fatua*), common cocklebur (*Xanthium pensylvanicum*), common lambsquarters (*Chenopodium album*), morning glory (*Ipomoea coccinea*), pigweed (*Amaranthus spp.*), velvetleaf (*Abutilion theophrasti*), common barnyardgrass (*Echinochloa crus-galli*), bermudagrass (*Cynodon dactylon*), downy brome (*Bromus tectorum*), goosegrass (*Eleusine indica*), green foxtail (*Setaria viridis*), Italian ryegrass (*Lolium multiflorum*), Johnsongrass (*Sorghum halepense*),

lesser canarygrass (*Phalaris minor*), windgrass (*Apera spica-venti*), wooly cupgrass (*Erichloa villosa*), yellow nutsedge (*Cyperus esculentus*), common chickweed (*Stellaria media*), common ragweed (*Ambrosia artemisiifolia*), *Kochia scoparia*, horseweed (*Conyza canadensis*), rigid ryegrass (*Lolium rigidum*), goosegrass (*Eleucine indica*), hairy fleabane (*Conyza bonariensis*), buckhorn plantain (*Plantago lanceolata*), tropical spiderwort (*Commelina benghalensis*), field bindweed (*Convolvulus arvensis*), purple nutsedge (*Cyperus rotundus*), redvine (*Brunnichia ovata*), hemp sesbania (*Sesbania exaltata*), sicklepod (*Senna obtusifolia*), Texas blueweed (*Helianthus ciliaris*), and Devil's claws (*Proboscidea louisianica*). In other embodiments, the weed comprises a herbicide-resistant ryegrass, for example, a glyphosate resistant ryegrass, a paraquat resistant ryegrass, a ACCase-inhibitor resistant ryegrass, and a non-selective herbicide resistant ryegrass. In some embodiments, the undesired plants are proximate the crop plants.

As used herein, by "selectively controlled" it is intended that the majority of weeds in an area of cultivation are significantly damaged or killed, while if crop plants are also present in the field, the majority of the crop plants are not significantly damaged. Thus, a method is considered to selectively control weeds when at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more of the weeds are significantly damaged or killed, while if crop plants are also present in the field, less than 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1% of the crop plants are significantly damaged or killed.

In some embodiments, a soybean 3560.4.3.5 plant is not significantly damaged by treatment with a particular herbicide applied to that plant at a dose equivalent to a rate of at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 150, 170, 200, 300, 400, 500, 600, 700, 800, 800, 1000, 2000, 3000, 4000, 5000 or more grams or ounces (1 ounce = 29.57ml) of active ingredient or commercial product or herbicide formulation per acre or per hectare, whereas an appropriate control plant is significantly damaged by the same treatment.

In specific embodiments, an effective amount of an ALS inhibitor herbicide comprises at least about 0.1, 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 750, 800, 850, 900, 950, 1000, 2000, 3000, 4000, 5000, or more grams or ounces (1 ounce = 29.57ml) of active ingredient per hectare. In other embodiments, an effective amount of an ALS inhibitor comprises at least about 0.1-

50, about 25-75, about 50-100, about 100-110, about 110-120, about 120-130, about 130-140, about 140-150, about 150-200, about 200-500, about 500-600, about 600-800, about 800-1000, or greater grams or ounces (1 ounce = 29.57ml) of active ingredient per hectare. Any ALS inhibitor, for example, those listed in Table 1 can be applied at these levels.

In other embodiments, an effective amount of a sulfonylurea comprises at least 0.1, 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900, 1000, 5000 or more grams or ounces (1 ounce = 29.57ml) of active ingredient per hectare. In other embodiments, an effective amount of a sulfonylurea comprises at least about 0.1-50, about 25-75, about 50-100, about 100-110, about 110-120, about 120-130, about 130-140, about 140-150, about 150-160, about 160-170, about 170-180, about 190-200, about 200-250, about 250-300, about 300-350, about 350-400, about 400-450, about 450-500, about 500-550, about 550-600, about 600-650, about 650-700, about 700-800, about 800-900, about 900-1000, about 1000-2000, or more grams or ounces (1 ounce = 29.57ml) of active ingredient per hectare. Representative sulfonylureas that can be applied at this level are set forth in Table 1.

In other embodiments, an effective amount of a sulfonylaminocarbonyltriazolinones, triazolopyrimidines, pyrimidinyloxy(thio)benzoates, and imidazolinones can comprise at least about 0.1, 1, 5, 10, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1500, 1550, 1600, 1650, 1700, 1800, 1850, 1900, 1950, 2000, 2500, 3500, 4000, 4500, 5000 or greater grams or ounces (1 ounce = 29.57ml) active ingredient per hectare. In other embodiments, an effective amount of a sulfonyluminocarbonyltriazolines, triazolopyrimidines, pyrimidinyloxy(thio)benzoates, or imidazolinones comprises at least about 0.1-50, about 25-75, about 50-100, about 100-110, about 110-120, about 120-130, about 130-140, about 140-150, about 150-160, about 160-170, about 170-180, about 190-200, about 200-250, about 250-300, about 300-350, about 350-400, about 400-450, about 450-500, about 500-550, about 550-600, about 600-650, about 650-700, about 700-800, about 800-900, about 900-1000, about 1000-2000, or more grams or ounces (1 ounce = 29.57 ml) active ingredient per hectare.

Additional ranges of the effective amounts of herbicides can be found, for example, in various publications from University Extension services. See, for

example, Bernards *et al.* (2006) *Guide for Weed Management in Nebraska* (www.ianrpubs.url.edu/sendlt/ec130); Regher *et al.* (2005) *Chemical Weed Control for Fields Crops, Pastures, Rangeland, and Noncropland*, Kansas State University Agricultural Extension Station and Corporate Extension Service; Zollinger *et al.* (2006) *North Dakota Weed Control Guide*, North Dakota Extension Service, and the Iowa State University Extension at www.weeds.iastate.edu, each of which is herein incorporated by reference.

In some embodiments, glyphosate is applied to an area of cultivation and/or to at least one plant in an area of cultivation at rates between 8 and 32 ounces of acid equivalent per acre, or at rates between 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30 ounces of acid equivalent per acre at the lower end of the range of application and between 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, and 32 ounces of acid equivalent per acre at the higher end of the range of application (1 ounce = 29.57 ml). In other embodiments, glyphosate is applied at least at 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or greater ounce of active ingredient per hectare (1 ounce = 29.57 ml). In some embodiments, a sulfonylurea herbicide is applied to a field and/or to at least one plant in a field at rates between 0.04 and 1.0 ounces of active ingredient per acre, or at rates between 0.1, 0.2, 0.4, 0.6, and 0.8 ounces of active ingredient per acre at the lower end of the range of application and between 0.2, 0.4, 0.6, 0.8, and 1.0 ounces of active ingredient per acre at the higher end of the range of application. (1 ounce = 29.57 ml)

Glyphosate herbicides as a class contain the same active ingredient, but the active ingredient is present as one of a number of different salts and/or formulations. However, herbicides known to inhibit ALS vary in their active ingredient as well as their chemical formulations. One of skill in the art is familiar with the determination of the amount of active ingredient and/or acid equivalent present in a particular volume and/or weight of herbicide preparation.

In some embodiments, an ALS inhibitor herbicide is employed. Rates at which the ALS inhibitor herbicide is applied to the crop, crop part, seed or area of cultivation can be any of the rates disclosed herein. In specific embodiments, the rate for the ALS inhibitor herbicide is about 0.1 to about 5000 g ai/hectare, about 0.5 to about 300 g ai/hectare, or about 1 to about 150 g ai/hectare.

Generally, a particular herbicide is applied to a particular field (and any plants growing in it) no more than 1, 2, 3, 4, 5, 6, 7, or 8 times a year, or no more than 1, 2, 3, 4, or 5 times per growing season.

By "treated with a combination of" or "applying a combination of" herbicides to a crop, area of cultivation or field" it is intended that a particular field, crop or weed is treated with each of the herbicides and/or chemicals indicated to be part of the combination so that a desired effect is achieved, *i.e.*, so that weeds are selectively controlled while the crop is not significantly damaged. In some embodiments, weeds which are susceptible to each of the herbicides exhibit damage from treatment with each of the herbicides which is additive or synergistic. The application of each herbicide and/or chemical may be simultaneous or the applications may be at different times, so long as the desired effect is achieved. Furthermore, the application can occur prior to the planting of the crop.

The proportions of herbicides used with other herbicidal active ingredients in herbicidal compositions are generally in the ratio of 5000:1 to 1:5000, 1000:1 to 1:1000, 100:1 to 1:100, 10:1 to 1:10 or 5:1 to 1:5 by weight. The optimum ratios can be easily determined by those skilled in the art based on the weed control spectrum desired. Moreover, any combinations of ranges of the various herbicides disclosed in Table 1 can also be applied in the methods.

Thus, in some embodiments, improved methods for selectively controlling weeds in a field are provided wherein the total herbicide application may be less than 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1% of that used in other methods. Similarly, in some embodiments, the amount of a particular herbicide used for selectively controlling weeds in a field may be less than 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1% of the amount of that particular herbicide that would be used in other methods, *i.e.*, methods not utilizing a plant of the invention.

In some embodiments, a 3560.4.3.5 soybean plant benefits from a synergistic effect wherein the herbicide tolerance conferred by the GLYAT polypeptide and the GM-HRA polypeptide is greater than expected from simply combining the herbicide tolerance conferred by each gene separately to a transgenic plant containing them individually. See, *e.g.*, McCutchen *et al.* (1997) *J. Econ. Entomol.* 90: 1170-1180; Priesler *et al.* (1999) *J. Econ. Entomol.* 92: 598-603. As used herein, the terms "synergy," "synergistic," "synergistically" and derivations thereof, such as in a "synergistic effect" or a "synergistic herbicide combination" or a "synergistic herbicide composition" refer to circumstances under which the biological activity of a

combination of herbicides, such as at least a first herbicide and a second herbicide, is greater than the sum of the biological activities of the individual herbicides. Synergy, expressed in terms of a "Synergy Index (SI)," generally can be determined by the method described by Kull *et al. Applied Microbiology* 9, 538 (1961). See also Colby "Calculating Synergistic and Antagonistic Responses of Herbicide Combinations," *Weeds* 15, 20-22 (1967).

In other instances, the herbicide tolerance conferred on a 3560.4.3.5 plant is additive; that is, the herbicide tolerance profile conferred by the herbicide tolerance genes is what would be expected from simply combining the herbicide tolerance conferred by each gene separately to a transgenic plant containing them individually. Additive and/or synergistic activity for two or more herbicides against key weed species will increase the overall effectiveness and/or reduce the actual amount of active ingredient(s) needed to control said weeds. Where such synergy is observed, the plant may display tolerance to a higher dose or rate of herbicide and/or the plant may display tolerance to additional herbicides or other chemicals beyond those to which it would be expected to display tolerance. For example, a 3560.4.3.5 soybean plant may show tolerance to organophosphate compounds such as insecticides and/or inhibitors of 4-hydroxyphenylpyruvate dioxygenase.

Thus, for example, the 3560.4.3.5 soybean plants can exhibit greater than expected tolerance to various herbicides, including but not limited to glyphosate, ALS inhibitor chemistries, and sulfonylurea herbicides. The 3560.4.3.5 soybean plants may show tolerance to a particular herbicide or herbicide combination that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 15%, 17%, 20%, 22%, 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 300%, 400%, or 500% or more higher than the tolerance of an appropriate control plant that contains only a single herbicide tolerance gene which confers tolerance to the same herbicide or herbicide combination. Thus, 3560.4.3.5 soybean plants may show decreased damage from the same dose of herbicide in comparison to an appropriate control plant, or they may show the same degree of damage in response to a much higher dose of herbicide than the control plant. Accordingly, in specific embodiments, a particular herbicide used for selectively containing weeds in a field is more than 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 100% or greater than the amount of that

particular herbicide that would be used in other methods, i.e., methods not utilizing a plant of the invention.

In the same manner, in some embodiments, a 3560.4.3.5 soybean plant shows improved tolerance to a particular formulation of a herbicide active ingredient in comparison to an appropriate control plant. Herbicides are sold commercially as formulations which typically include other ingredients in addition to the herbicide active ingredient; these ingredients are often intended to enhance the efficacy of the active ingredient. Such other ingredients can include, for example, safeners and adjuvants (see, *e.g.*, Green and Foy (2003) "Adjuvants: Tools for Enhancing Herbicide Performance," in *Weed Biology and Management*, ed. Inderjit (Kluwer Academic Publishers, The Netherlands)). Thus, a 3560.4.3.5 soybean plant can show tolerance to a particular formulation of a herbicide (*e.g.*, a particular commercially available herbicide product) that is at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 15%, 17%, 20%, 22%, 25%, 27%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 100%, 125%, 150%, 175%, 200%, 300%, 400%, 500%, 600%, 700%, 800%, 900%, 1000%, 1100%, 1200%, 1300%, 1400%, 1500%, 1600%, 1700%, 1800%, 1900%, or 2000% or more higher than the tolerance of an appropriate control plant that contains only a single herbicide tolerance gene which confers tolerance to the same herbicide formulation.

In some embodiments, a 3560.4.3.5 soybean plant shows improved tolerance to a herbicide or herbicide class to which at least one other herbicide tolerance gene confers tolerance as well as improved tolerance to at least one other herbicide or chemical which has a different mechanism or basis of action than either glyphosate or the herbicide corresponding to said at least one other herbicide tolerance gene. This surprising benefit finds use in methods of growing crops that comprise treatment with various combinations of chemicals, including, for example, other chemicals used for growing crops. Thus, for example, a 3560.4.3.5 soybean plant may also show improved tolerance to chlorpyrifos, a systemic organophosphate insecticide. Thus, further provided is a 3560.4.3.5 soybean plant that confers tolerance to glyphosate (i.e., a *glyat* gene) and a sulfonylurea herbicide tolerance gene which shows improved tolerance to chemicals which affect the cytochrome P450 gene, and methods of use thereof. In some embodiments, the 3560.4.3.5 soybean plants also show improved tolerance to dicamba. In these embodiments, the improved tolerance to dicamba may be evident in the presence of glyphosate and a sulfonylurea herbicide.

In other methods, a herbicide combination is applied over a 3560.4.3.5 soybean plant, where the herbicide combination produces either an additive or a synergistic effect for controlling weeds. Such combinations of herbicides can allow the application rate to be reduced, a broader spectrum of undesired vegetation to be controlled, improved control of the undesired vegetation with fewer applications, more rapid onset of the herbicidal activity, or more prolonged herbicidal activity.

An "additive herbicidal composition" has a herbicidal activity that is about equal to the observed activities of the individual components. A "synergistic herbicidal combination" has a herbicidal activity higher than what can be expected based on the observed activities of the individual components when used alone. Accordingly, the presently disclosed subject matter provides a synergistic herbicide combination, wherein the degree of weed control of the mixture exceeds the sum of control of the individual herbicides. In some embodiments, the degree of weed control of the mixture exceeds the sum of control of the individual herbicides by any statistically significant amount including, for example, about 1% to 5%, about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to 40%, about 40% to about 50%, about 50% to about 60%, about 60% to about 70%, about 70% to about 80%, about 80% to about 90%, about 90% to about 100%, about 100% to 120% or greater. Further, a "synergistically effective amount" of a herbicide refers to the amount of one herbicide necessary to elicit a synergistic effect in another herbicide present in the herbicide composition. Thus, the term "synergist," and derivations thereof, refer to a substance that enhances the activity of an active ingredient (ai), i.e., a substance in a formulation from which a biological effect is obtained, for example, a herbicide.

Accordingly, in some embodiments, the presently disclosed subject matter provides a method for controlling weeds in an area of cultivation. In some embodiments, the method comprises: (a) planting the area with a 3560.4.3.5 crop seeds or crop plants; and (b) applying to the weed, the crop plants, a crop part, the area of cultivation, or a combination thereof, an effective amount of a herbicide composition comprising at least one of a synergistically effective amount of glyphosate and a synergistically effective amount of an ALS inhibitor (for example, but not limited to, a sulfonylurea herbicide), or agriculturally suitable salts thereof, wherein at least one of: (i) the synergistically effective amount of the glyphosate is lower than an amount of glyphosate required to control the weeds in the absence of

the sulfonylurea herbicide; (ii) the synergistically effective amount of the ALS inhibitor herbicide is lower than an amount of the ALS inhibitor required to control the weeds in the absence of glyphosate; and (iii) combinations thereof; and wherein the effective amount of the herbicide composition is tolerated by the crop seeds or crop plants and controls the weeds in the area of cultivation.

In some embodiments, the herbicide composition used in the presently disclosed method for controlling weeds comprises a synergistically effective amount of glyphosate and a sulfonylurea herbicide. In further embodiments, the presently disclosed synergistic herbicide composition comprises glyphosate and a sulfonylurea herbicide selected from the group consisting of metsulfuron-methyl, chlorsulfuron, and triasulfuron.

In particular embodiments, the synergistic herbicide combination further comprises an adjuvant such as, for example, an ammonium sulfate-based adjuvant, e.g., ADD-UP® (Wenkem S.A., Halfway House, Midrand, South Africa). In additional embodiments, the presently disclosed synergistic herbicide compositions comprise an additional herbicide, for example, an effective amount of a pyrimidinyloxy(thio)benzoate herbicide. In some embodiments, the pyrimidinyloxy(thio)benzoate herbicide comprises bispyribac, e.g., (VELOCITY®, Valent U.S.A. Corp., Walnut Creek, California, United States of America), or an agriculturally suitable salt thereof.

In some embodiments of the presently disclosed method for controlling undesired plants, the glyphosate is applied pre-emergence, post-emergence or pre- and post-emergence to the undesired plants or plant crops; and/or the ALS inhibitor herbicide (i.e., the sulfonylurea herbicide) is applied pre-emergence, post-emergence or pre- and post-emergence to the undesired plants or plant crops. In other embodiments, the glyphosate and/or the ALS inhibitor herbicide (i.e., the sulfonylurea herbicide) are applied together or are applied separately. In yet other embodiments, the synergistic herbicide composition is applied, e.g. step (b) above, at least once prior to planting the crop(s) of interest, e.g., step (a) above.

Weeds that can be difficult to control with glyphosate alone in fields where a crop is grown (such as, for example, a soybean crop) include but are not limited to the following: horseweed (*e.g., Conyza canadensis*); rigid ryegrass (*e.g., Lolium rigidum*); goosegrass (*e.g., Eleusine indica*); Italian ryegrass (*e.g., Lolium multiflorum*); hairy fleabane (*e.g., Conyza bonariensis*); buckhorn plantain (*e.g.,*

Plantago lanceolata); common ragweed (*e.g., Ambrosia artemisifolia*); morning glory (*e.g., Ipomoea spp.*); waterhemp (*e.g., Amaranthus spp.*); field bindweed (*e.g., Convolvulus arvensis*); yellow nutsedge (*e.g., Cyperus esculentus*); common lambsquarters (*e.g., Chenopodium album*); wild buckwheat (*e.g., Polygonium convolvulus*); velvetleaf (*e.g., Abutilon theophrasti*); kochia (*e.g., Kochia scoparia*); and Asiatic dayflower (*e.g., Commelina spp.*). In areas where such weeds are found, the 3560.4.3.5 soybeans are particularly useful in allowing the treatment of a field (and therefore any crop growing in the field) with combinations of herbicides that would cause unacceptable damage to crop plants that did not contain both of these polynucleotides. Plants that are tolerant to glyphosate and other herbicides such as, for example, sulfonylurea, imidazolinone, triazolopyrimidine, pyrimidinyl(thio)benzoate, and/or sulfonylaminocarbonyltriazolinone herbicides in addition to being tolerant to at least one other herbicide with a different mode of action or site of action are particularly useful in situations where weeds are tolerant to at least two of the same herbicides to which the plants are tolerant. In this manner, plants of the invention make possible improved control of weeds that are tolerant to more than one herbicide.

For example, some commonly used treatments for weed control in fields where current commercial crops (including, for example, soybeans) are grown include glyphosate and, optionally, 2,4-D; this combination, however, has some disadvantages. Particularly, there are weed species that it does not control well and it also does not work well for weed control in cold weather. Another commonly used treatment for weed control in soybean fields is the sulfonylurea herbicide chlorimuron-ethyl, which has significant residual activity in the soil and thus maintains selective pressure on all later-emerging weed species, creating a favorable environment for the growth and spread of sulfonylurea-resistant weeds. However, the 3560.4.3.5 soybean can be treated with herbicides (*e.g.*, chlorimuron-ethyl) and combinations of herbicides that would cause unacceptable damage to standard plant varieties. Thus, for example, fields containing the 3560.4.3.5 soybean can be treated with sulfonylurea, imidazolinone, triazolopyrimidines, pyrimidiny(thio)benzoates, and/or sulfonylaminocarbonyltriazonlinone such as the sulfonylurea chlorimuron-ethyl, either alone or in combination with other herbicides. For example, fields containing soybean plants of the invention can be treated with a combination of glyphosate and tribenuron-methyl (available commercially as Express®). This

combination has several advantages for weed control under some circumstances, including the use of herbicides with different modes of action and the use of herbicides having a relatively short period of residual activity in the soil. A herbicide having a relatively short period of residual activity is desirable, for example, in situations where it is important to reduce selective pressure that would favor the growth of herbicide-tolerant weeds. Of course, in any particular situation where weed control is required, other considerations may be more important, such as, for example, the need to prevent the development of and/or appearance of weeds in a field prior to planting a crop by using a herbicide with a relatively long period of residual activity. The 3560.4.3.5 soybean plants can also be treated with herbicide combinations that include at least one of nicosulfuron, metsulfuron-methyl, tribenuron-methyl, thifensulfuron-methyl, and/or rimsulfuron. Treatments that include both tribenuron-methyl and thifensulfuron-methyl may be particularly useful.

Other commonly used treatments for weed control in fields where current commercial varieties of crops (including, for example, soybeans) are grown include the sulfonylurea herbicide thifensulfuron-methyl (available commercially as Harmony GT®). However, one disadvantage of thifensulfuron-methyl is that the higher application rates required for consistent weed control often cause injury to a crop growing in the same field. The 3560.4.3.5 soybean plants can be treated with a combination of glyphosate and thifensulfuron-methyl, which has the advantage of using herbicides with different modes of action. Thus, weeds that are resistant to either herbicide alone are controlled by the combination of the two herbicides, and the 3560.4.3.5 soybean plants are not significantly damaged by the treatment.

Other herbicides which are used for weed control in fields where current commercial varieties of crops (including, for example, soybeans) are grown are the triazolopyrimidine herbicide cloransulam-methyl (available commercially as FirstRate®) and the imidazolinone herbicide imazaquin (available commercially as Sceptor®). When these herbicides are used individually they may provide only marginal control of weeds. However, fields containing the 3560.4.3.5 soybean can be treated, for example, with a combination of glyphosate (*e.g.*, Roundup® (glyphosate isopropylamine salt)), imazapyr (currently available commercially as Arsenal®), chlorimuron-ethyl (currently available commercially as Classic®), quizalofop-P-ethyl (currently available commercially as Assure II®), and fomesafen (currently available commercially as Flexstar®). This combination has the advantage of using herbicides

with different modes of action. Thus, weeds that are tolerant to just one or several of these herbicides are controlled by the combination of the five herbicides, and the 3560.4.3.5 soybeans are not significantly damaged by treatment with this herbicide combination. This combination provides an extremely broad spectrum of protection against the type of herbicide-tolerant weeds that might be expected to arise and spread under current weed control practices.

Fields containing the 3560.4.3.5 soybean plants may also be treated, for example, with a combination of herbicides including glyphosate, rimsulfuron, and dicamba or mesotrione. This combination may be particularly useful in controlling weeds which have developed some tolerance to herbicides which inhibit ALS. Another combination of herbicides which may be particularly useful for weed control includes glyphosate and at least one of the following: metsulfuron-methyl (commercially available as Ally®), imazapyr (commercially available as Arsenal®), imazethapyr, imazaquin, and sulfentrazone. It is understood that any of the combinations discussed above or elsewhere herein may also be used to treat areas in combination with any other herbicide or agricultural chemical.

Some commonly-used treatments for weed control in fields where current commercial crops (including, for example, maize) are grown include glyphosate (currently available commercially as Roundup®), rimsulfuron (currently available commercially as Resolve® or Matrix®), dicamba (commercially available as Clarity®), atrazine, and mesotrione (commercially available as Callisto®). These herbicides are sometimes used individually due to poor crop tolerance to multiple herbicides. Unfortunately, when used individually, each of these herbicides has significant disadvantages. Particularly, the incidence of weeds that are tolerant to individual herbicides continues to increase, rendering glyphosate less effective than desired in some situations. Rimsulfuron provides better weed control at high doses which can cause injury to a crop, and alternatives such as dicamba are often more expensive than other commonly-used herbicides. However, 3560.4.3.5 soybean can be treated with herbicides and combinations of herbicides that would cause unacceptable damage to standard plant varieties, including combinations of herbicides that comprise rimsulfuron and/or dicamba. Other suitable combinations of herbicides for use with 3560.4.3.5 soybean plants include glyphosate, sulfonylurea, imidazolinone, triazolopyrimidine, pryimidinyloxy(thio)benzoates, and/or sulfonylaminocarbonyltriazonlinone herbicides, including, for example, and at least

one of the following: metsulfuron-methyl, tribenuron-methyl, chlorimuron-ethyl, imazethapyr, imazapyr, and imazaquin.

For example, 3560.4.3.5 soybean plants can be treated with a combination of glyphosate and rimsulfuron, or a combination or rimsulfuron and at least one other herbicide. 3560.4.3.5 soybean plants can also be treated with a combination of glyphosate, rimsulfuron, and dicamba, or a combination of glyphosate, rimsulfuron, and at least one other herbicide. In some embodiments, at least one other herbicide has a different mode of action than both glyphosate and rimsulfuron. The combination of glyphosate, rimsulfuron, and dicamba has the advantage that these herbicides have different modes of action and short residual activity, which decreases the risk of incidence and spread of herbicide-tolerant weeds.

Some commonly-used treatments for weed control in fields where current commercial crops are grown include glyphosate (currently available commercially as Roundup®), chlorimuron-ethyl, tribenuron-methyl, rimsulfuron (currently available commercially as Resolve® or Matrix®), imazethapyr, imazapyr, and imazaquin. Unfortunately, when used individually, each of these herbicides has significant disadvantages. Particularly, the incidence of weeds that are tolerant to individual herbicides continues to increase, rendering each individual herbicide less effective than desired in some situations. However, 3560.4.3.5 soybean can be treated with a combination of herbicides that would cause unacceptable damage to standard plant varieties, including combinations of herbicides that include at least one of those mentioned above.

In the methods, a herbicide may be formulated and applied to an area of interest such as, for example, a field or area of cultivation, in any suitable manner. A herbicide may be applied to a field in any form, such as, for example, in a liquid spray or as solid powder or granules. In specific embodiments, the herbicide or combination of herbicides that are employed in the methods comprises a tankmix or a premix. A herbicide may also be formulated, for example, as a "homogenous granule blend" produced using blends technology (see, *e.g.*, U.S. Pat. No. 6,022,552, entitled "Uniform Mixtures of Pesticide Granules"). The blends technology of U.S. Patent No. 6,022,552 produces a nonsegregating blend (*i.e.*, a "homogenous granule blend") of formulated crop protection chemicals in a dry granule form that enables delivery of customized mixtures designed to solve specific problems. A homogenous granule blend can be shipped, handled, subsampled, and applied in the same manner as

traditional premix products where multiple active ingredients are formulated into the same granule.

Briefly, a "homogenous granule blend" is prepared by mixing together at least two extruded formulated granule products. In some embodiments, each granule product comprises a registered formulation containing a single active ingredient which is, for example, a herbicide, a fungicide, and/or an insecticide. The uniformity (homogeneity) of a "homogenous granule blend" can be optimized by controlling the relative sizes and size distributions of the granules used in the blend. The diameter of extruded granules is controlled by the size of the holes in the extruder die, and a centrifugal sifting process may be used to obtain a population of extruded granules with a desired length distribution (see, *e.g.*, U.S. Pat. No. 6,270,025).

A homogenous granule blend is considered to be "homogenous" when it can be subsampled into appropriately sized aliquots and the composition of each aliquot will meet the required assay specifications. To demonstrate homogeneity, a large sample of the homogenous granule blend is prepared and is then subsampled into aliquots of greater than the minimum statistical sample size.

In non-limiting embodiments, the 3560.4.5.3 soybean plant can be treated with herbicides (*e.g.*, chlorimuron-ethyl and combinations of other herbicides that without the 3560.4.3.5 event would have caused unacceptable crop response to plant varieties without the glyphosate/ALS inhibitor genetics). Thus, for example, fields planted with and containing 3560.4.3.5 soybeans can be treated with sulfonylurea, imidazolinone, triazolopyrimidine, pyrimidinyl(thio)benzoate, and/or sulfonylaminocarbonyltriazonlinone herbicides, either alone or in combination with other herbicides. Since ALS inhibitor chemistries have different herbicidal attributes, blends of ALS inhibitor plus other chemistries will provide superior weed management strategies including varying and increased weed spectrum, the ability to provide specified residual activity (SU/ALS inhibitor chemistry with residual activity leads to improved foliar activity which leads to a wider window between glyphosate applications, as well as, an added period of control if weather conditions prohibit timely application).

Blends also afford the ability to add other agrochemicals at normal, labeled use rates such as additional herbicides (a 3rd/4th mechanism of action), fungicides, insecticides, plant growth regulators and the like thereby saving costs associated with additional applications.

Any herbicide formulation applied over the 3560.4.3.5 soybean plant can be prepared as a "tank-mix" composition. In such embodiments, each ingredient or a combination of ingredients can be stored separately from one another. The ingredients can then be mixed with one another prior to application. Typically, such mixing occurs shortly before application. In a tank-mix process, each ingredient, before mixing, typically is present in water or a suitable organic solvent. For additional guidance regarding the art of formulation, see T. S. Woods, "The Formulator's Toolbox--Product Forms for Modern Agriculture" *Pesticide Chemistry and Bioscience, The Food-Environment Challenge*, T. Brooks and T. R. Roberts, Eds., Proceedings of the 9th International Congress on Pesticide Chemistry, The Royal Society of Chemistry, Cambridge, 1999, pp. 120-133. See also U.S. Pat. No. 3,235,361, Col. 6, line 16 through Col. 7, line 19 and Examples 10-41; U.S. Pat. No. 3,309,192, Col. 5, line 43 through Col. 7, line 62 and Examples 8, 12, 15, 39, 41, 52, 53, 58, 132, 138-140, 162-164, 166, 167 and 169-182; U.S. Pat. No. 2,891,855, Col. 3, line 66 through Col. 5, line 17 and Examples 1-4; Klingman, *Weed Control as a Science*, John Wiley and Sons, Inc., New York, 1961, pp 81-96; and Hance *et al., Weed Control Handbook*, 8th Ed., Blackwell Scientific Publications, Oxford, 1989, each of which is incorporated herein by reference in their entirety.

The methods further allow for the development of herbicide combinations to be used with the 3560.4.3.5 soybean plants. In such methods, the environmental conditions in an area of cultivation are evaluated. Environmental conditions that can be evaluated include, but are not limited to, ground and surface water pollution concerns, intended use of the crop, crop tolerance, soil residuals, weeds present in area of cultivation, soil texture, pH of soil, amount of organic matter in soil, application equipment, and tillage practices. Upon the evaluation of the environmental conditions, an effective amount of a combination of herbicides can be applied to the crop, crop part, seed of the crop or area of cultivation.

In some embodiments, the herbicide applied to the 3560.4.3.5 soybean plants serves to prevent the initiation of growth of susceptible weeds and/or serve to cause damage to weeds that are growing in the area of interest. In some embodiments, the herbicide or herbicide mixture exert these effects on weeds affecting crops that are subsequently planted in the area of interest (*i.e.*, field or area of cultivation). In the methods, the application of the herbicide combination need not occur at the same time. So long as the field in which the crop is planted contains detectable amounts of

the first herbicide and the second herbicide is applied at some time during the period in which the crop is in the area of cultivation, the crop is considered to have been treated with a mixture of herbicides according to the invention. Thus, methods encompass applications of herbicide which are "preemergent," "postemergent," "preplant incorporation" and/or which involve seed treatment prior to planting.

In one embodiment, methods are provided for coating seeds. The methods comprise coating a seed with an effective amount of a herbicide or a combination of herbicides (as disclosed elsewhere herein). The seeds can then be planted in an area of cultivation. Further provided are seeds having a coating comprising an effective amount of a herbicide or a combination of herbicides (as disclosed elsewhere herein).

"Preemergent" refers to a herbicide which is applied to an area of interest (*e.g.*, a field or area of cultivation) before a plant emerges visibly from the soil. "Postemergent" refers to a herbicide which is applied to an area after a plant emerges visibly from the soil. In some instances, the terms "preemergent" and "postemergent" are used with reference to a weed in an area of interest, and in some instances these terms are used with reference to a crop plant in an area of interest. When used with reference to a weed, these terms may apply to only a particular type of weed or species of weed that is present or believed to be present in the area of interest. While any herbicide may be applied in a preemergent and/or postemergent treatment, some herbicides are known to be more effective in controlling a weed or weeds when applied either preemergence or postemergence. For example, rimsulfuron has both preemergence and postemergence activity, while other herbicides have predominately preemergence (metolachlor) or postemergence (glyphosate) activity. These properties of particular herbicides are known in the art and are readily determined by one of skill in the art. Further, one of skill in the art would readily be able to select appropriate herbicides and application times for use with the transgenic plants of the invention and/or on areas in which transgenic plants of the invention are to be planted.

"Preplant incorporation" involves the incorporation of compounds into the soil prior to planting.

Thus, improved methods of growing a crop and/or controlling weeds are provided such as, for example, "pre-planting burn down," wherein an area is treated with herbicides prior to planting the crop of interest in order to better control weeds. Further provided are methods of growing a crop and/or controlling weeds which are "no-till" or "low-till" (also referred to as "reduced tillage"). In such methods, the soil

is not cultivated or is cultivated less frequently during the growing cycle in comparison to traditional methods; these methods can save costs that would otherwise be incurred due to additional cultivation, including labor and fuel costs.

The methods encompass the use of simultaneous and/or sequential applications of multiple classes of herbicides. In some embodiments, the methods involve treating a plant of the invention and/or an area of interest (*e.g.*, a field or area of cultivation) and/or weed with just one herbicide or other chemical such as, for example, a sulfonylurea herbicide.

The time at which a herbicide is applied to an area of interest (and any plants therein) may be important in optimizing weed control. The time at which a herbicide is applied may be determined with reference to the size of plants and/or the stage of growth and/or development of plants in the area of interest, *e.g.*, crop plants or weeds growing in the area. The stages of growth and/or development of plants are known in the art. For example, soybean plants normally progress through vegetative growth stages known as V_E (emergence), V_C (unifoliolate), V_1 (first trifoliolate), and V_2 to V_N. Soybeans then switch to the reproductive growth phase in response to photoperiod cues; reproductive stages include R_1 (beginning bloom), R_2 (full bloom), R_3 (beginning pod), R_4 (full pod), R_5 (beginning seed), R_6 (full seed), R_7 (beginning maturity), and R_8 (full maturity). Thus, for example, the time at which a herbicide or other chemical is applied to an area of interest in which plants are growing may be the time at which some or all of the plants in a particular area have reached at least a particular size and/or stage of growth and/or development, or the time at which some or all of the plants in a particular area have not yet reached a particular size and/or stage of growth and/or development.

