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CA 2786301 A1 2011/07/14

(21) 2 786 301

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2011/01/07
(87) Date publication PCT/PCT Publication Date: 2011/07/14
(85) Entrée phase nationale/National Entry: 2012/07/03
(86) N° demande PCT/PCT Application No.: US 2011/020546
(87) N° publication PCT/PCT Publication No.: 2011/085221
(30) Priorité/Priority: 2010/01/07 (US61/293,165)

(51) Cl.Int./Int.Cl. *A01H 5/00* (2006.01), *A01H 5/10* (2006.01), *C12N 15/29* (2006.01), *C12N 15/82* (2006.01)

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(54) Titre : PLANTES TOLERANTES AUX HERBICIDES
(54) Title: HERBICIDE-TOLERANT PLANTS

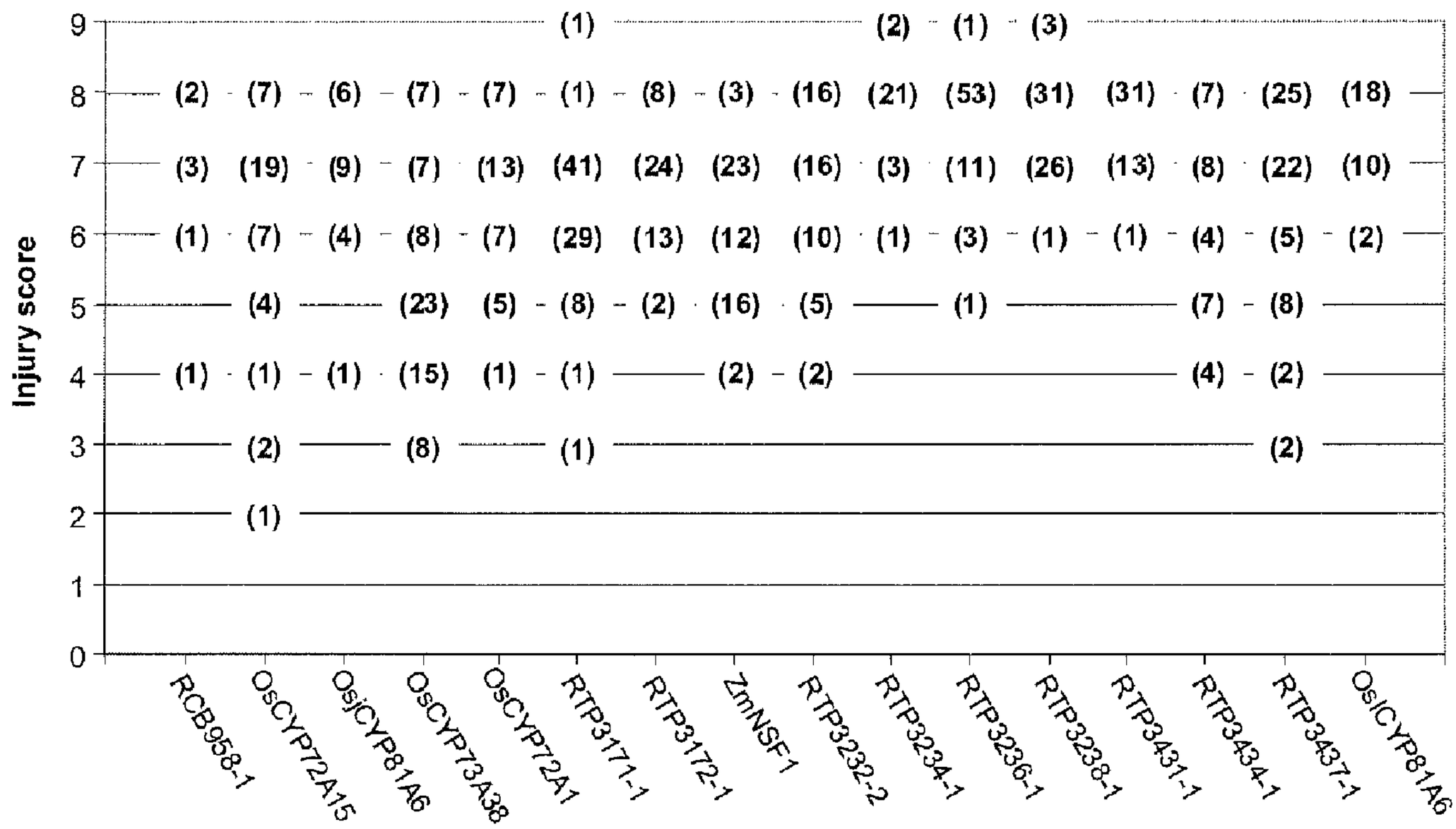

FIG. 3

(57) Abrégé/Abstract:
The present invention provides saflufenacil-tolerant plants. The present invention also provides methods for controlling the growth of weeds by applying saflufenacil to which the saflufenacil-tolerant plants of the invention are tolerant. Plants of the invention express a cytochrome P450 polypeptide, the expression of which confers, to the plants, tolerance to the saflufenacil.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 July 2011 (14.07.2011)

PCT

(10) International Publication Number
WO 2011/085221 A3

(51) International Patent Classification:
A01H 5/00 (2006.01) *C12N 15/29* (2006.01)
A01H 5/10 (2006.01) *C12N 15/82* (2006.01)

(21) International Application Number:
PCT/US2011/020546

(22) International Filing Date:
7 January 2011 (07.01.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/293,165 7 January 2010 (07.01.2010) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
19 January 2012

(54) Title: HERBICIDE-TOLERANT PLANTS

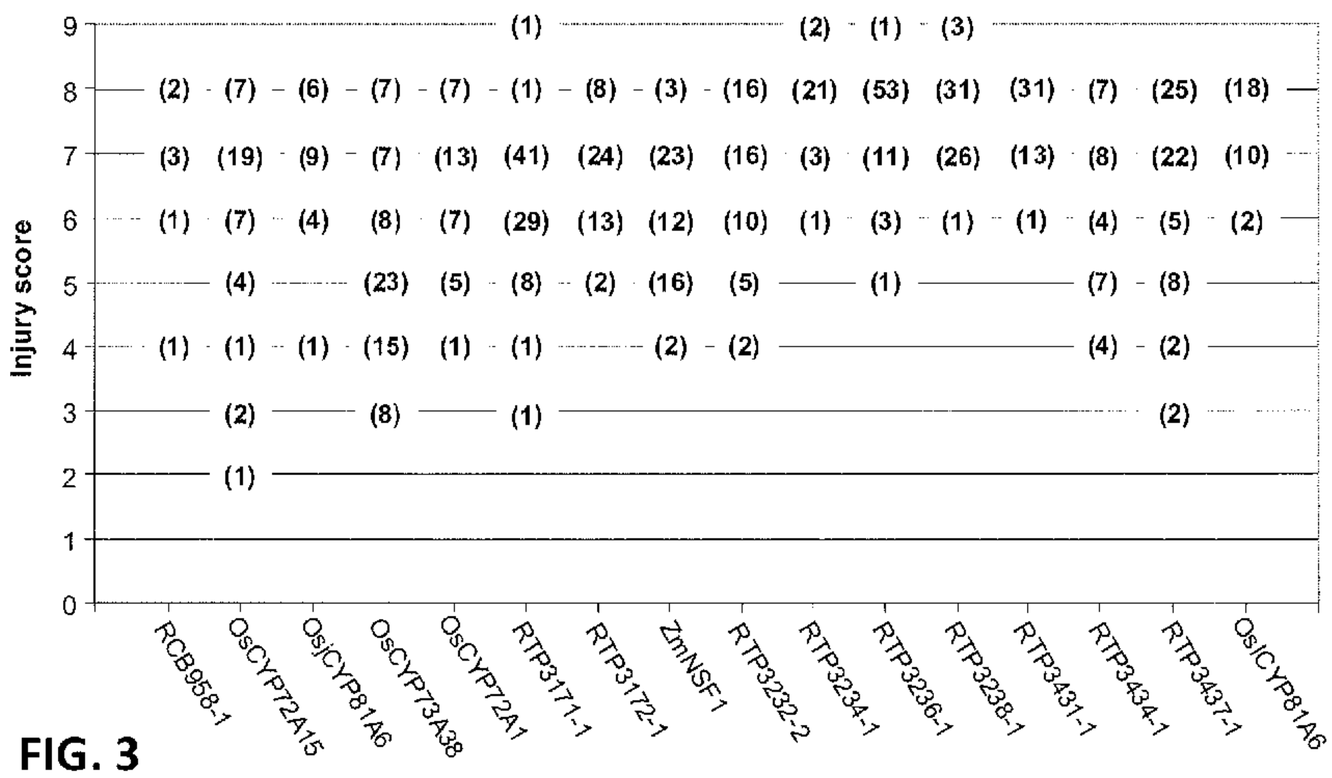

FIG. 3

(57) Abstract: The present invention provides saflufenacil-tolerant plants. The present invention also provides methods for controlling the growth of weeds by applying saflufenacil to which the saflufenacil-tolerant plants of the invention are tolerant. Plants of the invention express a cytochrome P450 polypeptide, the expression of which confers, to the plants, tolerance to the saflufenacil.

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HERBICIDE-TOLERANT PLANTS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/293,165 filed on January 7, 2010, which is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to herbicide-tolerant plants.

BACKGROUND OF THE INVENTION

[0003] P450's form a large diverse gene family with about 246 isoforms in *Arabidopsis* and 372 identified in rice. P450s are hemoproteins that convert a broad range of substrates to more or less bioactive products. P450s are critical in numerous metabolic pathways, including lignin and pigment biosynthesis, detoxification of harmful compounds, and are considered important in the evolution of land plants. Inhibitors of P450 activity include 1-aminobenzo-triazole, tetcyclacis, piperonyl butoxide, and tridiphane.

[0004] Saflufenacil is an herbicide active ingredient (A.I.) of the pyrimidinedione chemical class. Saflufenacil is an active ingredient that is similar to flumioxazin and sulfentrazone and is readily absorbed by foliage, root, and shoot tissue of plants. It is believed that saflufenacil inhibits the pigment biosynthesis pathway at protoporphyrinogen oxidase (PPO), which causes an accumulation of photodynamic, toxic compounds that rapidly damage cell membranes and results in cell death. Herbicidal compositions can be used that have saflufenacil as the sole A.I. or that are supplemented with glyphosate to manage a wide spectrum of dicot weeds. Herbicidal compositions comprising saflufenacil have been labeled for pre-plant or pre-emergence treatment in corn, sorghum, wheat, barley, oats, rye, triticale, soybean, and tree/nut/vine cropping systems. Saflufenacil-containing herbicidal compositions have good foliar and residual activity on broadleaf weeds in both no-

till and tilled cropping systems. However, application of saflufenacil after emergence can result in rapid and significant crop injury.

[0005] There remains a need in the art for plants or plants parts that exhibit tolerance to herbicidal compositions comprising saflufenacil.

BRIEF SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention provides a plant or plant part comprising a recombinant polynucleotide encoding a CYP81A or CYP73A polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil.

[0007] In some aspects, the present invention provides a seed capable of germination into a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant tolerance to saflufenacil.