In some embodiments, the 3560.4.3.5 soybean plants show improved tolerance to postemergence herbicide treatments. For example, the 3560.4.3.5 plants may be tolerant to higher doses of herbicide, tolerant to a broader range of herbicides (i.e., tolerance to more ALS inhibitor chemistries), and/or may be tolerant to doses of herbicide applied at earlier or later times of development in comparison to an appropriate control plant. For example, in some embodiments, the 3560.4.3.5 soybean plants show an increased resistance to morphological defects that are known to result from treatment at particular stages of development.

Different chemicals such as herbicides have different "residual" effects, *i.e.*, different amounts of time for which treatment with the chemical or herbicide

continues to have an effect on plants growing in the treated area. Such effects may be desirable or undesirable, depending on the desired future purpose of the treated area (*e.g.*, field or area of cultivation). Thus, a crop rotation scheme may be chosen based on residual effects from treatments that will be used for each crop and their effect on the crop that will subsequently be grown in the same area. One of skill in the art is familiar with techniques that can be used to evaluate the residual effect of a herbicide; for example, generally, glyphosate has very little or no soil residual activity, while herbicides that act to inhibit ALS vary in their residual activity levels. Residual activities for various herbicides are known in the art, and are also known to vary with various environmental factors such as, for example, soil moisture levels, temperature, pH, and soil composition (texture and organic matter). The 3560.4.3.5 soybean plants find particular use in methods of growing a crop where improved tolerance to residual activity of a herbicide is beneficial.

For example, in one embodiment, the 3560.4.3.5 soybean plants have an improved tolerance to glyphosate as well as to ALS inhibitor chemistries (such as sulfonylurea herbicides) when applied individually, and further provide improved tolerance to combinations of herbicides such as glyphosate and/or ALS inhibitor chemistries. Moreover, the transgenic plants disclosed herein provide improved tolerance to treatment with additional chemicals commonly used on crops in conjunction with herbicide treatments, such as safeners, adjuvants such as ammonium sulfonate and crop oil concentrate, and the like.

The term "safener" refers to a substance that when added to a herbicide formulation eliminates or reduces the phytotoxic effects of the herbicide to certain crops. One of ordinary skill in the art would appreciate that the choice of safener depends, in part, on the crop plant of interest and the particular herbicide or combination of herbicides included in the synergistic herbicide composition. Exemplary safeners suitable for use with the presently disclosed herbicide compositions include, but are not limited to, those disclosed in U.S. Patent Nos. 4,808,208; 5,502,025; 6,124,240 and U.S. Patent Application Publication Nos. 2006/0148647; 2006/0030485; 2005/0233904; 2005/0049145; 2004/0224849; 2004/0224848; 2004/0224844; 2004/0157737; 2004/0018940; 2003/0171220; 2003/0130120; 2003/0078167, the disclosures of which are incorporated herein by reference in their entirety. The methods can involve the use of herbicides in combination with herbicide safeners such as benoxacor, BCS (1-bromo-4-

[(chloromethyl) sulfonyl]benzene), cloquintocet-mexyl, cyometrinil, dichlormid, 2-(dichloromethyl)-2-methyl-1,3-dioxolane (MG 191), fenchlorazole-ethyl, fenclorim, flurazole, fluxofenim, furilazole, isoxadifen-ethyl, mefenpyr-diethyl, methoxyphenone ((4-methoxy-3-methylphenyl)(3-methylphenyl)-methanone), naphthalic anhydride (1,8-naphthalic anhydride) and oxabetrinil to increase crop safety. Antidotally effective amounts of the herbicide safeners can be applied at the same time as the compounds, or applied as seed treatments. Therefore an aspect of the present invention relates to the use of a mixture comprising glyphosate, at least one other herbicide, and an antidotally effective amount of a herbicide safener.

Seed treatment is particularly useful for selective weed control, because it physically restricts antidoting to the crop plants. Therefore a particularly useful embodiment is a method for selectively controlling the growth of weeds in a field comprising treating the seed from which the crop is grown with an antidotally effective amount of safener and treating the field with an effective amount of herbicide to control weeds. Antidotally effective amounts of safeners can be easily determined by one skilled in the art through simple experimentation. An antidotally effective amount of a safener is present where a desired plant is treated with the safener so that the effect of a herbicide on the plant is decreased in comparison to the effect of the herbicide on a plant that was not treated with the safener; generally, an antidotally effective amount of safener prevents damage or severe damage to the plant treated with the safener. One of skill in the art is capable of determining whether the use of a safener is appropriate and determining the dose at which a safener should be administered to a crop.

In specific embodiments, the herbicide or herbicide combination applied to the 3560.4.3.5 plant acts as a safener. In this embodiment, a first herbicide or a herbicide mixture is applied at an antidotally effect amount to the plant. Accordingly, a method for controlling weeds in an area of cultivation is provided. The method comprises planting the area with crop seeds or plants which comprise a first polynucleotide encoding a polypeptide that can confer tolerance to glyphosate operably linked to a promoter active in a plant; and, a second polynucleotide encoding an ALS inhibitor-tolerant polypeptide operably linked to a promoter active in a plant. A combination of herbicides comprising at least an effective amount of a first and a second herbicide is applied to the crop, crop part, weed or area of cultivation thereof. The effective amount of the herbicide combination controls weeds; and, the effective amount of the

first herbicide is not tolerated by the crop when applied alone when compared to a control crop that has not been exposed to the first herbicide; and, the effective amount of the second herbicide is sufficient to produce a safening effect, wherein the safening effect provides an increase in crop tolerance upon the application of the first and the second herbicide when compared to the crop tolerance when the first herbicide is applied alone.

In specific embodiments, the combination of safening herbicides comprises a first ALS inhibitor and a second ALS inhibitor. In other embodiments, the safening effect is achieved by applying an effective amount of a combination of glyphosate and at least one ALS inhibitor chemistry. In still other embodiments, a safening affect is achieved when the 3560.4.3.5 soybean crops, crop part, crop seed, weed, or area of cultivation is treated with at least one herbicide from the sulfonylurea family of chemistries in combination with at least one herbicide from the ALS family of chemistries (such as, for example, an imidazolinone).

Such mixtures provide increased crop tolerance (i.e., a decrease in herbicidal injury). This method allows for increased application rates of the chemistries post or pre-treatment. Such methods find use for increased control of unwanted or undesired vegetation. In still other embodiments, a safening affect is achieved when the 3560.4.3.5 soybean crops, crop part, crop seed, weed, or area of cultivation is treated with at least one herbicide from the sulfonylurea family of chemistry in combination with at least one herbicide from the imidazolinone family. This method provides increased crop tolerance (i.e., a decrease in herbicidal injury). In specific embodiments, the sulfonylurea comprises rimsulfuron and the imidazolinone comprises imazethapyr. In other embodiments, glyphosate is also applied to the crop, crop part, or area of cultivation.

As used herein, an "adjuvant" is any material added to a spray solution or formulation to modify the action of an agricultural chemical or the physical properties of the spray solution. See, for example, Green and Foy (2003) "Adjuvants: Tools for Enhancing Herbicide Performance," in *Weed Biology and Management*, ed. Inderjit (Kluwer Academic Publishers, The Netherlands). Adjuvants can be categorized or subclassified as activators, acidifiers, buffers, additives, adherents, antiflocculants, antifoamers, defoamers, antifreezes, attractants, basic blends, chelating agents, cleaners, colorants or dyes, compatibility agents, cosolvents, couplers, crop oil concentrates, deposition agents, detergents, dispersants, drift control agents,

emulsifiers, evaporation reducers, extenders, fertilizers, foam markers, formulants, inerts, humectants, methylated seed oils, high load COCs, polymers, modified vegetable oils, penetrators, repellants, petroleum oil concentrates, preservatives, rainfast agents, retention aids, solubilizers, surfactants, spreaders, stickers, spreader stickers, synergists, thickeners, translocation aids, uv protectants, vegetable oils, water conditioners, and wetting agents.

In addition, methods can comprise the use of a herbicide or a mixture of herbicides, as well as, one or more other insecticides, fungicides, nematocides, bactericides, acaricides, growth regulators, chemosterilants, semiochemicals, repellents, attractants, pheromones, feeding stimulants or other biologically active compounds or entomopathogenic bacteria, virus, or fungi to form a multi-component mixture giving an even broader spectrum of agricultural protection. Examples of such agricultural protectants which can be used in methods include: insecticides such as abamectin, acephate, acetamiprid, amidoflumet (S-1955), avermectin, azadirachtin, azinphos-methyl, bifenthrin, bifenazate, buprofezin, carbofuran, cartap, chlorfenapyr, chlorfluazuron, chlorpyrifos, chlorpyrifos-methyl, chromafenozide, clothianidin, cyflumetofen, cyfluthrin, beta-cyfluthrin, cyhalothrin, lambda-cyhalothrin, cypermethrin, cyromazine, deltamethrin, diafenthiuron, diazinon, dieldrin, diflubenzuron, dimefluthrin, dimethoate, dinotefuran, diofenolan, emamectin, endosulfan, esfenvalerate, ethiprole, fenothiocarb, fenoxycarb, fenpropathrin, fenvalerate, fipronil, flonicamid, flubendiamide, flucythrinate, tau-fluvalinate, flufenerim (UR-50701), flufenoxuron, fonophos, halofenozide, hexaflumuron, hydramethylnon, imidacloprid, indoxacarb, isofenphos, lufenuron, malathion, metaflumizone, metaldehyde, methamidophos, methidathion, methomyl, methoprene, methoxychlor, metofluthrin, monocrotophos, methoxyfenozide, nitenpyram, nithiazine, novaluron, noviflumuron (XDE-007), oxamyl, parathion, parathion-methyl, permethrin, phorate, phosalone, phosmet, phosphamidon, pirimicarb, profenofos, profluthrin, pymetrozine, pyrafluprole, pyrethrin, pyridalyl, pyriprole, pyriproxyfen, rotenone, ryanodine, spinosad, spirodiclofen, spiromesifen (BSN 2060), spirotetramat, sulprofos, tebufenozide, teflubenzuron, tefluthrin, terbufos, tetrachlorvinphos, thiacloprid, thiamethoxam, thiodicarb, thiosultap-sodium, tralomethrin, triazamate, trichlorfon and triflumuron; fungicides such as acibenzolar, aldimorph, amisulbrom, azaconazole, azoxystrobin, benalaxyl, benomyl, benthiavalicarb, benthiavalicarb-isopropyl, binomial, biphenyl, bitertanol, blasticidin-

S, Bordeaux mixture (Tribasic copper sulfate), boscalid/nicobifen, bromuconazole, bupirimate, buthiobate, carboxin, carpropamid, captafol, captan, carbendazim, chloroneb, chlorothalonil, chlozolinate, clotrimazole, copper oxychloride, copper salts such as copper sulfate and copper hydroxide, cyazofamid, cyflunamid, cymoxanil, cyproconazole, cyprodinil, dichlofluanid, diclocymet, diclomezine, dicloran, diethofencarb, difenoconazole, dimethomorph, dimoxystrobin, diniconazole, diniconazole-M, dinocap, discostrobin, dithianon, dodemorph, dodine, econazole, etaconazole, edifenphos, epoxiconazole, ethaboxam, ethirimol, ethridiazole, famoxadone, fenamidone, fenarimol, fenbuconazole, fencaramid, fenfuram, fenhexamide, fenoxanil, fenpiclonil, fenpropidin, fenpropimorph, fentin acetate, fentin hydroxide, ferbam, ferfurazoate, ferimzone, fluazinam, fludioxonil, flumetover, fluopicolide, fluoxastrobin, fluquinconazole, fluquinconazole, flusilazole, flusulfamide, flutolanil, flutriafol, folpet, fosetyl-aluminum, fuberidazole, furalaxyl, furametapyr, hexaconazole, hymexazole, guazatine, imazalil, imibenconazole, iminoctadine, iodicarb, ipconazole, iprobenfos, iprodione, iprovalicarb, isoconazole, isoprothiolane, kasugamycin, kresoxim-methyl, mancozeb, mandipropamid, maneb, mapanipyrin, mefenoxam, mepronil, metalaxyl, metconazole, methasulfocarb, metiram, metominostrobin/fenominostrobin, mepanipyrim, metrafenone, miconazole, myclobutanil, neo-asozin (ferric methanearsonate), nuarimol, octhilinone, ofurace, orysastrobin, oxadixyl, oxolinic acid, oxpoconazole, oxycarboxin, paclobutrazol, penconazole, pencycuron, penthiopyrad, perfurazoate, phosphonic acid, phthalide, picobenzamid, picoxystrobin, polyoxin, probenazole, prochloraz, procymidone, propamocarb, propamocarb-hydrochloride, propiconazole, propineb, proquinazid, prothioconazole, pyraclostrobin, pryazophos, pyrifenox, pyrimethanil, pyrifenox, pyrolnitrine, pyroquilon, quinconazole, quinoxyfen, quintozene, silthiofam, simeconazole, spiroxamine, streptomycin, sulfur, tebuconazole, techrazene, tecloftalam, tecnazene, tetraconazole, thiabendazole, thifluzamide, thiophanate, thiophanate-methyl, thiram, tiadinil, tolclofos-methyl, tolyfluanid, triadimefon, triadimenol, triarimol, triazoxide, tridemorph, trimoprhamide tricyclazole, trifloxystrobin, triforine, triticonazole, uniconazole, validamycin, vinclozolin, zineb, ziram, and zoxamide; nematocides such as aldicarb, oxamyl and fenamiphos; bactericides such as streptomycin; acaricides such as amitraz, chinomethionat, chlorobenzilate, cyhexatin, dicofol, dienochlor, etoxazole, fenazaquin, fenbutatin oxide, fenpropathrin, fenpyroximate, hexythiazox, propargite, pyridaben and

tebufenpyrad; and biological agents including entomopathogenic bacteria, such as *Bacillus thuringiensis* subsp. *Aizawai, Bacillus thuringiensis* subsp. *Kurstaki,* and the encapsulated delta-endotoxins of *Bacillus thuringiensis* (e.g., Cellcap, MPV, MPVII); entomopathogenic fungi, such as green muscardine fungus; and entomopathogenic virus including baculovirus, nucleopolyhedro virus (NPV) such as HzNPV, AfNPV; and granulosis virus (GV) such as CpGV. The weight ratios of these various mixing partners to other compositions (*e.g.*, herbicides) used in the methods typically are between 100:1 and 1:100, or between 30:1 and 1:30, between 10:1 and 1:10, or between 4:1 and 1:4.

Further provide are compositions comprising a biologically effective amount of a herbicide of interest or a mixture of herbicides, and an effective amount of at least one additional biologically active compound or agent and can further comprise at least one of a surfactant, a solid diluent or a liquid diluent. Examples of such biologically active compounds or agents are: insecticides such as abamectin, acephate, acetamiprid, amidoflumet (S-1955), avermectin, azadirachtin, azinphos-methyl, bifenthrin, binfenazate, buprofezin, carbofuran, chlorfenapyr, chlorfluazuron, chlorpyrifos, chlorpyrifos-methyl, chromafenozide, clothianidin, cyfluthrin, beta-cyfluthrin, cyhalothrin, lambda-cyhalothrin, cypermethrin, cyromazine, deltamethrin, diafenthiuron, diazinon, diflubenzuron, dimethoate, diofenolan, emamectin, endosulfan, esfenvalerate, ethiprole, fenothicarb, fenoxycarb, fenpropathrin, fenvalerate, fipronil, flonicamid, flucythrinate, tau-fluvalinate, flufenerim (UR-50701), flufenoxuron, fonophos, halofenozide, hexaflumuron, imidacloprid, indoxacarb, isofenphos, lufenuron, malathion, metaldehyde, methamidophos, methidathion, methomyl, methoprene, methoxychlor, monocrotophos, methoxyfenozide, nithiazin, novaluron, noviflumuron (XDE-007), oxamyl, parathion, parathion-methyl, permethrin, phorate, phosalone, phosmet, phosphamidon, pirimicarb, profenofos, pymetrozine, pyridalyl, pyriproxyfen, rotenone, spinosad, spiromesifin (BSN 2060), sulprofos, tebufenozide, teflubenzuron, tefluthrin, terbufos, tetrachlorvinphos, thiacloprid, thiamethoxam, thiodicarb, thiosultap-sodium, tralomethrin, trichlorfon and triflumuron; fungicides such as acibenzolar, azoxystrobin, benomyl, blasticidin-S, Bordeaux mixture (tribasic copper sulfate), bromuconazole, carpropamid, captafol, captan, carbendazim, chloroneb, chlorothalonil, copper oxychloride, copper salts, cyflufenamid, cymoxanil, cyproconazole, cyprodinil, (*S*)-3,5-dichloro-*N*-(3-chloro-1-ethyl-1-methyl-2-

oxopropyl)-4-methylbenzamide (RH 7281), diclocymet (S-2900), diclomezine, dicloran, difenoconazole, (*S*)-3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenyl-amino)-4*H*-imidazol-4-one (RP 407213), dimethomorph, dimoxystrobin, diniconazole, diniconazole-M, dodine, edifenphos, epoxiconazole, famoxadone, fenamidone, fenarimol, fenbuconazole, fencaramid (SZX0722), fenpiclonil, fenpropidin, fenpropimorph, fentin acetate, fentin hydroxide, fluazinam, fludioxonil, flumetover (RPA 403397), flumorf/flumorlin (SYP-L190), fluoxastrobin (HEC 5725), fluquinconazole, flusilazole, flutolanil, flutriafol, folpet, fosetyl-aluminum, furalaxyl, furametapyr (S-82658), hexaconazole, ipconazole, iprobenfos, iprodione, isoprothiolane, kasugamycin, kresoxim-methyl, mancozeb, maneb, mefenoxam, mepronil, metalaxyl, metconazole, metomino-strobin/fenominostrobin (SSF-126), metrafenone (AC375839), myclobutanil, neo-asozin (ferric methane-arsonate), nicobifen (BAS 510), orysastrobin, oxadixyl, penconazole, pencycuron, probenazole, prochloraz, propamocarb, propiconazole, proquinazid (DPX-KQ926), prothioconazole (JAU 6476), pyrifenox, pyraclostrobin, pyrimethanil, pyroquilon, quinoxyfen, spiroxamine, sulfur, tebuconazole, tetraconazole, thiabendazole, thifluzamide, thiophanate-methyl, thiram, tiadinil, triadimefon, triadimenol, tricyclazole, trifloxystrobin, triticonazole, validamycin and vinclozolin; nematocides such as aldicarb, oxamyl and fenamiphos; bactericides such as streptomycin; acaricides such as amitraz, chinomethionat, chlorobenzilate, cyhexatin, dicofol, dienochlor, etoxazole, fenazaquin, fenbutatin oxide, fenpropathrin, fenpyroximate, hexythiazox, propargite, pyridaben and tebufenpyrad; and biological agents including entomopathogenic bacteria, such as *Bacillus thuringiensis* subsp. *Aizawai, Bacillus thuringiensis* subsp. *Kurstaki,* and the encapsulated delta-endotoxins of *Bacillus thuringiensis* (*e.g.*, Cellcap, MPV, MPVII); entomopathogenic fungi, such as green muscardine fungus; and entomopathogenic virus including baculovirus, nucleopolyhedro virus (NPV) such as HzNPV, AfNPV; and granulosis virus (GV) such as CpGV. Methods may also comprise the use of plants genetically transformed to express proteins toxic to invertebrate pests (such as *Bacillus thuringiensis* delta-endotoxins). In such embodiments, the effect of exogenously applied invertebrate pest control compounds may be synergistic with the expressed toxin proteins.

General references for these agricultural protectants include *The Pesticide Manual, 13th Edition*, C. D. S. Tomlin, Ed., British Crop Protection Council,

Farnham, Surrey, U.K., 2003 and *The BioPesticide Manual*, *2nd Edition*, L. G. Copping, Ed., British Crop Protection Council, Farnham, Surrey, U.K., 2001.

In certain instances, combinations with other invertebrate pest control compounds or agents having a similar spectrum of control but a different mode of action will be particularly advantageous for resistance management. Thus, compositions can further comprise a biologically effective amount of at least one additional invertebrate pest control compound or agent having a similar spectrum of control but a different mode of action. Contacting a plant genetically modified to express a plant protection compound (*e.g.*, protein) or the locus of the plant with a biologically effective amount of a compound can also provide a broader spectrum of plant protection and be advantageous for resistance management.

Thus, methods can employ a herbicide or herbicide combination and may further comprise the use of insecticides and/or fungicides, and/or other agricultural chemicals such as fertilizers. The use of such combined treatments can broaden the spectrum of activity against additional weed species and suppress the proliferation of any resistant biotypes.

Methods can further comprise the use of plant growth regulators such as aviglycine, *N*-(phenylmethyl)-1*H*-purin-6-amine, ethephon, epocholeone, gibberellic acid, gibberellin A_4 and A_7, harpin protein, mepiquat chloride, prohexadione calcium, prohydrojasmon, sodium nitrophenolate and trinexapac-methyl, and plant growth modifying organisms such as *Bacillus cereus* strain BP01.

Embodiments are further defined in the following Examples. It should be understood that these Examples are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics, and without departing from the spirit and scope thereof, can make various changes and modifications of the embodiments of the invention to adapt it to various usages and conditions. Thus, various modifications of the embodiments of the invention, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

EXPERIMENTAL

Example 1. Insert and Flanking Border Sequence Characterization of Soybean Event 3560.4.3.5

Soybean event 3560.4.3.5, hereafter referred to as 3560.4.3.5 soybean, was obtained by microprojectile bombardment with the *Not* I-*Asc* I fragment from plasmid PHP20163. This fragment, PHP20163A, contains the *glyphosate acetlytransferase (glyat 4601)* gene under the control of the SCP1 promoter that is a synthetic constitutive promoter comprising a portion of the CaMV 35S promoter (Odell *et al.* (1985) Nature 313:810-812) and the Rsyn7-Syn II Core synthetic consensus promoter (US Patent No. 6,072,050 and 6555673). See also, for example, US20030226166, Table 13, and SEQ ID NO:3. Downstream of this element is the Tobacco Mosaic Virus (TMV) omega 5'UTR translational enhancer element (Gallie *et al.* (1992) *Nucleic Acid Research* 20:4631-4638) and the proteinase inhibitor II (*pin*II) terminator from *Solanum tuberosum* (Keil *et al.* (1986) *Nucleic Acid Research* 14:5641-5650 and An *et al.* (1989) *Plant Cell* 1:115-122). PHP20163A also contains the *gm-hra* gene that is a modified version of the acetolactate synthase gene from soybean with 15 additional nucleotides on the 5' end (2697-2711) derived from the *als* 5' UTR and two nucleotides changes within the coding sequence under the control of the S-adenosyl-L-methionine synthetase (SAMS) promoter (US Application Publication 2003/0226166) with its 5'UTR and intron (SAMS Pro) and the acetolactate synthase (*gm-als*) terminator, both from soybean.

To characterize the integrity of the inserted DNA from PHP20163A and the genomic insertion site, the sequence of the insert and flanking genomic DNA border regions of 3560.4.3.5 soybean was determined. In total, 10849 base pairs (bp) of 3560.4.3.5 soybean was sequenced, comprising 5362 bp of DNA insert from PHP20163A, 3317 bp of 5' flanking genomic border sequence, and 2170 bp of 3' flanking genomic border sequence. When compared to the expected sequence from the DNA fragment used for transformation, the insert was confirmed to be intact and identical to PHP20163A and precisely integrated into the soy genome. PCR amplification from the 3560.4.3.5 soybean insert and border sequences confirmed that the border regions were of soybean origin and that the junction regions could be used for identification of 3560.4.3.5 soybean.

BLASTn analysis of the border regions resulted in significant identities to public and proprietary soybean genomic sequences. Overall, characterization of the

insert and genomic border sequence of 3560.4.3.5 soybean along with Southern blot data (data not show) indicated that a single insertion of the DNA fragment, PHP20613A, was present in the soybean genome.

The following abbreviations are used in describing the present invention.

ALS	acetolactate synthase protein
bp	base pair
glyat	glyphosate acetyltransferase gene
GLYAT	glyphosate acetyltransferase protein
GLYAT4601 gene	glyphosate acetyltransferase protein derived from *glyat4601*
gm-als	wild type acetolactate synthase gene from soybean
gm-hra	modified version of acetolactate synthase gene from soybean
kb	kilobase
PCR	polymerase chain reaction
UTR	untranslated region
als	acetolactate synthase gene
AMS	ammonium sulfate
DAT	days after treatment
glyat4601	glyphosate acetyl transferase gene from the 7th round of DNA shuffling a *glyat* gene family isolated from *Bacillus licheniformis* (Castle *et al.* (2004) *Science* 304:1151-1154; Siehl *et al.* (2005) *Pest Manag. Sci.* 61:235-240).
GLYAT4601	glyphosate acetyl transferase protein from the 7th round of DNA shuffling a *glyat* gene family isolated from *Bacillus licheniformis* (Castle *et al.* (2004) *Science* 304:1151-1154; Siehl *et al.* (2005) *Pest Manag. Sci.* 61:235-240).
hra	highly resistant allele of the acetolactate synthase gene
glyat+gm-hra	transgenic event expressing both the *glyat4601* and *gm-hra* genes
NILs	near isogenic lines
NIS	non-ionic surfactant
SAMS	S-adenosyl-L-methionine synthase promoter (US Patent Application No 2003/0226166)

SCP1	Synthetic constitutive promoter 1 (US Patent Nos. 6072050 and 6555673 B1.

Soybean (*Glycine max*) has been modified by the insertion of the glyphosate acetyltransferase (*glyat4601*) gene derived from *Bacillus licheniformis* and a modified version of the soybean acetolactate synthase gene (*gm-hra*). The *glyat4601* gene was functionally improved by a gene shuffling process to optimize the kinetics of glyphosate acetyltransferase (GLYAT) activity for acetylating the herbicide glyphosate. The insertion of the *glya4601t* gene in the plant confers tolerance to the herbicidal active ingredient glyphosate through the conversion of glyphosate to the non-toxic acetylated form. The insertion of the *gm-hra* gene produces a modified form of the acetolactate synthase enzyme. ALS is essential for branched chain amino acid biosynthesis and the modification in the *gm-hra* gene overcomes this inhibition and thus provides tolerance to a wide range of ALS-inhibiting herbicides.

The publicly available cultivar Jack was used as the recipient line for generation of 3560.4.3.5 soybean. The 3560.4.3.5 soybean was obtained by microprojectile bombardment with the *Not* I-*Asc* I fragment from plasmid PHP20163 (Figure 1). This fragment, PHP20163A (Figure 2), contains the *glyat4601* gene under the control of the SCP1 promoter and Tobacco Mosaic Virus (TMV) omega 5' UTR translational enhancer element and the proteinase inhibitor II (*pin*II) terminator from *Solanum tuberosum*. PHP20163A also contains the *gm-hra* gene under the control of the S-adenosyl-L-methionine synthetase (SAMS) promoter and the acetolactate synthase (*gm-als*) terminator, both from soybean.

The transgenic 3560.4.3.5 soybean was generated using the Biolistics PDS-1000/He particle gun, manufactured by Bio-Rad (Hercules, CA), essentially as described by Klein *et al*. (1987). The targets for transformation were clumps of secondary somatic embryos derived from explants from small, immature soybean seeds of the cultivar Jack. The secondary somatic embryos were excised from immature explants, transferred to a liquid soybean culture maintenance medium, and subcultured at regular intervals until prepared for bombardment.

Soybean somatic embryogenic cultures were used in transformation experiments 2-4 months after initiation. On the day of transformation, microscopic gold particles were coated with the purified fragment PHP20163A DNA and accelerated into the embryogenic soybean cultures. Only PHP20163A DNA was

used, and no additional DNA (*e.g.,* carrier DNA) was used in the transformation process.

Following the transformation, the soybean tissue was transferred to flasks of fresh liquid culture maintenance medium for recovery. After seven days, the liquid culture medium was changed to culture maintenance medium supplemented with chlorsulfuron as the selection agent. Chlorsulfuron belongs to a family of ALS-inhibiting herbicides, and therefore only soybean cells that had stably inherited the *gm-hra* transgene continued to grow.

After several weeks in the culture maintenance medium supplemented with chlorsulfuron, small islands of healthy, chlorsulfuron-tolerant green tissue became visible and started to grow out of pieces of dying somatic embryogenic tissue. Green embryogenic clumps were excised from associated pieces of dying or dead tissue and received regular changes of fresh liquid selection medium until the start of the regeneration process. Embryogenic tissue samples were analyzed to confirm the presence of the *glyat4601* and *gm-hra* transgenes by Southern blot hybridization. T0 plants were regenerated and transferred to the greenhouse for seed production. Figure 5 describes a breeding diagram.

In the microprojectile bombardment transformation the 3560.4.3.5 soybean, DNA is inserted into the plant genome. The integration of the DNA fragment can occur at virtually any site in the plant genome. Once inserted, the genes that contain plant expression elements are recognized by the plant and may be expressed. Various molecular techniques are then used to specifically characterize the integration site in the 3560.4.3.5 soybean.

Southern blot analyses indicated that a single, intact PHP20163A fragment inserted into the soybean genome to produce the 3560.4.3.5 soybean (data not shown). Cloning and sequencing of the flanking genomic border regions of 3560.4.3.5 soybean and the inserted DNA was undertaken to characterize the insertion site in the soybean genome and obtain sequence that could be used to uniquely identify 3560.4.3.5 soybean.

Leaf tissue from the T5 generation of 3560.4.3.5 soybean was used as for additional sequence characterization. The T5 generation represents transformation of a Jack soybean variety, followed by two self-crossings. A single plant from this second self-crossing was selected fro three subsequent rounds of self-crossing and

seed bulking. Southern blot analysis was used for event confirmation on plant leaf tissue of 3560.4.3.5 soybean (data not shown).

Leaf tissue from soybean plants that were not genetically modified was used as a control for sequence characterization. The unmodified soybean plants have a genetic background representative of 3560.4.3.5 soybean background; however, they do not contain the plant transcription units for the *glyat4601* and *gm-hra* genes.

The 100 bp and 1 kb step DNA Ladders (Promega, Madison, WI) were used to estimate DNA fragment sizes on agarose gels.

Soybean seed for 3560.4.3.5 soybean and unmodified control seed were planted to produce sufficient numbers of plants for DNA analysis. For characterization of 3560.4.3.5 soybean line, eight T5 seeds were planted. Eight seeds were planted for control soybean line as well. One seed was planted per pot, and the pot was uniquely identified. Planting and growing conditions were conducive to healthy plant growth including regulated light and water.

Leaf samples were collected for each of the control and 3560.4.3.5 soybean plants. For each sample, sufficient leaf material from above the growing point was collected and placed in a pre-labeled sample bag. The samples were placed on dry ice and were transferred to an ultra low freezer following collection. All samples were maintained frozen until processing.

Frozen leaf samples (1-2 gram quantities) were ground, and the genomic DNA was isolated using a modified Urea Extraction Buffer procedure (Chen *et al*. (1994) Urea-based plant DNA miniprep in Freeling M. and Walbot V., eds, *The Maize Handbook*, Springer-Verlag, New York, p 526-527). Genomic DNA was extracted from leaf tissue harvested from individual plants as described above. Specifically, the tissue was pulverized in tubes containing grinding beads using a Geno/Grinder™ (SPEX CertiPrep, Inc., Metuchen, NJ) instrument and the genomic DNA isolated using a standard procedure. Approximately 1 gram ground tissue was extracted with 5 mL Urea Extraction Buffer (7 M Urea, 0.34 M NaCl, 0.05 M Tris-HCl, pH 8.0, 0.02 M EDTA, 1% N-Lauroylsarcosine) for 12-30 minutes at 37°C, followed by two extractions with phenol/chloroform/isoamyl alcohol (25:24:1) and one extraction with water saturated chloroform. The DNA was precipitated from the aqueous phase by the addition of 1/10 volume of 3 M NaOAc (pH 5.2) and 1 volume of isopropyl alcohol, followed by centrifugation to pellet the DNA. After washing the pellet twice

with 70% ethanol, the DNA was dissolved in 0.5 mL TE buffer (10mM Tris, 1 mM EDTA, pH 7.5) and treated with 10 μg Ribonuclease A for 15 minutes at 37°C. The sample was extracted once with phenol:chloroform:isoamyl alcohol (25:24:1) and once with water saturated chloroform, followed by precipitation with isopropyl alcohol and washing with 70% ethanol. After drying, the DNA was re-dissolved with 0.5 mL TE buffer and stored at 4°C. Following extraction, the DNA was visualized on an agarose gel to determine the DNA quality, and was quantified using Pico Green® reagent (Molecular Probes, Inc., Eugene, OR) and spectrofluorometric analysis.

Phenotypic analysis of 3560.4.3.5 soybean plants and control plants was carried out by western blot analysis using antibodies to the GLYAT4601 protein to confirm the absence or presence of the GLYAT4601 protein in material used for Southern blot analysis and sequence characterization. Total protein was extracted by grinding several leaf punches to homogeneity in 150 μl of protein extraction buffer (50mM Tris-HCl (pH 7.5), 0.1% SDS, and 10mM β-mercaptoethanol). An aliquot of each crude extract was mixed with LDS Sample Buffer and reducing agent (Invitrogen) and heated to approximately 95°C for 5 minutes. Proteins were separated by size under denaturing conditions through a NuPAGE™ Bis/Tris Gel system as described (Invitrogen). Selected molecular weight standards were used to determine sufficient migration in the gel and for molecular weight determination on the western blot (Invitrogen). The Bis/Tris gel was transferred to a nitrocellulose membrane using the method as described for the Novex™ XCell II™ Blot Module Western Transfer (Invitrogen). Alternatively, gels were stained with SimplyBlue™ SafeStain (Invitrogen) to visualize the proteins to verify equivalent sample loading.

The GLYAT4601 protein band was detected using the WesternBreeze® Chemiluminescent Western Blot Immunodetection Kit as described (Invitrogen). Primary monoclonal antibodies specific for the GLYAT4601 protein were used with the WesternBreeze® Kit. Bands were then visualized using a chemiluminescent substrate. Blots were exposed to X-ray film for one or more time points to detect protein bands. Purified GLYAT4601 protein was used as a positive control on the western blots. Plants were scored as positive for GLYAT4601 when a band of the appropriate size was present and scored as negative when the band was absent on the western blots.

A preliminary Southern blot analysis of DNA isolated from all 3560.4.3.5 soybean plants was used to verify the presence of both the *glyat4601* and *gm-hra* genes. Methods for this preliminary characterization are described below. Final Southern blot analysis was carried out on a subset of 3560.4.3.5 soybean plants (data not shown).

Genomic DNA samples extracted from selected 3560.4.3.5 soybean and control soybean plants were digested with restriction enzymes following a standard procedure. Approximately 2 µg of genomic DNA was digested in a volume of 100 µL using 50 units of enzyme according to manufacturer's recommendations. The digestions were carried out at 37°C for three hours, followed by ethanol precipitation with 1/10 volume of 3 M NaOAc (pH 5.2) and 2 volumes of 100% ethanol. After incubation at 4°C and centrifugation, the DNA was allowed to dry and re-dissolved in TE buffer. The reference plasmid, PHP20163, was spiked into a control plant DNA sample in an amount equivalent to approximately one or three gene copies per soybean genome and digested with the same enzyme to serve as a positive control for probe hybridization and to verify sizes of internal fragments on the Southern blot.

Following restriction enzyme digestion, the DNA fragments produced were electrophoretically separated by size through an agarose gel and a molecular weight standard ΦX174 RF DNA/*Hae* III Fragments (Invitrogen) was used to determine sufficient migration and separation of the fragments on the gel. DIG labeled DNA Molecular Weight Marker VII (Roche), visible after DIG detection as described below, was used to determine hybridizing fragment size on the Southern blots.