[0008] In one aspect, the present invention provides a plant cell of or capable of regenerating a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the plant cell comprises the recombinant polynucleotide operably linked to a promoter. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant tolerance to saflufenacil.

[0009] In another aspect, the present invention provides a plant cell comprising a recombinant polynucleotide operably linked to a promoter operable in the cell, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the cell tolerance to saflufenacil. In some

embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the cell tolerance to saflufenacil.

[0010] In other aspects, the present invention provides a plant product prepared from a plant or plant part comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A, or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil.

[0011] In some aspects, the present invention provides a progeny or descendant plant derived from a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the progeny or descendant plant comprises in at least some of its cells the recombinant polynucleotide operably linked to the promoter, the expression of the polypeptide in the progeny or descendant plant conferring to the progeny or descendant plant tolerance to the saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant tolerance to saflufenacil.

[0012] In other aspects, the present invention provides a method for controlling weeds at a locus for growth of a plant, the method comprising: (a) applying an herbicide composition comprising saflufenacil to the locus; and (b) planting a seed at the locus, wherein the seed is capable of producing a plant that comprises in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to the saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant tolerance to saflufenacil.

[0013] In some aspects, the present invention provides a method for controlling weeds at a locus for growth of a plant, the method comprising: applying an herbicidal composition

comprising saflufenacil to the locus; wherein said locus is: (a) a locus that contains: a plant or a seed capable of producing said plant; or (b) a locus that is to be after said applying is made to contain the plant or the seed; wherein the plant or the seed comprises in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to the saflufenacil. In one embodiment, the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A.

[0014] In other aspects, the present invention provides a method of producing a plant having tolerance to saflufenacil, the method comprising regenerating a plant from a plant cell transformed with a recombinant polynucleotide operably linked to a promoter operable in the cell, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide, conferring to the plant tolerance to the saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers to the plant tolerance to saflufenacil.

[0015] In one aspect, the present invention provides a method of producing a progeny plant having tolerance to saflufenacil, the method comprising: crossing a first saflufenacil-tolerant plant with a second plant to produce a saflufenacil-tolerant progeny plant, wherein the first plant and the progeny plant comprise in at least some of their cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring tolerance to saflufenacil. In some embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of which confers tolerance to saflufenacil.

[0016] In still further aspects, the present invention provides a plant or plant part comprising in of at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil, wherein the plant or plant part further exhibits a second herbicide-tolerant trait. In some embodiments, the recombinant polynucleotide

encodes a CYP72A15 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1: Graph presenting safener and saflufenacil-induced expression of rice and maize P450 genes. Expression of rice, wheat, and maize P450 genes are shown as the fold change (log2 transformed) over that of unsprayed check controls.

[0018] Figure 2: Schematic guide tree. Sequences identified for testing in the maize, rice, and soybean transgenic systems were aligned and a guide tree prepared. Genes designated as OsCYP72A1, OsCYP72A15, NSF1 conferred tolerance to saflufenacil in one or more transgenic plant systems as disclosed herein. The guide tree was generated using the AlignX program, with the Neighbor Joining method (NJ) which works on a matrix of distances between all pairs of and these distances are related to the level of divergence between the sequences. The calculated distance values for each molecule are displayed in parenthesis.

[0019] Figure 3: Herbicide injury scores for independent T0 maize events treated with 200 g ai/ha saflufenacil + 1 % MSO. Numbers indicate number of events that fell within a particular injury score. Injury scores are from 0-9, 0 being no injury and 9 being death. Untransformed J553 is the control.

[0020] Figure 4: Herbicide injury scores for segregating independent T1 maize events treated with 240 g ai/ha Kixor + 1% MSO. Numbers indicate the number of plants that fell within a particular injury score. Injury scores are from 0-9, 0 being no injury and 9 being death. Untransformed J553 is the control.

[0021] Figure 5: Herbicide injury scores for cuttings of independent T0 transgenic and wild type (cultivar Williams82) soybean events treated with 25 g ai/ha saflufenacil + 1% MSO. Numbers indicate number of events that fell within a particular injury score. Injury scores are from 0-9, 0 being no injury and 9 being death.

[0022] Figure 6: Herbicide injury of cuttings from T0 soybean events treated as indicated. Application rate of saflufenacil is indicated.

[0023] Figure 7: Saflufenacil tolerance spray test of T0 soybean cuttings from RTP3173 (ZmNSF1) transformants. Saflufenacil spray rates are indicated below the plants as g ai/ha.

DETAILED DESCRIPTION OF THE INVENTION

[0024] Definitions

[0025] As used herein, "tolerant" or "herbicide-tolerant" indicates a plant or plant part thereof capable of growing in the presence of an amount of herbicide that normally causes growth inhibition in a non-tolerant (*e.g.*, a wild-type) plant or portion thereof. Levels of herbicide that normally inhibit growth of a non-tolerant plant are known and readily determined by those skilled in the art. Examples include the amounts recommended by manufacturers for application. The maximum rate is an example of an amount of herbicide that would normally inhibit growth of a non-tolerant plant.

For the present invention, the terms "herbicide-tolerant" and "herbicide-resistant" are used interchangeably and are intended to have an equivalent meaning and an equivalent scope. Similarly, the terms "herbicide-tolerance" and "herbicide-resistance" are used interchangeably and are intended to have an equivalent meaning and an equivalent scope. Similarly, the terms "tolerant" and "resistant" are used interchangeably and are intended to have an equivalent meaning and an equivalent scope.

[0026] As used herein, in regard to an herbicidal composition useful in various embodiments hereof, terms such as PPO inhibitor (*e.g.,* saflufenacil), acetohydroxyacid synthase (AHAS) inhibitor, acetyl-Coenzyme A carboxylase (ACCase) inhibitor, 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS) inhibitor, imidazolinone, sulfonylurea, and the like, refer to those agronomically acceptable herbicide active ingredients (A.I.) recognized in the art. Similarly, terms such as fungicide, nematicide, pesticide, and the like, refer to other agronomically acceptable active ingredients recognized in the art.

[0027] When used in reference to a particular mutant enzyme or polypeptide, terms such as herbicide-tolerant and herbicide-tolerance refer to the ability of such enzyme or polypeptide to perform its physiological activity in the presence of an amount of an herbicide A.I. that would normally inactivate or inhibit the activity of the wild-type (non-mutant) version of said enzyme or polypeptide. For example, when used specifically in regard to an

AHAS enzyme, or AHASL polypeptide, it refers specifically to the ability to tolerate an AHAS-inhibitor. Classes of AHAS-inhibitors include sulfonylureas, imidazolinones, triazolopyrimidines, sulfonylaminocarbonyltriazolinones, and pyrimidinyloxy[thio]benzoates. In some embodiments in which an imidazolinone herbicide is to be used, the AHASL is preferably one that comprises at least one herbicide tolerance mutation located at amino acid residue position 122, 205, 574, or 653 (*Arabidopsis thaliana* AHASL numbering); and in some embodiments in which a sulfonylurea herbicide is to be used, the AHASL is preferably one that comprises at least one herbicide tolerance mutation located at amino acid residue position 197 or 574 (*Arabidopsis thaliana* AHASL numbering).

[0028] As used herein, "recombinant," when referring to nucleic acid or polypeptide, indicates that such material has been altered as a result of human application of a recombinant technique, such as by polynucleotide restriction and ligation, by polynucleotide overlap-extension, or by genomic insertion or transformation. A gene sequence open reading frame is recombinant if that nucleotide sequence has been removed from it natural text and cloned into any type of artificial nucleic acid vector. The term recombinant also can refer to an organism having a recombinant material, *e.g.,* a plant that comprises a recombinant nucleic acid can be considered a recombinant plant.

[0029] The term "transgenic plant" refers to a plant that comprises a heterologous polynucleotide. Preferably, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to refer to any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been so altered by the presence of heterologous nucleic acid including those transgenic organisms or cells initially so altered, as well as those created by crosses or asexual propagation from the initial transgenic organism or cell. In some embodiments, a "recombinant" organism is a "transgenic" organism. The term "transgenic" as used herein is not intended to encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods (*e.g.*, crosses) or by naturally occurring events such as, *e.g.*, self-fertilization, random cross-

fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

[0030] As used herein, "mutagenized" refers to an organism or DNA thereof having alteration(s) in the biomolecular sequence of its native genetic material as compared to the sequence of the genetic material of a corresponding wild-type organism or DNA, wherein the alteration(s) in genetic material were induced and/or selected by human action. Examples of human action that can be used to produce a mutagenized organism or DNA include, but are not limited to, as illustrated in regard to herbicide tolerance: tissue culture of plant cells (*e.g.*, calli) and selection thereof with herbicides (*e.g.*, saflufenacil), treatment of plant cells with a chemical mutagen and subsequent selection with herbicide(s); or by treatment of plant cells with x-rays and subsequent selection with herbicide(s). Any method known in the art can be used to induce mutations. Methods of inducing mutations can induce mutations in random positions in the genetic material or can induce mutations in specific locations in the genetic material (*i.e.*, can be directed mutagenesis techniques), such as by use of a genoplasty technique.

[0031] As used herein, a "genetically modified organism" (GMO) is an organism whose genetic characteristics contain alteration(s) that were produced by human effort causing transfection that results in transformation of a target organism with genetic material from another or "source" organism, or with synthetic or modified-native genetic material, or an organism that is a descendant thereof that retains the inserted genetic material. The source organism can be of a different type of organism (*e.g.*, a GMO plant can contain bacterial genetic material) or from the same type of organism (*e.g.*, a GMO plant can contain genetic material from another plant). As used herein in regard to plants and other organisms, "recombinant," "transgenic," and "GMO" are considered synonyms and indicate the presence of genetic material from a different source; in contrast, "mutagenized" is used to refer to a plant or other organism, or the DNA thereof, in which no such transgenic material is present, but in which the native genetic material has become mutated so as to differ from a corresponding wild-type organism or DNA.

[0032] As used herein, "wild-type" or "corresponding wild-type plant" means the typical form of an organism or its genetic material, as it normally occurs, as distinguished from, *e.g.*, mutagenized and/or recombinant forms.

[0033] As used herein, "descendant" refers to any generation plant. In some embodiments, a descendant is a first, second, third, fourth, fifth, sixth, seventh, eight, ninth, or tenth generation plant.

[0034] As used herein, "progeny" refers to a first generation plant.

[0035] The term "seed" comprises seeds of all types, such as, for example, true seeds, caryopses, achenes, fruits, tubers, seedlings and similar forms. In the context of *Brassica* and *Sinapis* species, "seed" refers to true seed(s) unless otherwise specified. For example, the seed can be seed of transgenic plants or plants obtained by traditional breeding methods. Examples of traditional breeding methods can include cross-breeding, selfing, back-crossing, embryo rescue, in-crossing, out-crossing, inbreeding, selection, asexual propagation, and other traditional techniques as are known in the art.

[0036] Although exemplified with reference to specific plants or plant varieties and their hybrids, in various embodiments, the presently described methods using saflufenacil can be employed with a variety of commercially valuable plants. Saflufenacil-tolerant plant lines described as useful herein can be employed in weed control methods either directly or indirectly, *i.e.* either as crops for herbicide treatment or as saflufenacil-tolerance trait donor lines for development, as by traditional plant breeding, to produce other varietal and/or hybrid crops containing such trait or traits. All such resulting variety or hybrids crops, containing the ancestral saflufenacil-tolerance trait or traits can be referred to herein as progeny or descendant of the ancestral, saflufenacil-tolerant line(s). Such resulting plants can be said to retain the "herbicide tolerance characteristic(s)" of the ancestral plant, *i.e.* meaning that they possess and express the ancestral genetic molecular components responsible for the trait.

[0037] In one aspect, the present invention provides a plant or plant part comprising a recombinant polynucleotide encoding a cytochrome P450 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil.

[0038] In some embodiments, the cytochrome P450 polypeptide is a CYP81A isoform or a CY73A isoform polypeptide. In other embodiments, the cytochrome P450 polypeptide is CYP81A6, NSF1, CYP73A38, or CYP72A15. In one embodiment, the CYP81A isoform is CYP81A6 or NSF1.

[0039] In other embodiments, the present invention provides a plant or plant part comprising a recombinant polynucleotide encoding a cytochrome P450 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil, with the provisio that the P450 polypeptide is a cytochrome P450 other than NSF1. In another embodiment, the NSF1 is other than a maize NSF1.

[0040] A CYP81A6 amino acid sequence is disclosed by, *e.g.,* GenBank Accession No. AAK63920.1, which is herein incorporated by reference in its entirety.

[0041] In one embodiment, the plant or plant part of the present invention comprises a recombinant polynucleotide encoding a CYP81A6 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil. In another embodiment, the CYP81A6 polypeptide is a rice polypeptide. In other embodiments, the CYP81A6 polypeptide has the amino acid sequence set forth in SEQ ID NO:1 (Table 1).

[0042] Table 1. SEQ ID NO:1.

OsjCYP81A6

```
  1 maflgwavdi ardsgasssv vltcdgygsa lyfspwdsvp lpataspddg fllprfpdvc
 61 vqrsqftnhl apangtgggg srtgvkeeas evlswpptsk qsvrrlevae hwyrlyktdn
121 qrlspdsqqv svlaeshcdl asgnwkeisi hhkkmpsstt tktttpsrda wivsarsdpf
181 hllleaqapl gikadalsqi aavhqshrnt shirelslam dnayiiails vailfllhyy
241 llgrgnggaa rlppgppavp ilghlhlvkk pmhatmsrla erygpvfslr lgsrravvvs
301 spgcarecft ehdvtfanrp rfesqllvsf ngaalatasy gahwrnlrri vavqllsahr
361 vglmsgliag evramvrrmy raaaaspaga ariqlkrrlf evslsvlmet iahtkatrpe
421 tdpdtdmsve aqefkqvvde iiphigaanl wdylpalrwf dvfgvrrkil aavsrrdafl
481 rrlidaerrr lddgdegekk smiavlltlq ktepevytdn mitaltanlf gagtettstt
541 sewamsllln hpdtlkkaqa eidasvgnsr litaddvtrl gylqcivret lrlypaapml
601 lphessadck vggyniprgs mllinayaih rdpavweepe kfmperfedg gcdgnllmpf
661 gmgrrrcpge tlalrtvglv lgtliqcfdw ervdgvevdm tegggltipk vvpleamcrp
721 rdamggvlre lv
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[0043] A nucleic acid sequence encoding a CYP81A6 polypeptide is shown in Table 2.

[0044] Table 2. SEQ ID NO:2.

OsjCYP81A6

atggcgttcttgggatgggcggtcgacatcgcccgcgactccggcgcgtcgagctccgtcgtgctcac
ctgcgacggctacggctcggcgctctacttctcgccgtgggacagtgtcccgcttccggcgacagctt
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cgcggctgccgccgggtccaccggccgtcccgatcctgggacacctccacctcgtcaagaagccgatg
cacgccaccatgtcccgcctcgccgagcggtacgggccggtgttctcgctgcgcctcgggtcgcggcg
tgccgtggtggtgtcgtcgccggggtgcgccagggagtgcttcaccgagcacgacgtgaccttcgcga
accggcccaggttcgagtcgcagctgctggtctcgttcaacggcgccgcgctcgccacggcgagctac
ggcgcgcactggcgcaacctccgccggatcgtcgccgtgcagctgctctccgcgcaccgcgtcggcct
catgtcggggctcatcgccggcgaggtccgcgccatggtgcggaggatgtaccgcgccgcggccgcgt
ccccgccggcgccgcgcgcatccagctgaagcggaggctgttcgaggtctccctcagcgtgctcatg
gagaccatcgcccacaccaaggcgacccgccccgagacggacccggacaccgacatgtccgtggaagc
ccaggagtttaagcaggtcgtcgacgagatcatcccgcacatcggcgcggccaacctgtgggactact
tgccggcgctccggtggttcgacgtgttcggcgtcaggaggaagatcctcgccgctgtaagccggagg
gacgcgttccttcgccgcctgatcgacgcggagcggcggaggctggacgacggcgacgagggcgagaa
gaagagcatgatcgccgtgctgctcactctgcagaagacagagccggaggtgtacaccgataacatga
tcacagctctaacggcgaacttgttcggagcaggaacagagacaacctcgacgacatcagaatgggcg
atgtcgctactgctgaaccaccccgacacactcaagaaagcgcaagccgagatcgacgcatccgtcgg
caactctcgcctgatcaccgccgacgacgtgactcgcctcggctacctccagtgcatcgtcagggaga
cgctccgcctgtaccccgccgcgccgatgctcctcccgcacgagtcctccgccgactgcaaggtcggc
ggctacaacatcccgcgcgggtcgatgttgctcatcaacgcgtacgccatccaccgtgacccggcggt
gtgggaggagccggagaagttcatgccggagaggttcgaggacggcgggtgcgacggcaatctcttga
tgccgttcgggatggggaggcggaggtgccccggcgagacgctggcgctgcgcacagtggggttggtg
ctgggcacgctgatccagtgcttcgactgggagagggtcgacggcgtggaggtcgacatgactgaagg
tggcgggctcaccatccccaaggtcgtgccgttggaggccatgtgcaggccgcgcgacgccatgggtg
gtgttcttcgcgagctcgtctga

[0045] In another embodiment, the recombinant polynucleotide encoding the CYP81A6 polypeptide has the nucleic acid sequence set forth in SEQ ID NO:2.

[0046] An NSF1 amino acid sequence is disclosed by, *e.g.,* GenBank Accession No. ACG28028.1, which is herein incorporated by reference in its entirety.

[0047] In one embodiment, the plant or plant part of the present invention comprises a recombinant polynucleotide encoding an NSF1 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil. In another embodiment, the NSF1

polypeptide is a corn polypeptide. In other embodiments, the NSF1 polypeptide has the amino acid sequence set forth in SEQ ID NO:3 (Table 3).

[0048] Table 3. SEQ ID NO:3.

```
NSF1

  1 mdkayiaals aaalfllhyl lgrraggegk akakgsrrrl ppsppaipfl ghlhlvkapf
 61 hgalarlaar hgpvfsmrlg trravvvssp dcarecfteh dvnfanrplf psmrlasfdg
121 amlsvssygp ywrnlrrvaa vqllsahrvg cmapaieaqv ramvrrmdra aaaggggvar
181 vqlkrrlfel slsvlmetia htktsraead adsdmsteah efkqivdelv pyigtanrwd
241 ylpvlrwfdv fgvrnkilda vgrrdaflgr lidgerrrld agdesesksm iavlltlqks
301 epevytdtvi talcanlfga gtettsttte wamslllnhr ealkkaqaei daavgtsrlv
361 taddvphlty lqcivdetlr lhpaaplllp hesaadctvg gydvprgtml lvnvhavhrd
421 pavwedpdrf vperfegagg kaegrllmpf gmgrrkcpge tlalrtvglv latllqcfdw
481 dtvdgaqvdm kasggltmpr avpleamcrp rtamrgvlkr l
```

[0049] A nucleic acid sequence encoding an NSF1 polypeptide is shown in Table 4.

[0050] Table 4. SEQ ID NO:4.

```
NSF1

atggataaggcctacatcgccgccctctccgccgccgccctcttcttgctccactacctcctgggccg
gcgggccggcggcgagggcaaggccaaggccaagggctcgcggcggcggctcccgccgagccctccgg
cgatcccgttcctgggccacctccacctcgtcaaggccccgttccacggggcgctggcccgcctcgct
gcgcgccacggcccggtgttctccatgcgcctggggacccggagagccgtggtcgtgtcgtcgccgga
ctgcgccagggagtgcttcacggagcacgacgtgaacttcgcgaaccggccgctgttcccgtcgatgc
ggctggcgtccttcgacggcgccatgctctccgtgtccagctacggcccgtactggcgcaacctgcgc
cgcgtcgccgccgtgcagctcctctccgcgcaccgcgtcgggtgcatggccccgccatcgaagcgca
ggtgcgcgccatggtgcggaggatggaccgcgccgccgcggccggcggcggcggcgtcgcgcgcgtcc
agctcaagcggcggctgttcgagctctccctcagcgtgctcatggagaccatcgcgcacaccaagacg
tcccgcgccgaggccgacgccgactcggacatgtcgaccgaggcccacgagttcaagcagatcgtcga
cgagctcgtgccgtacatcggcacggccaaccgctgggactacctgccggtgctgcgctggttcgacg
tgttcggcgtgaggaacaagatcctcgacgccgtgggcagaagggacgcgttcctggggcggctcatc
gacggggagcggcggaggctggacgctggcgacgagagcgaaagtaagagcatgattgcggtgctgct
cactctgcagaagtccgagccagaggtctacactgacactgtgatcactgctctttgcgcgaacctat
tcggcgccggaacggagaccacgtccaccacgacggaatgggccatgtcactgctgctgaaccaccgg
gaggcgctcaagaaggcgcaggccgagatcgacgcggcggtgggcacctcccgcctggtgaccgcgga
cgacgtgccccacctcacctacctgcagtgcatcgtcgacgagacgctgcgcctgcacccggccgcgc
cgctgctgctgccgcacgagtccgccgcggactgcacggtcggcggctacgacgtgccgcgcggcacg
atgctgctggtcaacgtgcacgcggtccacagggaccccgcggtgtgggaggacccggacaggttcgt
gccggagcggttcgagggcgccggcggcaaggccgaggggcgcctgctgatgccgttcgggatggggc
ggcgcaagtgccccggggagacgctcgcgctgcggaccgtcgggctggtgctcgccacgctgctccag
tgcttcgactgggacacggttgatggagctcaggttgacatgaaggctagcggcgggctgaccatgcc
ccgggccgtcccgttggaggccatgtgcaggccgcgtacagctatgcgtggtgttcttaagaggctct
ga
```

[0051] In one embodiment, the recombinant polynucleotide encoding the NSF1 polypeptide has the nucleic acid sequence set forth in SEQ ID NO:4.

[0052] A CYP73A38 amino acid sequence is disclosed by, *e.g.*, GenBank Accession No. NP_001055190.1, which is herein incorporated by reference in its entirety.

[0053] In one embodiment, the plant or plant part of the present invention comprises a recombinant polynucleotide encoding a CYP73A38 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil. In another embodiment, the CYP73A38 polypeptide is a rice polypeptide. In other embodiments, the CYP73A38 polypeptide has the amino acid sequence set forth in SEQ ID NO:5 (Table 5).

[0054] Table 5. SEQ ID NO:5.

OsjCYP73A38