Agarose gels containing the separated DNA fragments were depurinated, denatured, and neutralized *in situ*, and transferred to a nylon membrane in 20x SSC buffer (3M NaCl, 0.3 M Sodium Citrate) using the method as described for the TURBOBLOTTER™ Rapid Downward Transfer System (Schleicher & Schuell, Keene, NH). Following transfer to the membrane, the DNA was bound to the membrane by UV crosslinking (Stratalinker, Stratagene, La Jolla, CA). Probes for the SCP1 promoter, *glyat4601*, *pin*II terminator, SAMS, *gm-hra*, and *als* terminator were used to detect genes and elements within the insertion (Table 3). Backbone and hygromycin resistance gene cassette regions (*backbone 20163* and *hyg20163* probes) of the PHP20163 plasmid were used to verify absence of plasmid backbone DNA in 3560.4.3.5 soybean (Table 3). DNA fragments of the probe elements were generated

by PCR from plasmid PHP20163 (Figure 1) or a plasmid with equivalent elements using specific primers. PCR fragments were electrophoretically separated on an agarose gel, excised and purified using a gel purification kit (Qiagen, Valencia, CA). DNA probes were generated from these fragments by PCR that incorporated a DIG labeled nucleotide, [DIG-11]-dUTP, into the fragment. PCR labeling of isolated fragments was carried out according to the procedures supplied in the PCR DIG Probe Synthesis Kit (Roche).

Table 3. Description of DNA Probes Used for Southern Blot Hybridization

Probe Name	Genetic Element	Figure *Probe*	Position on PHP20163A (bp to bp)	Position on PHP20163 (bp to bp)	Length (bp)
SCP1 promoter	SCP1 promoter	Figure 6 *probe 1*	12 to 479 (SEQ ID NO:29)	12 to 479	486
glyat4601	*glyat4601* gene	Figure 6 *probe 2*	597 to 1012 (SEQ ID NO:30)	597 to 1012	416
*pin*II terminator	*pin*II terminator	Figure 6 *probe 3*	1107 to 1340 (SEQ ID NO:31)	1107 to 1340	234
SAMS[1]	SAMS promoter and intron elements	Figure 6 *probe 4*	1702 to 2146 (SEQ ID NO:32) 2147 to 2638 (SEQ ID NO:33)	1702 to 2146 2147 to 2638	445 492
gm-hra[1]	*gm-hra* gene	Figure 6 *probe 5*	2700 to 3629 (SEQ ID NO:34) 3635 to 4664 (SEQ ID NO:35)	2700 to 3629 3635 to 4664	930 1030
gm-als terminator	*gm-als* terminator	Figure 6 *probe 6*	4670 to 5318 (SEQ ID NO:36)	4670 to 5318	649
backbone 20163[1]	plasmid backbone of PHP20163	Figure 7 *probe A*	N/A[2]	7427-7954 6665-7416	528 752
hyg 20163[1]	hygromycin resistance gene of PHP20163	Figure 7 *probe B*	N/A	6097-6619 5389-6091	523 703

[1]Two non-overlapping segments were generated for this probe and were combined for hybridization.

[2]Not Applicable; these are not present on the PHP20163A fragment.

Genomic DNA isolated from 3560.4.3.5 soybean plants was digested with *Hin*d III and *Xba* I, and electrophoretically separated, transferred to nylon membranes, and hybridized to the *glyat4601* and *gm-hra* gene probes. Labeled probes were hybridized to the target DNA on nylon membranes for detection of the specific fragments using the procedures essentially as described for DIG Easy Hyb solution (Roche). After stringent washes, the hybridized DIG-labeled probes and DIG-labeled DNA standards were visualized using CDP-Star Chemiluminescent Nucleic Acid Detection System with DIG Wash and Block Buffer Set (Roche). Blots were exposed to X-ray film for one or more time points to detect hybridizing fragments and to visualize molecular weight standards. Images were then digitally captured by detection with the Luminescent Image Analyzer LAS-3000 (Fujifilm Medical Systems, Stamford, CT). The sizes of detected bands were documented for each digest and each probe.

Following hybridization and detection, membranes were stripped of DIG-labeled probe to prepare the blot for subsequent re-hybridization to additional probes. Membranes were rinsed briefly in distilled, de-ionized water and then stripped in a solution of 0.2 M NaOH and 1.0% SDS at 40°C with constant shaking. The membranes were then rinsed in 2x SSC and either used directly for subsequent hybridizations or stored at 4°C or -20°C for later use. The alkali-based stripping procedure effectively removes probes labeled with the alkali-labile DIG. This preliminary Southern blot analysis showed the presence of the insertion in 3560.4.3.5 soybean plants and confirmed that 3560.4.3.5 soybean plants used for this study contained the same insertion (Table 4).

Table 4. Summary of Preliminary Southern Screen Data for 3560.4.3.5 Soybean Line.

Plant ID	Sample ID	Southern Blot *glyat4601* Probe[2]	Southern Blot *gm-hra* Probe[2]
T-F-05-140S-17	T-17	+	+
T-F-05-140S-18	T-18	+	+
T-F-05-140S-19	T-19	+	+
T-F-05-140S-20	T-20	+	+
T-F-05-140S-21	T-21	+	+
T-F-05-140S-22	T-22	+	+
T-F-05-140S-23	T-23	+	+
T-F-05-140S-24	T-24	+	+

+ indicates hybridization signal on Southern blot.

Four plants were chosen for insert and border sequence analysis: 3560.4.3.5 soybean plants T17 and T18, and control plants C1 and C2. PCR primers were synthesized by Sigma-Genosys, Inc. (The Woodlands, TX) and MWG (Ebersberg, Germany), and used at concentrations of 0.3-0.4uM. For amplification of the PHP20163A insert region, both the Advantage-GC-2 PCR system (Clontech) and the Expand Long Template PCR System (Roche) were used to amplify genomic DNA (100-500ng); and for the 5' and 3' flanking genomic border PCR, the Advantage-GC-2 PCR system was used to amplify from genomic DNA (100ng). The PCR products were visualized under UV light following electrophoresis through a 1% agarose gel with 1x TAE and ethidium bromide; excised, and purified from the gel using Qiaquick gel purification kit (Qiagen, Valencia, CA).

The PCR products were cloned using the TOPO TA-cloning Kit (pCR2.1-TOPO vector, Invitrogen, Carlsbad, CA). Plasmids were isolated using QIAprep Spin Miniprep Kit (Qiagen), screened by restriction enzyme digestions, and sequenced.

For complete sequence coverage of the cloned PCR products representing the insert of the 3560.4.3.5 soybean, the clones were sequenced by a transposon-based sub-cloning method to facilitate bi-directional sequencing of a cloned insert from the site of the transposition event (MJ Research TGS system; Happa *et al.* (1999) *Nucleic Acid Research* 27:2777-2784. Since unintended mutations can occur during the generation of the PCR products, we cloned and sequenced products from two separate PCR reactions with T17 and T18 DNA. In all cases, any PCR-induced sequence error was present in only one of the four clones, allowing reliable consensus calls to be made on every base of the sequence.

Initial sequence characterization of the 5' flanking border was carried out using the BD GenomeWalker Universal Kit (Clontech, Mountain View, CA). The GenomeWalker protocol involves digesting the genomic DNA with various restriction enzymes and ligating these digests with an adaptor, thereby creating “libraries” to be used as template for two rounds of PCR. The PCR primers for the 2 rounds of PCR consisted of an insert-specific (SCP1 promoter) primer, and an adaptor-specific primer. Based on sequence information generated from the Genome Walker experiments, primers were designed to perform PCR on genomic DNA from 3560.4.3.5 and control soybean plants, using primers that spanned the 5' junction.

(Figure 3 and data not shown). To extend the 5' border sequence further, we used the DNA Walking SpeedUp Kit (Seegene, Inc. Rockville, MD) was used, which is another PCR-based genomic DNA walking approach based on the DNA Walking Annealing Control Primer PCR Technology (Ochman *et al.* (1988) *Genetics* 120:621; Silver *et al.* (1989) *Journal of Virology* 63:1924; Triglia *et al.* (1988) *NAR* 16:8186). The last round of border extension was performed by anchoring within the 5'-most end of the border and again using the Genome Walker kit. The final 5' flanking border sequence was verified by cloning and sequencing of 5' flanking genomic border PCR products. In order to demonstrate that the identified 5' flanking genomic border sequence is of soybean origin, PCR was performed within this 5' flanking genomic region on genomic DNA from 3560.4.3.5 soybean and control soybean plants (Figure 3 and data not shown).

Initial sequence characterization of the 3' flanking border was carried out using inverse PCR (Silver *et al.* (1989) *Journal of Virology* 63:1924; Ochman *et al.* (1988) *Genetics* 120:621; Triglia *et al.* (1988) *NAR* 16:8186), with insert-specific primers anchored in the *glyat*4601 and *gm-hra* genes. Sequence obtained from products generated using inverse PCR was then used to design primers for PCR on genomic DNA from 3560.4.3.5 and control soybean plants, using primers that spanned the 3' junction. (Figure 3 and data not shown). The 3' flanking genomic border sequence was verified by cloning and sequencing the 3' flanking genomic border PCR products. The genomic flanking border was extended further using the sequence information from proprietary soybean sequence that matched this flanking border to design PCR primers. Cloning and sequencing the resulting PCR products confirmed this additional flanking border sequence. In order to demonstrate that the identified 3' flanking genomic border sequences were of soybean origin, PCR was performed within this 3' genomic region on DNA from 3560.4.3.5 soybean and control soybean plants (Figure 3 and data not shown).

For verification of the DNA sequence that inserted into the soybean genome, PCR was performed to amplify, clone, and sequence the inserted DNA from 3560.4.3.5 soybean. PCR primers just outside the insert (primer pair 1297/1298) were used to amplify the entire insert from DNA of 3560.4.3.5 soybean plants. This amplification produced products of the expected size (5.4 kb) from two separate reactions from each of two test samples; these were cloned and sequenced. In addition, the DNA fragment PHP20163A used for creating 3560.4.3.5 soybean line

was sequenced, and compared with the insert sequence generated from 3560.4.3.5 soybean genomic DNA.

Both the 5' and 3' flanking border sequences of the 3560.4.3.5 soybean were subjected to BLASTn analysis in order to identify the nature and potential function of the flanking sequences in the soybean genome. The searches were performed against the NCBI Genbank Nucleotide ("nt") dataset (www.ncbi.nlm.nih.gov/), Release 154, last updated 7/31/06, 4,302,011 total sequences), as well as to Genome Survey Sequence dataset (GSS, Release 07/28/06). Finally, sequences were compared to a dataset consisting of all proprietary soybean genomic and EST sequences generated by Pioneer. Default parameters were used in all cases. Both 5' and 3' border/insert junctions were also screened for the presence of novel open reading frames (ORFs) ≥ 100 amino acids (300 bp) in length using Vector NTI 9.1 (Invitrogen, Carlsbad, CA).

DNA isolated from eight 3560.4.3.5 soybean plants was digested with *Xba* I and *Hin*d III, and hybridized to *gm-hra* and *glyat*4601 gene probes to verify the presence of the insertion and to verify the molecular equivalence of the insertion among all 8 plants analyzed. The preliminary Southern blot screen indicated that 3560.4.3.5 soybean plants exhibited identical hybridization patterns (Table 4 and data not shown). In the *Xba* I digests, the *glyat4601* probe hybridized to a 1.4 kb band and the *gm-hra* probe hybridized to a 3.9 kb band as expected (Figure 2), demonstrating the intact insertion of PHP20163A. In the *Hind* III digests, the *glyat4601* probe hybridized to a 5' flanking 6.1 kb band, the *gm-hra* probe hybridized to 3' flanking 7.4 kb band, and an internal 2.4 kb band, demonstrating that the insertion exists as a single copy in the genome (Figure 2). The *gm-hra* probe also hybridizes to other bands corresponding to endogenous sequences. Four plants were chosen for insert and border sequence analysis: 3560.4.3.5 soybean plants T17 and T18, and control Jack plants C1 and C2.

In the initial characterization of the 3560.4.3.5 soybean line, the flanking genomic border regions were cloned and sequenced using the GenomeWalker and inverse PCR methods. This preliminary sequence information was used to design primers for PCR to generate products in the three regions of interest from 3560.4.3.5 soybean genomic DNA: 5' flanking border, entire insert, and 3' flanking border (Table 11, Table 4; Table 10; and, Figure 3). Using information from the flanking border sequence, PCR was performed on 3560.4.3.5 soybean genomic DNA and unmodified control genomic DNA.

For the 5' flanking sequence, PCR was performed with a forward primer in the 5' border (primer 1679) and a reverse primer in the SCP1 Promoter (primer 1658), resulting in the expected products in 3560.4.3.5 soybean plants (396 bp), and not in the control DNAs (Figure 3 and data not shown). Following two more rounds of walking (with the DNA Walking SpeedUp and GenomeWalker Kits), additional 5' flanking border sequence was obtained. To verify the 5' flanking border sequence, PCR products were generated from 3560.4.3.5 DNA, cloned and sequenced. The complete sequence information is presented in Figure 4A-E.

For the 3' flanking sequence, PCR was performed with a forward primer in the *gm-hra* (primer 1439) and a reverse primer in the 3' border sequence (primer 1666), resulting in the expected products in 3560.4.3.5 soybean plants (1029 bp) and not the control DNAs (Figure 3 and data not shown). The 3' flanking border PCR products were generated from 3560.4.3.5 soybean DNA, cloned and sequenced. This sequence showed the same identity to a proprietary soybean DNA sequence, which was used to design primers to further extend the flanking border. Again, the resulting 3' flanking border PCR products generated from 3560.4.3.5 soybean DNA were cloned and sequenced. The complete sequence information is presented in Figure 4A-E.

For amplification of the insert, PCR primers situated in each flanking region were used to amplify the entire insert from DNA of event 3560.4.3.5 plants (primer pair: 1297/1298). As expected, the predicted PCR products of 5.5 kb were generated only from 3560.4.3.5 soybean DNA, and not from the control DNA. The insert PCR products were cloned and sequenced. The sequence information is presented in Figure 4A-E.

In addition, the DNA fragment PHP20163A used for creating 3560.4.3.5 soybean line was sequenced, and compared with the insert sequence generated from 3560.4.3.5 soybean genomic DNA. The sequence of the insert in 3560.4.3.5 soybean is identical to the sequence of the PHP20163A DNA fragment.

In total, 10849 bp of sequence from genomic DNA of 3560.4.3.5 soybean was confirmed: 3317 bp of the 5' flanking genomic border, 2170 bp of the 3' flanking genomic border, and the 5362 bp comprising the inserted DNA (Figure 3 and Figure 4A-E).

To demonstrate that the identified 5' and 3' flanking border sequences are of soybean origin, PCR was performed within the 5' and 3' flanking genomic regions (primer pairs 1679/1514 and 1660/1666, respectively) from 3560.4.3.5 soybean DNA

samples and unmodified control samples. The expected size PCR products (246 bp for 5' genomic region and 297 bp for 3' genomic region) were generated using genomic DNA from both 3560.4.3.5 soybean and control soybean plants, indicating that the sequences were of soybean genomic origin and not specific to 3560.4.3.5 soybean (Figure 3 and data not data shown). These PCR products from both the 3560.4.3.5 and control soybean DNAs were cloned and sequenced and shown to have identical sequence.

When the 3317 bp sequence from the 5' flanking region of 3560.4.3.5 soybean was compared to the Genbank Nucleotide ("nt") dataset (www.ncbi.nlm.nih.gov/), no significant alignments were returned. Analysis using the GSS subset returned a two areas of the flanking region (nt 2723-2863; 2881-3203) with significant similarity (98% and 92%, respectively) to genomic sequences from the legume *Medicago truncatula* (barrel medic). The BLASTn search against Pioneer proprietary soybean genomic and EST sequences returned a 98% identity alignment encompassing nt 2860-3317 to a single soybean genomic sequence. An additional region (nt 531 - 2144) returned lower, yet significant identities (84%-92%) to several different soybean genomic clones. The 3' flanking sequence produced two highly significant alignments (97-99% identity) to two different public soybean genomic sequences (accessions CL868338.1 and CL867466.1) and a single proprietary genomic sequence in the region from nt 9772-10849. An additional region (nt 9250-9554) displayed significant identity (92-94%) to wheat genomic and mitochondrial sequences. The 5' and 3' junction regions between the soybean genomic border sequence and the insertion in 3560.4.3.5 soybean were analyzed for the presence of novel open reading frames. No open reading frames greater than or equal to 100 amino acids were identified in the 5' or 3' border junction regions, indicating that no novel open reading frames were generated as a result of the insertion.

Southern blot analysis also confirmed that the DNA insertion remained stable during traditional soybean breeding procedures. The analysis was conducted on two self-crossed generations, T4 and T5, and verified that the insertion remained intact and stably integrated as demonstrated by identical hybridization patterns in the two generations. The F3 generation was also analyzed by Southern blot analysis and confirmed the same stable, event-specific hybridization pattern as exhibited by the T4 and T5 generations. These results confirmed the stability of the insertion in 3560.4.3.5 soybean across multiple breeding generations.

As discussed below, the *Bgl* II restriction enzyme has a single site (bp position 2254) located within the PHP20163A fragment (Figure 6) and will generate a unique event-specific hybridization pattern for 3560.4.3.5 soybean when hybridized to the *glyat4601* and *gm-hra* probes. This analysis confirms event stability across generations as changes to the insertion structure in 3560.4.3.5 soybean would be detected. As discussed below, a band of approximately 2500 bp would be expected with the *glyat4601* probe to confirm stability across generations (Table 5). Likewise, for the *gm-hra* probe, a band of approximately 3500 bp would be expected to confirm stability across generations (Table 6).

Table 5. Predicted and Observed Hybridizing Bands on Southern Blots with *glyat4601* Cassette Probes

Probe	Restriction Enzyme	Predicted Fragment Size from PHP20163A[1] (bp)	Predicted Fragment Size from PHP20163[2] (bp)	Observed Fragment Size in GLYAT 3560.4.3.5 soybean[3] (bp)
SCP1 promoter	*Bgl* II	>2300[4]	3485	~2500
glyat4601	*Bgl* II	>2300[4]	3485	~2500
*pin*II terminator	*Bgl* II	>2300[4]	3485	~2500
SCP1 promoter	*Xba* I	1379	1379[5]	1379[6]
glyat4601	*Xba* I	1379	1379[5]	1379[6]
*pin*II terminator	*Xba* I	1379	1379[5]	1379[6]

[1] Predicted fragment sizes for 3560.4.3.5 soybean are based on the map of PHP20163A as shown in Figure 6.

[2] Predicted fragment sizes for hybridization to samples containing the plasmid positive control are based on the PHP20163 plasmid map as shown in Figure 7.

[3] Observed fragment sizes are considered approximate from these analyses and are based on the indicated sizes of the DIG-labeled DNA Molecular Weight Marker VII fragments on the Southern blots. Due to incorporation of DIG molecules for visualization, the marker fragments typically run approximately 5-10% larger than their actual indicated molecular weight.

[4] Minimum fragment size predicted based on an intact insertion of PHP20163A (data not shown).

[5] Predicted hybridizing *Xba* I fragment size from plasmid PHP20163 grown in a Dam^- strain. For plasmid grown in a Dam^+ strain, the hybridizing *Xba* I fragment size is predicted to be 5307 bp.

[6] Observed fragment is equal to the predicted size based on blot (data not shown) showing comparison to plasmid PHP20163 grown in a Dam^- strain.

Table 6. Predicted and Observed Hybridizing Bands on Southern Blots with *gm-hra* Cassette Probes

Probe	Restriction Enzyme	Predicted Fragment Size from PHP20163A[1] (bp)	Predicted Fragment Size from PHP20163[2] (bp)	Observed Fragment Size in GLYAT 3560.4.3.5 Soybean[3] (bp)
SAMS	*Bgl* II	>3100[4] >2300[4]	4468 3485	~3500 ~2500 ~6900* ~4700*
gm-hra	*Bgl* II	>3100[4]	4468	~3500 >8600* >8600*
gm-als terminator	*Bgl* II	>3100[4]	4468	~3500 >8600*
SAMS	*Xba* I	3928	3928[5]	3928[6] >8600*
gm-hra	*Xba* I	3928	3928[5]	3928[6] >8600* ~8600* ~7400* ~6800* ~5800* ~4500*
gm-als terminator	*Xba* I	3928	3928[5]	3928[6] ~5800*

An asterisk (*) and gray shading indicates the designated band is due to probe hybridization to endogenous soybean genome sequences, as can be determined by the presence of the same band in all lanes, both 3560.4.3.5 soybean and control.

[1] Predicted fragment sizes for 3560.4.3.5 soybean are based on the map of PHP20163A as shown in Figure 6.

[2] Predicted fragment sizes for hybridization in samples containing the plasmid positive control are based on the PHP20163 plasmid map as shown in Figure 7.

[3] Observed fragment sizes are considered approximate from these analyses and are based on the indicated sizes of the DIG-labeled DNA Molecular Weight Marker VII fragments on the Southern blots. Due to incorporation of DIG molecules for visualization, the marker fragments typically run approximately 5-10% larger than their actual indicated molecular weight.

[4] Minimum fragment size predicted based on an intact insertion of PHP20163A (data not shown).

[5] Predicted hybridizing *Xba* I fragment size from plasmid PHP20163 grown in a Dam^- strain. For plasmid grown in a Dam^+ strain, the hybridizing *Xba* I fragment size is predicted to be 5307 bp.

[6] Observed fragment is equal to the predicted size based on blot (data not shown) showing comparison to plasmid PHP20163 grown in a Dam^- strain.

Genomic DNA of T4 and T5 generations of 3560.4.3.5 soybean was digested with *Bgl* II and hybridized to the *glyat4601* and *gm-hra* probes to confirm stability

across generations (data not shown). A band of approximately 2500 bp specific to 3560.4.3.5 soybean hybridized to the *glyat4601* probe in both the T4 and T5 generations (Table 5 and data not shown). With the *gm-hra* probe, a single band of approximately 3500 bp specific to 3560.4.3.5 soybean was present in both generations (Table 6 and data not shown). In addition to the 3500 bp band, the *gm-hra* probe also hybridized to additional bands that were determined to be endogenous to the soybean genome since these bands were present in both 3560.4.3.5 soybean and control soybean plants (data not shown). Hybridization results from both the *glyat4601* and *gm-hra* probes confirmed that the insertion of the PHP20163A DNA fragment in 3560.4.3.5 soybean remained stable across the self-crossed T4 and T5 generations.

Southern blot analysis of the F3 generation of 3560.4.3.5 soybean was also conducted. A total of 77 individual plants were analyzed (data not shown). Genomic DNA of the F3 generation was digested with *Bgl* II and hybridized to the *glyat4601* and *gm-hra* probes. A band of approximately 2500 bp was observed with the *glyat4601* probe (data not shown) and a single band of approximately 3500 bp specific to 3560.4.3.5 soybean was observed with the *gm-hra* probe (data not shown). As with the previous analysis conducted, the *gm-hra* probe hybridized to additional bands in 3560.4.3.5 soybean and control samples which were due to endogenous sequences within the soybean genome (data not shown). Hybridizations results from both the *glyat4601* and *gm-hra* probes were consistent with the results from the T4 and T5 generations described above and confirmed the stability of inheritance of the insertion during traditional soybean breeding.

Both the T4 and T5 generations were analyzed to confirm the absence of plasmid sequence from plasmid PHP20163 outside of the transformation fragment PHP20163A, *i.e.* the plasmid backbone sequence removed prior to transformation. The results verified the absence of backbone sequences in 3560.4.3.5 soybean.

The *backbone 20163* and *hyg 20163* probes (Table 3 and Figure 7) were designed to hybridize to areas of plasmid PHP20163 outside of the transformation fragment (data not shown) and were hybridized to *Xba* I-digested genomic DNA to confirm absence of these sections of the plasmid in 3560.4.3.5 soybean. Neither of the two backbone probes hybridized to 3560.4.3.5 soybean samples (data not shown), confirming the absence of these sequences.

Genomic DNA from leaf material of the Jack soybean variety was used as a negative control for all Southern blot analyses. Genomic DNA from an elite soybean

variety (Elite 1) was included as an additional negative control for analysis of the F3 generation. Plasmid PHP20163 was used as a positive control for probe hybridization and to verify fragment sizes internal to the transformation fragment PHP20163A. All probes used for the analysis are indicated on the schematic maps of PHP20163A and PHP20163 (Figures 6 and 7, respectively) and outlined in Table 3.

The integration pattern of the insertion in 3560.4.3.5 soybean was investigated with *Bgl* II digestion to determine copy number and with *Xba* I digestion to determine insertion integrity. Southern blots were hybridized to several probes to confirm copy number and integrity of each genetic element. SCP1 promoter, *glyat4601*, and *pin*II terminator probes were used to characterize the *glyat4601* cassette (Table 3 data not shown). SAMS, *gm-hra*, and *als* terminator probes were used to characterize the *gm-hra* cassette (Table 3 and data not shown).

The *Bgl* II digest provides information about number of copies integrated into the genome of 3560.4.3.5 soybean as there is a single restriction enzyme site in the PHP20163A fragment at base pair (bp) position 2254 (data not shown) and additional sites outside the fragment in the soybean genome. Hybridization with the probes from each cassette, except for the SAMS probe, would indicate the number of copies of each element found in 3560.4.3.5 soybean based on the number of hybridizing bands (*e.g.* one hybridizing band indicates one copy of the element). For the SAMS probe, since the *Bgl* II site is located within the probe region, two hybridizing bands would be expected for every one copy of the element. Predicted and observed fragment sizes for 3560.4.3.5 soybean with *Bgl* II are given in Table 5 for the *glyat4601* cassette and in Table 6 for the *gm-hra* cassette.

Based on the Southern blot analyses as discussed below, it was determined that a single, intact PHP20163A fragment has been inserted into the genome of 3560.4.3.5 soybean as diagramed in the insertion map (Figure 8).

A single copy of all the elements of the *glyat4601* cassette was inserted into 3560.4.3.5 soybean. SCP1 promoter, *glyat4601*, and *pin*II terminator probes were hybridized to *Bgl* II-digested genomic DNA from individual 3560.4.3.5 soybean plants of the T4 generation (Table 5 and data not shown). Each of the probes hybridized to the same single fragment of approximately 2500 base pairs (bp) (Table 5 and data not shown), indicating the expected arrangement of genetic elements on the fragment inserted in 3560.4.3.5 soybean.

Likewise, a single copy of all the elements of the *gm-hra* cassette was inserted into 3560.4.3.5 soybean. The elements comprising this cassette - the SAMS promoter region, *gm-hra* gene, and *als* terminator - were used as probes to determine number of copies inserted. The probes of this cassette are homologous to elements endogenous to the soybean genome and therefore each probe hybridized to bands in control soybean samples. The hybridizing bands in 3560.4.3.5 soybean from the endogenous soybean genome are indicated by asterisks in the shaded boxes of Table 6 and were determined by their presence in control soybean samples and are thus not associated with the insertion.

The SAMS probe hybridized to one band of approximately 2500 bp and a second band of approximately 3500 bp in 3560.4.3.5 soybean (Table 6 and data not shown, SAMS probe). Two bands would be expected to indicate one insertion of this element with this probe as the *Bgl* II site is located within the SAMS region of PHP20163A (Figure 6) and the results indicated one copy of the element. The 2500 bp fragment was determined to be the same fragment containing the *glyat4601* cassette as described above.

The *gm-hra* and *als* terminator probes hybridized to the same 3500 bp fragment as the SAMS probe (Table 6 and data not shown, *gm-hra* and *gm-als* terminator probes). The hybridization of all three probes to the same 3500 bp fragment and of the SAMS probe to the 2500 bp fragment confirmed the expected arrangement of the genetic elements in the DNA insertion in 3560.4.3.5 soybean.

Xba I digestion was used to verify that the *glyat4601* and *gm-hra* cassettes were complete and intact in 3560.4.3.5 soybean as there are three sites in the PHP20163A fragment (base pair positions 8, 1387, and 5315) which precisely flank each gene expression cassette (Figure 6). Hybridization with the probes of the *glyat4601* and *gm-hra* cassettes confirmed that all the elements were found on the appropriate internal fragments containing the cassette. Expected and observed fragment sizes with *Xba* I are given in Table 6 for the *glyat4601* cassette and Table 6 for the *gm-hra* cassette.

The SCP1 promoter, *glyat4601*, and *pin*II terminator probes each hybridized to the expected internal band of 1378 bp (Table 5 and data not shown) and the size was confirmed by additional hybridizations as described in the section below and data not shown. Because these probes hybridized to the same internal fragment of the

predicted size, the *glyat4601* cassette in 3560.4.3.5 soybean was determined to be intact and all elements of the cassette were confirmed on this fragment.

The SAMS, *gm-hra*, and *als* terminator probes each hybridized to the expected internal band of 3927 bp band (Table 6 and data not shown) and the size was confirmed by additional hybridization described in the section below. Because these probes hybridized to the same fragment, the *gm-hra* cassette in 3560.4.3.5 soybean was determined to be intact and all elements were present.

Plasmid PHP20163 was prepared from a strain of *E. coli* that expresses a DNA methylase (a Dam^+ strain). This plasmid did not produce the expected bands when digested with *Xba* I and probed with the *glyat4601* and *gm-hra* cassette probes. A band of approximately 5300 bp was observed in lanes containing plasmid PHP20163 for all probes (data not shown) instead of the predicted 1379 bp and 3928 bp size bands (Tables 5 and 6). Based on the plasmid sequence, it was determined that the *Xba* I site at bp position 1387 of PHP20163 (Figure 7) overlaps a Dam methylation recognition sequence. The final adenine in this site is methylated, thus blocking digestion by *Xba* I.

The inability for *Xba* I enzyme to cut at this site affected the size prediction from fragment PHP20163A in 3560.4.3.5 soybean (Tables 5 and 6, Figure 6). In order to confirm the size in 3560.4.3.5 soybean and the plasmid result, plasmid PHP20163 was prepared from a strain of *E. coli* lacking Dam methylase (Dam^- strain) and was compared to the plasmid PHP20163 (data not shown). Southern hybridization results (data not shown) show the plasmid comparison alongside samples of the T4 and T5 generations of 3560.4.3.5 soybean digested with *Xba* I. The blot probed with *glyat4601* and *gm-hra* probes demonstrated that plasmid PHP20163 prepared from the Dam^- strain digested as expected by *Xba* I and produced the predicted size bands of 1379 bp and 3927 bp (data not shown), while the original plasmid from the Dam^+ strain again produced a band of approximately 5300 bp for both probes (data not shown). These results confirmed that the central *Xba* I site at position 1387 was blocked from digestion due to Dam methylation. Furthermore, the hybridizing bands in 3560.4.3.5 soybean were of the equivalent size as those in the unmethylated plasmid PHP20163 from the Dam^- strain confirming that 3560.4.3.5 soybean contained a complete and intact insertion (data not shown).

A Chi squared analysis of trait inheritance data from five different generations (T1, F2, F3, BC1F2, C2F2) was performed to determine the Mendelian heritability

and stability of the *glyat4601* and *gm-hra* genes in the progeny of 3560.4.3.5 soybean. The breeding history of the five generations evaluated for Mendelian inheritance is described in Figure 5. For each of the generations tested, the plants were expected to segregate 1:2:1 (homozygous positive plants:hemizygous plants:homozygous negative [null] plants). Various approaches, as outlined below, were used to confirm this segregation ratio in the progeny of 3560.4.3.5 soybean.

In some studies, homozygous positive plants were not differentiated from hemizygous plants, resulting in a 3:1 positive:negative segregation pattern (Table 7). For the three generations listed in Table 7, three different methods, respectively, were used to score the plants as positive or negative:

- qualitative PCR analysis to identify the plants containing the *glyat4601* gene (T1 generation); or
- western analysis to score plants for GLYAT4601 protein expression followed by confirmation of those same plants by Southern analysis with both *glyat4601* and *gm-hra* probes (F3 generation), or
- an ALS seed soak assay (BC1F2 generation). In this assay, seeds are soaked in an ALS-inhibiting herbicide containing the active ingredient chlorsulfuron. Only seed expressing the *gm-hra* gene will emerge after planting.

In certain studies, plants that did not contain either the *glyat4601* or *gm-hra* genes were removed from the study prior to the conduct of segregation analysis. Remaining plants were then scored as homozygous positive or hemizygous, resulting in a 1:2 homozygous positive:hemizygous segregation pattern (Table 8).

For the F2 generation listed in Table 8, two methods were used to remove the negative plants and score the remaining plants as homozygous positive or hemizygous:

- A glyphosate spray was applied after the plants had emerged, removing all of the homozygous negative (null) plants. This was followed by an ALS-inhibiting herbicide "ragdoll" assay for the *gm-hra* gene, where progeny seed from the F2 plants were screened to determine the F2 parent plant genotype. In the ragdoll assay, paper towels were wetted with an ALS-inhibiting herbicide containing the active ingredient chlorsulfuron. Ten progeny seeds from a single F2 parent plant were rolled into the wetted towel and allowed to germinate. An F2 parent plant was scored as homozygous positive for the *gm-*

hra gene if all ten progeny seeds germinated and grew normally. An F2 parent plant was scored as homozygous negative for the *gm-hra* gene if all ten progeny seeds did not germinate and grow normally. A hemizygous F2 parent genotype was characterized by having a mixture of resistant and susceptible plants within the ten seed sample.

- An ALS seed soak assay of the seeds prior to planting removed all of the homozygous negative (null) plants, followed by a quantitative real time PCR (qPCR) assay to distinguish plants that were homozygous positive or hemizygous for the *glyat4601* gene.

In some studies, all plants were identified as homozygous positive, hemizygous, or homozygous negative to confirm a 1:2:1 expected segregation ratio (Table 9). Segregation analysis was conducted for the C2F2 generation using quantitative real time PCR (qPCR) assays for both the *glyat4601* and *gm-hra* genes. Because the *glyat4601* and *gm-hra* genes were physically linked in the DNA fragment used for transformation and are expected to have identical segregation ratios in the progeny of 3560.4.3.5 soybean, the *glyat4601* results are applicable to the inheritance of *gm-hra* and vice versa. In generations where both traits were analyzed in the same plants (F3 and C2F2), identical segregation data would experimentally confirm co-segregation of the *glyat4601* and *gm-hra* genes

Results from the Mendelian analysis are summarized in Tables 7, 8 and 9. All P-values were greater than 0.05, indicating no statistically significant differences between the observed and expected frequencies of the *glyat4601* and/or *gm-hra* genes in five generations of 3560.4.3.5 soybean. The results of this analysis are consistent with the finding of a single locus of insertion of the *glyat4601* and *gm-hra* genes that segregates in 3560.4.3.5 soybean progeny according to Mendel's laws of genetics. The stability of the insert has been demonstrated in five generations of self- and cross-pollinations.