```
  1 mdallvekvl lglfvaavla lvvakltgkr lrlppgpaga pivgnwlqvg ddlnhrnlma
 61 larrfgdill lrmgvrnlvv vsspdlakev lhtqgvefgs rtrnvvfdif tgkgqdmvft
121 vygdhwrkmr rimtvpfftn kvvaqnragw eeearlvved vrrdpaaats gvvirrrlql
181 mmyndmfrim fdrrfdsvdd plfnklkafn aersrlsqsf eynygdfipv lrpflrryla
241 rchqlksqrm klfedhfvqe rkrvmeqtge ircamdhile aerkgeinhd nvlyivenin
301 vaaiettlws iewgiaelvn hpsiqskvre emasvlggaa vtepdlerlp ylqavvketl
361 rlrmaipllv phmnladgkl agydipaesk ilvnawflan dpkrwvrpde frperfleee
421 kaveahgndf rfvpfgvgrr scpgiilalp iigitlgrlv qsfdllpppg mdkvdttekp
481 gqfsnqilkh atvvckpida
```

[0055] A nucleic acid sequence encoding a CYP73A38 polypeptide is shown in Table 6.

[0056] Table 6. SEQ ID NO:6.

OsjCYP73A38

```
atggacgccctcctcgtggagaaggtcctcctgggcctgttcgtggcggcggtgctggccctagtggt
ggccaagctcaccgggaagaggctccgcctcccgcccggccccgccggcgctcccatcgtcggcaact
ggctccaggtcggcgacgacctcaaccaccgcaacctgatggcgctggcgcggcggttcggcgacatc
ctcctcctccgcatgggcgtccgcaacctggtggtggtgtccagcccggacctcgccaaggaggtgct
ccacacccagggcgtcgagttcggctcccgcacccgcaacgtggtgttcgacatcttcaccgggaagg
ggcaggacatggtgttcaccgtgtacggcgaccactggcgcaagatgcggcggatcatgacggtgccc
ttcttcaccaacaaggtggtggcccagaaccgcgcgggttgggaggaggaggcgaggctggtggtgga
ggacgtccgccgcgaccccgccgcggcgacctccggcgtggtgatccggcgaaggttgcagctgatga
tgtacaacgacatgttccgcatcatgttcgaccgccgtttcgacagcgtggacgacccgctcttcaac
aagctcaaggccttcaacgcggagcgcagccgcctctcgcagagcttcgagtacaactacggtgactt
catccccgtcctccgcccccttcctccgccgctacctcgcacgctgccaccagctcaagtcccagcgca
tgaagctcttcgaggaccacttcgtccaggaacgcaagagagtgatggaacagactggtgagatccgg
tgcgccatggaccacatcctcgaggccgagaggaagggcgagatcaaccacgacaacgtcctctacat
cgtcgagaacatcaacgttgctgctatcgagacgacgctgtggtcgatcgaatggggaatcgcggagc
tggtgaaccacccgagcatccagtcgaaggtgcgggaggagatggcgtcggtgctgggcggcgcggcg
gtgacggagccggacctggagcggctgccgtaccttcaggcggtggtgaaggagacgctgcggttgcg
catggcgatcccgctgctggtgccgcacatgaacctcgccgacggcaagctcgccggctacgacatcc
ccgccgagtccaagatcctggtgaacgcgtggttcctcgccaacgaccccaagcggtgggtgcgcccc
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gacgagtttaggccggagaggttcctggaggaggagaaggccgtggaggcgcacggcaacgacttccg
cttcgtgcccttcggcgtcggccgccgcagctgccccgggatcatcctcgcgctgcccatcatcggga
tcacgctcggccgcctcgtccagagcttcgacctgctgccgccgcccgggatggacaaggtggacacc
accgagaagcccggccagttcagcaaccagatcctcaagcacgccaccgtcgtctgcaagcccatcga
cgcctag
```

[0057] In another embodiment, the recombinant polynucleotide encoding the CYP73A38 polypeptide has the nucleic acid sequence set forth in SEQ ID NO:7.

[0058] A CYP72A15 amino acid sequence is disclosed by, *e.g.*, GenBank Accession No. NP_001043632.1, which is herein incorporated by reference in its entirety. In one embodiment, the plant or plant part of the present invention comprises a recombinant polynucleotide encoding a CYP72A15 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil. In another embodiment, the CYP72A15 polypeptide is a rice polypeptide. In other embodiments, the CYP72A15 polypeptide has the amino acid sequence set forth in SEQ ID NO:7 (Table 7).

[0059] Table 7. SEQ ID NO:7.

```
OsCYP72A15

  1 mlmmlgaasq wilaaaaaaa vaallwlavs tlewawwtpr rleralraqg irgnryrlft
 61 gdvpenvrln rearkkplpl gchdiiprvl pmfskaveeh gkpsftwfgp tprvmisdpe
121 sirevmsnkf ghygkpkptr lgkllasgvv syegekwakh rrilnpafhh ekikrmlpvf
181 sncctemvtr wensmsiegm sevdvwpefq nltgdviskt afgssyeegr rifqlqaesa
241 eriiqafrti fipgywflpt knnrrlreie revskllrgi igkreraikn getsngdllg
301 llvesnmres ngkaelgmtt deiieecklf yfagmettsv lltwtlivls mhpewqerar
361 eevlhhfgrt tpdydslsrl kivtmilyev lrlyppvvfl trrtykemel ggikypaevt
421 lmlpilfihh dpdiwgkdag efnpgrfadg isnatkyqts ffpfgwgpri cigqnfalle
481 akmaictilq rfsfelspsy ihapftvitl hpqhgaqikl kki
```

[0060] A nucleic acid sequence encoding a CYP72A15 polypeptide is shown in Table 8.

[0061] Table 8. SEQ ID NO:8.

```
OsCYP72A15

atgctgatgatgctaggggcggcctcccagtggatcctggccgccgccgcggcggcggccgtggcggc
gctgctgtggctggccgtgtcgacgctggagtgggcctggtggacgccgcggcggctggagcgcgccc
tccgggcgcagggcatccggggcaaccggtaccgcctcttcaccggcgacgtgccggagaacgtccgg
ctcaaccgggaggcccggaagaagccgctgccgctcggctgccacgacatcatcccgcgcgtgctgcc
gatgttcagcaaagccgttgaggagcacgggaaaccatcattcacttggtttggcccaacgccaagag
tgatgatttcagaccctgaatcaataagggaagttatgtctaataagtttggccactatggcaaacca
aagcctacccgtctcggaaagttgctagcctccggagttgtaagctatgaaggcgagaaatgggcaaa
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gcaccggagaattctgaatcctgcctttcaccacgagaaaataaagcggatgctgccagtttttctaactgctgcacggaaatggttacaagatgggagaattcaatgtctattgaaggaatgtcagaggtagatgtttggcctgagttccaaaatcttacaggagatgtcatatcaaagacagcattcggtagcagctatgaggaaggaaggagaattttcagctgcaagcagagtcagccgaacgcataatacaagcctttcggacaattttataccaggatattggttcttaccaactaaaaacaacagaaggttgagagaaattgaaagagaggtcagcaaacttctacgaggaataattggaaagagagagcgggctattaaaaatggtgaaaccagtaatggtgacttgttgggcttattggtggagtcaaatatgagggagtcaaatgggaaagcagaactaggaatgactacggacgaaattattgaggaatgcaagctattttattttgcaggaatggagacaacatcagtattgctcacttggacattaattgtgctaagtatgcacccagagtggcaagagcgagcaagagaagaagtgctacaccactttggaagaaccacaccagactatgatagcttaagtcgtctgaagattgtaacaatgattctgtacgaggttcttaggttgtatccgccagtggtgttcttgaccagacgaacatacaaggaaatggagctcggcggcatcaaatatcccgctgaagtgacccttatgttgcccatttttatttattcaccatgatcccgatatttggggaaaagatgcaggtgaattcaatccagggaggtttgctgatggcatctccaacgcaacgaagtatcagacctctttctttccatttggatggggtccccgaatctgcatcggccagaactttgcactattggaagccaagatggctatctgtacaatccttcagcggttctcctttgagctttcaccatcgtacatccacgcaccattcactgtgataactctccacccacagcatggtgcacaaattaagctgaagaaaatctaa

[0062] In another embodiment, the recombinant polynucleotide encoding the CYP72A15 polypeptide has the nucleic acid sequence set forth in SEQ ID NO:8.

[0063] Additional examples, without limitation, of cytochrome P450 amino acid and nucleic acid sequences are shown in Table 9 and 10, respectively.

[0064] Table 9. Examples of CYP450 polypeptide amino acid sequences.

TaCYP709C1 (SEQ ID NO:9) MGLVWMVAAAVAAVLASWAFDALVYLVWRPRAITRQLRAQGVGGPGYRFFAGNLAEIKQLRADSAGAALD IGDHDFVPRVQPHFRKWIPIHGRTFLYWFGAKPTLCIADVNVVKQVLSDRGGLYPKSIGNPHIARLLGKG LVLTDGDDWKRHRKVVHPAFNMDKLKMMTVTMSDCAGSMMSEWKAKMDKGGSVEIDLSSQFEELTADVIS HTAFGSSYEQGKKVFLAQRELQFLAFSTVFNVQIPSFRYLPTEKNLKIWKLDKEVRTMLMNIIKGRLATK DTMGYGNDLLGLMLEACAPEDGQNPLLSMDEIIDECKTFFFAGHDTSSHLLTWTMFLLSTHPEWQEKLRE EVLRECGNGIPTGDMLNKLQLVNMFLLETLRLYAPVSAIQRKAGSDLEVGGIKVTEGTFLTIPIATIHRD KEVWGEDANKFKPMRFENGVTRAGKHPNALLSFSSGPRSCIGQNFAMIEAKAVIAVILQRFSFSLSPKYV HAPMDVITLRPKFGLPMILKSLEM
Os07g0635500 (SEQ ID NO:10) MGNLGWMVAAAVAAVVASWAFDAVVKLVWRPRAITRRLRAQGVGGPGYRFFSGNLGEIRRLRDEGAGVVL DVSSHDFVPIVQPHFRKWIPLYGKTFMYWFGARPTICLADVSMVRQVLSDRTGMYPKNVSNPYFARLLGK GLVLTDGDEWKRHRKVVHPAFNMDKLKMMTVTMSDCAQSMISEWESELGTKGDIVEIELSRRFEELTADV ISHTAFGSSYKEGKQVFLAQRELQFLAFSTFLSIQIPGSSYLPTKKNLKTWSVDKKVRSMLTDIIKSRLN NKDVAGYGNDLLGLMLEACAPEHGESQPQLSMDEIIAECKTFFFAGHDTTSHLLTWTMFLLSTHPEWQEK LREEVATECDGKVPTGDMLNKLKLVNMFLLETLRLYGPVAFIQRRVNAELELGGITVPEGTVLSIPIATI HRDKEVWGEDADIFKPERFKNGVSKAGKYPNALLSFSSGPRACIGQNFAMIEAKAVIAMILQRFSFTLSP KYVHVPTDVITLRPKYGLPMILKSLKV
Os02g0467600 (SEQ ID NO:11)