Table 7. Comparison of Observed and Expected 3:1 Segregation Ratios for 3560.4.3.5 Soybean

Generation	Method	Observed		Expected		Chi-squared test
		Positives +/+ or +/-	Negatives -/-	Positives +/+ or +/-	Negatives -/-	P-value
T1	*Glyat4601* PCR	59	23	61.5	20.5	0.610
F3 Elite 1 background	GLYAT 4601westerns followed by Southern analyses with *glyat4601* and *gm-hra* probes	75	15	67.5	22.5	0.088
BC1F2	ALS seed soak					
Elite 7 background		700	222	691.5	230.5	0.543
Elite 8 background		761	273	775.5	258.5	0.315
Elite 9 background		160	54	160.5	53.5	1.000
Elite 10 background		205	79	213	71	0.304

Table 8. Comparison of Observed and Expected 1:2 Segregation Ratios for 3560.4.3.5 Soybean

Generation	Method	Observed		Expected		Chi-squared test
		Homo-zygous +/+	Hemi-zygous +/-	Homo-zygous +/+	Hemi-zygous +/-	P-value
F2						
Elite 1 background	Glyphosate spray to remove nulls and followed by ALS inhibitor ragdoll test	16	24	13.3	26.7	0.467
Elite 2 background		32	53	28.3	56.7	0.466
F2						
Elite 3 background	ALS seed soak to remove nulls followed by qPCR for *glyat4601*	110	182	97.3	194.7	0.131
Elite 4 background		124	284	136	272	0.227
Elite 5 background		27	61	29.3	58.7	0.678
Elite 6 background		22	29	17	34	0.181

Table 9. Comparison of Observed and Expected 1:2:1 Segregation Ratios for 3560.4.3.5 Soybean

Generation	Method	Observed			Expected			Chi-squared test
		+/+	+/-	-/-	+/+	+/-	-/-	P-value
C2F2								
Elite 44 background	*Glyat4601* and *gm-hra* qPCR	41	76	43	40	80	40	0.799
Elite 45 background		160	294	142	149	298	149	0.550

Table 10. Description and Sizes of PCR Products

Forward Primer	Reverse Primer	Description of amplified region	Size (bp) of PCR product
1297	1298	Inserted DNA	5457
1679	1658	5' flanking genomic border to SCP1 Promoter	396
1679	1514	5' flanking genomic border	246
1439	1666	*gm-hra* to 3' flanking genomic border	1029
1660	1666	3' flanking genomic border	297

Table 11. List of Primer Sequences Used in PCR Reactions

Primer Name	**Abbreviated Primer Name**	**Sequence (5'-3')**	Target Sequence Location **(bp to bp)**[1]	SEQ ID NO
05-0-1227	1227	TGGTCTTCTGAGACTGTATCTTTGATATTC	3402-3373	24
05-0-1297	1297	TGCCCGAGGTCGTTAGGTCGAATAGGCTAG	3268-3297	25
05-0-1298	1298	TCCTATTCAAGATGGGCAGTGTCTTCCTAATGATG	8724-8690	17
06-0-1439	1439	GATAACTGAGGGTGATGGTAGAACGAGGTACTGATTG	7951-7987	18
06-0-1440	1440	TGTGATAACTGAGGGTGATGGTAGAACGAGGTACTG	7948-7983	26
06-0-1473	1473	TTATTTCCGATCGGATCCTGCCAGTGGAG	10849-10821	50
06-0-1504	1504	CCACCATGTTGACGGATCTCTAG	3347-3325	51
06-0-1505	1505	GCAATGGAATCCGAGGAGGTTTC	3463-3441	52
06-0-1506	1506	GCAATGATGGCATTTGTAGGTG	3532-3511	53
06-0-1514	1514	TCGATCGGTCAAGAATCCGGTTCTC	3263-3239	19
06-0-1549	1549	AAACTGAAGCGATGGCAGAACCGCACAG	1962-1935	54
06-0-1550	1550	TCGAAGTGGCAATAGAGCCACACAATATCGATAAG	2012-1978	55
06-0-1610	1610	AGCAATTGTTTTGTGCATTTCCAAATTTCAATCTG	1-35	40
06-0-1658	1658	CAATAGCCCTTTGGTCTTCTGAGACTGTATCTTTG	3413-3379	20
06-0-1660	1660	GTAATATCATCATTAGGAAGACACTGCCCATCTTG	8683-8717	21
06-0-1666	1666	CCATATTTGAAAGCCTAAGCAGATGGCATAATTC	8979-8946	22
06-0-1679	1679	TTCAGCAACAAACTCTCATCGTGAGCAG	3018-3045	23

1. Location in sequence of 356043 soybean. (See figure 4)
Bases 1 - 3317 = 5' genomic border, bases 3318 - 8679 = insert, bases 8680 - 10849 = 3' genomic border.

Table 12. Additional Primers for Detecting 3560.4.3.5 soybean

Primer	Characteristics	SEQ ID NO
GGTCGAATAGGCTAGGTTTACGAA	(start 751, length 24 nt, tm 59, %GC 59)	7
AAGAGACTAAGGCCGCTC	(taqman mgb probe, start 776, lenth 18, tm 68, % gc 56)	9
CCACCATGTTGACGGATCTCT	(start 815, length 21, tm 58)	8
GTCGAATAGGCTAGGTTTACGAAAAA	primer DP-356-f1	37
TTTGATATTCTTGGAGTAGACGAGAGTGT	Primer Dp-356-r1	38
6FAM-CTCTAGAGATCCGTCAACATGGTG GAGCAC-TAMRA	Dp356-p (Taqman probe)	39

Example 2. Additional methods to identify a 3560.4.3.5 Event

Oligonucleotide PCR reagents:

Forward Primer: 5' GGTCGAATAGGCTAGGTTTACGAA (SEQ ID NO:7)

Reverse Primer: 5' CCACCATGTTGACGGATCTCT (SEQ ID NO:8)

Taqman MGB probe: 5' Fam-AAGAGACTAAGGCCGCTC-MGB 3' (SEQ ID NO:9)

Each primer is used at a concentration of 900 nM in the PCR. The MGB probe was used at a concentration of 80 nM in the PCR. The PCR mixture used was "Extract-N-Amp PCR Ready Mix" (Cat. No. E3004) from Sigma-Aldrich. Rox reference dye was also included in the PCR mixture by adding 0.01 volumes of Sigma-Aldrich "Reference Dye for Quantitative PCR" (Cat. No. R4526). PCR was performed for 40 cycles with one cycle consisting of the following two steps: Step 1: 15 seconds at 95° C and Step 2: 60 seconds at 60° C. Amplicon product had a size of 85 bp.

Additional primers that can be used to identify a 3560.4.3.5 soybean event are set forth in Table 13.

Table 13

Primer	Sequence	Product Size (bp)	SEQ ID NO
104312	AGATCCGTCAACATGGTGGAGCAC		44
104314	TGACAGATAGCTGGGCAATGGAATCC	150	45
Probe 125323	6FAM-TATCGGGAAACCTC-MGB		46
109893 (endogenous control)	CTTTGCTGTTTGATTGCTGGGTTGTC		47
109894 (endogenous control)	TGTGTGGACCCATTGGCCTTTAGATTAT	144	48
Probe 125322 (endogenous control)	VIC-ACTCTGCAGTTGCCTT-MGB		49

These primers were used in a presence/absence assay to detect a 3560.4.3.5 soybean event. Primers 104312+104314/SCP1TP10 detect the transgenic insertion; control primers, such as, 109893+109894/probe P94032A2 were used to detect house-keeping gene (aspartate aminotransferase gene) and were used as internal control. A 3560.4.3.5 soybean event will show heterozygous signals (FAM and VIC) and the non-transgenic genotypes will show homozygous P94032A2 (VIC) positive. PCR conditions employed in this method of dectecting the 3560.4.3.5 soybean are shown below.

Table 14

PCR Conditions		
Initial denat	120 sec	95C
Anneal	90 sec	66C
Extend	90 sec	72C
Denat	30 sec	95C
	14 cycles	
Final extend	120	72C
Initial denat	120 sec	95C
Anneal	60 sec	60C
Extend	10 sec	82C
Denat	30 sec	95C
Final extend	0	82C
	32 cycles	

Those skilled in the art would also include a control PCR using an endogenous gene to verify that the isolated genomic DNA was suitable for PCR amplification.

Soyb ean endogenous genes that have been used successfully with soybean samples are the following: Lectin gene (Schmidt, M and Parrott, W, 2001) and conglycinin α'-subunit gene (Shirai (1998) *Biosci Biotechnol Biochem* 62:1461-1464). Another location to find endogenous gene targets for PCR is the web site (www.//biotech.jrc.it) which is sponsored by the Joint Research Centre (JRC) of the European Commission. See, also, Schmidt *et al.* (2001) *Plant Cell Rep* 20:422-428.

Example 3

Soybean event 3560.4.3.5 was selected as a lead event and BC1F3 lines were created by backcrossing 3560.4.3.5 soybean into four different conventional lines. Across these four backcross populations, lines with the 3560.4.3.5 event (sprayed with glyphosate) were not significantly different for yield compared to negative null segregant lines (sprayed with conventional herbicides). Soybean event 3560.4.3.5 confers a high level of tolerance to glyphosate and ALS inhibitor herbicides with no yield impact.

The first objective of this study was to evaluate 3560.4.3.5 soybean to determine the level of tolerance to different application rates of glyphosate and ALS inhibitor herbicides. The second objective was to develop homozygous positive and homozygous negative null segregants (NILs) of 3560.4.3.5 soybean event to determine if yield was impacted due to presence of the 3560.4.3.5 event. The third objective was to integrate event 3560.4.3.5 into different genetic backgrounds to determine if there was any impact on herbicide tolerance or yield performance.

MATERIALS AND METHODS

For early generation transgenic event testing, T0 plants were sprayed at the V2 to V6 growth stage (Fehr *et al.* (1977) Coop. Ext. Ser. Special Report 80. Iowa State Univ., Ames IA) with 1.68 kg ae ha^{-1} or 3.36 kg ae ha^{-1} glyphosate plus adjuvants (0.25% v/v nonionic surfactant (NIS) + 2.24 kg ai ha^{-1} ammonium sulfate (AMS)) in the Newark, DE greenhouses. Plants were rated using a 1 to 9 scale ten days after treatment (DAT), where 1 = dead plant to 9 = no observed spray response.

Selected events were advanced at the T1 generation for herbicide tolerance confirmation in greenhouses. Plants were sprayed at the V3 growth stage with either 1.68 kg ae ha^{-1} glyphosate, 60 g ai ha^{-1} thifensulfuron methyl (methyl 3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]-2-

thiophenecarboxylate), or a sequential application of 70 g ai ha^{-1} thifensulfuron methyl at V3 followed by 2.24 kg ai ha-1 glyphosate at V6. All spray treatments included 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS. At ten days after spray application, plants were rated using a 1 to 9 scale. Since the T1 events were segregating, only the non-susceptible plants were scored, and an average rating of tolerant plants within each event was calculated.

Plants from individual T1 events were grown separately in 2.4 m rows (76.2 cm row spacing). Presence or absence of *glyat+hra* in each individual T1 plant was determined by using polymerase chain reaction (PCR) amplification of the *glyat4601* insert. PCR results for each event were analyzed using chi square analyses to identify events with Mendelian segregation. For each of the events evaluated, T1 plants that were positive for the *glyat+hra* were harvested separately and advanced as plant-to-row T2 short rows (2.4 m rows with 76.2 cm row spacing). Twelve remnant seed from each T2field entry were grown and V3 plants were sprayed with 3.36 kg ae ha^{-1} glyphosate plus 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS to determine zygosity of the corresponding short row. Lines were considered homozygous positive if all 12 plants were tolerant, homozygous negative if all 12 plants were susceptible, and heterozygous if the 12 plants were a mixture of tolerant and susceptible phenotypes.

Chi square was used to analyze T2 results to identify events with Mendelian segregation across generations. Twenty four different lines at the T3 generation with the *glyat+hra* were selected for advancement to herbicide tolerance trials. Homozygous positive and homozgygous negative NILs from ten events were advanced to isoline yield trials. 3560.4.3.5 soybean event was one of the events selected for advancement.

The 3560.4.3.5 soybean event was evaluatead for glyphosate tolerance. 3560.4.3.5 soybean was grown in a randomized complete block (RCB) design in a split plot arrangement with three replications of paired 3.7 m rows (76.2 cm row spacing). Treatments consisted of an unsprayed control, and three spray rates applied at the V5 growth stage using either 3.36 kg ae ha^{-1}, 6.72 kg ae ha^{-1}, or 13.44 kg ae ha^{-1} glyphosate. Plots were evaluated for crop response at 7 and 14 days after treatment (DAT), and rated using a 1-9 score. Crop response data were analyzed using the general linear model (GLM) and mixed model analysis of variance (ANOVA) procedures of SAS. (The SAS System is a registered trademark of SAS Institute, Inc., Cary, NC, USA)

Selected T4 lines were tested for glyphosate tolerance. This experiment was a RCB design in a split plot arrangement with two replications of individual 1.2 m rows (76.2 cm row spacing). At the V3 growth stage, 6.72 kg ae ha^{-1} glyphosate was applied to one block, while another was unsprayed for use as a control. The third block received a sequential application of 6.72 kg ae ha^{-1} glyphosate at V3 followed by 6.72 kg ae ha^{-1} glyphosate at R1. Plots were evaluated for crop response at 7 DAT and 14 DAT, and assigned a visual response score from 0% to 100% response, where 0% = no response observed to 100% = plot completely dead. Crop response data were analyzed using the GLM and mixed model ANOVA procedures of SAS.

ALS inhibitor herbicide tolerance of the *gm-hra* gene was compared to sulfonylurea tolerant soybeans (STS®) by growing T4 plants of event 3560.4.3.5 and a STS® cultivar. The experiment was designed as a RCB design in a split plot arrangement with two replications of paired 3.7 m rows (76.2 cm row spacing). Spray treatments were applied at the V3 growth stage and consisted of one of the following: an unsprayed control, 8.8 g ai ha^{-1} rimsulfuron (*N*-[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]-3-(ethylsulfonyl)-2-pyridinesulfonamide), 8.8 g ai ha^{-1} tribenuron methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)methylamino]carbonyl]amino]sulfonyl]benzoate), or 30.0 g ai ha^{-1} chlorimuron ethyl (ethyl 2-[[[[(4-chloro-6-methoxypyrimidin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate) + 9.67 g ai ha^{-1} thifensulfuron methyl. All spray treatments listed had 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS added. Plots were evaluated for crop response at 7 DAT and 14 DAT, and assigned a visual response score from 0% to 100% response. Crop response data were analyzed using the mixed model ANOVA procedure of SAS.

ALS inhibitor herbicide tolerance trials were completed at two separate locations. Plants from the T5 generation of event 3560.4.3.5 and a STS® cultivar were grown in a RCB design in a split plot arrangement with three replications of paired 2.4 m rows (76.2 cm row spacing). Treatments were applied at the V3 growth stage and consisted of an unsprayed control, 4.2 g ai ha^{-1} metsulfuron methyl (methyl 2-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate), 70.0 g ai ha^{-1} thifensulfuron methyl, and 17.5 g ai ha^{-1} tribenuron methyl. All spray treatments listed had 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS added. Visual response ratings (0% to 100%) were assigned to

each plot at 7 DAT, 14 DAT, and 28 DAT. Crop response data were analyzed using the mixed model ANOVA procedure of SAS.

Multiple trials were conducted with the lead event 3560.4.3.5 soybean to observe the level of tolerance to different rates and application timings of glyphosate with and without other pesticide mixtures. The first experiments were grown in an RCB design of three replications of paired 3.7 m rows (76.2 cm row spacing), blocked by replication with randomized spray treatments within each replication. Treatments were applied at the V2 or R2 growth stage, and consisted of the following: unsprayed control, 3.36 kg ae ha^{-1} glyphosate, 3.36 kg ae ha^{-1} glyphosate + 560.4 g ai ha^{-1} chlorpyrifos (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate), 3.36 kg ae ha^{-1} glyphosate + 1120.9 g ai ha^{-1} bentazon (3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one 2,2-dioxide), 3.36 kg ae ha^{-1} glyphosate + 141.3 g ai ha^{-1} imazethapyr (2-[4.5-dihydro-4-methyl-4-(1 -methylethyl)-5-oxo-lH-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid), 3.36 kg ae ha^{-1} glyphosate + 263.4 g ai ha^{-1} fomesafen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-*N*-(methylsulfonyl)-2-nitrobenzamide), 3.36 kg ae ha^{-1} glyphosate + 17.5 g ai ha^{-1} thifensulfuron methyl + 17.5 g ai ha^{-1} tribenuron methyl, 3.36 kg ae ha^{-1} glyphosate + 17.5 g ai ha^{-1} thifensulfuron methyl + 17.5 g ai ha^{-1} tribenuron methyl + 560.4 g ai ha^{-1} chlorpyrifos, 3.36 kg ae ha^{-1} glyphosate + 17.5 g ai ha^{-1} thifensulfuron methyl + 17.5 g ai ha^{-1} tribenuron methyl + 1120.9 g ai ha^{-1} bentazon, 3.36 kg ae ha^{-1} glyphosate + 17.5 g ai ha^{-1} thifensulfuron methyl + 17.5 g ai ha^{-1} tribenuron methyl + 141.3 g ai ha^{-1} imazethapyr, and 3.36 kg ae ha^{-1} glyphosate + 17.5 g ai ha^{-1} thifensulfuron methyl + 17.5 g ai ha^{-1} tribenuron methyl + 263.4 g ai ha^{-1} fomesafen. All spray treatments listed had 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS added. Visual response ratings (0% to 100%) were assigned to each plot at 7 DAT, 14 DAT, and 28 DAT. Crop response data were analyzed using the GLM and mixed model ANOVA procedures of SAS.

The second set of tolerance experiments were designed to measure yield potential of 3560.4.3.5soybean after application of common field use rates of glyphosate and/or ALS inhibitor herbicides. These experiments were six replications of paired 3.7 m rows (76.2 cm row spacing), grown in a RCB design, blocked by replication, with randomized spray treatments within each replication. One experiment was grown at field A and two experiments were grown at field B and C (which were separated by planting date and physical distance of field locations.) In addition, the two fields had different environmental influence; one field experienced

drought stress through the spring and early summer, the other was well irrigated throughout the growing season. Treatments were randomized within each of the replications and sprayed at the VC, V2, R2, or R5 growth stages. Treatments consisted of 1.68 kg ai ha^{-1} glyphosate, 5.8 g ai ha^{-1} chlorimuron ethyl + 4.4 g ai ha^{-1} thifensulfuron methyl, and 1.68 kg ai ha^{-1} glyphosate + 5.8 g ai ha^{-1} chlorimuron ethyl + 4.4 g ai ha^{-1} thifensulfuron methyl. All spray treatments listed had 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS) added. Visual response ratings (0% to 100%) were assigned to each plot at 7 DAT, 14 DAT, and 28 DAT. Plots were harvested and yield was calculated for each plot. Crop response data were analyzed using the GLM and mixed model ANOVA procedures of SAS.

Homozygous positive and homozygous negative null segregants (NILs) of 3560.4.3.5 soybean from T3 generation events were grown in preliminary yield trials to determine if yield was impacted due to the presence of 3560.4.3.5 event. Isoline yield trials were designed as a RCB (blocked by event), with a single replication of paired 3.7 m rows (76.2 cm row spacing). Trials were mechanically cultivated and/or had labeled use rates of conventional soybean herbicides applied, as needed, to ensure weed-free conditions. Maturity scores and yield data were collected for each entry and subject to ANOVA using the mixed model procedure of SAS.

T3 seed for each isoline entry was also advanced to the T4 generation. Remnant T3 seed from each line was confirmed to be either homozygous positive or homozygous negative by evaluating 12 seedlings that were germinated in chlorsulfuron herbicide solution. Conventional soybean lines are not tolerant to spray application of chlorsulfuron, which is currently utilized in small grain production to control broadleaf weeds. The chlorsulfuron herbicide stock solution was created by mixing 66.7 mg of chlorsulfuron with 1 liter of water. The mixture was buffered to a pH of 7.5 using 1 mM phosphate buffer. From this stock solution, 20 ml was mixed with 1 liter of water, which was then used to saturate germination paper. Seeds were added to the germination paper and observed 10 days after initial treatment for their response. Seedlings that possessed the 3560.4.3.5 event germinated and grew normally, while seedlings that lacked the event created hooked unifoliolate leaves that did not expand and develop any further (data not shown). Lines were scored as homozygous positive if all 12 seedlings grew normally through the solution (data not shown). Lines were scored as homozygous negative if all 12 plants had the inhibited hooked unifoliolate phenotype (data not shown).

Homozygous positive 3560.4.3.5 event and homozygous negative NILs were grown in isoline yield trial experiments. Trials were mechanically cultivated and/or had labeled use rates of conventional soybean herbicides applied, as needed, to ensure weed-free conditions. Maturity scores and yield data were collected for each entry and subject to ANOVA using the mixed model procedure of SAS.

Remnant T4 seed from each line was confirmed to be either homozygous positive or homozygous negative by evaluating 12 seedlings using the chlorsulfuron herbicide screen. T4 seed for each selected isoline entry was advanced to the T5 generation.

Event 3560.4.3.5 was selected for additional isoline yield trial evaluations. Homozygous positive and homozygous negative NILs from event 3560.4.3.5 were grown using the same experimental yield test design implemented above. Experiments were planted and trials were mechanically cultivated and/or had labeled use rates of conventional soybean herbicides applied, as needed, to ensure weed-free conditions. Maturity scores and yield data were collected for each entry and subject to ANOVA using the mixed model procedure of SAS.

Event 3560.4.3.5 was selected for introgression into four different Pioneer® conventional (non-transgenic) elite lines with different genetic parentage. The four elite lines had relative maturities (RM) of 22, 27, 30, and 38. After the initial cross, F1 seed was backcrossed to the respective recurrent parent and BC1F1 seed was advanced to the F2 generation. The four BC1F2 populations created were grown and harvested as individual plants. BC1F3 lines were created using single plant-to-row 2.4 m increase rows. Homozygous positive or homozygous negative sister lines were selected for yield testing based upon screening of 12 remnant seedlings for each line using the chlorsulfuron herbicide seedling test. Based upon the remnant screening across the four populations, 328 positive 3560.4.3.5 soybean lines, and 116 negative lines were selected for preliminary yield trials

For the 3560.4.3.5 x elite preliminary yield trials, BC1F3 lines were blocked by the recurrent parent and presence or absence of the 3560.4.3.5 event. Positive and negative blocks were planted adjacent to each other at different locations, based upon expected maturity of the lines within the population. The 3560.4.3.5 event positive blocks had a V3 application of 2.24 kg ai ha^{-1} glyphosate plus 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS, while negative blocks had conventional herbicides applied (as needed) to maintain weed free plots. Maturity scores and yield data were collected

for each entry at each location and subject to multiple regression and ANOVA using the GLM and mixed model procedures of SAS.

RESULTS AND DISCUSSION

T0 3560.4.3.5 soybean were evaluated for glyphosate tolerance. The 3560.4.3.5 soybean were sprayed with 2.24 kg ai ha^{-1} glyphosate and 4.48 kg ae ha^{-1} glyphosate (Table 15). The 2.24 kg ai ha^{-1} treatment would correspond to 2X the typical labeled field application rate of glyphosate, while the 4.48 kg ae ha^{-1} treatment would correspond to 4X the typical labeled field application rate of glyphosate. The untransformed parental line (sprayed as a control) did not survive either glyphosate application rate and was consistently rated a 1.

The 3560.4.3.5 event was advanced to the T1 generation in a greenhouse to confirm herbicide tolerance. The 3560.4.3.5 event had tolerance after application of 1.68 kg ae ha^{-1} glyphosate, after application of 60.0 g ai ha^{-1} thifensulfuron methyl, and after a sequential application of 70 g ai ha^{-1} thifensulfuron methyl at V3, followed by 1.68 kg ae ha^{-1} glyphosate at V5 as shown in Table 15. These data indicate event 3560.4.3.5 confers tolerance to both glyphosate and thifensulfuron methyl application at early generations after transformation.

The 3560.4.3.5 event was analyzed for Mendelian segregation across early generations (Table 16).

Table 15. Southern copy number estimates and average visual response ratings† ten days after herbicide application on T0 and T1 generation transgenic event 3560.4.3.5

			Generation					
	Southern bands‡		T0		T1			
Event	*glyat4601*	*hra*	1.68§	3.36§	1.68§	60.0¶	70.0+1.68#	
3560.4.3.5	1	1	7.0	6.5	7.0	7.0	8.0	8.0

† Average visual response rating of tolerant plants 10 days after herbicide application in the greenhouse (0 = dead plant to 9 = no response detected)

‡ Estimated number of copies based upon Southern analysis using *HindIII* and the *glyat*4601 gene or gm-*hra* gene as a probe

§ Glyphosate application (1.68 kg ae ha^{-1}, or 3.36 kg ae ha^{-1}) + 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS applied at the V3 growth stage

¶ Thifensulfuron methyl application (60.0 g ai ha^{-1} or 70.0 g ai ha^{-1}) + 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS applied at the V3 growth stage.

Sequential application; initially sprayed at the V3 growth stage with 70 g ai ha^{-1} thifensulfuron methyl + 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS, followed by spray of 1.68 kg ae ha^{-1} glyphosate + 0.25% v/v NIS + 2.24 kg ae ha^{-1} AMS at the V6 growth stage. The first score is 10 days after the V3 thifensulfuron methyl application, the second score is 10 days after the V6 glyphosate application

:gation ratios of T1 plants and T2 progeny rows of event 3560.4.3.5.

Generation								
T1 plants[†]					T2 lines[‡]			
Observed		Expected			Observed		Expected	
Positive	Negative	Positive	Negative	chi^2 P[§]	Positive	Negative	Positive	Negative
59	23	61.5	21.1	0.605	34	9	32.3	10.3

·e identified to be positive or negative for the *glyat* transgene insert using PCR amplification
ved from individual *glyat* positive T1 plants) were screened by spraying 3.36 kg ae ha^{-1} glyphosate plus 0.25% v.
MS on 12 remnant V3 plants in a greenhouse.

ty that deviation from expected model is due to chance alone

The T3 plants from the 3560.4.3.5 plants were sprayed in a glyphosate herbicide tolerance field trial. Table 17 shows the herbicide response at seven DAT after application of 3.36 kg ae ha^{-1} glyphosate, after application of 6.72 kg ae ha^{-1} glyphosate, and after application of 13.44 kg ae ha^{-1} glyphosate. The untransformed control line was susceptible to any glyphosate application and was consistently rated as a 1. 3560.4.3.5 soybean T4 plants were advanced for additional glyphosate tolerance testing. Table 17 shows the spray ratings at seven and fourteen DAT with 6.72 kg ae ha^{-1} glyphosate.

LSMeans for visual ratings[†] of selected T3 and T4 3560.4.3.5 plants, sprayed with experimental rates of glyphosate in field ts.

2004 (T3 plants)[‡]				2005 (T4 plants)[§]					
Glyphosate spray rate (kg ae ha^{-1})				Glyphosate spray rate (kg ae ha^{-1})					
Unsprayed	3.36	6.72	13.44	Unsprayed		6.72		6.72 sequential[¶]	
7 DAT	7 DAT	7 DAT	7 DAT	7 DAT	14 DAT	7 DAT	14 DAT	7 DAT	14 DAT
9.0	8.0	6.7	5.7	9.0	9.0	6.8	7.8	7.8	7.7

:sponse ratings on a 1 to 9 scale, where 1 = completely dead plot to 9 = no response observed
isted of three randomized replications of paired 3.7 m rows (76.2 cm row spacing) sprayed at the V3 growth stage
isted of two randomized replications of paired 1.2 m rows (76.2 cm row spacing) sprayed at the V3 growth stage
al spray; 6.72 kg ae ha^{-1} glyphosate sprayed at V3, followed by 6.72 kg ae ha^{-1} glyphosate sprayed on the same plots at R1 (i w this treatment is after the R1 application)

In addition to the glyphosate herbicide tolerance trials, event 3560.4.3.5 was compared to a STS® cultivar in two experiments to determine if the *hra* gene had better tolerance to different ALS inhibitor herbicides(Table 18). Across these two experiments, the 3560.4.3.5 soybean had significantly lower crop response compared to STS® at 7 DAT and 14 DAT after application of 8.8 g ai ha^{-1} rimsulfuron, 8.8 g ai ha^{-1} tribenuron methyl, 4.2 g ai ha^{-1} metsulfuron methyl, and 17.5 g ai ha^{-1} tribenuron methyl (Table 18). These chemistries are currently not labeled for use on soybean, and will cause high levels of crop response on current STS® and conventional soybean cultivars. The tolerance data obtained indicate that 3560.4.3.5 soybean had significantly higher tolerance to multiple ALS inhibitor chemistries when compared directly to the STS® trait.

ence of LSMeans for crop response ratings† of 3560.4.3.5 soybean compared to STS® across different ALS inhit periments.

Rate			7 DAT				14 DAT				28 DAT¶		
(g ai ha^{-1})	Location§	Reps	*3560.4.3.5*	STS®	Difference	Pr>|t|	*3560.4.3.5*	STS®	Difference	Pr>|t|	*3560.4.3.5*	STS®	Difference
8.8	A	2	10.0	50.0	-40.0	<.0001	5.0	55.0	-50.0	<.0001			
8.8	A	2	5.0	35.0	-30.0	<.0001	5.0	42.5	-37.5	<.0001			
30.0 + 9.67	A	2	0.0	5.0	-5.0	0.200	0.0	5.0	-5.0	0.171			
0	A	2	0.0	0.0	0.0	1.000	0.0	0.0	-45.0	<.0001			
4.2	B	6	58.3	79.2	-20.8	<.0001	64.2	90.8	-26.7	<.0001	29.2	95.0	-65.8
70	B	6	23.3	27.5	-4.2.0	0.549	6.7	8.3	-1.7	0.797	8.3	6.7	1.7
17.5	B	6	11.7	36.7	-25.0	<.0001	15.0	48.3	-33.3	<.0001	5.0	21.7	-16.7
0	B	6	0.0	0.0	0.0	1.000	1.7	0.0	1.7	0.747	1.7	0.0	1.7

se ratings from 0% to 100%, where 0% = no response observed to 100% = plot completely dead.
S + 2.24 kg ai ha^{-1} AMS was added to all spray treatments
nsisted of two replications of paired 3.7 m rows (76 cm row spacing); B location consisted of three replications o
n row spacing), grown at two different locations.
al response of 35604.3.5 at 14 DAT, the 28 DAT ratings were not recorded for the A experiment

Event 3560.4.3.5 was evaluated for herbicide tolerance and yield performance in more extensive testing. For the first set of experiments, event 3560.4.3.5 was sprayed at a vegetative stage and a reproductive stage with different tank mixes of glyphosate and other pesticides. Across the treatments evaluated, there was minimal crop response at 7 DAT and 14 DAT (10% or less) for the glyphosate and all glyphosate plus pesticide mixtures applied at the V2 growth stage, except those containing bentazon or fomesafen (Table 19). The treatments with bentazon or fomesafen caused up to 20% initial phytotoxicity at 7 DAT, which diminished to less than 12% by 14 DAT. These results were expected, as the leaf bronzing observed was equivalent to phytotoxicity that is typically observed on commodity soybeans up to 14 days after application with either bentazon of fomesafen chemistry. It should also be noted that herbicide response ratings are very subjective, and in general, a 10% response cannot be easily distinguished unless there is an unsprayed control right next to the plot being evaluated. By 28 DAT, there was essentially no crop response to any of the treatments applied at the V2 growth stage (Table 19). For the mixtures applied at R2, all of the treatments had 10% or less response at 7 DAT, 14 DAT, and 28 DAT except for the treatment containing bentazon (Table 19). For this treatment an average crop response of 11.4% was recorded at 7 DAT, which diminished to less than 5% at 14 DAT and 0% at 28 DAT (Table 19). The crop response observed was the typical leaf bronzing that is commonly observed up to 14 DAT after application of bentazon on commodity soybeans. In general, 3560.4.3.5 soybean had excellent tolerance to different mixtures of glyphosate with and without other pesticides across two different growth stages evaluated in different environments.

ıns for crop response ratings† of 3560.4.3.5 soybean treated at two different growth stages with different tank mi

	Glyphosate rate (kg ae ha-1)	Other pesticides	Application rate (g ai ha^{-1})	Stage	7 DAT Reps	14 DAT Average	28 DAT Average
	Average						
	3.36		V2	9	3.9	3.3	0.6
	3.36	560.4	V2	9	5.6	3.3	0.0
	3.36	1120.9	V2	9	16.6	6.3	0.6
	3.36	141.3	V2	9	5.0	2.2	0.6
	3.36	263.4	V2	9	20.3	12.0	0.6
thyl + thifensulfuron-methyl	3.36	17.5 + 17.5	V2	9	5.0	8.3	0.0
thyl + thifensulfuron-methyl + chlorpyrifos	3.36	17.5 + 17.5 + 560.4	V2	9	7.6	10.8	0.0
thyl + thifensulfuron-methyl + bentazon	3.36	17.5 + 17.5 + 1120.9	V2	9	17.9	5.0	0.0
thyl + thifensulfuron-methyl + imazethapyr	3.36	17.5 + 17.5 + 141.3	V2	9	5.1	7.8	0.0
thyl + thifensulfuron-methyl + fomesafen	3.36	17.5 + 17.5 + 263.4	V2	9	19.8	11.4	1.1
	3.36		R2	9	1.7	0.6	0.0
	3.36	560.4	R2	9	1.7	0.6	0.0
	3.36	1120.9	R2	9	11.4	2.2	0.0
	3.36	141.3	R2	9	0.6	0.0	0.0
	3.36	263.4	R2	9	9.6	1.7	0.0
thyl + thifensulfuron-methyl	3.36	17.5 + 17.5	R2	9	1.1	0.0	0.0
thyl + thifensulfuron-methyl + chlorpyrifos	3.36	17.5 + 17.5 + 560.4	R2	9	2.8	0.0	0.0
thyl + thifensulfuron-methyl + bentazon	3.36	17.5 + 17.5 + 1120.9	R2	9	7.0	1.7	0.0
thyl + thifensulfuron-methyl + imazethapyr	3.36	17.5 + 17.5 + 141.3	R2	9	1.7	0.0	0.0
thyl + thifensulfuron-methyl + fomesafen	3.36	17.5 + 17.5 + 263.4	R2	9	5.3	2.8	0.0
	3.36		R2	9	0.0	0.0	0.0
					3.3	3.3	2.0

: ratings from 0% to 100%, where 0% = no response observed to 100% = plot completely dead.
ıad 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS added.

The second set of experiments conducted with event 3560.4.3.5 were designed to measure yield potential after application of different herbicides at field use rates that would be commonly utilized for soybean production. Across the glyphosate, ALS inhibitor, and glyphosate plus ALS inhibitor treatments there was minimal (10% or less) to essentially no response at 7, 14, and 28 DAT (Table 20). When yield data was collected, there were no significant yield differences detected for any of the herbicide treatments applied at different the growth stages (Table 20). In addition, the replications that received herbicide treatments were not statistically different from the unsprayed control replications for yield (Table 20). Based upon multiple year herbicide tolerance trials, it can be concluded that event 3560.4.3.5 had excellent tolerance to field use rates of glyphosate, ALS inhibitor, and glyphosate plus ALS inhibitor herbicides. In addition, yield potential was not affected when event 3560.4.3.5 was sprayed with glyphosate, ALS inhibitor, and glyphosate plus ALS inhibitor chemistries at different vegetative and reproductive growth stages.

eans for crop response ratings† and yield (kg ha^{-1}) for 3560.4.3.5 soybean treated at four different growth stages v
nations.