MAASAMRVAIATGASLAVHLFVKSFVQAQHPALTLLLPVAVFVGIAVGAKGGSGGDGKAPPGPAAVPVFG NWLQVGNDLNHRFLAAMSARYGPVFRLRLGVRNLVVVSDPKLATEVLHTQGVEFGSRPRNVVFDIFTANG ADMVFTEYGDHWRRMRRVMTLPFFTARVVQQYKAMWEAEMDAVVDDVRGDAVAQGTGFVVRRRLQLMLYN IMYRMMFDARFESVDDPMFIEATRFNSERSRLAQSFEYNYGDFIPILRPFLRGYLNKCRDLQSRRLAFFN NNYVEKRRKVMDTPGDRNKLRCAIDHILEAEKNGELTAENVIYIVENINVAAIETTLWSIEWALAEVVNH PAVQSKVRAEINDVLGDDEPITESSIHKLTYLQAVIKETLRLHSPIPLLVPHMNLEEAKLGGYTIPKGSK VVVNAWWLANNPALWENPEEFRPERFLEKESGVDATVAGKVDFRFLPFGVGRRSCPGIILALPILALIVG KLVRSFEMVPPPGVEKLDVSEKGGQFSLHIAKHSVVAFHPISA
Os06g0569500 (SEQ ID NO:12) MESMLVAGAGAAAVAAVGGLVAAAALADKLVAAPPPRKNRANPPPAVPGLPIIGNLHQLKEKKPHQTFAK WSETYGPIYTIKTGASPVVVLNSTEVAKEAMIDKFSSISTRKLPKAMSVLTRKSMVAISDYGDYQKMAKR NIMIGMLGFNAQKQFRGTRERMISNVLSTLHKLVSLDPHSPLNFRDVYINELFSLSLIQSLGEDVSSVYV EEFGREISKDEIFDVLVHEMMMCAVEADWRDYFPYLSWLPNKSFDTIVSTTEFRRDAIMNALIKKQKERI ARGEARASYIDFLLEAERSAQLTDDQLMLLLSESILAAADTVLVTTEWTMYEIAKNPDKQELLYQEIREA CGGEAVTEDDLPRLPYLNAVFHETLRLHSPVPVLPPRFVHDDTTLAGYDIAAGTQMMINVYACHMDEKVW ESPGEWSPERFLGEGFEVADRYKTMAFGAGRRTCAGSLQAMNIACVAVARLVQELEWRLREGDGDKEDTM QFTALKLDPLHVHLKPRGRM
Os08g0508000 (SEQ ID NO:13) MERDAWLLCAALAAATVVYYLACTTSRRAQRRRLPPGPTPLPVIGNVLSLRGNMHHALARLARERYGPVM ALKLGLVTAVVVSSPDAAREAFTKHDRRLAARAVPDTSRVRGFADRSMIWLPSSDTRWKTLRGVVATHVF SPRSIAAARGVRERKVRDIVGYFAAHVGEVVDVGEAVYSGVVNLVSNAFFSGDVVDVGEESAHGLREAVE DIILAIAKPNVSDLFPFLRPLDLQGWRRWAEKRYDTVFDILDNITNSRLADASAGNHAGDFLDSLLGLMS YGKIARDDVTTIMFDVFGAGTDTIAITVQWAMAELLRNPSIMAKARTEMEDVLAGKKTIEENDTEKLPYL RAVIKEAMRLHPVAPILLPHQAAEDGVEIGGYAVPKGSTVIFNVWAIMRDPTAWERPDEFMPERFLQRAE VDFRGKDFEFMPFGAGRRLCPGLPMAERVVPFILASLLHAFEWRLPDGMSAEELDVSEKFTTANVLTVPL KAVPILASSASELQAS
Os12g0150200 (SEQ ID NO:14) MEVELPWGARCAAAAFFVSSLCVAALGVVLLLLRRWPWCGCHVCRAYLAGSWRREFANLGDWYADLLRRS PTGTVHVHVLGCTVTANPANVEYMLKTRFDNFPKGRPFAALLGDLLGDGIFNVDGDAWRHQRKMASLELG SVAVRSYAYKIVAQEVEARLMPVLANAADSGAVVDLQDVFRRFAFDTICKISFGLDPGCLDREMPVSELA DAFDAASRLSAMRGAAASPLLWKMKRFLNVGSERELKKAIKLIDGLAAAMIRERRKLGVANSHDLLSRFM ASSGDDARGAADDKFLRDIVVSFLLAGRDTVSSALTTLFMILSKNPDVAAAMRAEAGAAAGESAAVSYEH LKRLNYTHAVLYENMRLFPPVQFDSKFCAAADVLPDGTYVDGGARVMYHPYAMGRMPRIWGADCDAFRPE RWLTGAGGAFVPESLFKYPVFQAGLRVCLGKELAITEMKAVSVAVVRAFDVEVVGENGRCGGGAAAAPRF VPGLTASISGGLPVKIRRV
ZM1S60596158 (SEQ ID NO:15) MERFYYVAAATFVLVFLLHHLLTRKKQQRLPPGPRFAYPILGHLPLVKKPLQTSFADLVSRHGPIIHLRL GRRHAVVVGSAAVAKECFSGELDVAIANRPHFPSAREVTFDYSVLTAVNYGALWRTMRRVSTVHLLSAHR VNVMSDTVIARELRVMVRRLARASASAPGDAARVELKRRLFDLSHSVLMETMAQTKNTYSDDPEEDMSRE AREMKDIIEEIIPLVGAANLWNYVPLLRWLDLYGAKRKLADVVNRRDLIFDNMIGAERQKLRQLERKKGE AHASESDKMGMIGVMLSLQKTEPDVYTDTFINALVSNLLAAGTETTSTTLEWAMSLLLNHPDVLKRAQEE IESNVGRDRLLDKNDLPRLPYLHCIISETLRLYPPTPMLLPHEASTDCKIHGYDVPAGSMVLVNAYAIHR DPAMWEDPEEFRPERFELGRAEGKFMMPFGMGRRRCPGENLAMRTMGLVLGALLQCFDWTRVGDREVDMA TATGTIMSKAVPLEAQCKPRANMSAVLQKI
ZM4s40785 (SEQ ID NO:16) MDKAYVAVLSVAFLFLVHYLVGRAAPGGGKGRKRLPPSPLAIPFLGHLHLVKTPFHSALGRLAERHGPVF

SLRMGCRRAVVVSSPECARACFTEHDMSFANRPRFESMRLVSFDGAMLSVSSYGPYWRTLRRVAAVQLLS
AHRVACMSPVICAEVRAMVRRMARLAAGGAARVQLRRRLFELSLGVLMETIARTKTSRSEACAADTDVSP
EASELTRISEEIMPYLGTANLWDYLPFLRWFDVFGVRNKLMAAVRWRDAFLRRLIDAERRRMDGDGDGEK
KSMIAVLLSLQKSEPELYTDTMIMALCGDLFGAGTETTSVTTEWAMSLLLSHPEALKKAQAEIDAVVGNS
RRLITADDVPRLGYLHCVINETLRMYPAAPLLLPHESAADCKVGGYDVPRGTLLIVNAYAIHRDPAVWED
PGSFLPERFEDGKAEGRLLMPFGMGRRKCPGETLALRTVGLVLATLLQCFDWDTVDGAEVDMTESGGLTM
PRAVPLEAMCKPRAAMCDVLREL

ZM1s57311919 (SEQ ID NO:17)

MDKAYIAALSAAALFLLHYLLGRRAGGEGKAKAKGSRRRLPPSPPAIPFLGHLHLVKAPFHGALARLAAR
HGPVFSMRLGTRRAVVVSSPDCARECFTEHDVNFANRPLFPSMRLASFDGAMLSVSSYGPYWRNLRRVAA
VQLLSAHRVGCMAPAIEAQVRAMVRRMDRAAAAGGGGVARVQLKRRLFELSLSVLMETIAHTKTSRAEAD
ADSDMSTEAHEFKQIVDELVPYIGTANRWDYLPVLRWFDVFGVRNKILDAVGRRDAFLGRLIDGERRRLD
AGDESESKSMIAVLLTLQKSEPEVYTDTVITALCAVSASSSTIRHSLILTKYKKSCPFSQFSRQHSGLYY
PPKYRIR

[0065] Table 10. Examples of nucleic acid sequences encoding CYP450 polypeptides.

TaCYP709C1 (SEQ ID NO:18)

atgggtcttgtctggatggtggcggccgccgtggcggcggtgctggcctcgtgggcgttcgacgcgctgg
tgtacctcgtgtggaggccgcgggccatcacccggcagctccgcgcgcagggcgtcggcggtccgggcta
caggttcttcgccgggaacctcgccgagatcaagcagctccgcgccgacagcgccggcgccgcgctggac
atcggcgaccacgacttcgtccccagggtccagccgcacttccgcaaatggatccccatccacgggcgca
cgttcttgtactggttcggagccaagccgacactgtgcatcgccgacgtgaacgtggtgaagcaggtgct
ctccgaccgcggcgggctgtaccccaagagcatcgggaacccgcacatcgcccgcctgctcggcaagggg
ctcgtgctcaccgacggcgacgactggaagcgccaccgcaaggtcgtccacccggccttcaacatggaca
agctcaagatgatgacggtgaccatgtccgactgtgccgggtcaatgatgtccgagtggaaggcaaagat
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[0066] In other embodiments, the cytochrome P450 polypeptide for use according to the present invention is a functional variant having, over the full-length of the variant, at least about 80%, illustratively, at least about 80%, 90%, 95%, 98%, 99% or more amino acid sequence identity to SEQ ID NO:1, 3, 5, 7, or 29.

[0067] In another embodiment, the cytochrome P450 polypeptide for use according to the present invention is a functional fragment of a polypeptide having the amino acid sequence set forth in SEQ ID NO:1, 3, 5, 7, or 29.

[0068] For polynucleotides, degenerate and codon-optimized sequences that encode the cytochrome P450 polypeptides also are within the scope of the present invention.

[0069] Thus, functional variants and fragments of the cytochrome polypeptides, and nucleic acid molecules encoding them, also are within the scope of the present invention, and

unless specifically described otherwise, irrespective of the origin of the P450 polypeptide and irrespective of whether it occurs naturally.

[0070] Various assays for functionality of a P450 polypeptide can be employed. For example, a functional variant or fragment of the P450 polypeptide can be assayed to determine its ability to confer saflufenacil detoxification. By way of illustration, a saflufenacil detoxification rate can be defined as a catalytic rate sufficient to provide a determinable increase in tolerance to saflufenacil in a plant or plant part comprising a recombinant polynucleotide encoding the variant or fragment of the P450 polypeptide, wherein the plant or plant part expresses the variant or fragment at up to about 0.5%, illustratively, about 0.05 to about 0.5%, about 0.1 to about 0.4%, and about 0.2 to about 0.3%, of the total cellular protein relative to a similarly treated control plant that does not express the variant or fragment.