	Glyphosate rate (kg ae ha^{-1})	ALS inhibitor rate (g ai ha^{-1})	Application Stage	Reps
	7 DAT	14 DAT	28 DAT	Yield
	1.68		VC	18
	1.6	0.8	0.6	2745.7
	1.68		V2	18
	5.3	2.8	0.6	2851.7
	1.68		R2	18
	2.0	0.6	0.0	2702.9
	1.68		R5	18
	0.3	0.3	0.0	2718.8
+ thifensulfuron methyl		5.8 + 4.4	VC	18
	0.7	0.6	0.6	2756.4
+ thifensulfuron methyl		5.8 + 4.4	V2	18
	0.6	0.0	0.3	2864.0
+ thifensulfuron methyl		5.8 + 4.4	R2	18
	0.0	0.0	0.3	2899.2
+ thifensulfuron methyl		5.8 + 4.4	R5	18
	0.0	0.0	0.6	2861.1
rimuron ethyl + thifensulfuron methyl	1.68	5.8 + 4.4	VC	18
	2.6	1.7	0.0	2647.3
rimuron ethyl + thifensulfuron methyl	1.68	5.8 + 4.4	V2	18
	6.9	3.8	2.2	2829.7
rimuron ethyl + thifensulfuron methyl	1.68	5.8 + 4.4	R2	18
	0.8	0.0	0.3	2841.6
rimuron ethyl + thifensulfuron methyl	1.68	5.8 + 4.4	R5	18
	1.1	0.0	0.0	2768.3
rol			Control	18
	0.0	0.0	0.0	2813.1
			Control	18
	0.0	0.0	0.0	2742.4
	1.3	1.1	0.8	155.6

: ratings from 0% to 100%, where 0% = no response observed to 100% = plot completely dead
ield locations were utilized; one field had severe drought stress during the spring and early summer, the other was well irri
ıad 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS added

Isoline yield test data were collected in five additional environments for event 3560.4.3.5 soybean. When isoline yield data were subject to mixed model ANOVA, the year, and location (nested within year) were significantly different (Table 21). Positive 3560.4.3.5 soybean and negative NILs, and the interactions with the 3560.4.3.5 event were not significantly different for yield (Table 21). These data indicate that presence of event 3560.4.3.5 does not impact final yield potential when NILs were tested across multiple years and environments.

Table 21. Mixed model ANOVA for yield (kg ha^{-1}) of positive and negative NILs of event 3560.4.3.5, tested in fourteen environments.

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Year	**2**	**80**	**26.92**	**<.0001**
Location(Year)	**11**	**80**	**7.82**	**<.0001**
glyat+hra	1	80	0.04	0.850
Year* *glyat+hra*	2	80	0.63	0.533
Location(Year)**glyat+hra*	11	80	1.75	0.077

Event 3560.4.3.5 was backcrossed into four different Pioneer® conventional elite lines to confirm Mendelian segregation, and to measure yield impact of the event in different genetic backgrounds. For each of the four different BC1F2 populations tested, the seedlings segregated in a 3:1 (tolerant:susceptible) ratio, when analyzed using chlorsulfuron screening (Table 22). When event 3560.4.3.5 was forward crossed into 31 different genetic backgrounds, all F2 populations examined segregated in a 3:1 (tolerant:susceptible) ratio when sprayed in the field with glyphosate (Table 22). These data suggest that in different genetic backgrounds, event 3560.4.3.5 will confer tolerance to glyphosate and ALS inhibitor herbicides, and will segregate as a single dominant gene.

Table 22. F2 segregation ratios of event 3560.4.3.5 backcrossed into four different elite genetic backgrounds, and forward crossed into 31 different elite genetic backgrounds.

		plants observed		plants expected		
Population	Generation†	Resistant	Susceptible	Resistant	Susceptible	P‡
3560.4.3.5x Elite7	BC1F2	700	222	691.5	230.5	0.518
3560.4.3.5x Elite8	BC1F2	761	273	775.5	258.5	0.298
3560.4.3.5x Elite9	BC1F2	160	54	160.5	53.5	0.937
3560.4.3.5x Elite10	BC1F2	205	79	213.0	71.0	0.273
3560.4.3.5x PHI1	F2	53	23	57.0	19.0	0.289
3560.4.3.5x PHI2	F2	76	20	72.0	24.0	0.346
3560.4.3.5x PHI3	F2	66	24	67.5	22.5	0.715
3560.4.3.5x PHI4	F2	75	23	73.5	24.5	0.726
3560.4.3.5x PHI5	F2	99	23	91.5	30.5	0.117
3560.4.3.5x PHI6	F2	96	24	90.0	30.0	0.206
3560.4.3.5x PHI11	F2	72	18	67.5	22.5	0.273
3560.4.3.5x PHI12	F2	115	36	113.3	37.8	0.742
3560.4.3.5x PHI13	F2	91	22	84.8	28.3	0.175
3560.4.3.5x PHI14	F2	97	26	92.3	30.8	0.323
3560.4.3.5x PHI15	F2	88	28	87.0	29.0	0.830
3560.4.3.5x PHI16	F2	66	24	67.5	22.5	0.715
3560.4.3.5x PHI17	F2	75	34	81.8	27.3	0.135
3560.4.3.5x PHI18	F2	108	25	99.8	33.3	0.099
3560.4.3.5x PHI19	F2	78	26	78.0	26.0	1.000
3560.4.3.5x PHI20	F2	95	29	93.0	31.0	0.678
3560.4.3.5x PHI21	F2	109	37	109.5	36.5	0.924
3560.4.3.5x PHI22	F2	75	26	75.8	25.3	0.863
3560.4.3.5x PHI23	F2	85	19	78.0	26.0	0.113
3560.4.3.5x PHI24	F2	81	18	74.3	24.8	0.117
3560.4.3.5x PHI25	F2	76	23	74.3	24.8	0.685
3560.4.3.5x PHI26	F2	97	36	99.8	33.3	0.582
3560.4.3.5x PHI27	F2	98	28	94.5	31.5	0.471
3560.4.3.5x PHI28	F2	89	22	83.3	27.8	0.208
3560.4.3.5x PHI29	F2	71	24	71.3	23.8	0.953
3560.4.3.5x PHI30	F2	54	15	51.8	17.3	0.532
3560.4.3.5x PHI31	F2	73	20	69.8	23.3	0.436
3560.4.3.5x PHI32	F2	53	12	48.8	16.3	0.223
3560.4.3.5x PHI33	F2	96	26	91.5	30.5	0.347
3560.4.3.5x PHI34	F2	76	20	72.0	24.0	0.346
3560.4.3.5x PHI35	F2	69	18	65.3	21.8	0.353

† BC1F2 populations were screened using chlorsulfuron solution on emerging seedlings. F2 populations were grown in the field and sprayed with 2.24 kg ai ha^{-1} glyphosate + 0.25% v/v NIS + 2.24 kg ai ha^{-1} AMS at the V4 growth stage. Resistant and susceptible plants were counted approximately 7 days after treatment for all populations examined.

‡ chi^2 probability that deviation from expected model is due to chance alone

To test the yield impact of event 3560.4.3.5 in different genetic backgrounds, BC1F3 lines were developed that were either homozygous positive or homozygous

negative for the 3560.4.3.5 event (Table 23). Since these lines are not true NILs, there may be some confounding error associated with the preliminary yield tests evaluated. For example, later maturing soybean lines typically have higher yield compared to earlier maturing lines within the same population. Therefore, maturity for each BC1F3 line was calculated as the number of days from planting to the estimated R8 growth stage (date when 95% of the pods had reached their mature pod color). Maturity estimates for each line were developed based upon direct comparison at each location to several non-transgenic Pioneer® experimental lines of known maturity. For the preliminary yield trail data analysis, maturity was analyzed as a covariate by nesting within the 3560.4.3.5 event to allow for a yield comparison between 3560.4.3.5 event positive and 3560.4.3.5 event negative lines at the same estimated maturity. It should be noted that the BC1F3 *glyat+hra* positive versus *glyat+hra* negative comparisons reported may also be confounded by the different herbicide programs utilized. However, blocking conventional cultivar trials from glyphosate tolerant trials has been a common practice in soybean cultivar development programs. In addition, for the 3560.4.3.5x elite data analysis presented, it was assumed that segregation of background maturity alleles, disease resistance alleles, and all other background genetic effects would occur at the same frequency within a population of 3560.4.3.5 event positive lines and within a population of 3560.4.3.5 event negative lines derived from the same initial BC1F1 plant.

Yield LSMeans of 3560.4.3.5 event positive and 3560.4.3.5 event negative lines were examined for each of the four BC1F3 populations tested across different locations (Table 23). For the BC1F3 population of 3560.4.3.5 x RM22 Elite, the 3560.4.3.5 event positive lines within maturity group 113 had significantly higher yield compared to the 3560.4.3.5 event negative lines within maturity group 113 (Table 23). This difference was most likely due to environmental effect, as for all the other maturity groupings, 3560.4.3.5 event positive and 3560.4.3.5 event negative lines were not statistically different for yield (Table 23). In addition, at a specific location, and when yield data from all locations tested were pooled, 3560.4.3.5 event positive lines were not significantly different for yield compared to 3560.4.3.5 event negative lines within the 3560.4.3.5 x RM22 Elite population (Table 23).

For the population of 3560.4.3.5 x RM27 Elite BC1F3 lines, the 3560.4.3.5 event positive lines tested had a significant yield advantage compared to the 3560.4.3.5 event

negative lines (Table 23). This observation is most likely due to an environmental effect, as when all locations were pooled, there were no significant differences detected for yield between 3560.4.3.5 event positive and 3560.4.3.5 event negative lines (Table 23). In addition, there were no significant differences detected for yield when 3560.4.3.5 event positive lines were compared to 3560.4.3.5 event negative lines at each specific maturity grouping (Table 23).

At two locations, there were significant yield differences observed within the population of 3560.4.3.5 x RM30 Elite BC1F3 lines. 3560.4.3.5 event positive lines had significantly higher yield compared to 3560.4.3.5 event negative lines, while at a different location, the opposite effect was noted (Table 23). When all locations are pooled, there was not a significant difference detected for yield between 3560.4.3.5 event positive and 3560.4.3.5 event negative lines (Table 23). These results suggest environmental influence was most likely causing the effect, as at each specific maturity grouping, there were no significant differences detected for yield when 3560.4.3.5 event positive lines were directly compared to 3560.4.3.5 event negative sister lines (Table 23).

Two significant yield differences were also observed within the population of 3560.4.3.5 x RM38 Elite BC1F3 lines at specific locations. In one of the replications, 3560.4.3.5 event positive lines had significantly higher yield compared to *glyat+hra* negative lines, while in another location, 3560.4.3.5 event negative lines had a yield advantage (Table 23). There was not a significant difference detected for yield when all locations were pooled for this population, which suggested environmental influence within a single location is causing the difference. At each of the maturity groupings, there was only a significant yield effect detected at maturity group 125, and in that case the 3560.4.3.5 event positive lines had significantly higher yield compared to 3560.4.3.5 event negative lines (Table 23). No distinct yield trends were evident across the four populations tested, indicating presence of the 3560.4.3.5 event does not impact yield potential when integrated into different genetic backgrounds.

Table 23. LSMeans for yield (kg ha^{-1}) at a location and a specific maturity for homozygous 3560.4.3.5 event positive and 3560.4.3.5 event negative BC1F3 lines within four different populations of event 3560.4.3.5x elite backgrounds.

Population	Location†	Maturity‡	3560.4.3.5 event Positive	Negative	Difference§	Pr>|t|
3560.4.3.5x RM22 ELITE	A	All	2471.74	2439.77	31.97	0.675
3560.4.3.5x RM22 ELITE	B	All	3491.77	3393.32	98.45	0.193
3560.4.3.5x RM22 ELITE	C	All	2683.63	2747.03	-63.40	0.402
3560.4.3.5x RM22 ELITE	D	All	3180.61	3144.42	36.19	0.632
3560.4.3.5x RM22 ELITE	E	All	2996.18	2912.63	83.55	0.271
3560.4.3.5x RM22 ELITE	All	All	2964.78	2927.43	37.35	0.341
3560.4.3.5x RM22 ELITE	All	111	2801.79	2836.18	-34.39	0.807
3560.4.3.5x RM22 ELITE	All	112	2927.15	2704.48	222.68	0.072
3560.4.3.5x RM22 ELITE	**All**	**113**	**2881.95**	**2686.65**	**195.30***	**0.041**
3560.4.3.5x RM22 ELITE	All	114	2625.92	2811.09	-185.17	0.285
3560.4.3.5x RM22 ELITE	All	115	2899.19	2839.28	59.91	0.633
3560.4.3.5x RM22 ELITE	All	116	2848.24	2859.77	-11.53	0.920
3560.4.3.5x RM22 ELITE	All	125	3183.30	2969.74	213.56	0.231
3560.4.3.5x RM22 ELITE	All	126	2976.31	3045.30	-69.00	0.770
3560.4.3.5x RM22 ELITE	All	127	2853.20	3029.97	-176.77	0.327
3560.4.3.5x RM22 ELITE	All	128	3031.55	3030.07	1.48	0.986
3560.4.3.5x RM22 ELITE	All	129	3088.60	2994.95	93.65	0.350
3560.4.3.5x RM22 ELITE	All	130	3198.18	3166.12	32.06	0.803
3560.4.3.5x RM22 ELITE	All	131	3117.01	2981.93	135.08	0.435
3560.4.3.5x RM22 ELITE	All	132	3074.62	3028.53	46.09	0.705
3560.4.3.5x RM27 ELITE	B	All	3331.34	3406.61	-75.26	0.640
3560.4.3.5x RM27 ELITE	**C**	**All**	**3218.79**	**2886.77**	**332.02***	**0.014**
3560.4.3.5x RM27 ELITE	D	All	3475.08	3417.17	57.91	0.656
3560.4.3.5x RM27 ELITE	E	All	3028.48	3149.72	-121.24	0.530
3560.4.3.5x RM27 ELITE	F	All	3099.48	3021.84	77.64	0.600
3560.4.3.5x RM27 ELITE	All	All	3230.63	3176.42	54.21	0.487
3560.4.3.5x RM27 ELITE	All	122	3311.82	3145.60	166.22	0.497
3560.4.3.5x RM27 ELITE	All	123	3226.68	3164.15	62.53	0.668
3560.4.3.5x RM27 ELITE	All	125	3148.80	3238.51	-89.71	0.660
3560.4.3.5x RM27 ELITE	All	126	3086.18	3213.06	-126.88	0.449
3560.4.3.5x RM27 ELITE	All	127	3271.69	3168.42	103.28	0.670
3560.4.3.5x RM27 ELITE	All	128	3237.08	3106.81	130.27	0.436
3560.4.3.5x RM27 ELITE	All	130	3127.15	2987.23	139.92	0.594
3560.4.3.5x RM27 ELITE	All	133	3435.66	3387.58	48.08	0.843
3560.4.3.5x RM30 ELITE	**C**	**All**	**3298.88**	**2983.11**	**315.77***	**<.0001**
3560.4.3.5x RM30 ELITE	**D**	**All**	**3544.23**	**3693.09**	**-148.86***	**0.029**
3560.4.3.5x RM30 ELITE	G	All	3472.84	3481.88	-9.05	0.888
3560.4.3.55 x RM30 ELITE	E	All	3177.69	3229.61	-51.92	0.418
3560.4.3.5x RM30 ELITE	F	All	3002.89	2975.75	27.13	0.673
3560.4.3.5x RM30 ELITE	All	All	3299.30	3272.69	26.62	0.512
3560.4.3.5x RM30 ELITE	All	123	3356.46	3010.13	346.33	0.063
3560.4.3.5x RM30 ELITE	All	125	3276.10	3172.47	103.63	0.194
3560.4.3.5x RM30 ELITE	All	126	3266.42	3168.61	97.81	0.228
3560.4.3.5x RM30 ELITE	All	127	3297.75	3299.10	-1.35	0.984
3560.4.3.5x RM30 ELITE	All	128	3285.84	3282.21	3.63	0.934

Population	Location†	Maturity‡	3560.4.3.5 event Positive	Negative	Difference§	Pr>|t|
3560.4.3.5x RM30 ELITE	All	129	3278.27	3407.86	-129.59	0.051
3560.4.3.5x RM30 ELITE	All	130	3334.29	3568.44	-234.15	0.118
3560.4.3.5x RM38 ELITE	C	All	2734.84	2659.09	75.75	0.256
3560.4.3.5x RM38 ELITE	G	All	3228.83	3305.91	-77.08	0.248
3560.4.3.5x RM38 ELITE	**E**	**All**	**3049.84**	**3190.55**	**-140.71***	**0.040**
3560.4.3.5x RM38 ELITE	F1	All	2678.11	2611.45	66.65	0.344
3560.4.3.5x RM38 ELITE	**F2**	**All**	**2687.71**	**2507.70**	**180.02***	**0.008**
3560.4.3.5x RM38 ELITE	All	All	2875.87	2854.94	20.93	0.608
3560.4.3.5x RM38 ELITE	All	122	2700.19	2803.14	-102.96	0.547
3560.4.3.5x RM38 ELITE	All	124	2743.61	2824.11	-80.50	0.564
3560.4.3.5x RM38 ELITE	**All**	**125**	**3206.30**	**2880.53**	**325.77***	**0.001**
3560.4.3.5x RM38 ELITE	All	126	2788.26	2864.36	-76.10	0.550
3560.4.3.5x RM38 ELITE	All	127	2849.14	2929.84	-80.71	0.514
3560.4.3.5x RM38 ELITE	All	128	2842.31	2989.39	-147.07	0.306
3560.4.3.5x RM38 ELITE	All	129	2928.44	2884.00	44.44	0.490
3560.4.3.5x RM38 ELITE	All	130	2918.29	2864.10	54.19	0.325
3560.4.3.5x RM38 ELITE	All	131	2906.27	2655.01	251.26	0.057

‡ Maturity is calculated as the average number of days between planting and R8 growth stage for the plot.

§ Estimated yield LSMean difference between 3560.4.3.5 event positive and 3560.4.3.5 event negative lines.

* Indicates estimated yield difference is significant at P = 0.05.

Table 24. Description of Genetic Elements in Fragment PHP20163A

Location on fragment PHP20163A (base pair position)	Genetic Element	Size (base pairs)	Description
1 to 16	polylinker region	16	Region for cloning genetic elements
17 to 502	SCP1 promoter	486	Constitutive synthetic promoter comprising a portion of the CaMV 35S promoter (Odell *et al.* (1985) Nature 313:801-812 and the Rsyn7-Syn II Core consensus promoter (US 6,0720,050 and 6,555673).
503 to 504	polylinker region	2	Region for cloning genetic elements
505 to 571	TMV omega 5'-UTR	67	An enhancer element derived from the Tobacco Mosaic Virus omega 5' untranslated leader (Gallie and Walbot (1992) NAR 20:4631-4638.
572 to 596	polylinker region	25	Region for cloning genetic elements
597 to 1037	*glyat4601* gene	441	Synthetic glyphosate N-acetyltransferase (*glyat*) gene (Castle *et al.* (2004) Science 304:1151-1154).
1038 to 1053	polylinker region	16	Region for cloning genetic elements
1054 to 1369	*pin*II terminator	316	Terminator region from *Solanum tuberosum* proteinase inhibitor II (*pin*II) gene (Keil *et al.* (1986) NAR 14:5641-5650; An *et al.* (1989) Plant Cell 1:115-122.
1370 to 1385	polylinker region	16	Region for cloning genetic elements
1386 to 2030	SAMS promoter	645	Promoter of the S-adenosyl-L-methionine synthetase (SAMS) gene from soybean (Falco and Li (2003) US publication 2003/0226166.
2031 to 2089	SAMS 5'-UTR	59	5' untranslated region of the SAMS gene from soybean (Falco and Li, 2003 US publication 2003/0226166).
2090 to 2680	SAMS intron	591	Intron within the 5'-untranslated region of the SAMS gene from soybean (Falco and Li, 2003 US publication 2003/0226166).
2681 to 2696	SAMS 5'-UTR	16	5' untranslated region (UTR) of the SAMS gene from soybean (Falco and Li, 2003 US publication 2003/0226166).
2697 to 4667	*gm-hra* gene	1971	Modified version of the acetolactate synthase gene from soybean with 15 additional nucleotides on the 5' end (2697 to 2711) derived from the SAMS gene and two nucleotide changes within the coding sequence.
4668 to 5319	*als* terminator	652	Native terminator from the soybean acetolactate synthase gene.
5319 to 5362	polylinker region	43	Region for cloning genetic elements

Table 25. Summary Table of SEQ ID NOS

SEQ ID NO	Description
1	Left sequence junction SC36
2	Right sequence junction D32ALS
3	complete inserted transgene
4	Left genomic border
5	Right genomic border
6	complete flanking and complete transgene insert
7	Forward primer
8	Reverse primer
9	Taqman MGB probe
10	Left flanking genomic/left border transgene (10 nt/10 nt)
11	Right flanking genomic/right border transgene (10 nt/ 10 nt)
12	Left flanking genomic/left bordertransgene (20 nt / 20 nt)
13	Right flanking genomic/right border transgene (20 nt / 20 nt)
14	Left flanking genomic/5' transgene
15	Right flanking genomic/3' transgene
16	Primer 1297
17	Primer 1298
18	Primer 1439
19	Primer 1514
20	Primer 1558
21	Primer 1660
22	Primer 1666
23	Primer 1679
24	Primer 1227
25	Primer 1297
26	Primer 1440
27	left flanking genomic/left border transgene (30 nt / 30 nt)

SEQ ID NO	Description
28	Right flanking genomic/right border transgene (30 nt / 30 nt)
29	SCP1 promoter probe
30	*glyat*4601 probe
31	*pinII* terminator probe
32	SAMS probe (5' end)
33	SAMS probe (3' end)
34	*gm-hra* probe (5' end)
35	*gm-hra* probe (3' end)
36	*als* probe
37	Primer DP-356-f1
38	Primer DP-356-r1
39	Primer DP-356-p
40	Primer 1610
41	Left flanking genomic/transgene
42	Right flanking genomic/transgene
43	181 nucleotides of the 5' end of transgene insert
44	Primer 104312
45	Primer 104314
46	Probe 125323
47	Primer 109893 (endogenous control)
48	Primer 109894 (endogenous control)
49	Probe 125322 (endogenous control)
50	Primer 1473
51	Primer 1504
52	Primer 1505
53	Primer 1506
54	Primer 1549
55	Primer 1550

The article "a" and "an" are used herein to refer to one or more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one or more element.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

THAT WHICH IS CLAIMED:

1. An isolated polynucleotide comprising SEQ ID NO: 10 or 11.

2. The isolated polynucleotide of claim 1, wherein said polynucleotide is selected from the group consisting of:

(a) a nucleotide sequence set forth in SEQ ID NO:6, 12, 13, 14, 15, 27, 28, 41, or 42; and,

(b) a nucleotide sequence comprising a fragment of SEQ ID NO: 6, 12, 13, 14, 15, 27, 28, 41 or 42.

3. A kit for identifying event 3560.4.3.5 in a biological sample, said kit comprising a first and a second primer, wherein said first and said second primer amplify a polynucleotide comprising a 3560.4.3.5 specific region.

4. The kit of claim 3, wherein said kit further comprises a polynucleotide for the detection of the 3560.4.3.5 specific region.

5. The kit of claim 3, wherein said first primer comprises a first fragment of SEQ ID NO: 6 and the second primer comprises a second fragment of SEQ ID NO:6, wherein said first and said second primer flank said 3560.4.3.5 specific region and share sufficient sequence homology or complementarity to said polynucleotide to amplify said 3560.4.3.5 specific region.

6. The kit of claim 5, wherein

a) said first primer comprises a fragment of SEQ ID NO:3 and said second primer comprises a fragment of SEQ ID NO:5;

b) said first primer comprises a fragment of SEQ ID NO:3 and said second primer comprises a fragment of SEQ ID NO:4;

c) said first primer comprises a fragment of SEQ ID NO: 4 and said second primer comprises a fragment of SEQ ID NO:5.

7. The kit of claim 5, wherein said first and said second primer comprises at least 8 consecutive polynucleotides of SEQ ID NO: 6.

8. The kit of claim 6, wherein said first or said second primer comprises at least 8 consecutive polynucleotides of SEQ ID NO:3, 4, or 5.

9. The kit of claim 3 or 4, wherein said first or said second primer comprise SEQ ID NO:7, 8, 9, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 37, 38, 39, 40, 44, 45, 46, 51, 52, 53, 54, 55.

10. A DNA detection kit comprising at least one polynucleotide that can specifically detect a 3560.4.3.5 specific region, wherein said polynucleotide comprises at least one DNA molecule of a sufficient length of contiguous nucleotides identical or complementary to SEQ ID NO: 6.

11. The DNA detection kit of claim 10, wherein said polynucleotide that can specifically detect a 3560.4.3.5 specific region comprises a polynucleotide having SEQ ID NO:10 or 11.

12. The DNA detection kit of claim 10, wherein said polynucleotide comprises a sequence which hybridizes under stringent conditions with sequences selected from the group consisting of:

(a) the sequences of SEQ ID NO:5 and SEQ ID NO:3; and,

(b) the sequences of SEQ ID NO:4 and SEQ ID NO: 3.

13. A soybean plant having stably integrated into its genome the polynucleotide of claim 1 or 2.

14. The soybean plant of claim 13, wherein said plant is a progeny of a plant grown from seeds of ATCC Seed Deposit PTA-8287.

15. A transgenic seed from the plant of claim 13.

16. A transgenic seed of ATCC Seed Deposit PTA-8287.

17. A method for identifying event 3560.4.3.5 in a biological sample, comprising

(a) contacting said sample with a first and a second primer; and,

(b) amplifying a polynucleotide comprising a 3560.4.3.5 specific region.

18. The method of claim 17, wherein the polynucleotide comprising the 3560.4.3.5 specific region comprises SEQ ID NO:11.

19. The method of claim 17, wherein the polynucleotide comprising the 3560.4.3.5 specific region comprises SEQ ID NO:10.

20. The method of claim 17, 18, or 19, further comprising detecting the 3560.4.3.5 specific region.

21. The method of claim 17, 18, or 19 , wherein said first primer comprises a first fragment of SEQ ID NO: 6 and the second primer comprises a second fragment of SEQ ID NO:6, wherein said first and said second primer flank said 3560.4.3.5 specific region and share sufficient sequence homology or complementarity to said polynucleotide to amplify said 3560.4.3.5 specific region.

22. The method of claim 21 , wherein

a) said first primer comprises a fragment of SEQ ID NO:3 and said second primer comprises a fragment of SEQ ID NO:5;

b) said first primer comprises a fragment of SEQ ID NO:3 and said second primer comprises a fragment of SEQ ID NO:4;

c) said first primer comprises a fragment of SEQ ID NO: 5 and said second primer comprises a fragment of SEQ ID NO:4.

23. The method of claim 21, wherein said first and said second primer comprise at least 8 consecutive polynucleotides of SEQ ID NO: 6.

24. The method of claim 22, wherein said first and said second primer comprise at least 8 consecutive polynucleotides of SEQ ID NO:5, 4 or 3.

25. The method of claim 17 , wherein said first and said second primer comprise SEQ ID NO:7, 8, 9, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 37, 38, 39 40, 44, 45, 46, 51, 52, 53, 54, or 55.

26. A method for identifying event 3560.4.3.5 in a biological sample, comprising

(a) contacting said sample with a first and a second primer;

(b) performing a DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(c) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of soybean event 3560.4.3.5.

27. The method of claim 26, wherein said first primer comprises a first fragment of SEQ ID NO: 6 and the second primer comprises a second fragment of SEQ ID NO:6, wherein said first and said second primer flank said 3560.4.3.5 specific region and share sufficient sequence homology or complementarity to said polynucleotide to amplify said 3560.4.3.5 specific region.

28. The method of claim 26, wherein said first and second primer are selected from the group consisting of:

(a) the sequences comprising SEQ ID NO:16 and SEQ ID NO:17;

(b) the sequences comprising SEQ ID NO:7 and SEQ ID NO:8;

(c) the sequences comprising SEQ ID NO:23 and SEQ ID NO:20;

(d) the sequences comprising SEQ ID NO:23 and SEQ ID NO:19;

(e) the sequences comprising SEQ ID NO:18 and SEQ ID NO:22;

(f) the sequences comprising SEQ ID NO:21 and SEQ ID NO:22;

(g) the sequences comprising SEQ ID NO:7 and SEQ ID NO:9;

(h) the sequences comprising SEQ ID NO:8 and SEQ ID NO:9;

and,

(i) the sequences comprising SEQ ID NO:44 and SEQ ID NO:45.

29. A method of detecting the presence of DNA corresponding to a 3560.4.3.5 event in a sample, the method comprising:

(a) contacting the sample with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from soybean event 3560.4.3.5 and specifically detects the 3560.4.3.5 event;

(b) subjecting the sample and probe to stringent hybridization conditions; and

(c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the 3560.4.3.5 event.

30. The method of claim 29, wherein said sample comprises soybean tissue.

31. A polynucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 7, 8, 9, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 37, 38, 39, 40, 43, 44, 45, 46, 51, 52, 53, 54, 55, or its complement.

32. A pair of DNA molecules comprising a first DNA molecule and a second DNA molecule, wherein said first DNA molecule comprising a first fragment of SEQ ID NO: 6 and the second DNA molecule comprising a second fragment of SEQ ID NO:6, wherein said first and said second DNA molecule flank said 3560.4.3.5 specific region of SEQ ID NO:6 and share sufficient sequence homology or complementarity to said polynucleotide to amplify said 3560.4.3.5 specific region.

33. A method for confirming seed purity or a method for screening seeds in a seed lot for a 3560.4.3.5 event comprising

(a) contacting said sample with a first and a second primer;

(b) performing a DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(c) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of soybean event 3560.4.3.5.

34. The method of claim 33, wherein said first primer comprises a first fragment of SEQ ID NO: 6 and the second primer comprises a second fragment of SEQ ID NO:6, wherein said first and said second primer flank said 3560.4.3.5 specific region and share sufficient sequence homology or complementarity to said polynucleotide to amplify said 3560.4.3.5 specific region.

35. The method of claim 34, wherein

a) said first primer comprises a fragment of SEQ ID NO:3 and said second primer comprises a fragment of SEQ ID NO:5;

b) said first primer comprises a fragment of SEQ ID NO:3 and said second primer comprises a fragment of SEQ ID NO:4;

c) said first primer comprises a fragment of SEQ ID NO: 4 and said second primer comprises a fragment of SEQ ID NO:5.

36. The method of claim 34, wherein said first and said second primer comprises at least 8 consecutive polynucleotides of SEQ ID NO: 6.

37. The method of claim 35, wherein said second primer and said first primer comprise at least 8 consecutive polynucleotides of SEQ ID NO:3, 4, or 5.

38. The method of claim 33, wherein said first or said second primer comprise SEQ ID NO: 7, 8, 9, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 37, 38, 39, 40, 44, 45, 46, 51, 52, 53, 54, or 55.

39. A method for confirming seed purity or for screening for the presence of a 3560.4.3.5 event in a seed lot comprising

(a) contacting a sample comprising soybean DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from soybean event 3560.4.3.5 and specifically detects the 3560.4.3.5 event;

(b) subjecting the sample and probe to stringent hybridization conditions; and

(c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the 3560.4.3.5 event.

40. A method of producing a glyphosate tolerant and ALS inhibitor tolerant plant comprising breeding a plant of claim 13 or 14 and selecting progeny by analyzing for at least one polynucleotide comprising a 3560.4.3.5 specific region.

41. The method of claim 40, wherein said polynucleotide comprises SEQ ID NO:11.

42. The method of claim 40, wherein said polynucleotide comprises SEQ ID NO:10.

43. The method of claim 39, wherein said polynucleotide is selected from the group consisting of:

(a) a nucleotide sequence set forth in SEQ ID NO:6, 10, 11, 12, 13, 14, 15, 27, 28, 41, 42, or 43;

(b) a nucleotide sequence comprising a fragment of SEQ ID NO: 6, 10, 11, 12, 13, 14, 15, 27, 28, 41, 42, or 43;.

44. A method for controlling weeds in an area of cultivation comprising applying an effective amount of glyphosate and/or an ALS inhibitor to the area of cultivation having a plant of claim 13 or 14.