[0071] In some embodiments, the P450 polypeptide is a functional variant or fragment of a cytochrome having the amino acid sequence set forth in SEQ ID NO:1, 3, 5, 7 or 29, wherein the functional variant or fragment has at least about 80% amino acid sequence identity to SEQ ID NO:1, 3, 5, 7 or 29. In other embodiments, the functional variant or fragment further has a saflufenacil detoxification rate defined as a catalytic rate sufficient to provide a determinable increase in tolerance to saflufenacil in a plant or plant part comprising a recombinant polynucleotide encoding the variant or fragment, wherein the plant or plant part expresses the variant or fragment at up to about 0.5% of the total cellular protein to a similarly treated control plant that does not express the variant or fragment.

[0072] In addition, one of ordinary skill in the art will further appreciate that changes can be introduced by mutation into the nucleotide sequences of the invention thereby leading to changes in the amino acid sequence of the encoded proteins without altering the biological activity of the proteins. Thus, for example, an isolated polynucleotide molecule encoding a P450 polypeptide having an amino acid sequence that differs from that of SEQ ID NO: 1, 3, 5, 7, or 29 can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR- mediated

mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention. For example, preferably, conservative amino acid substitutions may be made at one or more predicted preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif.

[0073] The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. For example, amino acid sequence variants can be prepared by mutations in the DNA. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) PNAS, 82:488-492; Kunkel *et al.* (1987) Methods in Enzymol. 154:367-382; U.S. Patent No. 4,873,192; Walker and Gaastra, eds. (1983) Techniques in Molecular Biology (MacMillan Publishing Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) Atlas of Protein Sequence and Structure (Natl. Biomed. Res. Found., Washington, D. C), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferable.

[0074] Alternatively, variant nucleotide sequences can be made by introducing mutations randomly along all or part of a coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened to identify mutants that encode proteins that retain activity.

For example, following mutagenesis, the encoded protein can be expressed recombinantly, and the activity of the protein can be determined using standard assay techniques.

[0075] In other aspects, the present invention encompasses a progeny or a descendant of a saflufenacil-tolerant plant of the present invention as well as seeds derived from the saflufenacil-tolerant plants of the invention and cells derived from the saflufenacil-tolerant plants of the invention.

[0076] In some embodiments, the present invention provides a progeny or descendent plant derived from a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the progeny or descendant plant comprises in at least some of its cells the recombinant polynucleotide operably linked to the regulatory sequence, the expression of the polypeptide in the progeny or descendant plant conferring to the progeny or descendant plant tolerance to the saflufenacil. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil.

[0077] In one embodiment, seeds of the present invention preferably comprise the saflufenacil-tolerance characteristics of the saflufenacil-tolerant plant. In other embodiments, a seed is capable of germination into a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil.

[0078] In some embodiments, plant cells of the present invention are capable of regenerating a plant or plant part. In other embodiments, plant cells are not capable of regenerating a plant or plant part. Examples of cells not capable of regenerating a plant include, but are not limited to, endosperm, seed coat (testa & pericarp), and root cap.

[0079] In another embodiment, the present invention provides a plant cell of or capable of regenerating a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the plant cell comprises the recombinant polynucleotide operably linked to a promoter. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil.

[0080] In other embodiments, the present invention provides a plant cell comprising a recombinant polynucleotide operably linked to a promoter operable in the cell, the recombinant polynucleotide being effective in the cell to express a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the cell tolerance to saflufenacil. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring to the cell tolerance to saflufenacil.

[0081] In some aspects, the present invention provides a plant product prepared from the saflufenacil-tolerant plants hereof. In some embodiments, examples of plant products include, without limitation, grain, oil, and meal. In one embodiment, a plant product is plant grain (*e.g.*, grain suitable for use as feed or for processing), plant oil (*e.g.*, oil suitable for use as food or biodiesel), or plant meal (*e.g.*, meal suitable for use as feed).

[0082] In one embodiment, a plant product prepared from a plant or plant part is provided, wherein the plant or plant part comprises in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the recombinant polynucleotide being effective in the cells of the plant or plant part to express a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil.

[0083] In some aspects, the present invention provides a method for producing a saflufenacil-tolerant plant. In one embodiment, the method comprises: regenerating a plant

from a plant cell transformed with a recombinant polynucleotide operably linked to a promoter operable in the cell, the recombinant polynucleotide being effective in the cell to express a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to the saflufenacil. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil.

[0084] Where appropriate, nucleic acid sequences may be optimized for increased expression in a transformed plant. For example, coding sequences can be provided that comprise plant-preferred codons for improved expression in a plant. See, for example, Campbell and Gowri (1990) Plant Physiol., 92:1-11 for a discussion of host-preferred codon usage. Methods also are known in the art for preparing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) Nucleic Acids Res. 17:477-498, herein incorporated by reference. Further, additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. Also, if desired, sequences can be readily modified to avoid predicted hairpin secondary mRNA structures. Nucleotide sequences for enhancing gene expression can also be used in the plant expression vectors. These include, for example, the introns of the maize Adhl, intronl gene (Callis et al. Genes and Development 1:1183-1200, 1987), and leader sequences, (W-sequence) from the Tobacco Mosaic virus (TMV), Maize Chlorotic Mottle Virus and Alfalfa Mosaic Virus (Gallie et al. Nucleic Acid Res. 15:8693-8711, 1987 and Skuzeski et al. Plant MoI. Biol. 15:65-79, 1990). The first intron from the shrunken-1 locus of maize, has been shown to increase expression of genes in chimeric gene constructs. U.S. Pat. Nos. 5,424,412 and 5,593,874 disclose the use of specific introns in gene expression constructs, and Gallie et al. (Plant Physiol. 106:929-939, 1994) also have shown that introns are useful for regulating gene expression on a tissue specific basis. To further enhance or to optimize gene expression, the plant expression vectors of the invention also may contain DNA sequences containing matrix attachment regions (MARs). Plant cells transformed with

such modified expression systems, then, may exhibit overexpression or constitutive expression of a nucleotide sequence of the invention.

[0085] Expression vectors may additionally contain 5' leader sequences in the expression construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyo carditis 5' noncoding region) (Elroy-Stein et al. (1989) PNAS, 86:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Gallie et al. (1995) Gene 165(2):233-238), MDMV leader (Maize Dwarf Mosaic Virus) (Virology 154:9-20), and human immunoglobulin heavy-chain binding protein (BiP) (Macejak et al. (1991) Nature 353:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling et al. (1987) Nature 325:622-625); tobacco mosaic virus leader (TMV) (Gallie et al. (1989) in Molecular Biology of RNA, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel et al. (1991) Virology 81:382-385). See also, Della-Cioppa et al. (1987) Plant Physiol. 84:965-968.

[0086] Other methods known to enhance translation also can be utilized, for example, introns, and the like. In preparing an expression vector, the various nucleic acid fragments may be manipulated, so as to provide for the nucleic acid 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 nucleic acid fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous nucleic acid, 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.

[0087] A number of promoters can be used in the practice of the invention. The promoters can be selected based on the desired outcome. The nucleic acids can be combined with constitutive, tissue-preferred, or other promoters for expression in plants.

[0088] Constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Patent No. 6,072,050; the core CaMV 35S promoter (Odell et al. (1985) Nature 313:810-812); rice actin (McElroy et al. (1990) Plant Cell 2:163-171); ubiquitin (Christensen et al. (1989) Plant MoI. Biol. 12:619-632 and Christensen et al. (1992) Plant MoI. Biol. 18:675-689); pEMU (Last et

al. (1991) Theor. Appl. Genet. 81:581- 588); MAS (Velten et al. (1984) EMBO J. 3:2723-2730); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

[0089] Tissue-preferred promoters can be utilized to target enhanced expression within a particular plant tissue. Such tissue-preferred promoters include, but are not limited to, leaf-preferred promoters, root-preferred promoters, seed- preferred promoters, and stem-preferred promoters. Some examples of tissue-preferred promoters are described by, *e.g.,* Yamamoto et al. (1997) Plant J. 12(2):255-265; Kawamata et al. (1997) Plant Cell Physiol. 38(7):792-803; Hansen et al. (1997) MoI. Gen Genet. 254(3):337-343; Russell et al. (1997) Transgenic Res. 6(2): 157-168; Rinehart et al. (1996) Plant Physiol. 112(3): 1331-1341; Van Camp et al. (1996) Plant Physiol. 112(2):525-535; Canevascini et al. (1996) Plant Physiol. 112(2):513-524; Yamamoto et al. (1994) Plant Cell Physiol. 35(5):773-778; Lam (1994) Results Probl. Cell Differ. 20:181- 196; Orozco ef α/. (1993) Plant MoI Biol. 23(6): 1129-1138; Matsuoka et al. (1993) Voc Natl. Acad. ScL USA 90(20):9586-9590; and Guevara-Garcia et al. (1993) Plant J 4(3):495-505. Promoters can be modified, if necessary, for weak expression.

[0090] In some embodiments, the nucleic acids of interest can be targeted to the chloroplast for expression. In this manner, where the nucleic acid of interest is not directly inserted into the chloroplast, the expression vector will additionally contain a chloroplast-targeting sequence comprising a nucleotide sequence that encodes a chloroplast transit peptide to direct the gene product of interest to the chloroplasts. Such transit peptides are known in the art. With respect to chloroplast-targeting sequences, "operably linked" means that the nucleic acid sequence encoding a transit peptide (i.e., the chloroplast-targeting sequence) is linked to the desired coding sequence of the invention such that the two sequences are contiguous and in the same reading frame. See, for example, Von Heijne et al. (1991) Plant MoI. Biol. Rep. 9:104-126; Clark et al. (1989) J Biol. Chem. 264:17544-17550; Della-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys. Res. Commun. 196:1414-1421; and Shah et al. (1986) Science 233:478-481. For example, a chloroplast transit peptide known in the art can be fused to the amino acid sequence of a

P450 polypeptide of the invention by operably linking a choloroplast-targeting sequence to the 5'-end of a nucleotide sequence encoding the P450.

[0091] Chloroplast targeting sequences are known in the art and include the chloroplast small subunit of ribulose-l,5-bisphosphate carboxylase (Rubisco) (de Castro Silva Filho et al. (1996) Plant Mol. Biol. 30:769-780; Schnell et al. (1991) J Biol. Chem. 266(5):3335-3342); EPSPS (Archer et al. (1990) J Bioenerg. Biomemb. 22(6):789-810); tryptophan synthase (Zhao et al. (1995) J Biol. Chem. 270(11):6081-6087); plastocyanin (Lawrence et al. (1997) J Biol. Chem. 272(33):20357-20363); chorismate synthase (Schmidt et al. (1993) J Biol. Chem. 268(36):27447-27457); and the light harvesting chlorophyll a/b binding protein (LHBP) (Lamppa et al. (1988) J Biol. Chem. 263:14996-14999). See also Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9:104- 126; Clark et al. (1989) J Biol. Chem. 264:17544-17550; Della-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys. Res. Commun. 196:1414-1421; and Shah et al. (1986) Science 233:478-481.

[0100] Methods for transformation of chloroplasts are known in the art. See, for example, Svab et al. (1990) Proc. Natl. Acad. ScL USA 87:8526-8530; Svab and Maliga (1993) Proc. Natl. Acad. Sci. USA 90:913-917; Svab and Maliga (1993) EMBO J. 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation can be accomplished by transactivation of a silent plastid-borne transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91:7301-7305.

[0101] The nucleic acids of interest to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be synthesized using chloroplast-preferred codons. See, for example, U.S. Patent No. 5,380,831, herein incorporated by reference.

[0102] Numerous plant transformation vectors and methods for transforming plants are available. See, for example, An, G. et al. (1986) Plant PysioL, 81:301-305; Fry, J., et al. (1987) Plant Cell Rep. 6:321-325; Block, M. (1988) Theor. Appl. Genet .16:161 -11 A;

Hinchee, et al. (1990) Stadler. Genet. Symp.2032\2.203-2\2; Cousins, et al. (1991) Aust. J. Plant Physiol. 18:481-494; Chee, P. P. and Slightom, J. L. (1992) Gene.118:255-260; Christou, et al. (1992) Trends. Biotechnol. 10:239-246; Halluin, et al. (1992) Bio/Technol. 10:309-314; Dhir, et al. (1992) Plant Physiol. 99:81-88; Casas et al. (1993) Proc. Nat. Acad Sd. USA 90:11212-11216; Christou, P. (1993) In Vitro Cell. Dev. Biol.-Plant; 29P:119-124; Davies, et al. (1993) Plant Cell Rep. 12:180-183; Dong, J. A. and Mchughen, A. (1993) Plant ScL 91:139-148; Franklin, C. I. and Trieu, T. N. (1993) Plant. Physiol. 102:167; Golovkin, et al. (1993) Plant ScL 90:41-52; Guo Chin ScL Bull. 38:2072-2078; Asano, et al. (1994) Plant Cell Rep. 13; Ayeres N. M. and Park, W. D. (1994) Crit. Rev. Plant. Sci. 13:219-239; Barcelo, et al. (1994) Plant. J. 5:583-592; Becker, et al. (1994) Plant. J. 5:299-307; Borkowska et al. (1994) Acta. Physiol Plant. 16:225-230; Christou, P. (1994) Agro. Food. Ind. Hi Tech. 5: 17-27; Eapen et al. (1994) Plant Cell Rep. 13:582-586; Hartman, et al. (1994) Bio-Technology 12: 919923; Ritala, et al. (1994) Plant. Mol. Biol. 24:317-325; and Wan, Y. C. and Lemaux, P. G. (1994) Plant Physiol. 104:3748.

[0103] In some embodiments, the methods of the invention involve introducing a polynucleotide construct into a plant. By "introducing" is intended presenting to the plant the polynucleotide construct in such a manner that the construct gains access to the interior of a cell of the plant. The methods of the invention do not depend on a particular method for introducing a polynucleotide construct to a plant, only that the polynucleotide construct gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotide constructs into plants are known in the art including, but not limited to, stable transformation methods, transient transformation methods, and virus-mediated methods.

[0104] By "stable transformation" is intended that the polynucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by descendent thereof. By "transient transformation" is intended that a polynucleotide construct introduced into a plant does not integrate into the genome of the plant.

[0105] For the transformation of plants and plant cells, the nucleotide sequences of the invention are inserted using standard techniques into any vector known in the art that is suitable for expression of the nucleotide sequences in a plant or plant cell. The selection of

the vector depends on the preferred transformation technique and the target plant species to be transformed. In an embodiment of the invention, the encoding nucleotide sequence is operably linked to a plant promoter, e.g. a promoter known in the art for high-level expression in a plant cell, and this construct is then introduced into a plant cell that is susceptible to saflufenacil; and a transformed plant is regenerated. In some embodiments, the transformed plant is tolerant to exposure to a level of saflufenacil that would kill or significantly injure a plant regenerated from an untransformed cell. This method can be applied to any plant species or crops.

[0106] Methodologies for constructing plant expression vectors and introducing foreign nucleic acids into plants are generally known in the art. For example, foreign DNA can be introduced into plants, using tumor-inducing (Ti) plasmid vectors. Other methods utilized for foreign DNA delivery involve the use of PEG mediated protoplast transformation, electroporation, microinjection whiskers, and biolistics or microprojectile bombardment for direct DNA uptake. Such methods are known in the art. (U.S. Pat. No. 5,405,765 to Vasil et ah; Bilang et a (1991) Gene 100: 247-250; Scheid et ah, (1991) MoL Gen. Genet., 228: 104-112; Guerche et ah, (1987) Plant Science 52: 111-116; Neuhause et ah, (1987) Theor. Appl Genet. 75: 30-36; Klein et ah, (1987) Nature 327: 70-73; Howell et ah, (1980) Science 208:1265; Horsch et ah, (1985) Science 227: 1229-1231; DeBlock et ah, (1989) Plant Physiology 91: 694-701; Methods for Plant Molecular Biology (Weissbach and Weissbach, eds.) Academic Press, Inc. (1988) and Methods in Plant Molecular Biology (Schuler and Zielinski, eds.) Academic Press, Inc. (1989).

[0107] Other suitable methods of introducing nucleotide sequences into plant cells include microinjection as described by, e.g., Crossway et ah (1986) Biotechniques 4:320-334, electroporation as described by e.g., Riggs et ah (1986) Proc. Natl. Acad. ScL USA 83:5602-5606, Agrobacterium-mediated transformation as described by e.g., Townsend et. al., U.S. Patent No. 5,563,055, Zhao et. al., U.S. Patent No. 5,981,840, direct gene transfer as described by, *e.g.,* Paszkowski et. al. (1984) EMBO J. 3:2717-2722, and ballistic particle acceleration as described by, e.g., U.S. Patent Nos. 4,945,050; 5,879,918; 5,886,244; and 5,932,782; Tomes et. al. (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in Plant Cell, Tissue, and Organ Culture: Fundamental

Methods, ed. Gamborg and Phillips (Springer- Verlag, Berlin); McCabe et ah (1988) Biotechnology 6:923-926); and Lecl transformation (WO 00/28058). Also see, Weissinger et. al., (1988) Ann. Rev. Genet. 22:421-477; Sanford et. al., (1987) Particulate Science and Technology 5:27-37 (onion); Christou et. al., (1988) Plant Physiol. 87:671-674 (soybean); McCabe et. al., (1988) Bio/Technology 6:923-926 (soybean); Finer and McMullen (1991) In Vitro Cell Dev. Biol. 27P:175-182 (soybean); Singh et. al., (1998) Theor. Appl. Genet. 96:319-324 (soybean); Datta et. al., (1990) Biotechnology 8:736-740 (rice); Klein et. al., (1988) PNAS, 85:4305-4309 (maize); Klein et. al., (1988) Biotechnology 6:559-563 (maize); U.S. Patent Nos. 5,240,855; 5,322,783; and 5,324,646; Tomes et. al., (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg (Springer- Verlag, Berlin) (maize); Klein et. al., (1988) Plant Physiol. 91:440-444 (maize); Fromm et. al., (1990) Biotechnology 8:833-839 (maize); Hooykaas-Van Slogteren et. al., (1984) Nature (London) 311:763-764; Bowen et. al., U.S. Patent No. 5,736,369 (cereals); Bytebier et. al., (1987) PNAS 84:5345-5349 (Liliaceae); De Wet et. al., (1985) in The Experimental Manipulation of Ovule Tissues, ed. Chapman et. al., (Longman, New York), pp. 197-209 (pollen); Kaeppler et. al., (1990) Plant Cell Reports 9:415-418 and Kaeppler et. al., (1992) Theor. Apph Genet. 84:560-566 (whisker-mediated transformation); D'Halluin et. al., (1992) Plant Cell 4:1495-1505 (electroporation); Li et. al., (1993) Plant Cell Reports 12:250- 255 and Christou and Ford (1995) Annals of Botany 75:407-413 (rice); Osjoda et. al., (1996) Nature Biotechnology 14:745-750 (maize via Agrobacterium tumefaciens); each of which is herein incorporated by reference.

[0108] In some embodiments, polynucleotides of the present invention may be introduced into plants by contacting plants with a virus or viral nucleic acids. Generally, such methods involve incorporating a polynucleotide construct of the invention within a viral DNA or RNA molecule. It is recognized that the polypeptides of the invention may be initially synthesized as part of a viral polyprotein, which later may be processed by proteolysis *in vivo* or *in vitro* to produce the desired recombinant poylpeptide. Further, it is recognized that promoters of the invention also encompass promoters utilized for transcription by viral RNA polymerases. Methods for introducing polynucleotide constructs into plants and expressing a protein encoded therein, involving viral DNA or RNA

molecules, are known in the art. See, for example, U.S. Patent Nos. 5,889,191, 5,889,190, 5,866,785, 5,589,367 and 5,316,931; herein incorporated by reference. The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick et a (1986) Plant Cell Reports 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved.

[0109] The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plant species of interest include, but are not limited to, corn or maize (*Zea mays*), Brassica sp. (e.g., *B. napus*, *B. rapa*, *B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), millet (*e.g.*, pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), saffiower (*Carthamus tinctorius*), wheat (*Triticum aestivum*, *T. Turgidum* ssp. *durum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solarium tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense*, *Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables, ornamentals, and conifers. Preferably, plants of the present invention are crop plants (for example, sunflower, *Brassica* sp., cotton, sugar, beet, soybean, peanut, alfalfa, saffiower, tobacco, corn, rice, wheat, rye, barley triticale, sorghum, millet, etc.).

[0110] In other aspects, saflufenacil-tolerant plants of the present invention can be employed as saflufenacil-tolerance trait donor lines for development, as by traditional plant breeding, to produce other varietal and/or hybrid crops containing such trait or traits. All such resulting variety or hybrids crops, containing the ancestral saflufenacil-tolerance trait or traits can be referred to herein as progeny or descendant of the ancestral, saflufenacil-tolerant line(s).

[0111] In other embodiments, the present invention provides a method for producing a saflufenacil-tolerant plant. The method comprises: crossing a first saflufenacil-tolerant plant with a second plant to produce a saflufenacil-tolerant progeny plant, wherein the first plant and the progeny plant comprise in at least some of their cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the recombinant polynucleotide being effective in the cells of the first plant to express a CYP81A or CYP73A polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring tolerance to saflufenacil. In other embodiments, the recombinant polynucleotide encodes a CYP72A15 polypeptide, the expression of the polypeptide conferring tolerance to saflufenacil.

[0112] For example, in the case of *Brassica* A-, B-, and C-genome saflufenacil trait(s), these can be bred into *Brassica* species having a corresponding genome, *e.g.*: *B. napus* (AACC), *B. juncea*.(AABB), *B. oleracea* (CC), *B. rapa* (AA), *B. nigra* (BB), *B. carinata* (BBCC), and *Raphanobrassica* varieties that are progeny of a cross between any of the foregoing and a *Raphanus* spp., *e.g.*, *Raphanobrassica* var. 'rabbage' (RRCC) from *B. oleracea* x *Raphanus sativus* or *Raphanobrassica* var. 'raparadish' (RRAA) from *B. rapa* x *Raphanus sativus*. Among these, *B. napus*, *B. rapa*, and *B. juncea* are of particular interest, with *B. napus* being preferred in some embodiments.