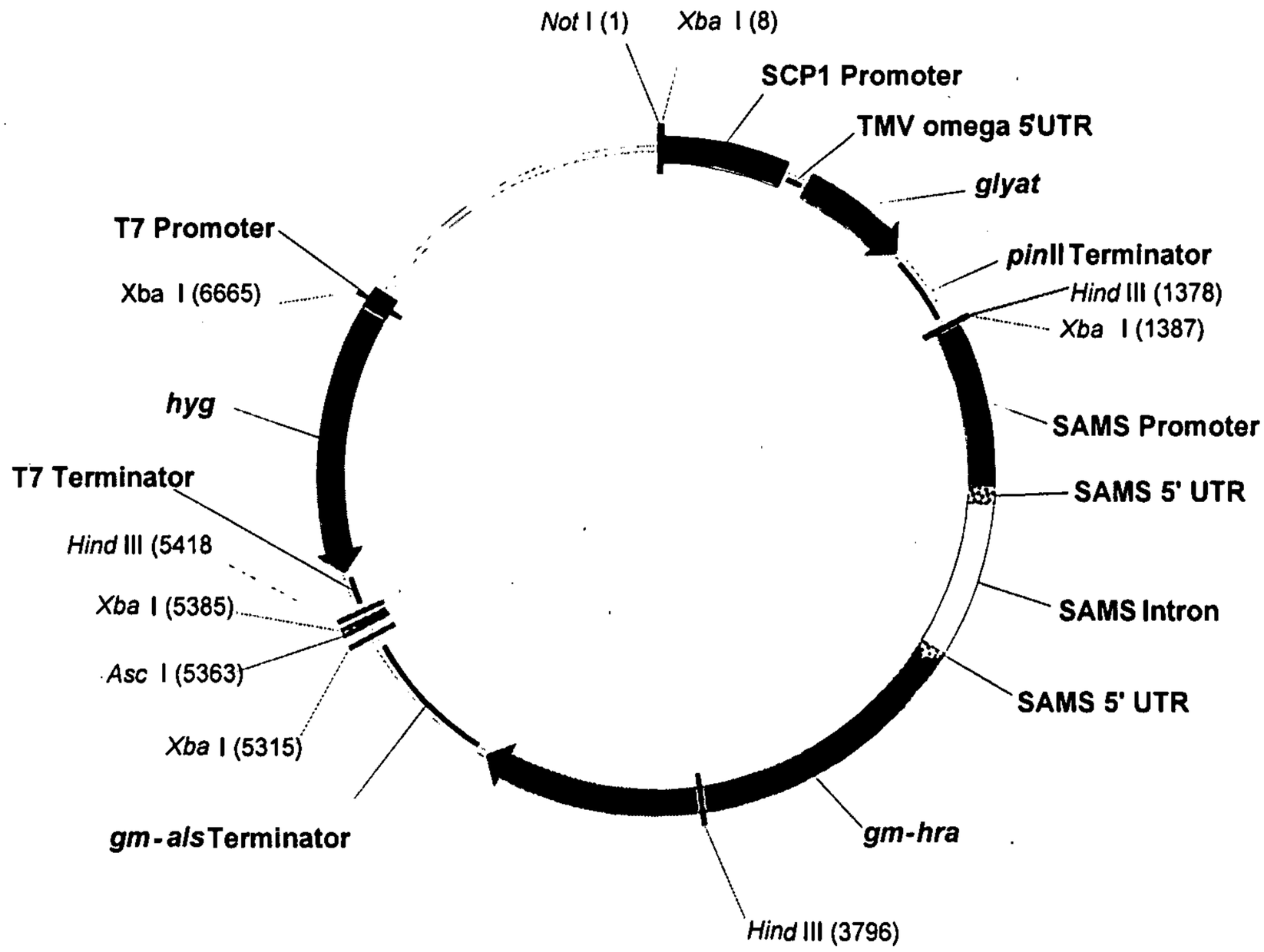
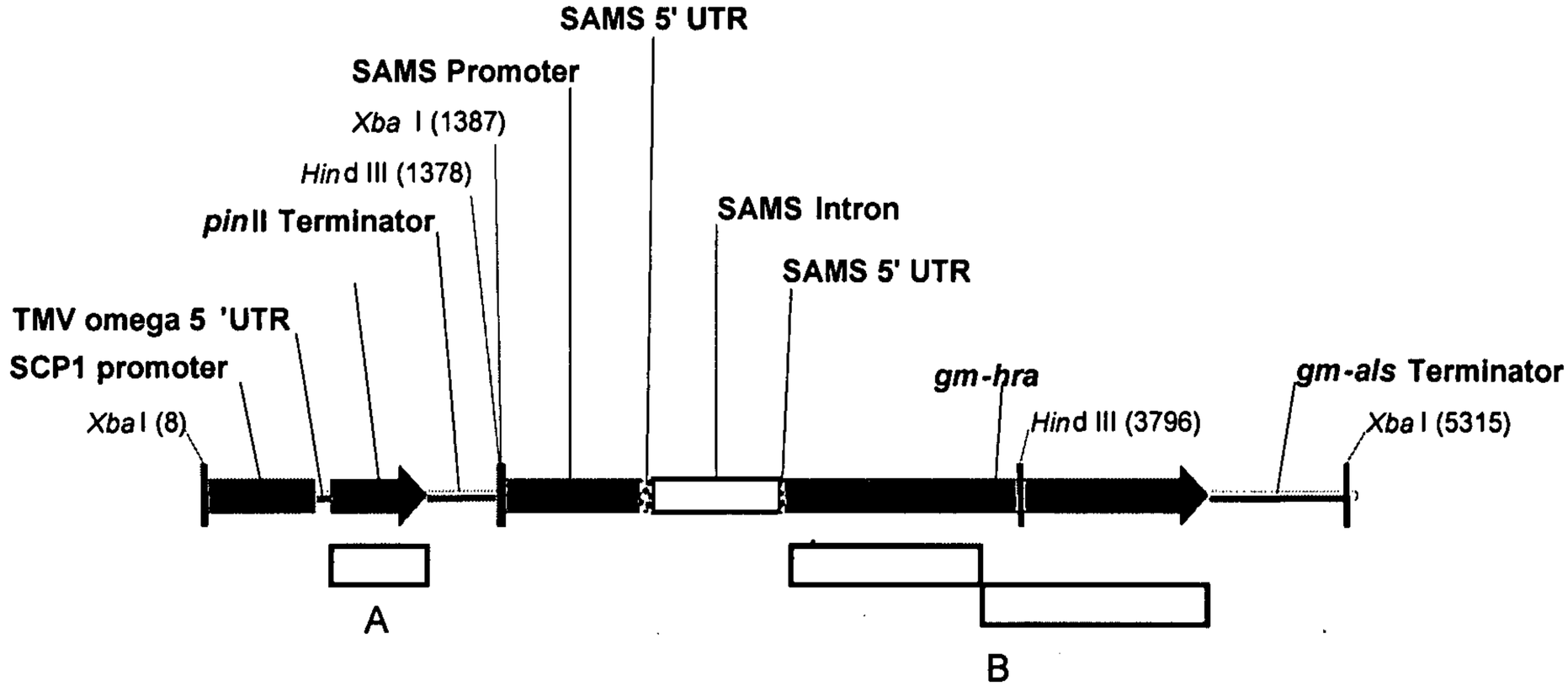

Not I (1)
Xba I (8)
SCP1 Promoter
TMV omega 5'UTR
glyat
pinII Terminator
Hind III (1378)
Xba I (1387)
SAMS Promoter
SAMS 5' UTR
SAMS Intron
SAMS 5' UTR
gm-hra
Hind III (3796)
gm-als Terminator
Xba I (5315)
Asc I (5363)
Xba I (5385)
Hind III (5418
T7 Terminator
hyg
Xba I (6665)
T7 Promoter

FIGURE 1



PHP20163A

5361 bp

FIGURE 2

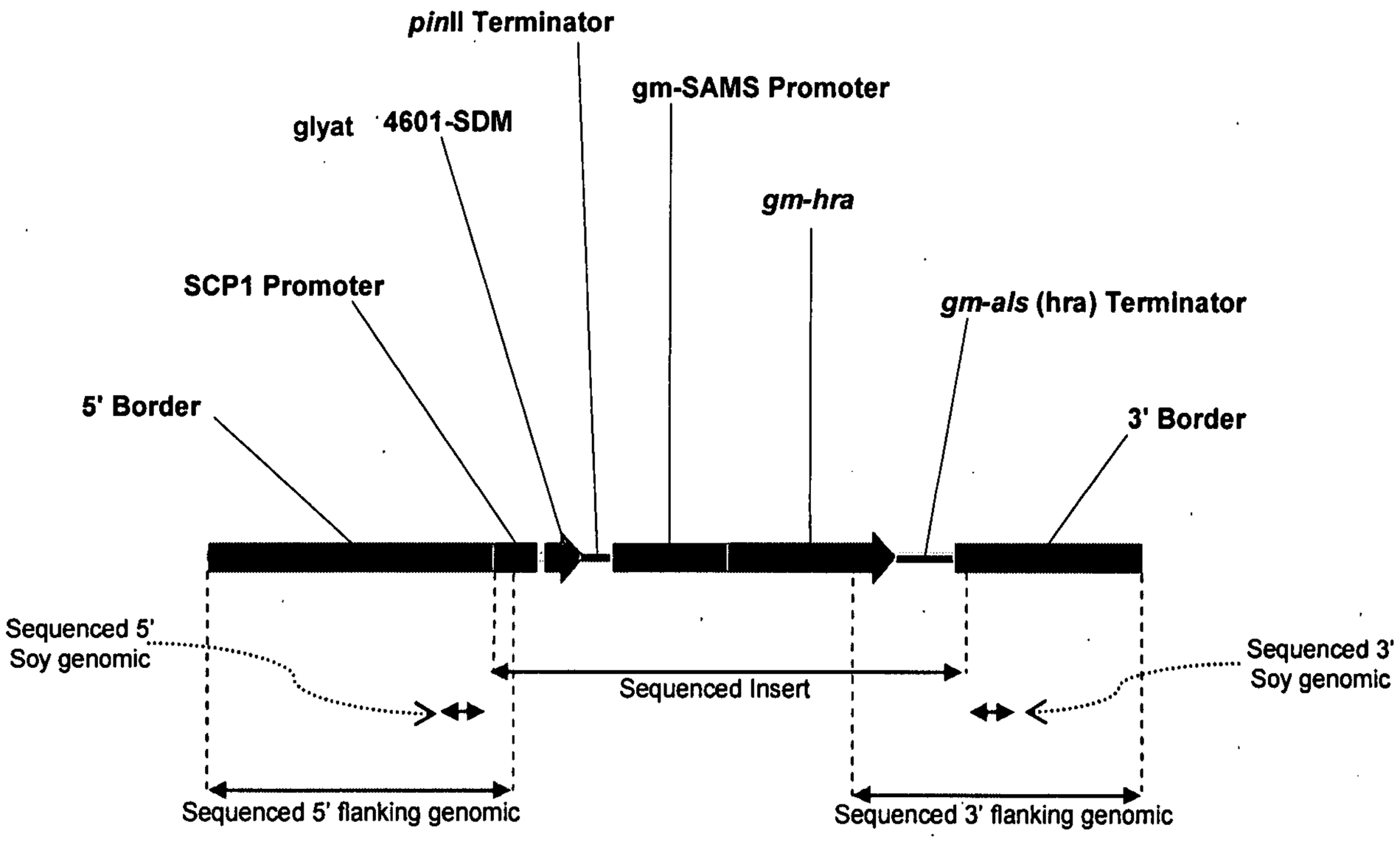

*pin*II Terminator
gm-SAMS Promoter
glyat
4601-SDM
gm-hra
SCP1 Promoter
gm-als (hra) Terminator
5' Border
3' Border
Sequenced 5'
Soy genomic
Sequenced Insert
Sequenced 3'
Soy genomic
Sequenced 5' flanking genomic
Sequenced 3' flanking genomic

FIGURE 3

Figure 4A


```
   1  AGCAATTGTT TTGTGCATTT CCAAATTTCA ATCTGATTTA TTAAATAAAA
  51  AAATTTTAAT TCGGGTTGAT TGTAAATCAG CTAAAGACAT TTTACAGAAA
 101  GACGTCAAAA ATCTTGGCTC CAAACAAATT TTTGCAAGAT GGCAAGCAAT
 151  ATTAAGTGTT TTTGATTTTG ACAGAATATA TCAAGGGCAC TTTAAACTCT
 201  CTACCTAACT ATCTTACACG TGAATTCTTA TAGACAAATT CTCATGCCAC
 251  CTAAGGCATC AGGCACCCCT CTCAGGGGTA GACGAAGCAC GAGTAAAGGA
 301  TTCAAGTTGG CCCTACCAGA GCCAACTATC AAAAAGAGTT CTTCCGCATC
 351  CTCTGGGTCA TCTACCCAAA AACCTAAAAT AAAAGGGTCA TCAACCCAAA
 401  TAGTCACCAT CAAACCTGAG TCATCCACTC AGGAATCCCC AAAACCTTCA
 451  ACCTCAAAAC AAACAAAGGC CGACTATACC TTCTCAGTAC AACACTCCAG
 501  GCCCTACAAG AAATTGGTCT AACCAAACTC CCAAAACTCG CCAAAAAGAC
 551  TTGGGCAGAC ATTGCCTCAG AATCTAATGA TGAATTTGAA ACTGATTTAC
 601  AAATCATGAT TCAAAACTCC AAACAATCCA AAACGATTGT CAATCCAAAA
 651  GGAAAACAGA CCTTATCCCA ACAAAAGACA CCATTACCAA AACCTACTAA
 701  CAGTTATATT TACAAAAATA AATTTCCAAC TGTTTTGTAG ATGGAGCCAA
 751  AATTTTGGGA CAAAAATCCC TTCAAGGCTA CAGCCAAGGC ATTTCCACCG
 801  GGCTTCCATT TCAAACCTAT TTCCACCAAT AAAACAAGAA TCTTTTACGA
 851  ATTCATACTG ATAGACACAA ACTCAGTGTC TATTAAACAC TTCAAAGACC
 901  CAAATGACAT AAATTTAAAC ACTCATTCAA CCATCCAGAT TTTAAAGTTC
 951  ATACAACCTC GACAATGTGG AACAAATATA AATCAAGCCA AACAATTCTT
1001  TGTACCCTTT GATCCTATAG GTTACACTAT TGGGATTATG TAGATGCATG
1051  GACCAATGTA TTCTGGCATC AAAATAACAA ATTCAAACAT TCTTGGCTTA
1101  TTTATTTCAA AACTAACACC GTCTATAATT TTCCAAATTG GTTCCTCCAA
1151  CGGTGGGACT TTTTTGGACC AAACTTTGAT ATCTACCCGG AGCAAGTCCA
1201  ACAAGGGTTT GATCAGTTCA AAAAAATGTT CAATTCTCAG GAATCACGAA
1251  TCCCTGTAGA CCTAAAATAC TTTTCCAATT TTGCATTGTC GTGGATATTT
1301  TCATGGCAAT ACAGATATGG GAAAACTGAA AACAACAAGC AGTTTCCATC
1351  ACTGCAACAT CATGCATTTA TCAAGTGGTG GAATTAGTTT GATACATCAA
1401  AAGCAGCACC AGATCAAGTG AGAATCTGGT TTCAAGCCCA TCCAGAATTT
1451  TTGAAAGTTG CTAATCCTGA GACTTCTTTA TTCCTCAATC AGAAGTCTCA
1501  ATTAGCTGCT TTCCTTTCTA GTTCCAAGTC AAAAGAAATT CTGGCACAAA
1551  ATCTAAAAGA AGTCCTACAG CTTCTCCAAC AAGAAGAAGA TAAAGGCTCT
1601  TCCTCAAAGA AGGAAGATAA CAATTCTTCA AAAGAAGATG ACGACCCTTT
1651  CTACCAAAAT GAAAATGATT GTTTTGGTAT TTCTCTAAAT GATGATTAAT
1701  TAAAAAATTA CATGTACTAT GTAAATAGTT TCGGTCACGA AACTGGCACT
1751  GTAGCTACAG TAAATTTTAT GGCTATTTAA GGAGTTCTCG GCCCATTTGT
1801  GAGGTACCTT TTCAGGTAGT CAGATCTCTA TTTTTAGAGA GAGAAACTCT
1851  AGGAAACAAT CTTTGTAAGT TTTTCTTTCG ATTTCAATAA ATTCAAAGTT
1901  TTCTCTTCAT ATCTCTTCTC CCTCTTGACC GGTCCTGTGC GGTTCTGCCA
1951  TCGCTTCAGT TTCTTCTCTT CTCTCCCCTT ATCGATATTG TGTGGCTCTA
2001  TTGCCACTTC GATTTCTTTT ATGTTGCTTT CATTTTAATT TGTTTTACCA
2051  TTTTCTTTTG ATATTATAAT TTCTATTTAA CTCTTGGTCA TACTGCATAT
2101  ATTCATAATA TATTCTTACA TCCTATCTAT CCGTTTGATC TCTTTTCACT
2151  GTTATATATA TATATATATA TATATATATC GTTTAACTTC ATGTTAGTAA
2201  TAAGATTAGA GTAAAAAATA TATATATAAC GAAGTTATTT TAACAAAAGG
2251  TATTTTTGTA AAAAAAAATT ATATGCTAAA AAAGTTTTAC TATATCTAAG
2301  CATGATTTTT TTTAATTCCC AAAACACGTG TAAATATTTT TAGGAATATT
2351  TTGTAAAAAA TCAAACATTT TTTTAATTAT TCGTATAAAA CATCAATCTT
2401  TAAGAATCAT AATTTTTAGA AATCATGATT TCTGGATATA AAAATACTTT
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FIGURE 4B

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2451  TTCTTGCAGC CAAACGTCTT CTAAAGCCAC ATGTTAATGG GTGTACAAAT
2501  TATAAAGTTT TTATAAACAT ATCACTTTTT AAAGTTAAAA ATATCACTAA
2551  TACGTTTCTA AATGCATTTT TTTTAGAAAT TATAAGTTTC TAGCACTCCA
2601  TTGGAGTTGT ATAGAACTAC TACTAATCTA CTATTAATAA CAATTTATCT
2651  CCTCCAAATA TTTAAGTAAA CTGCATATTT AGAGAATGTT GGACAGTAAA
2701  GCTAGCCACT CAATATTTAG GTGCTCCCCG AAAGAGGAAA GCAAACAAGC
2751  CACCAGCACT TCAATCAGTA AAGCTAGCCA CTCAACTCGC TCTCTTCAAA
2801  TTCCCTTTTA CATTTTATTT CAGATCCTCC ACCTAGCCAA GTAGGTCTCA
2851  AAAGGTTTAC CCCGCATATG CTTAGTCGCC GCAAGCTCCA TATAGGTTAC
2901  TTTGCGGGCT ACTGAATAGA ATCTTCGGTG AAAGGCGTCT ACCATATCGG
2951  CGCAACTATT GATCGAGTGC GTGTATACCA CGTGAATGCG ACACCCGAAA
3001  GACTAGCAGA AAAGTGCTTC AGCAACAAAC TCTCATCGTG AGCAGTGTCT
3051  CTGCTGGCAA TTTCGAAATT ACTAATATGC TGCTCTCGAG ATCTCCACTT
3101  CCATCATACA ACCGAAACCA GCTAAGGAAG GAGCGATCCA TAAGAATCGC
3151  CTCGAATAGC CATAACCTCA TCTCGCCTTC CACCGCACCA GCAAGAGGAA
3201  ACCGAATTAG AGCTGAAAGA ATACTAGAGC CATCGTAGGA GAACCGGATT
3251  CTTGACCGAT CGACTTTTGC CCGAGGTCGT TAGGTCGAAT AGGCTAGGTT
3301  TACGAAAAAG AGACTAAGGC CGCTCTAGAG ATCCGTCAAC ATGGTGGAGC
3351  ACGACACTCT CGTCTACTCC AAGAATATCA AAGATACAGT CTCAGAAGAC
3401  CAAAGGGCTA TTGAGACTTT TCAACAAAGG GTAATATCGG GAAACCTCCT
3451  CGGATTCCAT TGCCCAGCTA TCTGTCACTT CATCAAAAGG ACAGTAGAAA
3501  AGGAAGGTGG CACCTACAAA TGCCATCATT GCGATAAAGG AAAGGCTATC
3551  GTTCAAGATG CCTCTGCCGA CAGTGGTCCC AAAGATGGAC CCCCACCCAC
3601  GAGGAGCATC GTGGAAAAAG AAGACGTTCC AACCACGTCT TCAAAGCAAG
3651  TGGATTGATG TGATGATCCT ATGCGTATGG TATGACGTGT GTTCAAGATG
3701  ATGACTTCAA ACCTACCTAT GACGTATGGT ATGACGTGTG TCGACTGATG
3751  ACTTAGATCC ACTCGAGCGG CTATAAATAC GTACCTACGC ACCCTGCGCT
3801  ACCATCCCTA GAGCTGCAGC TTATTTTTAC AACAATTACC AACAACAACA
3851  AACAACAAAC AACATTACAA TTACTATTTA CAATTACAGT CGACCCGGGA
3901  TCCACACGAC ACCATGATAG AGGTGAAACC GATTAACGCA GAGGATACCT
3951  ATGAACTAAG GCATAGAATA CTCAGACCAA ACCAGCCGAT AGAAGCGTGT
4001  ATGTTTGAAA GCGATTTACT TCGTGGTGCA TTTCACTTAG GCGGCTTTTA
4051  CAGGGGCAAA CTGATTTCCA TAGCTTCATT CCACCAGGCC GAGCACTCGG
4101  AACTCCAAGG CCAGAAACAG TACCAGCTCC GAGGTATGGC TACCTTGGAA
4151  GGTTATCGTG AGCAGAAAGC GGGATCAACT CTAGTTAAAC ACGCTGAAGA
4201  AATCCTTCGT AAGAGGGGGG CGGACATGCT TTGGTGTAAT GCGAGGACAT
4251  CCGCCTCAGG CTACTACAAA AAGTTAGGCT TCAGCGAGCA GGGAGAGATA
4301  TTTGACACGC CGCCAGTAGG ACCTCACATC CTGATGTATA AAAGGATCAC
4351  ATAACTAGCT AGTCAGTTAA CCTAGACTTG TCCATCTTCT GGATTGGCCA
4401  ACTTAATTAA TGTATGAAAT AAAAGGATGC ACACATAGTG ACATGCTAAT
4451  CACTATAATG TGGGCATCAA AGTTGTGTGT TATGTGTAAT TACTAGTTAT
4501  CTGAATAAAA GAGAAAGAGA TCATCCATAT TTCTTATCCT AAATGAATGT
4551  CACGTGTCTT TATAATTCTT TGATGAACCA GATGCATTTC ATTAACCAAA
4601  TCCATATACA TATAAATATT AATCATATAT AATTAATATC AATTGGGTTA
4651  GCAAAACAAA TCTAGTCTAG GTGTGTTTTG CGAATTCGAT ATCAAGCTTT
4701  GCTCTAGATC AAACTCACAT CCAAACATAA CATGGATATC TTCCTTACCA
4751  ATCATACTAA TTATTTTGGG TTAAATATTA ATCATTATTT TTAAGATATT
4801  AATTAAGAAA TTAAAAGATT TTTTAAAAAA ATGTATAAAA TTATATTATT
4851  CATGATTTTT CATACATTTG ATTTTGATAA TAAATATATT TTTTTTAATT
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FIGURE 4C


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4901   TCTTAAAAAA TGTTGCAAGA CACTTATTAG ACATAGTCTT GTTCTGTTTA
4951   CAAAAGCATT CATCATTTAA TACATTAAAA AATATTTAAT ACTAACAGTA
5001   GAATCTTCTT GTGAGTGGTG TGGGAGTAGG CAACCTGGCA TTGAAACGAG
5051   AGAAAGAGAG TCAGAACCAG AAGACAAATA AAAAGTATGC AACAAACAAA
5101   TCAAAATCAA AGGGCAAAGG CTGGGGTTGG CTCAATTGGT TGCTACATTC
5151   AATTTTCAAC TCAGTCAACG GTTGAGATTC ACTCTGACTT CCCCAATCTA
5201   AGCCGCGGAT GCAAACGGTT GAATCTAACC CACAATCCAA TCTCGTTACT
5251   TAGGGGCTTT TCCGTCATTA ACTCACCCCT GCCACCCGGT TTCCCTATAA
5301   ATTGGAACTC AATGCTCCCC TCTAAACTCG TATCGCTTCA GAGTTGAGAC
5351   CAAGACACAC TCGTTCATAT ATCTCTCTGC TCTTCTCTTC TCTTCTACCT
5401   CTCAAGGTAC TTTTCTTCTC CCTCTACCAA ATCCTAGATT CCGTGGTTCA
5451   ATTTCGGATC TTGCACTTCT GGTTTGCTTT GCCTTGCTTT TTCCTCAACT
5501   GGGTCCATCT AGGATCCATG TGAAACTCTA CTCTTTCTTT AATATCTGCG
5551   GAATACGCGT TTGACTTTCA GATCTAGTCG AAATCATTTC ATAATTGCCT
5601   TTCTTTCTTT TAGCTTATGA GAAATAAAAT CACTTTTTTT TTATTTCAAA
5651   ATAAACCTTG GGCCTTGTGC TGACTGAGAT GGGGTTTGGT GATTACAGAA
5701   TTTTAGCGAA TTTTGTAATT GTACTTGTTT GTCTGTAGTT TTGTTTTGTT
5751   TTCTTGTTTC TCATACATTC CTTAGGCTTC AATTTTATTC GAGTATAGGT
5801   CACAATAGGA ATTCAAACTT TGAGCAGGGG AATTAATCCC TTCCTTCAAA
5851   TCCAGTTTGT TTGTATATAT GTTTAAAAAA TGAAACTTTT GCTTTAAATT
5901   CTATTATAAC TTTTTTTATG GCTGAAATTT TTGCATGTGT CTTTGCTCTC
5951   TGTTGTAAAT TTACTGTTTA GGTACTAACT CTAGGCTTGT TGTGCAGTTT
6001   TTGAAGTATA ACCATGCCAC ACAACACAAT GGCGGCCACC GCTTCCAGAA
6051   CCACCCGATT CTCTTCTTCC TCTTCACACC CCACCTTCCC CAAACGCATT
6101   ACTAGATCCA CCCTCCCTCT CTCTCATCAA ACCCTCACCA AACCCAACCA
6151   CGCTCTCAAA ATCAAATGTT CCATCTCCAA ACCCCCCACG GCGGCGCCCT
6201   TCACCAAGGA AGCGCCGACC ACGGAGCCCT TCGTGTCACG GTTCGCCTCC
6251   GGCGAACCTC GCAAGGGCGC GGACATCCTT GTGGAGGCGC TGGAGAGGCA
6301   GGGCGTGACG ACGGTGTTCG CGTACCCCGG CGGTGCGTCG ATGGAGATCC
6351   ACCAGGCGCT CACGCGCTCC GCCGCCATCC GCAACGTGCT CCCGCGCCAC
6401   GAGCAGGGCG GCGTCTTCGC CGCCGAAGGC TACGCGCGTT CCTCCGGCCT
6451   CCCCGGCGTC TGCATTGCCA CCTCCGGCCC CGGCGCCACC AACCTCGTGA
6501   GCGGCCTCGC CGACGCTTTA ATGGACAGCG TCCCAGTCGT CGCCATCACC
6551   GGCCAGGTCG CCCGCCGGAT GATCGGCACC GACGCCTTCC AAGAAACCCC
6601   GATCGTGGAG GTGAGCAGAT CCATCACGAA GCACAACTAC CTCATCCTCG
6651   ACGTCGACGA CATCCCCCGC GTCGTCGCCG AGGCTTTCTT CGTCGCCACC
6701   TCCGGCCGCC CCGGTCCGGT CCTCATCGAC ATTCCCAAAG ACGTTCAGCA
6751   GCAACTCGCC GTGCCTAATT GGGACGAGCC CGTTAACCTC CCCGGTTACC
6801   TCGCCAGGCT GCCCAGGCCC CCCGCCGAGG CCCAATTGGA ACACATTGTC
6851   AGACTCATCA TGGAGGCCCA AAAGCCCGTT CTCTACGTCG GCGGTGGCAG
6901   TTTGAATTCC AGTGCTGAAT TGAGGCGCTT TGTTGAACTC ACTGGTATTC
6951   CCGTTGCTAG CACTTTAATG GGTCTTGGAA CTTTTCCTAT TGGTGATGAA
7001   TATTCCCTTC AGATGCTGGG TATGCATGGT ACTGTTTATG CTAACTATGC
7051   TGTTGACAAT AGTGATTTGT TGCTTGCCTT TGGGGTAAGG TTTGATGACC
7101   GTGTTACTGG GAAGCTTGAG GCTTTTGCTA GTAGGGCTAA GATTGTTCAC
7151   ATTGATATTG ATTCTGCCGA GATTGGGAAG AACAAGCAGG CGCACGTGTC
7201   GGTTTGCGCG GATTTGAAGT TGGCCTTGAA GGGAATTAAT ATGATTTTGG
7251   AGGAGAAAGG AGTGGAGGGT AAGTTTGATC TTGGAGGTTG GAGAGAAGAG
7301   ATTAATGTGC AGAAACACAA GTTTCCATTG GGTTACAAGA CATTCCAGGA
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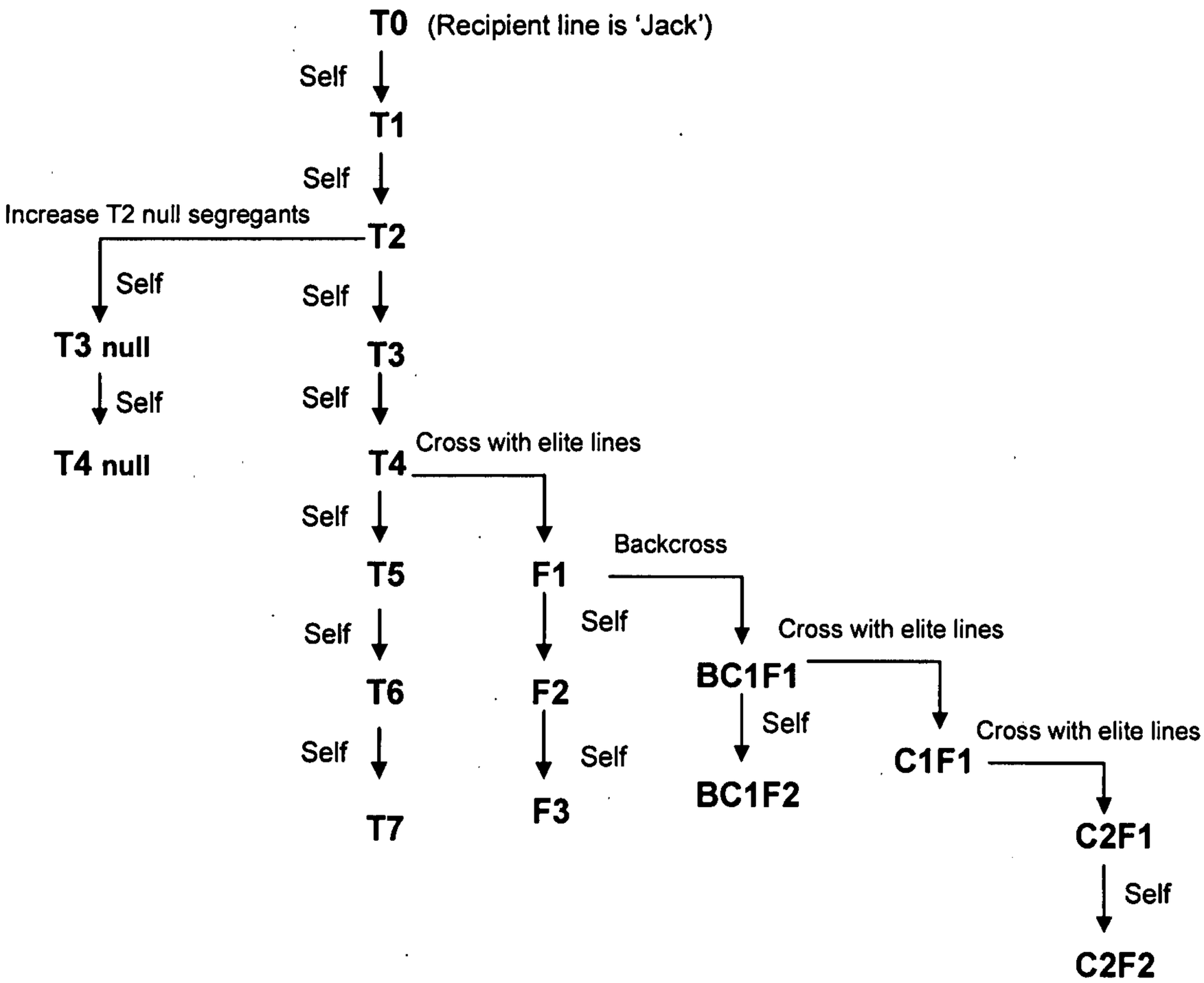
FIGURE 4D

7351 CGCGATTTCT CCGCAGCATG CTATCGAGGT TCTTGATGAG TTGACTAATG
7401 GAGATGCTAT TGTTAGTACT GGGGTTGGGC AGCATCAAAT GTGGGCTGCG
7451 CAGTTTTACA AGTACAAGAG ACCGAGGCAG TGGTTGACCT CAGGGGGTCT
7501 TGGAGCCATG GGTTTTGGAT TGCCTGCGGC TATTGGTGCT GCTGTTGCTA
7551 ACCCTGGGGC TGTTGTGGTT GACATTGATG GGGATGGTAG TTTCATCATG
7601 AATGTTCAGG AGTTGGCCAC TATAAGAGTG GAGAATCTCC CAGTTAAGAT
7651 ATTGTTGTTG AACAATCAGC ATTTGGGTAT GGTGGTTCAG TTGGAGGATA
7701 GGTTCTACAA GTCCAATAGA GCTCACACCT ATCTTGGAGA TCCGTCTAGC
7751 GAGAGCGAGA TATTCCCAAA CATGCTCAAG TTTGCTGATG CTTGTGGGAT
7801 ACCGGCAGCG CGAGTGACGA AGAAGGAAGA GCTTAGAGCG GCAATTCAGA
7851 GAATGTTGGA CACCCCTGGC CCCTACCTTC TTGATGTCAT TGTGCCCCAT
7901 CAGGAGCATG TGTTGCCGAT GATTCCCAGT AATGGATCCT TCAAGGATGT
7951 GATAACTGAG GGTGATGGTA GAACGAGGTA CTGATTGCCT AGACCAAATG
8001 TTCCTTGATG CTTGTTTTGT ACAATATATA TAAGATAATG CTGTCCTAGT
8051 TGCAGGATTT GGCCTGTGGT GAGCATCATA GTCTGTAGTA GTTTTGGTAG
8101 CAAGACATTT TATTTTCCTT TTATTTAACT TACTACATGC AGTAGCATCT
8151 ATCTATCTCT GTAGTCTGAT ATCTCCTGTT GTCTGTATTG TGCCGTTGGA
8201 TTTTTTGCTG TAGTGAGACT GAAAATGATG TGCTAGTAAT AATATTTCTG
8251 TTAGAAATCT AAGTAGAGAA TCTGTTGAAG AAGTCAAAAG CTAATGGAAT
8301 CAGGTTACAT ATTCAATGTT TTTCTTTTTT TAGCGGTTGG TAGACGTGTA
8351 GATTCAACTT CTCTTGGAGC TCACCTAGGC AATCAGTAAA ATGCATATTC
8401 CTTTTTTAAC TTGCCATTTA TTTACTTTTA GTGGAAATTG TGACCAATTT
8451 GTTCATGTAG AACGGATTTG GACCATTGCG TCCACAAAAC GTCTCTTTTG
8501 CTCGATCTTC ACAAAGCGAT ACCGAAATCC AGAGATAGTT TTCAAAAGTC
8551 AGAAATGGCA AAGTTATAAA TAGTAAAACA GAATAGATGC TGTAATCGAC
8601 TTCAATAACA AGTGGCATCA CGTTTCTAGT TCTAGACCCA TCAGCTGGGC
8651 CGGCCACTAG TGAGCTCGGT ACCCGGGGGC GCGTAATATC ATCATTAGGA
8701 AGACACTGCC CATCTTGAAT AGGATTTTAG CTACTAAATA TGTTGATGGT
8751 CTTTATGAAA AACTATTAAC TAGGAATATT ATGCTACCCA TATGGAAAGA
8801 AGACGCTAGG GGAATAGAAA GACCATCAAA TAAACGAAGT CAACACCAGG
8851 TCTTCCGAAG CATTAACAAT TACCTATTTA ATATGTACTC AGTCCGGGTG
8901 GATATCTCAC TACATTGACG CAGTTTGTTC AAAGACGAAC GCCCTGAATT
8951 ATGCCATCTG CTTAGGCTTT CAAATATGGT ACGCTCTAAT GCCAAGCCTT
9001 ATGCTGGTCT TAGGGTATTA TCATCAAATC TTTAAGCCAG AGGTAGTTAA
9051 ATACATCAAG GACACCATAG GAGTATGGCA CAACGATATT GTCAAGATCG
9101 CATCAGATCT AATAGGCAAT AATGAATTCT TCATGCAGCC CGACGTGGGA
9151 ACGCTCGAAA GCAGTGGGGC CTCTGGGACA GGGACCAGAC CTGAGTCGCT
9201 AACATTTGGG AATAAGAGAA GTAGATATAC CCAATTTTTT AACTAGCCAA
9251 GGAAGGAAAG CGGGAAAGGT CCGATACAAA GGAAAGGGTT GCGAGGCTTA
9301 ACGATTTAGA ATATAGCTGT TGAGGTGGCA CTTGTTCCCC CGGGGCGGGG
9351 GTATATGCCC GTAGCTTTAT TCTGTCACTT CTTTCAGATC AATGAAGTTG
9401 AAAAGTTATA GAGTAAGGGA CCCTTGTTTA CAAAGCTGTC ACTCCAAGAA
9451 CTCGAAGTCA AGCATCTTCG GGAATATCCA GATTAGTCTT CAACTAGAGA
9501 AAGGATAGGA ATCTCCTTTG CAGAGTTTTC TTCTCCTGCT GATGTAGCGG
9551 TAAAGTCAAA AGTTGGATGC CCTTTTTTCT TTATTTAATT AATTCCGTTG
9601 ATAGAGCTTT TGAGCGGATG CAAGCACTAG ATTCTTCAAC GAGTACCAAT
9651 AATAAATGAA TTCACCAGAC TAAGAGAAGA AAACAGAACA AAAAGATTAA
9701 GCCCAGCCGC CTTCGGGAAG ACCTATCTTC GTCGGGAGGA AGAGCCCTCT
9751 TTACACCATT GTGATTAGAA AAAACCGAAA AGTGGACCGG CCTAGTAACC

FIGURE 4E

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 9801  AATAGAGCGG GGCTTGATCC CCACTTTAAA TCTATTGGAT AGAGCCCTCA
 9851  GCCCAGGGCA AGCGATTGAA TTCTATTTGA TTATGGGTTA GGTGGAACCT
 9901  GAAACTAGCA CTTACAAATG AGTTAGCAAA AGGAAAAAGA CAATTCTCAA
 9951  ATGCGTACAA GACTTTCTTC CTTCTTTGTT TAAGAGGCCA GTCTGCGATG
10001  GATGCTCGTG CATGAAAAAG GGCTTTGATC TATTCACCAC TTATATAATA
10051  GAGCCAATCT CTGCAGGACA AGATATCTAT TTTGTCATTG GGAAGTAAGG
10101  CTTAAGTCGA CGAAAAAGTT AGGAAAGGGG ATCATATGGC TAGGGTTGCC
10151  CTCGGGGCTC AAGGGTTTAG CGATGAAGAG TGCCAAGCAA AAGGTCAATA
10201  CCGGTACGCC GATCAAAGAA GTCCAGTGGC AAGGCCCTTT CAGCCAAGCT
10251  AGCGTGCTGA ACAGAAAGTC GTAGAGTGAT GACAGCTTCT TCTTCTTGAG
10301  TCATTCGTGT GACAACATCA GGATCTCGTC GAAAGACCTC CTCTGCCTAT
10351  CTCTCCCGCA AGAGAGGACT CGTTATGGCG CACCTCTTTT TAGCAGTCTC
10401  GTCAATAAGA TAAGATTGCC CCTCCCTTCT TATTGATTTG ATAAAGGGCT
10451  TTGTCCACTC CCTCTCTTCT TAGCCGAGCG GAGTGACGGT TTAGTTTAGG
10501  CTTTAGATGC CACTGCGAAA GACTCTAGAG ATCCACTCTC ACAGCGTATA
10551  CGCGACATCC CTATGTATAC ACAATCCTTT CAAGCAGCTA GGACAGCTAG
10601  CAAGCAAGTT ATCTGTTCGC GGACAAGCTC TCTGGATGAC AAAAAACATG
10651  CTCTTTCATG CGGAAAAAAC ACGGTCTTTC GTGGAAGTTG GTCGATTTGA
10701  AGTCGCTTTA TGAGTGAAAA TGGGTCGATG ACGAAAAAGA CGGGGAAAAT
10751  GATCAACTGT CACATTTTGA TGCCAGTTTA GGGCTAAAAT GAACTTTCAT
10801  CCAAAAAGAC CGAGAAAACG CTCCACTGGC AGGATCCGAT CGGAAATAA
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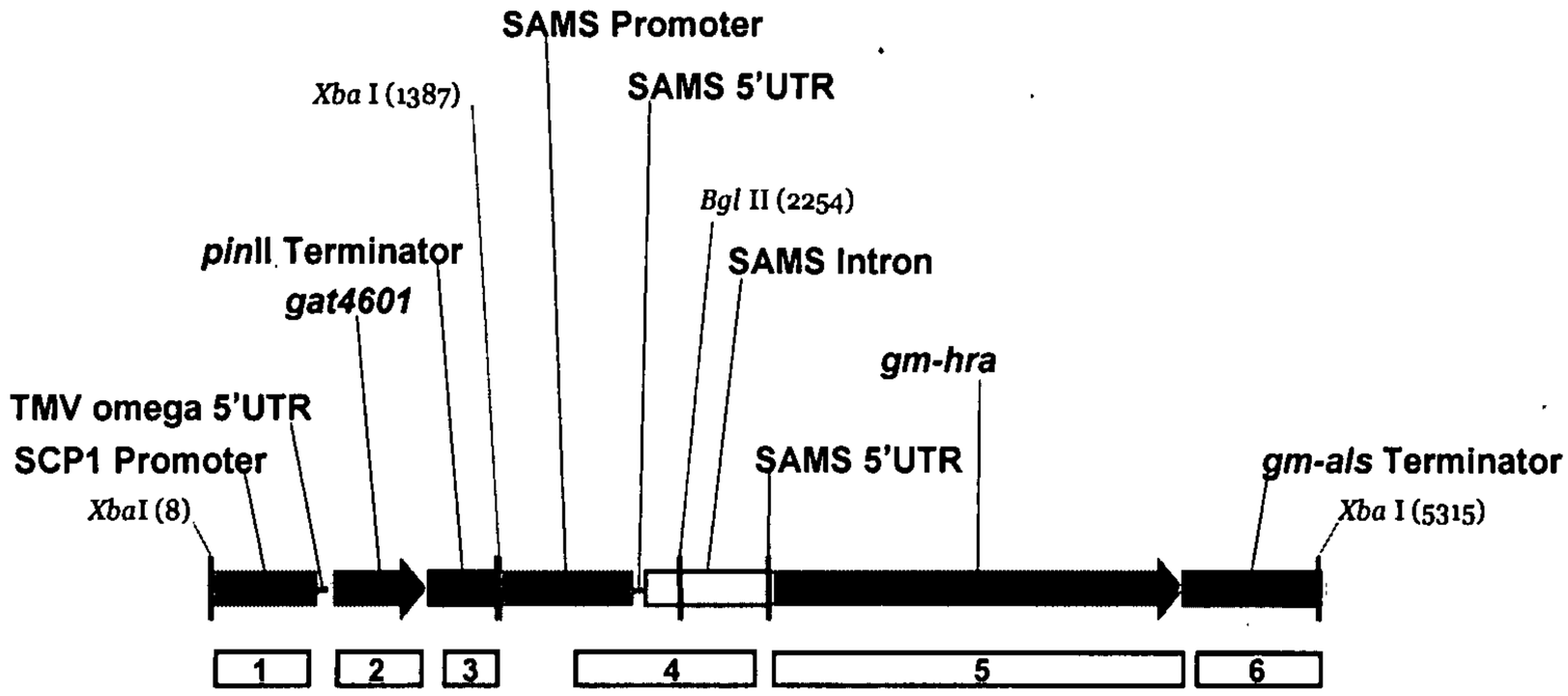


Analysis	GLYAT 3560.4.3.5 Soybean Generation Used	Control Used
Molecular	T4, T5 and F3	Jack and Elite 1*
Inheritance	T1, F2, F3, BC1F2 and C2F2	Not applicable
Levels of transgenic proteins	T5	Jack
Germination / dormancy	T7	Jack
Field agronomics	T3	T3 null segregants
	T4	T4 null segregants
	T5	Jack
Compositional assessment	T5	Jack and four commercial soybean varieties
Poultry study	T7	Jack and three commercial soybean varieties

FIGURE 5
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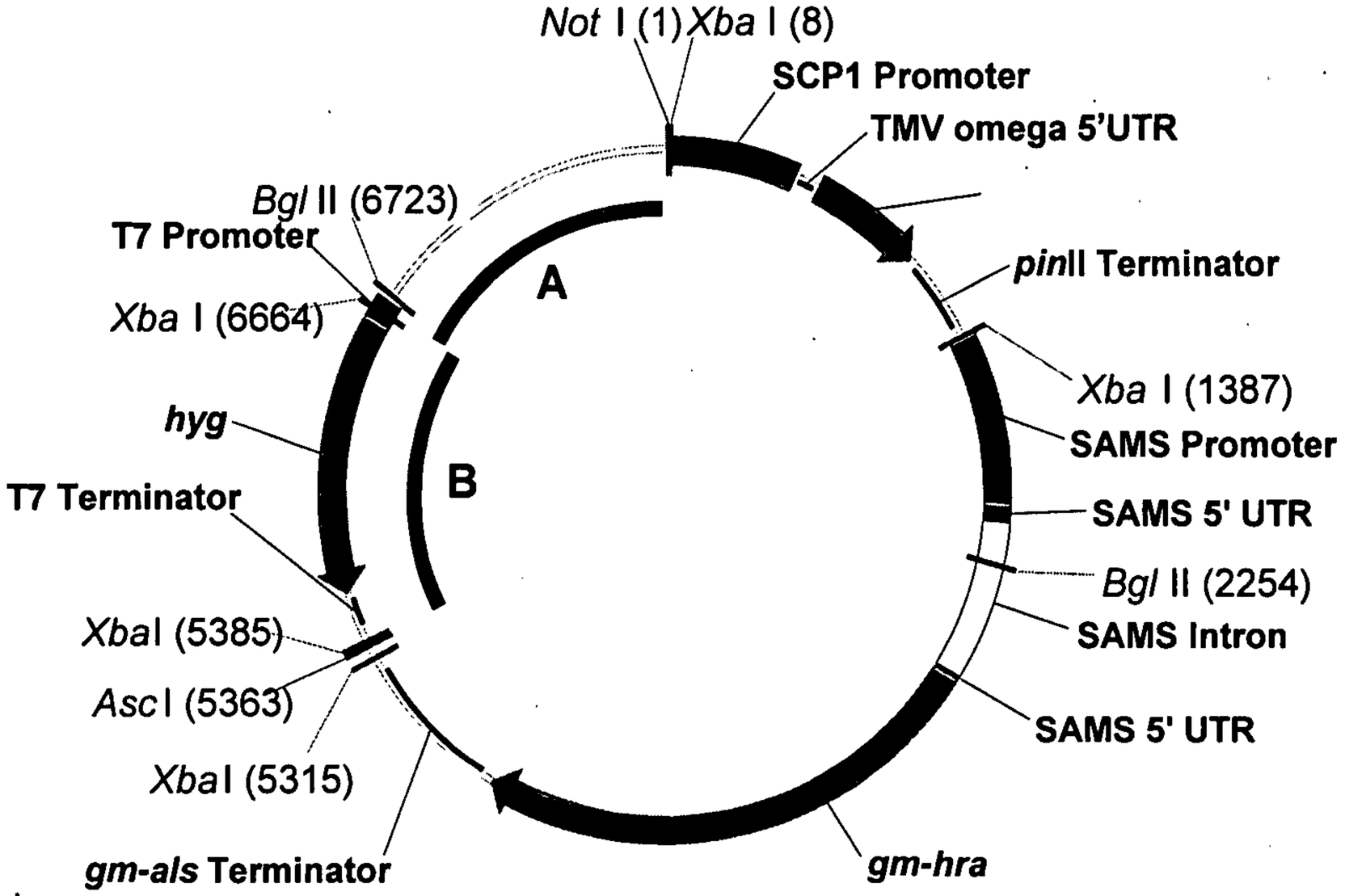
*Elite 1 is one of several elite soybean lines crossed with T4 plants to yield F1 plants.

FIGURE 6
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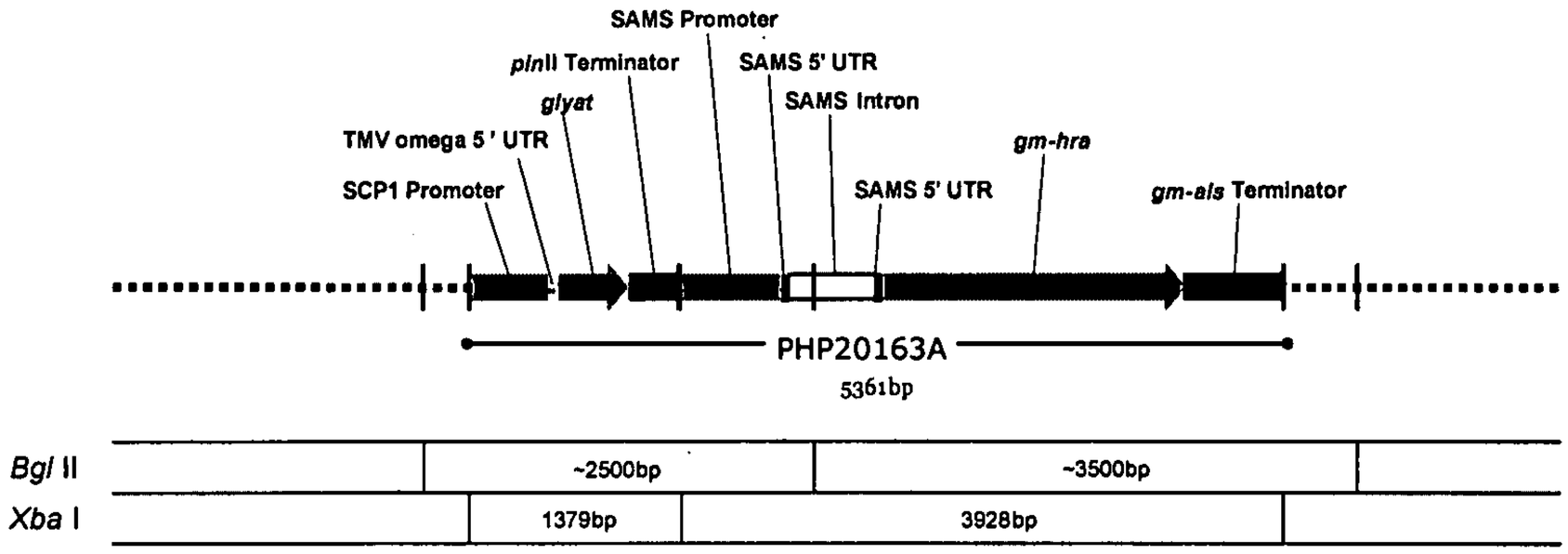
Number	Probe Identification
1	SCP1 promoter probe
2	*glyat 4601* probe
3	*pin*II terminator probe
4	SAMS probe
5	*gm-hra* probe
6	*gm-als* terminator probe

FIGURE 7
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Letter	Backbone Probe Identification
A	*backbone 20163* probe
B	*hyg 20163* probe

FIGURE 8
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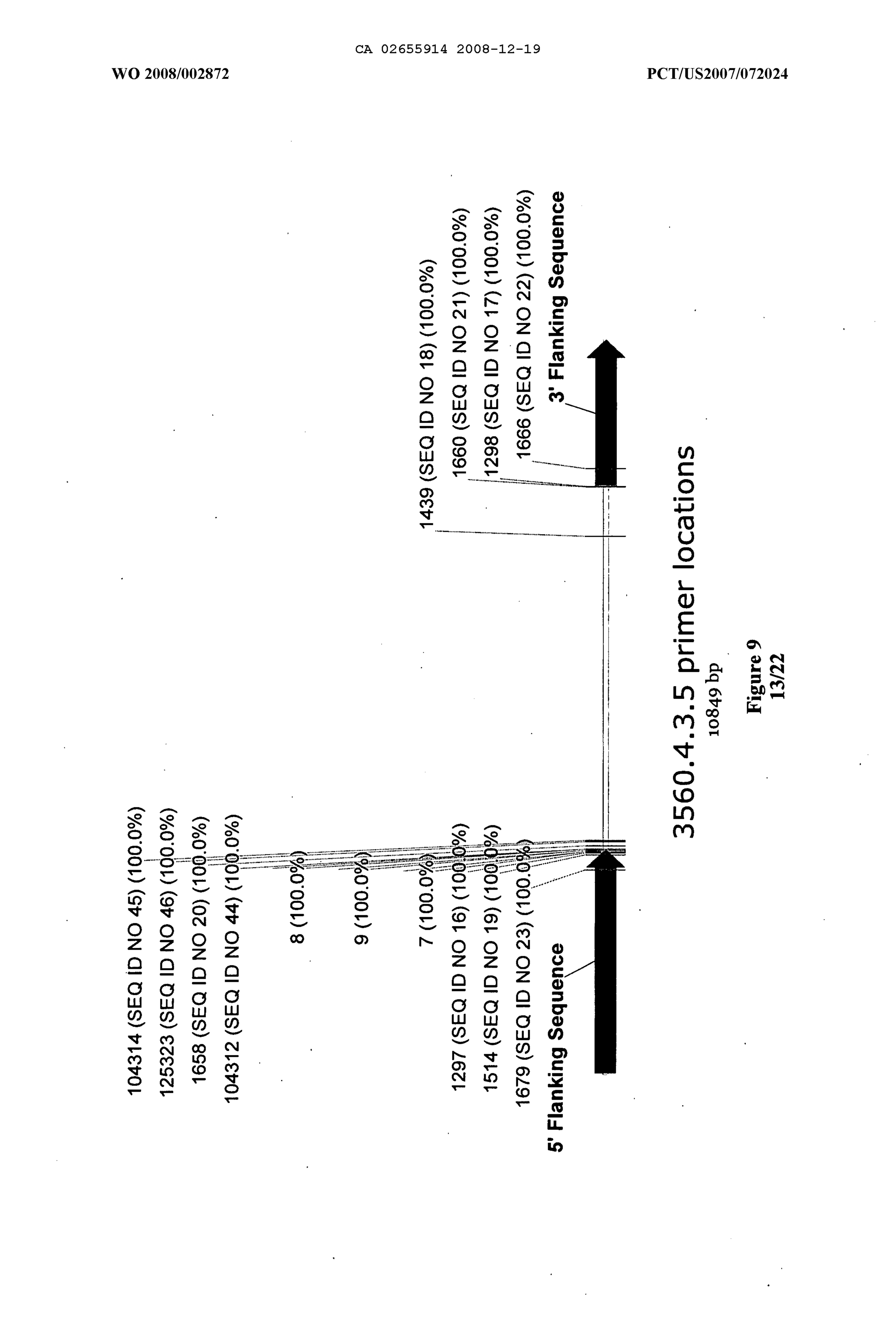


Figure 9
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Figure 10A
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   1 AGCAATTGTT TTGTGCATTT CCAAATTTCA ATCTGATTTA TTAAATAAAA AAATTTTAAT TCGGGTTGAT TGTAAATCAG CTAAAGACAT TTTACAGAAA
     TCGTTAACAA AACACGTAAA GGTTTAAAGT TAGACTAAAT AATTTATTTT TTTAAAATTA AGCCCAACTA ACATTTAGTC GATTTCTGTA AAATGTCTTT

 101 GACGTCAAAA ATCTTGGCTC CAAACAAATT TTTGCAAGAT GGCAAGCAAT ATTAAGTGTT TTTGATTTTG ACAGAATATA TCAAGGGCAC TTTAAACTCT
     CTGCAGTTTT TAGAACCGAG GTTTGTTTAA AAACGTTCTA CCGTTCGTTA TAATTCACAA AAACTAAAAC TGTCTTATAT AGTTCCCGTG AAATTTGAGA

 201 CTACCTAACT ATCTTACACG TGAATTCTTA TAGACAAATT CTCATGCCAC CTAAGGCATC AGGCACCCCT CTCAGGGGTA GACGAAGCAC GAGTAAAGGA
     GATGGATTGA TAGAATGTGC ACTTAAGAAT ATCTGTTTAA GAGTACGGTG GATTCCGTAG TCCGTGGGGA GAGTCCCCAT CTGCTTCGTG CTCATTTCCT

 301 TTCAAGTTGG CCCTACCAGA GCCAACTATC AAAAAGAGTT CTTCCGCATC CTCTGGGTCA TCTACCCAAA AACCTAAAAT AAAAGGGTCA TCAACCCAAA
     AAGTTCAACC GGGATGGTCT CGGTTGATAG TTTTTCTCAA GAAGGCGTAG GAGACCCAGT AGATGGGTTT TTGGATTTTA TTTTCCCAGT AGTTGGGTTT

 401 TAGTCACCAT CAAACCTGAG TCATCCACTC AGGAATCCCC AAAACCTTCA ACCTCAAAAC AAACAAAGGC CGACTATACC TTCTCAGTAC AACACTCCAG
     ATCAGTGGTA GTTTGGACTC AGTAGGTGAG TCCTTAGGGG TTTTGGAAGT TGGAGTTTTG TTTGTTTCCG GCTGATATGG AAGAGTCATG TTGTGAGGTC

 501 GCCCTACAAG AAATTGGTCT AACCAAACTC CCAAAACTCG CCAAAAAGAC TTGGGCAGAC ATTGCCTCAG AATCTAATGA TGAATTTGAA ACTGATTTAC
     CGGGATGTTC TTTAACCAGA TTGGTTTGAG GGTTTTGAGC GGTTTTTCTG AACCCGTCTG TAACGGAGTC TTAGATTACT ACTTAAACTT TGACTAAATG

 601 AAATCATGAT TCAAAACTCC AAACAATCCA AAACGATTGT CAATCCAAAA GGAAAACAGA CCTTATCCCA ACAAAAGACA CCATTACCAA AACCTACTAA
     TTTAGTACTA AGTTTTGAGG TTTGTTAGGT TTTGCTAACA GTTAGGTTTT CCTTTTGTCT GGAATAGGGT TGTTTTCTGT GGTAATGGTT TTGGATGATT

 701 CAGTTATATT TACAAAAATA AATTTCCAAC TGTTTTGTAG ATGGAGCCAA AATTTTGGGA CAAAAATCCC TTCAAGGCTA CAGCCAAGGC ATTTCCACCG
     GTCAATATAA ATGTTTTTAT TTAAAGGTTG ACAAAACATC TACCTCGGTT TTAAAACCCT GTTTTTAGGG AAGTTCCGAT GTCGGTTCCG TAAAGGTGGC

 801 GGCTTCCATT TCAAACCTAT TTCCACCAAT AAAACAAGAA TCTTTTACGA ATTCATACTG ATAGACACAA ACTCAGTGTC TATTAAACAC TTCAAAGACC
     CCGAAGGTAA AGTTTGGATA AAGGTGGTTA TTTTGTTCTT AGAAAATGCT TAAGTATGAC TATCTGTGTT TGAGTCACAG ATAATTTGTG AAGTTTCTGG

 901 CAAATGACAT AAATTTAAAC ACTCATTCAA CCATCCAGAT TTTAAAGTTC ATACAACCTC GACAATGTGG AACAAATATA AATCAAGCCA AACAATTCTT
     GTTTACTGTA TTTAAATTTG TGAGTAAGTT GGTAGGTCTA AAATTTCAAG TATGTTGGAG CTGTTACACC TTGTTTATAT TTAGTTCGGT TTGTTAAGAA

1001 TGTACCCTTT GATCCTATAG GTTACACTAT TGGGATTATG TAGATGCATG GACCAATGTA TTCTGGCATC AAAATAACAA ATTCAAACAT TCTTGGCTTA
     ACATGGGAAA CTAGGATATC CAATGTGATA ACCCTAATAC ATCTACGTAC CTGGTTACAT AAGACCGTAG TTTTATTGTT TAAGTTTGTA AGAACCGAAT

1101 TTTATTTCAA AACTAACACC GTCTATAATT TTCCAAATTG GTTCCTCCAA CGGTGGGACT TTTTTGGACC AAACTTTGAT ATCTACCCGG AGCAAGTCCA
     AAATAAAGTT TTGATTGTGG CAGATATTAA AAGGTTTAAC CAAGGAGGTT GCCACCCTGA AAAAACCTGG TTTGAAACTA TAGATGGGCC TCGTTCAGGT

1201 ACAAGGGTTT GATCAGTTCA AAAAAATGTT CAATTCTCAG GAATCACGAA TCCCTGTAGA CCTAAAAATAC TTTTCCAATT TTGCATTGTC GTGGATATTT
     TGTTCCCAAA CTAGTCAAGT TTTTTTACAA GTTAAGAGTC CTTAGTGCTT AGGGACATCT GGATTTTATG AAAAGGTTAA AACGTAACAG CACCTATAAA

1301 TCATGGCAAT ACAGATATGG GAAAACTGAA AACAACAAGC AGTTTCCATC ACTGCAACAT CATGCATTTA TCAAGTGGTG GAATTAGTTT GATACATCAA
     AGTACCGTTA TGTCTATACC CTTTTGACTT TTGTTGTTCG TCAAAGGTAG TGACGTTGTA GTACGTAAAT AGTTCACCAC CTTAATCAAA CTATGTAGTT
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Figure 10B
15/22

1401 AAGCAGCACC AGATCAAGTG AGAATCTGGT TTCAAGCCCA TCCAGAATTT TTGAAAGTTG CTAATCCTGA GACTTCTTTA TTCCTCAATC AGAAGTCTCA
TTCGTCGTGG TCTAGTTCAC TCTTAGACCA AAGTTCGGGT AGGTCTTAAA AACTTTCAAC GATTAGGACT CTGAAGAAAT AAGGAGTTAG TCTTCAGAGT

1501 ATTAGCTGCT TTCCTTTCTA GTTCCAAGTC AAAAGAAATT CTGGCACAAA ATCTAAAAGA AGTCCTACAG CTTCTCCAAC AAGAAGAAGA TAAAGGCTCT
TAATCGACGA AAGGAAAGAT CAAGGTTCAG TTTTCTTTAA GACCGTGTTT TAGATTTTCT TCAGGATGTC GAAGAGGTTG TTCTTCTTCT ATTTCCGAGA

1601 TCCTCAAAGA AGGAAGATAA CAATTCTTCA AAAGAAGATG ACGACCCTTT CTACCAAAAT GAAAATGATT GTTTTGGTAT TTCTCTAAAT GATGATTAAT
AGGAGTTTCT TCCTTCTATT GTTAAGAAGT TTTCTTCTAC TGCTGGGAAA GATGGTTTTA CTTTTACTAA CAAAACCATA AAGAGATTTA CTACTAATTA

1701 TAAAAAATTA CATGTACTAT GTAAATAGTT TCGGTCACGA AACTGGCACT GTAGCTACAG TAAATTTTAT GGCTATTTAA GGAGTTCTCG GCCCATTTGT
ATTTTTTAAT GTACATGATA CATTTATCAA AGCCAGTGCT TTGACCGTGA CATCGATGTC ATTTAAAATA CCGATAAATT CCTCAAGAGC CGGGTAAACA

1801 GAGGTACCTT TTCAGGTAGT CAGATCTCTA TTTTTAGAGA GAGAAACTCT AGGAAACAAT CTTTGTAAGT TTTTCTTTCG ATTTCAATAA ATTCAAAGTT
CTCCATGGAA AAGTCCATCA GTCTAGAGAT AAAAATCTCT CTCTTTGAGA TCCTTTGTTA GAAACATTCA AAAAGAAAGC TAAAGTTATT TAAGTTTCAA

1901 TTCTCTTCAT ATCTCTTCTC CCTCTTGACC GGTCCTGTGC GGTTCTGCCA TCGCTTCAGT TTCTTCTCTT CTCTCCCCTT ATCGATATTG TGTGGCTCTA
AAGAGAAGTA TAGAGAAGAG GGAGAACTGG CCAGGACACG CCAAGACGGT AGCGAAGTCA AAGAAGAGAA GAGAGGGGAA TAGCTATAAC ACACCGAGAT

2001 TTGCCACTTC GATTTCTTTT ATGTTGCTTT CATTTTAATT TGTTTTACCA TTTTCTTTTG ATATTATAAT TTCTATTTAA CTCTTGGTCA TACTGCATAT
AACGGTGAAG CTAAAGAAAA TACAACGAAA GTAAAATTAA ACAAAATGGT AAAAGAAAAC TATAATATTA AAGATAAATT GAGAACCAGT ATGACGTATA

2101 ATTCATAATA TATTCTTACA TCCTATCTAT CCGTTTGATC TCTTTTCACT GTTATATATA TATATATATA TATATATATC GTTTAACTTC ATGTTAGTAA
TAAGTATTAT ATAAGAATGT AGGATAGATA GGCAAACTAG AGAAAAGTGA CAATATATAT ATATATATAT ATATATATAG CAAATTGAAG TACAATCATT

2201 TAAGATTAGA GTAAAAAATA TATATATAAC GAAGTTATTT TAACAAAAGG TATTTTTGTA AAAAAAAATT ATATGCTAAA AAAGTTTTAC TATATCTAAG
ATTCTAATCT CATTTTTTAT ATATATATTG CTTCAATAAA ATTGTTTTCC ATAAAAACAT TTTTTTTTAA TATACGATTT TTTCAAAATG ATATAGATTC

2301 CATGATTTTT TTTAATTCCC AAAACACGTG TAAATATTTT TAGGAATATT TTGTAAAAAA TCAAACATTT TTTTAATTAT TCGTATAAAA CATCAATCTT
GTACTAAAAA AAATTAAGGG TTTTGTGCAC ATTTATAAAA ATCCTTATAA AACATTTTTT AGTTTGTAAA AAAATTAATA AGCATATTTT GTAGTTAGAA

2401 TAAGAATCAT AATTTTTAGA AATCATGATT TCTGGATATA AAAATACTTT TTCTTGCAGC CAAACGTCTT CTAAAGCCAC ATGTTAATGG GTGTACAAAT
ATTCTTAGTA TTAAAAATCT TTAGTACTAA AGACCTATAT TTTTATGAAA AAGAACGTCG GTTTGCAGAA GATTTCGGTG TACAATTACC CACATGTTTA

2501 TATAAAGTTT TTATAAACAT ATCACTTTTT AAAGTTAAAA ATATCACTAA TACGTTTCTA AATGCATTTT TTTTAGAAAT TATAAGTTTC TAGCACTCCA
ATATTTCAAA AATATTTGTA TAGTGAAAAA TTTCAATTTT TATAGTGATT ATGCAAAGAT TTACGTAAAA AAAATCTTTA ATATTCAAAG ATCGTGAGGT

2601 TTGGAGTTGT ATAGAACTAC TACTAATCTA CTATTAATAA CAATTTATCT CCTCCAAATA TTTAAGTAAA CTGCATATTT AGAGAATGTT GGACAGTAAA
AACCTCAACA TATCTTGATG ATGATTAGAT GATAATTATT GTTAAATAGA GGAGGTTTAT AAATTCATTT GACGTATAAA TCTCTTACAA CCTGTCATTT

2701 GCTAGCCACT CAATATTTAG GTGCTCCCCG AAAGAGGAAA GCAAACAAGC CACCAGCACT TCAATCAGTA AAGCTAGCCA CTCAACTCGC TCTCTTCAAA
CGATCGGTGA GTTATAAATC CACGAGGGGC TTTCTCCTTT CGTTTGTTCG GTGGTCGTGA AGTTAGTCAT TTCGATCGGT GAGTTGAGCG AGAGAAGTTT

Figure 10C
16/22

2801 TTCCCTTTTA CATTTTATTT CAGATCCTCC ACCTAGCCAA GTAGGTCTCA AAAGGTTTAC CCCGCATATG CTTAGTCGCC GCAAGCTCCA TATAGGTTAC
AAGGGAAAAT GTAAAATAAA GTCTAGGAGG TGGATCGGTT CATCCAGAGT TTTCCAAATG GGGCGTATAC GAATCAGCGG CGTTCGAGGT ATATCCAATG

2901 TTTGCGGGCT ACTGAATAGA ATCTTCGGTG AAAGGCGTCT ACCATATCGG CGCAACTATT GATCGAGTGC GTGTATACCA CGTGAATGCG ACACCCGAAA
AAACGCCCGA TGACTTATCT TAGAAGCCAC TTTCCGCAGA TGGTATAGCC GCGTTGATAA CTAGCTCACG CACATATGGT GCACTTACGC TGTGGGCTTT

1679 (SEQ ID NO 23) 100.0%
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3001 GACTAGCAGA AAAGTGCTTC AGCAACAAAC TCTCATCGTG AGCAGTGTCT CTGCTGGCAA TTTCGAAATT ACTAATATGC TGCTCTCGAG ATCTCCACTT
CTGATCGTCT TTTCACGAAG TCGTTGTTTG AGAGTAGCAC TCGTCACAGA GACGACCGTT AAAGCTTTAA TGATTATACG ACGAGAGCTC TAGAGGTGAA

3101 CCATCATACA ACCGAAACCA GCTAAGGAAG GAGCGATCCA TAAGAATCGC CTCGAATAGC CATAACCTCA TCTCGCCTTC CACCGCACCA GCAAGAGGAA
GGTAGTATGT TGGCTTTGGT CGATTCCTTC CTCGCTAGGT ATTCTTAGCG GAGCTTATCG GTATTGGAGT AGAGCGGAAG GTGGCGTGGT CGTTCTCCTT

1297 (SEQ ID NO 16) 100.0%
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7 100.0%
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3201 ACCGAATTAG AGCTGAAAGA ATACTAGAGC CATCGTAGGA GAACCGGATT CTTGACCGAT CGACTTTTGC CCGAGGTCGT TAGGTCGAAT AGGCTAGGTT
TGGCTTAATC TCGACTTTCT TATGATCTCG GTAGCATCCT CTTGGCCTAA GAACTGGCTA GCTGAAAACG GGCTCCAGCA ATCCAGCTTA TCCGATCCAA
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1514 (SEQ ID NO 19) 100.0%

9 100.0%
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7 100.0%
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104312 (SEQ ID NO 44) 100.0%
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3301 TACGAAAAAG AGACTAAGGC CGCTCTAGAG ATCCGTCAAC ATGGTGGAGC ACGACACTCT CGTCTACTCC AAGAATATCA AAGATACAGT CTCAGAAGAC
ATGCTTTTTC TCTGATTCCG GCGAGATCTC TAGGCAGTTG TACCACCTCG TGCTGTGAGA GCAGATGAGG TTCTTATAGT TTCTATGTCA GAGTCTTCTG
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8 100.0% 1658 (SEQ ID NO 20) 100.0%

Figure 10D
17/22

125323 (SEQ ID NO 46) 100.0%
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3401 CAAAGGGCTA TTGAGACTTT TCAACAAAGG GTAATATCGG GAAACCTCCT CGGATTCCAT TGCCCAGCTA TCTGTCACTT CATCAAAAGG ACAGTAGAAA
GTTTCCCGAT AACTCTGAAA AGTTGTTTCC CATTATAGCC CTTTGGAGGA GCCTAAGGTA ACGGGTCGAT AGACAGTGAA GTAGTTTTCC TGTCATCTTT
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1658 (SEQ ID NO 20) 100.0% 104314 (SEQ ID NO 45) 100.0%

3501 AGGAAGGTGG CACCTACAAA TGCCATCATT GCGATAAAGG AAAGGCTATC GTTCAAGATG CCTCTGCCGA CAGTGGTCCC AAAGATGGAC CCCCACCCAC
TCCTTCCACC GTGGATGTTT ACGGTAGTAA CGCTATTTCC TTTCCGATAG CAAGTTCTAC GGAGACGGCT GTCACCAGGG TTTCTACCTG GGGGTGGGTG

3601 GAGGAGCATC GTGGAAAAAG AAGACGTTCC AACCACGTCT TCAAAGCAAG TGGATTGATG TGATGATCCT ATGCGTATGG TATGACGTGT GTTCAAGATG
CTCCTCGTAG CACCTTTTTC TTCTGCAAGG TTGGTGCAGA AGTTTCGTTC ACCTAACTAC ACTACTAGGA TACGCATACC ATACTGCACA CAAGTTCTAC

3701 ATGACTTCAA ACCTACCTAT GACGTATGGT ATGACGTGTG TCGACTGATG ACTTAGATCC ACTCGAGCGG CTATAAATAC GTACCTACGC ACCCTGCGCT
TACTGAAGTT TGGATGGATA CTGCATACCA TACTGCACAC AGCTGACTAC TGAATCTAGG TGAGCTCGCC GATATTTATG CATGGATGCG TGGGACGCGA

3801 ACCATCCCTA GAGCTGCAGC TTATTTTTAC AACAATTACC AACAACAACA AACAACAAAC AACATTACAA TTACTATTTA CAATTACAGT CGACCCGGGA
TGGTAGGGAT CTCGACGTCG AATAAAAATG TTGTTAATGG TTGTTGTTGT TTGTTGTTTG TTGTAATGTT AATGATAAAT GTTAATGTCA GCTGGGCCCT

3901 TCCACACGAC ACCATGATAG AGGTGAAACC GATTAACGCA GAGGATACCT ATGAACTAAG GCATAGAATA CTCAGACCAA ACCAGCCGAT AGAAGCGTGT
AGGTGTGCTG TGGTACTATC TCCACTTTGG CTAATTGCGT CTCCTATGGA TACTTGATTC CGTATCTTAT GAGTCTGGTT TGGTCGGCTA TCTTCGCACA

4001 ATGTTTGAAA GCGATTTACT TCGTGGTGCA TTTCACTTAG GCGGCTTTTA CAGGGGCAAA CTGATTTCCA TAGCTTCATT CCACCAGGCC GAGCACTCGG
TACAAACTTT CGCTAAATGA AGCACCACGT AAAGTGAATC CGCCGAAAAT GTCCCCGTTT GACTAAAGGT ATCGAAGTAA GGTGGTCCGG CTCGTGAGCC

4101 AACTCCAAGG CCAGAAACAG TACCAGCTCC GAGGTATGGC TACCTTGGAA GGTTATCGTG AGCAGAAAGC GGGATCAACT CTAGTTAAAC ACGCTGAAGA
TTGAGGTTCC GGTCTTTGTC ATGGTCGAGG CTCCATACCG ATGGAACCTT CCAATAGCAC TCGTCTTTCG CCCTAGTTGA GATCAATTTG TGCGACTTCT

4201 AATCCTTCGT AAGAGGGGGG CGGACATGCT TTGGTGTAAT GCGAGGACAT CCGCCTCAGG CTACTACAAA AAGTTAGGCT TCAGCGAGCA GGGAGAGATA
TTAGGAAGCA TTCTCCCCCC GCCTGTACGA AACCACATTA CGCTCCTGTA GGCGGAGTCC GATGATGTTT TTCAATCCGA AGTCGCTCGT CCCTCTCTAT

4301 TTTGACACGC CGCCAGTAGG ACCTCACATC CTGATGTATA AAAGGATCAC ATAACTAGCT AGTCAGTTAA CCTAGACTTG TCCATCTTCT GGATTGGCCA
AAACTGTGCG GCGGTCATCC TGGAGTGTAG GACTACATAT TTTCCTAGTG TATTGATCGA TCAGTCAATT GGATCTGAAC AGGTAGAAGA CCTAACCGGT

4401 ACTTAATTAA TGTATGAAAT AAAAGGATGC ACACATAGTG ACATGCTAAT CACTATAATG TGGGCATCAA AGTTGTGTGT TATGTGTAAT TACTAGTTAT
TGAATTAATT ACATACTTTA TTTTCCTACG TGTGTATCAC TGTACGATTA GTGATATTAC ACCCGTAGTT TCAACACACA ATACACATTA ATGATCAATA

4501 CTGAATAAAA GAGAAAGAGA TCATCCATAT TTCTTATCCT AAATGAATGT CACGTGTCTT TATAATTCTT TGATGAACCA GATGCATTTC ATTAACCAAA
GACTTATTTT CTCTTTCTCT AGTAGGTATA AAGAATAGGA TTTACTTACA GTGCACAGAA ATATTAAGAA ACTACTTGGT CTACGTAAAG TAATTGGTTT

Figure 10E
18/22

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4601 TCCATATACA TATAAATATT AATCATATAT AATTAATATC AATTGGGTTA GCAAAACAAA TCTAGTCTAG GTGTGTTTTG CGAATTCGAT ATCAAGCTTT
     AGGTATATGT ATATTTATAA TTAGTATATA TTAATTATAG TTAACCCAAT CGTTTTGTTT AGATCAGATC CACACAAAAC GCTTAAGCTA TAGTTCGAAA

4701 GCTCTAGATC AAACTCACAT CCAAACATAA CATGGATATC TTCCTTACCA ATCATACTAA TTATTTTGGG TTAAATATTA ATCATTATTT TTAAGATATT
     CGAGATCTAG TTTGAGTGTA GGTTTGTATT GTACCTATAG AAGGAATGGT TAGTATGATT AATAAAACCC AATTTATAAT TAGTAATAAA AATTCTATAA

4801 AATTAAGAAA TTAAAAGATT TTTTAAAAAA ATGTATAAAA TTATATTATT CATGATTTTT CATACATTTG ATTTTGATAA TAAATATATT TTTTTTAATT
     TTAATTCTTT AATTTTCTAA AAAATTTTTT TACATATTTT AATATAATAA GTACTAAAAA GTATGTAAAC TAAAACTATT ATTTATATAA AAAAAATTAA

4901 TCTTAAAAAA TGTTGCAAGA CACTTATTAG ACATAGTCTT GTTCTGTTTA CAAAAGCATT CATCATTTAA TACATTAAAA AATATTTAAT ACTAACAGTA
     AGAATTTTTT ACAACGTTCT GTGAATAATC TGTATCAGAA CAAGACAAAT GTTTTCGTAA GTAGTAAATT ATGTAATTTT TTATAAATTA TGATTGTCAT

5001 GAATCTTCTT GTGAGTGGTG TGGGAGTAGG CAACCTGGCA TTGAAACGAG AGAAAGAGAG TCAGAACCAG AAGACAAATA AAAAGTATGC AACAAACAAA
     CTTAGAAGAA CACTCACCAC ACCCTCATCC GTTGGACCGT AACTTTGCTC TCTTTCTCTC AGTCTTGGTC TTCTGTTTAT TTTTCATACG TTGTTTGTTT

5101 TCAAAATCAA AGGGCAAAGG CTGGGGTTGG CTCAATTGGT TGCTACATTC AATTTTCAAC TCAGTCAACG GTTGAGATTC ACTCTGACTT CCCCAATCTA
     AGTTTTAGTT TCCCGTTTCC GACCCCAACC GAGTTAACCA ACGATGTAAG TTAAAAGTTG AGTCAGTTGC CAACTCTAAG TGAGACTGAA GGGGTTAGAT

5201 AGCCGCGGAT GCAAACGGTT GAATCTAACC CACAATCCAA TCTCGTTACT TAGGGGCTTT TCCGTCATTA ACTCACCCCT GCCACCCGGT TTCCCTATAA
     TCGGCGCCTA CGTTTGCCAA CTTAGATTGG GTGTTAGGTT AGAGCAATGA ATCCCCGAAA AGGCAGTAAT TGAGTGGGGA CGGTGGGCCA AAGGGATATT

5301 ATTGGAACTC AATGCTCCCC TCTAAACTCG TATCGCTTCA GAGTTGAGAC CAAGACACAC TCGTTCATAT ATCTCTCTGC TCTTCTCTTC TCTTCTACCT
     TAACCTTGAG TTACGAGGGG AGATTTGAGC ATAGCGAAGT CTCAACTCTG GTTCTGTGTG AGCAAGTATA TAGAGAGACG AGAAGAGAAG AGAAGATGGA

5401 CTCAAGGTAC TTTTCTTCTC CCTCTACCAA ATCCTAGATT CCGTGGTTCA ATTTCGGATC TTGCACTTCT GGTTTGCTTT GCCTTGCTTT TTCCTCAACT
     GAGTTCCATG AAAAGAAGAG GGAGATGGTT TAGGATCTAA GGCACCAAGT TAAAGCCTAG AACGTGAAGA CCAAACGAAA CGGAACGAAA AAGGAGTTGA

5501 GGGTCCATCT AGGATCCATG TGAAACTCTA CTCTTTCTTT AATATCTGCG GAATACGCGT TTGACTTTCA GATCTAGTCG AAATCATTTC ATAATTGCCT
     CCCAGGTAGA TCCTAGGTAC ACTTTGAGAT GAGAAAGAAA TTATAGACGC CTTATGCGCA AACTGAAAGT CTAGATCAGC TTTAGTAAAG TATTAACGGA

5601 TTCTTTCTTT TAGCTTATGA GAAATAAAAT CACTTTTTTT TTATTTCAAA ATAAACCTTG GGCCTTGTGC TGACTGAGAT GGGGTTTGGT GATTACAGAA
     AAGAAAGAAA ATCGAATACT CTTTATTTTA GTGAAAAAAA AATAAAGTTT TATTTGGAAC CCGGAACACG ACTGACTCTA CCCCAAACCA CTAATGTCTT

5701 TTTTAGCGAA TTTTGTAATT GTACTTGTTT GTCTGTAGTT TTGTTTTGTT TTCTTGTTTC TCATACATTC CTTAGGCTTC AATTTTATTC GAGTATAGGT
     AAAATCGCTT AAAACATTAA CATGAACAAA CAGACATCAA AACAAAACAA AAGAACAAAG AGTATGTAAG GAATCCGAAG TTAAAATAAG CTCATATCCA

5801 CACAATAGGA ATTCAAACTT TGAGCAGGGG AATTAATCCC TTCCTTCAAA TCCAGTTTGT TTGTATATAT GTTTAAAAAA TGAAACTTTT GCTTTAAATT
     GTGTTATCCT TAAGTTTGAA ACTCGTCCCC TTAATTAGGG AAGGAAGTTT AGGTCAAACA AACATATATA CAAATTTTTT ACTTTGAAAA CGAAATTTAA

5901 CTATTATAAC TTTTTTTATG GCTGAAATTT TTGCATGTGT CTTTGCTCTC TGTTGTAAAT TTACTGTTTA GGTACTAACT CTAGGCTTGT TGTGCAGTTT
     GATAATATTG AAAAAAATAC CGACTTTAAA AACGTACACA GAAACGAGAG ACAACATTTA AATGACAAAT CCATGATTGA GATCCGAACA ACACGTCAAA
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Figure 10F
19/22

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6001 TTGAAGTATA ACCATGCCAC ACAACACAAT GGCGGCCACC GCTTCCAGAA CCACCCGATT CTCTTCTTCC TCTTCACACC CCACCTTCCC CAAACGCATT
     AACTTCATAT TGGTACGGTG TGTTGTGTTA CCGCCGGTGG CGAAGGTCTT GGTGGGCTAA GAGAAGAAGG AGAAGTGTGG GGTGGAAGGG GTTTGCGTAA

6101 ACTAGATCCA CCCTCCCTCT CTCTCATCAA ACCCTCACCA AACCCAACCA CGCTCTCAAA ATCAAATGTT CCATCTCCAA ACCCCCCACG GCGGCGCCCT
     TGATCTAGGT GGGAGGGAGA GAGAGTAGTT TGGGAGTGGT TTGGGTTGGT GCGAGAGTTT TAGTTTACAA GGTAGAGGTT TGGGGGGTGC CGCCGCGGGA

6201 TCACCAAGGA AGCGCCGACC ACGGAGCCCT TCGTGTCACG GTTCGCCTCC GGCGAACCTC GCAAGGGCGC GGACATCCTT GTGGAGGCGC TGGAGAGGCA
     AGTGGTTCCT TCGCGGCTGG TGCCTCGGGA AGCACAGTGC CAAGCGGAGG CCGCTTGGAG CGTTCCCGCG CCTGTAGGAA CACCTCCGCG ACCTCTCCGT

6301 GGGCGTGACG ACGGTGTTCG CGTACCCCGG CGGTGCGTCG ATGGAGATCC ACCAGGCGCT CACGCGCTCC GCCGCCATCC GCAACGTGCT CCCGCGCCAC
     CCCGCACTGC TGCCACAAGC GCATGGGGCC GCCACGCAGC TACCTCTAGG TGGTCCGCGA GTGCGCGAGG CGGCGGTAGG CGTTGCACGA GGGCGCGGTG

6401 GAGCAGGGCG GCGTCTTCGC CGCCGAAGGC TACGCGCGTT CCTCCGGCCT CCCCGGCGTC TGCATTGCCA CCTCCGGCCC CGGCGCCACC AACCTCGTGA
     CTCGTCCCGC CGCAGAAGCG GCGGCTTCCG ATGCGCGCAA GGAGGCCGGA GGGGCCGCAG ACGTAACGGT GGAGGCCGGG GCCGCGGTGG TTGGAGCACT

6501 GCGGCCTCGC CGACGCTTTA ATGGACAGCG TCCCAGTCGT CGCCATCACC GGCCAGGTCG CCCGCCGGAT GATCGGCACC GACGCCTTCC AAGAAACCCC
     CGCCGGAGCG GCTGCGAAAT TACCTGTCGC AGGGTCAGCA GCGGTAGTGG CCGGTCCAGC GGGCGGCCTA CTAGCCGTGG CTGCGGAAGG TTCTTTGGGG

6601 GATCGTGGAG GTGAGCAGAT CCATCACGAA GCACAACTAC CTCATCCTCG ACGTCGACGA CATCCCCCGC GTCGTCGCCG AGGCTTTCTT CGTCGCCACC
     CTAGCACCTC CACTCGTCTA GGTAGTGCTT CGTGTTGATG GAGTAGGAGC TGCAGCTGCT GTAGGGGGCG CAGCAGCGGC TCCGAAAGAA GCAGCGGTGG

6701 TCCGGCCGCC CCGGTCCGGT CCTCATCGAC ATTCCCAAAG ACGTTCAGCA GCAACTCGCC GTGCCTAATT GGGACGAGCC CGTTAACCTC CCCGGTTACC
     AGGCCGGCGG GGCCAGGCCA GGAGTAGCTG TAAGGGTTTC TGCAAGTCGT CGTTGAGCGG CACGGATTAA CCCTGCTCGG GCAATTGGAG GGGCCAATGG

6801 TCGCCAGGCT GCCCAGGCCC CCCGCCGAGG CCCAATTGGA ACACATTGTC AGACTCATCA TGGAGGCCCA AAAGCCCGTT CTCTACGTCG GCGGTGGCAG
     AGCGGTCCGA CGGGTCCGGG GGGCGGCTCC GGGTTAACCT TGTGTAACAG TCTGAGTAGT ACCTCCGGGT TTTCGGGCAA GAGATGCAGC CGCCACCGTC

6901 TTTGAATTCC AGTGCTGAAT TGAGGCGCTT TGTTGAACTC ACTGGTATTC CCGTTGCTAG CACTTTAATG GGTCTTGGAA CTTTTCCTAT TGGTGATGAA
     AAACTTAAGG TCACGACTTA ACTCCGCGAA ACAACTTGAG TGACCATAAG GGCAACGATC GTGAAATTAC CCAGAACCTT GAAAAGGATA ACCACTACTT

7001 TATTCCCTTC AGATGCTGGG TATGCATGGT ACTGTTTATG CTAACTATGC TGTTGACAAT AGTGATTTGT TGCTTGCCTT TGGGGTAAGG TTTGATGACC
     ATAAGGGAAG TCTACGACCC ATACGTACCA TGACAAATAC GATTGATACG ACAACTGTTA TCACTAAACA ACGAACGGAA ACCCCATTCC AAACTACTGG

7101 GTGTTACTGG GAAGCTTGAG GCTTTTGCTA GTAGGGCTAA GATTGTTCAC ATTGATATTG ATTCTGCCGA GATTGGGAAG AACAAGCAGG CGCACGTGTC
     CACAATGACC CTTCGAACTC CGAAAACGAT CATCCCGATT CTAACAAGTG TAACTATAAC TAAGACGGCT CTAACCCTTC TTGTTCGTCC GCGTGCACAG

7201 GGTTTGCGCG GATTTGAAGT TGGCCTTGAA GGGAATTAAT ATGATTTTGG AGGAGAAAGG AGTGGAGGGT AAGTTTGATC TTGGAGGTTG GAGAGAAGAG
     CCAAACGCGC CTAAACTTCA ACCGGAACTT CCCTTAATTA TACTAAAACC TCCTCTTTCC TCACCTCCCA TTCAAACTAG AACCTCCAAC CTCTCTTCTC

7301 ATTAATGTGC AGAAACACAA GTTTCCATTG GGTTACAAGA CATTCCAGGA CGCGATTTCT CCGCAGCATG CTATCGAGGT TCTTGATGAG TTGACTAATG
     TAATTACACG TCTTTGTGTT CAAAGGTAAC CCAATGTTCT GTAAGGTCCT GCGCTAAAGA GGCGTCGTAC GATAGCTCCA AGAACTACTC AACTGATTAC
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Figure 10G
20/22

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7401 GAGATGCTAT TGTTAGTACT GGGGTTGGGC AGCATCAAAT GTGGGCTGCG CAGTTTTACA AGTACAAGAG ACCGAGGCAG TGGTTGACCT CAGGGGGTCT
     CTCTACGATA ACAATCATGA CCCCAACCCG TCGTAGTTTA CACCCGACGC GTCAAAATGT TCATGTTCTC TGGCTCCGTC ACCAACTGGA GTCCCCCAGA

7501 TGGAGCCATG GGTTTTGGAT TGCCTGCGGC TATTGGTGCT GCTGTTGCTA ACCCTGGGGC TGTTGTGGTT GACATTGATG GGGATGGTAG TTTCATCATG
     ACCTCGGTAC CCAAAACCTA ACGGACGCCG ATAACCACGA CGACAACGAT TGGGACCCCG ACAACACCAA CTGTAACTAC CCCTACCATC AAAGTAGTAC

7601 AATGTTCAGG AGTTGGCCAC TATAAGAGTG GAGAATCTCC CAGTTAAGAT ATTGTTGTTG AACAATCAGC ATTTGGGTAT GGTGGTTCAG TTGGAGGATA
     TTACAAGTCC TCAACCGGTG ATATTCTCAC CTCTTAGAGG GTCAATTCTA TAACAACAAC TTGTTAGTCG TAAACCCATA CCACCAAGTC AACCTCCTAT

7701 GGTTCTACAA GTCCAATAGA GCTCACACCT ATCTTGGAGA TCCGTCTAGC GAGAGCGAGA TATTCCCAAA CATGCTCAAG TTTGCTGATG CTTGTGGGAT
     CCAAGATGTT CAGGTTATCT CGAGTGTGGA TAGAACCTCT AGGCAGATCG CTCTCGCTCT ATAAGGGTTT GTACGAGTTC AAACGACTAC GAACACCCTA

7801 ACCGGCAGCG CGAGTGACGA AGAAGGAAGA GCTTAGAGCG GCAATTCAGA GAATGTTGGA CACCCCTGGC CCCTACCTTC TTGATGTCAT TGTGCCCCAT
     TGGCCGTCGC GCTCACTGCT TCTTCCTTCT CGAATCTCGC CGTTAAGTCT CTTACAACCT GTGGGGACCG GGGATGGAAG AACTACAGTA ACACGGGGTA

                                                    1439 (SEQ ID NO 18) 100.0%
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7901 CAGGAGCATG TGTTGCCGAT GATTCCCAGT AATGGATCCT TCAAGGATGT GATAACTGAG GGTGATGGTA GAACGAGGTA CTGATTGCCT AGACCAAATG
     GTCCTCGTAC ACAACGGCTA CTAAGGGTCA TTACCTAGGA AGTTCCTACA CTATTGACTC CCACTACCAT CTTGCTCCAT GACTAACGGA TCTGGTTTAC

8001 TTCCTTGATG CTTGTTTTGT ACAATATATA TAAGATAATG CTGTCCTAGT TGCAGGATTT GGCCTGTGGT GAGCATCATA GTCTGTAGTA GTTTTGGTAG
     AAGGAACTAC GAACAAAACA TGTTATATAT ATTCTATTAC GACAGGATCA ACGTCCTAAA CCGGACACCA CTCGTAGTAT CAGACATCAT CAAAACCATC

8101 CAAGACATTT TATTTTCCTT TTATTTAACT TACTACATGC AGTAGCATCT ATCTATCTCT GTAGTCTGAT ATCTCCTGTT GTCTGTATTG TGCCGTTGGA
     GTTCTGTAAA ATAAAAGGAA AATAAATTGA ATGATGTACG TCATCGTAGA TAGATAGAGA CATCAGACTA TAGAGGACAA CAGACATAAC ACGGCAACCT

8201 TTTTTTGCTG TAGTGAGACT GAAAATGATG TGCTAGTAAT AATATTTCTG TTAGAAATCT AAGTAGAGAA TCTGTTGAAG AAGTCAAAAG CTAATGGAAT
     AAAAAACGAC ATCACTCTGA CTTTTACTAC ACGATCATTA TTATAAAGAC AATCTTTAGA TTCATCTCTT AGACAACTTC TTCAGTTTTC GATTACCTTA

8301 CAGGTTACAT ATTCAATGTT TTTCTTTTTT TAGCGGTTGG TAGACGTGTA GATTCAACTT CTCTTGGAGC TCACCTAGGC AATCAGTAAA ATGCATATTC
     GTCCAATGTA TAAGTTACAA AAAGAAAAAA ATCGCCAACC ATCTGCACAT CTAAGTTGAA GAGAACCTCG AGTGGATCCG TTAGTCATTT TACGTATAAG

8401 CTTTTTTAAC TTGCCATTTA TTTACTTTTA GTGGAAATTG TGACCAATTT GTTCATGTAG AACGGATTTG GACCATTGCG TCCACAAAAC GTCTCTTTTG
     GAAAAAATTG AACGGTAAAT AAATGAAAAT CACCTTTAAC ACTGGTTAAA CAAGTACATC TTGCCTAAAC CTGGTAACGC AGGTGTTTTG CAGAGAAAAC

8501 CTCGATCTTC ACAAAGCGAT ACCGAAATCC AGAGATAGTT TTCAAAAGTC AGAAATGGCA AAGTTATAAA TAGTAAAACA GAATAGATGC TGTAATCGAC
     GAGCTAGAAG TGTTTCGCTA TGGCTTTAGG TCTCTATCAA AAGTTTTCAG TCTTTACCGT TTCAATATTT ATCATTTTGT CTTATCTACG ACATTAGCTG
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Figure 10H
21/22

1660 (SEQ ID NO 21) 100.0%
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8601 TTCAATAACA AGTGGCATCA CGTTTCTAGT TCTAGACCCA TCAGCTGGGC CGGCCACTAG TGAGCTCGGT ACCCGGGGGC GCGTAATATC ATCATTAGGA
AAGTTATTGT TCACCGTAGT GCAAAGATCA AGATCTGGGT AGTCGACCCG GCCGGTGATC ACTCGAGCCA TGGGCCCCCG CGCATTATAG TAGTAATCCT
~~~~~~~~~~~~~
1298 (SEQ ID NO 17) 100.0%

1660 (SEQ ID NO 21) 100.0%
~~~~~~~~~~~~~~~~~~
8701 AGACACTGCC CATCTTGAAT AGGATTTTAG CTACTAAATA TGTTGATGGT CTTTATGAAA AACTATTAAC TAGGAATATT ATGCTACCCA TATGGAAAGA
TCTGTGACGG GTAGAACTTA TCCTAAAATC GATGATTTAT ACAACTACCA GAAATACTTT TTGATAATTG ATCCTTATAA TACGATGGGT ATACCTTTCT
~~~~~~~~~~~~~~~~~~~~~~~~~
1298 (SEQ ID NO 17) 100.0%

8801 AGACGCTAGG GGAATAGAAA GACCATCAAA TAAACGAAGT CAACACCAGG TCTTCCGAAG CATTAACAAT TACCTATTTA ATATGTACTC AGTCCGGGTG
TCTGCGATCC CCTTATCTTT CTGGTAGTTT ATTTGCTTCA GTTGTGGTCC AGAAGGCTTC GTAATTGTTA ATGGATAAAT TATACATGAG TCAGGCCCAC

8901 GATATCTCAC TACATTGACG CAGTTTGTTC AAAGACGAAC GCCCTGAATT ATGCCATCTG CTTAGGCTTT CAAATATGGT ACGCTCTAAT GCCAAGCCTT
CTATAGAGTG ATGTAACTGC GTCAAACAAG TTTCTGCTTG CGGGACTTAA TACGGTAGAC GAATCCGAAA GTTTATACCA TGCGAGATTA CGGTTCGGAA
~~~~~~
1666 (SEQ ID NO 22) 100.0%

9001 ATGCTGGTCT TAGGGTATTA TCATCAAATC TTTAAGCCAG AGGTAGTTAA ATACATCAAG GACACCATAG GAGTATGGCA CAACGATATT GTCAAGATCG
TACGACCAGA ATCCCATAAT AGTAGTTTAG AAATTCGGTC TCCATCAATT TATGTAGTTC CTGTGGTATC CTCATACCGT GTTGCTATAA CAGTTCTAGC

9101 CATCAGATCT AATAGGCAAT AATGAATTCT TCATGCAGCC CGACGTGGGA ACGCTCGAAA GCAGTGGGGC CTCTGGGACA GGGACCAGAC CTGAGTCGCT
GTAGTCTAGA TTATCCGTTA TTACTTAAGA AGTACGTCGG GCTGCACCCT TGCGAGCTTT CGTCACCCCG GAGACCCTGT CCCTGGTCTG GACTCAGCGA

9201 AACATTTGGG AATAAGAGAA GTAGATATAC CCAATTTTTT AACTAGCCAA GGAAGGAAAG CGGGAAAGGT CCGATACAAA GGAAAGGGTT GCGAGGCTTA
TTGTAAACCC TTATTCTCTT CATCTATATG GGTTAAAAAA TTGATCGGTT CCTTCCTTTC GCCCTTTCCA GGCTATGTTT CCTTTCCCAA CGCTCCGAAT

9301 ACGATTTAGA ATATAGCTGT TGAGGTGGCA CTTGTTCCCC CGGGGCGGGG GTATATGCCC GTAGCTTTAT TCTGTCACTT CTTTCAGATC AATGAAGTTG
TGCTAAATCT TATATCGACA ACTCCACCGT GAACAAGGGG GCCCCGCCCC CATATACGGG CATCGAAATA AGACAGTGAA GAAAGTCTAG TTACTTCAAC

9401 AAAAGTTATA GAGTAAGGGA CCCTTGTTTA CAAAGCTGTC ACTCCAAGAA CTCGAAGTCA AGCATCTTCG GGAATATCCA GATTAGTCTT CAACTAGAGA
TTTTCAATAT CTCATTCCCT GGGAACAAAT GTTTCGACAG TGAGGTTCTT GAGCTTCAGT TCGTAGAAGC CCTTATAGGT CTAATCAGAA GTTGATCTCT

9501 AAGGATAGGA ATCTCCTTTG CAGAGTTTTC TTCTCCTGCT GATGTAGCGG TAAAGTCAAA AGTTGGATGC CCTTTTTTCT TTATTTAATT AATTCCGTTG
TTCCTATCCT TAGAGGAAAC GTCTCAAAAG AAGAGGACGA CTACATCGCC ATTTCAGTTT TCAACCTACG GGAAAAAAGA AATAAATTAA TTAAGGCAAC
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Figure 10I
22/22

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9601  ATAGAGCTTT TGAGCGGATG CAAGCACTAG ATTCTTCAAC GAGTACCAAT AATAAATGAA TTCACCAGAC TAAGAGAAGA AAACAGAACA AAAAGATTAA
      TATCTCGAAA ACTCGCCTAC GTTCGTGATC TAAGAAGTTG CTCATGGTTA TTATTTACTT AAGTGGTCTG ATTCTCTTCT TTTGTCTTGT TTTTCTAATT

9701  GCCCAGCCGC CTTCGGGAAG ACCTATCTTC GTCGGGAGGA AGAGCCCTCT TTACACCATT GTGATTAGAA AAAACCGAAA AGTGGACCGG CCTAGTAACC
      CGGGTCGGCG GAAGCCCTTC TGGATAGAAG CAGCCCTCCT TCTCGGGAGA AATGTGGTAA CACTAATCTT TTTTGGCTTT TCACCTGGCC GGATCATTGG

9801  AATAGAGCGG GGCTTGATCC CCACTTTAAA TCTATTGGAT AGAGCCCTCA GCCCAGGGCA AGCGATTGAA TTCTATTTGA TTATGGGTTA GGTGGAACCT
      TTATCTCGCC CCGAACTAGG GGTGAAATTT AGATAACCTA TCTCGGGAGT CGGGTCCCGT TCGCTAACTT AAGATAAACT AATACCCAAT CCACCTTGGA

9901  GAAACTAGCA CTTACAAATG AGTTAGCAAA AGGAAAAAGA CAATTCTCAA ATGCGTACAA GACTTTCTTC CTTCTTTGTT TAAGAGGCCA GTCTGCGATG
      CTTTGATCGT GAATGTTTAC TCAATCGTTT TCCTTTTTCT GTTAAGAGTT TACGCATGTT CTGAAAGAAG GAAGAAACAA ATTCTCCGGT CAGACGCTAC

10001 GATGCTCGTG CATGAAAAAG GGCTTTGATC TATTCACCAC TTATATAATA GAGCCAATCT CTGCAGGACA AGATATCTAT TTTGTCATTG GGAAGTAAGG
      CTACGAGCAC GTACTTTTTC CCGAAACTAG ATAAGTGGTG AATATATTAT CTCGGTTAGA GACGTCCTGT TCTATAGATA AAACAGTAAC CCTTCATTCC

10101 CTTAAGTCGA CGAAAAAGTT AGGAAAGGGG ATCATATGGC TAGGGTTGCC CTCGGGGCTC AAGGGTTTAG CGATGAAGAG TGCCAAGCAA AAGGTCAATA
      GAATTCAGCT GCTTTTTCAA TCCTTTCCCC TAGTATACCG ATCCCAACGG GAGCCCCGAG TTCCCAAATC GCTACTTCTC ACGGTTCGTT TTCCAGTTAT

10201 CCGGTACGCC GATCAAAGAA GTCCAGTGGC AAGGCCCTTT CAGCCAAGCT AGCGTGCTGA ACAGAAAGTC GTAGAGTGAT GACAGCTTCT TCTTCTTGAG
      GGCCATGCGG CTAGTTTCTT CAGGTCACCG TTCCGGGAAA GTCGGTTCGA TCGCACGACT TGTCTTTCAG CATCTCACTA CTGTCGAAGA AGAAGAACTC

10301 TCATTCGTGT GACAACATCA GGATCTCGTC GAAAGACCTC CTCTGCCTAT CTCTCCCGCA AGAGAGGACT CGTTATGGCG CACCTCTTTT TAGCAGTCTC
      AGTAAGCACA CTGTTGTAGT CCTAGAGCAG CTTTCTGGAG GAGACGGATA GAGAGGGCGT TCTCTCCTGA GCAATACCGC GTGGAGAAAA ATCGTCAGAG

10401 GTCAATAAGA TAAGATTGCC CCTCCCTTCT TATTGATTTG ATAAAGGGCT TTGTCCACTC CCTCTCTTCT TAGCCGAGCG GAGTGACGGT TTAGTTTAGG
      CAGTTATTCT ATTCTAACGG GGAGGGAAGA ATAACTAAAC TATTTCCCGA AACAGGTGAG GGAGAGAAGA ATCGGCTCGC CTCACTGCCA AATCAAATCC

10501 CTTTAGATGC CACTGCGAAA GACTCTAGAG ATCCACTCTC ACAGCGTATA CGCGACATCC CTATGTATAC ACAATCCTTT CAAGCAGCTA GGACAGCTAG
      GAAATCTACG GTGACGCTTT CTGAGATCTC TAGGTGAGAG TGTCGCATAT GCGCTGTAGG GATACATATG TGTTAGGAAA GTTCGTCGAT CCTGTCGATC

10601 CAAGCAAGTT ATCTGTTCGC GGACAAGCTC TCTGGATGAC AAAAAACATG CTCTTTCATG CGGAAAAAAC ACGGTCTTTC GTGGAAGTTG GTCGATTTGA
      GTTCGTTCAA TAGACAAGCG CCTGTTCGAG AGACCTACTG TTTTTTGTAC GAGAAAGTAC GCCTTTTTTG TGCCAGAAAG CACCTTCAAC CAGCTAAACT

10701 AGTCGCTTTA TGAGTGAAAA TGGGTCGATG ACGAAAAAGA CGGGGAAAAT GATCAACTGT CACATTTTGA TGCCAGTTTA GGGCTAAAAT GAACTTTCAT
      TCAGCGAAAT ACTCACTTTT ACCCAGCTAC TGCTTTTTCT GCCCCTTTTA CTAGTTGACA GTGTAAAACT ACGGTCAAAT CCCGATTTTA CTTGAAAGTA

10801 CCAAAAAGAC CGAGAAAACG CTCCACTGGC AGGATCCGAT CGGAAATAA
      GGTTTTTCTG GCTCTTTTGC GAGGTGACCG TCCTAGGCTA GCCTTTATT
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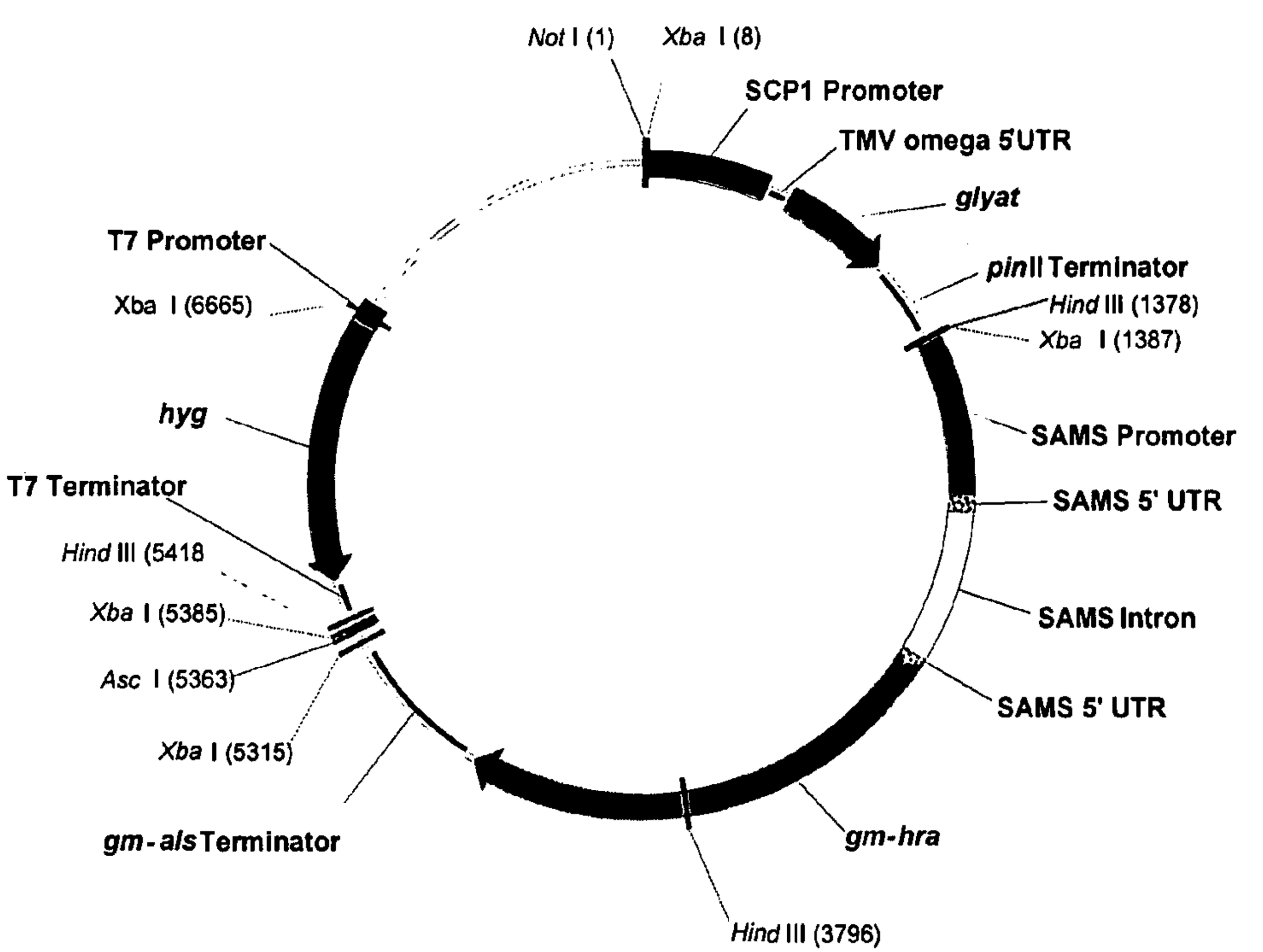

Not I (1)
Xba I (8)
SCP1 Promoter
TMV omega 5'UTR
glyat
pinII Terminator
Hind III (1378)
Xba I (1387)
SAMS Promoter
SAMS 5' UTR
SAMS Intron
SAMS 5' UTR
gm-hra
Hind III (3796)
gm-als Terminator
Xba I (5315)
Asc I (5363)
Xba I (5385)
Hind III (5418
T7 Terminator
hyg
Xba I (6665)
T7 Promoter

FIGURE 1