[0113] In some embodiments, traditional plant breeding is employed whereby the saflufenacil-tolerant trait is introduced in the progeny plant resulting therefrom. In one embodiment, the present invention provides a method for producing a saflufenacil-tolerant progeny plant, the method comprising: crossing a parent plant with a saflufenacil-tolerant plant to introduce the saflufenacil-tolerance characteristics of the saflufenacil-tolerant plant into the germplasm of the progeny plant, wherein the progeny plant has increased tolerance to the saflufenacil relative to the parent plant.

[0114] In other embodiments, the method further comprises the step of introgressing the saflufenacil-tolerance characteristics through traditional plant breeding techniques to obtain a descendent plant having the saflufenacil-tolerance characteristics.

[0115] In other embodiments, saflufenacil-tolerant characteristics/traits of the present invention can be stacked with any combination of plant characteristic(s)/trait(s) of interest to provide plants with a desired combination of characteristics/traits.

[0116] In other aspects, plants of the invention include those plants which, in addition to being saflufenacil-tolerant, have been subjected to further genetic modifications by breeding, mutagenesis or genetic engineering, *e.g.* have been rendered tolerant to applications of specific other classes of herbicides, such as AHAS inhibitors; auxinic herbicides; bleaching herbicides such as hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors or phytoene desaturase (PDS) inhibitors; EPSPS inhibitors such as glyphosate; glutamine synthetase (GS) inhibitors such as glufosinate; protoporphyrinogen-IX oxidase (PPO) inhibitors other than saflufenacil ("other PPO inhibitors") (*e.g.*, acifluorfen, butafenacil, carfentrazone, flufenpyr-ethyl, fomesafen, flumiclorac, flumioxazin, lactofen, oxadiargyl, oxadiazon, oxyfluorfen, sulfentrazone); lipid biosynthesis inhibitors such as acetyl CoA carboxylase (ACCase) inhibitors; or oxynil (*i.e.* bromoxynil or ioxynil) herbicides as a result of conventional methods of breeding or genetic engineering. Thus, saflufenacil-tolerant plants of the invention can be made resistant to multiple classes of herbicides through multiple genetic modifications, such as resistance to both glyphosate and glufosinate or to both glyphosate and a herbicide from another class such as HPPD inhibitors, AHAS inhibitors, or ACCase inhibitors. These herbicide resistance technologies are, for example, described in *Pest Management Science* (at volume, year, page): 61, 2005, 246; 61, 2005, 258; 61, 2005, 277; 61, 2005, 269; 61, 2005, 286; 64, 2008, 326; 64, 2008, 332; *Weed Science* 57, 2009, 108; *Australian Journal of Agricultural Research* 58, 2007, 708; *Science* 316, 2007, 1185; and references quoted therein.

[0117] For example, saflufenacil-tolerant plants of the invention, in some embodiments, may be tolerant to ACCase inhibitors, such as "dims" (*e.g.*, cycloxydim, sethoxydim, clethodim, or tepraloxydim), "fops" (*e.g.*, clodinafop, diclofop, fluazifop, haloxyfop, or quizalofop), and "dens" (such as pinoxaden); to auxinic herbicides, such as dicamba; to

EPSPS inhibitors, such as glyphosate; to other PPO inhibitors; and to GS inhibitors, such as glufosinate.

[0118] In addition to these classes of inhibitors, saflufenacil-tolerant plants of the invention may also be tolerant to herbicides having other modes of action, for example, chlorophyll/carotenoid pigment inhibitors, cell membrane disruptors, photosynthesis inhibitors, cell division inhibitors, root inhibitors, shoot inhibitors, and combinations thereof.

[0119] Such tolerance traits may be expressed, *e.g.*: as mutant AHASL proteins, mutant ACCase proteins, mutant EPSPS proteins, or mutant glutamine synthetase proteins; or as mutant native, inbred, or transgenic aryloxyalkanoate dioxygenase (AAD or DHT), haloarylnitrilase (BXN), 2,2-dichloropropionic acid dehalogenase (DEH), glyphosate-N-acetyltransferase (GAT), glyphosate decarboxylase (GDC), glyphosate oxidoreductase (GOX), glutathione-S-transferase (GST), phosphinothricin acetyltransferase (PAT or bar), or cytochrome P450 (CYP450) proteins having an herbicide-degrading activity. Saflufenacil-tolerant plants hereof can also be stacked with other traits including, but not limited to, pesticidal traits such as *Bt* Cry and other proteins having pesticidal activity toward coleopteran, lepidopteran, nematode, or other pests; nutrition or nutraceutical traits such as modified oil content or oil profile traits, high protein or high amino acid concentration traits, and other trait types known in the art.

[0120] Furthermore, in other embodiments, saflufenacil-tolerant plants are also covered which are, by the use of recombinant DNA techniques and/or by breeding and/or otherwise selected for such characteristics, rendered able to synthesize one or more insecticidal proteins, especially those known from the bacterial genus *Bacillus*, particularly from *Bacillus thuringiensis*, such as δ-endotoxins, *e.g.* CryIA(b), CryIA(c), CryIF, CryIF(a2), CryIIA(b), CryIIIA, CryIIIB(b1) or Cry9c; vegetative insecticidal proteins (VIP), *e.g.* VIP1, VIP2, VIP3 or VIP3A; insecticidal proteins of bacteria colonizing nematodes, *e.g. Photorhabdus* spp. or *Xenorhabdus* spp.; toxins produced by animals, such as scorpion toxins, arachnid toxins, wasp toxins, or other insect-specific neurotoxins; toxins produced by fungi, such streptomycete toxins; plant lectins, such as pea or barley lectins; agglutinins; proteinase inhibitors, such as trypsin inhibitors, serine protease inhibitors, patatin, cystatin or papain inhibitors; ribosome-inactivating proteins (RIP), such as ricin, maize-RIP, abrin, luffin,

saporin or bryodin; steroid metabolism enzymes, such as 3-hydroxy-steroid oxidase, ecdysteroid-IDP-glycosyl-transferase, cholesterol oxidases, ecdysone inhibitors or HMG-CoA-reductase; ion channel blockers, such as blockers of sodium or calcium channels; juvenile hormone esterase; diuretic hormone receptors (helicokinin receptors); stilben synthase, bibenzyl synthase, chitinases or glucanases. In the context of the present invention these insecticidal proteins or toxins are to be understood expressly also as pre-toxins, hybrid proteins, truncated or otherwise modified proteins. Hybrid proteins are characterized by a new combination of protein domains, (see, *e.g.* WO 02/015701). Further examples of such toxins or genetically modified plants capable of synthesizing such toxins are disclosed, *e.g.*, in EP-A 374 753, WO 93/007278, WO 95/34656, EP-A 427 529, EP-A 451 878, WO 03/18810 und WO 03/52073. The methods for producing such genetically modified plants are generally known to the person skilled in the art and are described, *e.g.* in the publications mentioned above. These insecticidal proteins contained in the genetically modified plants impart to the plants producing these proteins tolerance to harmful pests from all taxonomic groups of arthropods, especially to beetles (*Coeloptera*), two-winged insects (*Diptera*), and moths (*Lepidoptera*) and to nematodes (*Nematoda*).

[0121] In some embodiments, expression of one or more protein toxins (*e.g.*, insecticidal proteins) in the saflufenacil-tolerant plants is effective for controlling organisms that include, for example, members of the classes and orders: *Coleoptera* such as the American bean weevil *Acanthoscelides obtectus*; the leaf beetle *Agelastica alni*; click beetles (*Agriotes lineatus*, *Agriotes obscurus*, *Agriotes bicolor*); the grain beetle *Ahasverus advena*; the summer schafer *Amphimallon solstitialis*; the furniture beetle *Anobium punctatum*; *Anthonomus spp.* (weevils); the Pygmy mangold beetle *Atomaria linearis*; carpet beetles (*Anthrenus spp.*, *Attagenus spp.*); the cowpea weevil *Callosobruchus maculates*; the fried fruit beetle *Carpophilus hemipterus*; the cabbage seedpod weevil *Ceutorhynchus assimilis*; the rape winter stem weevil *Ceutorhynchus picitarsis*; the wireworms *Conoderus vespertinus* and *Conoderus falli*; the banana weevil *Cosmopolites sordidus*; the New Zealand grass grub *Costelytra zealandica*; the June beetle *Cotinis nitida*; the sunflower stem weevil *Cylindrocopturus adspersus*; the larder beetle *Dermestes lardarius*; the corn rootworms *Diabrotica virgifera*, *Diabrotica virgifera virgifera*, and *Diabrotica barberi*; the Mexican bean beetle *Epilachna varivestis*; the old house borer *Hylotropes bajulus*; the lucerne weevil

Hypera postica; the shiny spider beetle *Gibbium psylloides*; the cigarette beetle *Lasioderma serricorne*; the Colorado potato beetle *Leptinotarsa decemlineata*; Lyctus beetles (*Lyctus spp.*); the pollen beetle *Meligethes aeneus*; the common cockshafer *Melolontha melolontha*; the American spider beetle *Mezium americanum*; the golden spider beetle *Niptus hololeucus*; the grain beetles *Oryzaephilus surinamensis* and *Oryzaephilus Mercator*; the black vine weevil *Otiorhynchus sulcatus*; the mustard beetle *Phaedon cochleariae*, the crucifer flea beetle *Phyllotreta cruciferae*; the striped flea beetle *Phyllotreta striolata*; the cabbage steam flea beetle *Psylliodes chrysocephala*; *Ptinus spp.* (spider beetles); the lesser grain borer *Rhizopertha dominica*; the pea and been weevil *Sitona lineatus*; the rice and granary beetles *Sitophilus oryzae* and *Sitophilus granaries*; the red sunflower seed weevil *Smicronyx fulvus*; the drugstore beetle *Stegobium paniceum*; the yellow mealworm beetle *Tenebrio molitor*; the flour beetles *Tribolium castaneum* and *Tribolium confusum*; warehouse and cabinet beetles (*Trogoderma spp.*); the sunflower beetle *Zygogramma exclamationis*; *Dermaptera* (earwigs) such as the European earwig *Forficula auricularia* and the striped earwig *Labidura riparia*; *Dictyoptera* such as the oriental cockroach *Blatta orientalis*; the greenhouse millipede *Oxidus gracilis*; the beet fly *Pegomyia betae*; the frit fly *Oscinella frit*; fruitflies (*Dacus spp.*, *Drosophila spp.*); Isoptera (termites) including species from the familes *Hodotermitidae*, *Kalotermitidae*, *Mastotermitidae*, *Rhinotermitidae*, *Serritermitidae*, *Termitidae*, *Termopsidae*; the tarnished plant bug *Lygus lineolaris*; the black bean aphid *Aphis fabae*; the cotton or melon aphid *Aphis gossypii*; the green apple aphid *Aphis pomi*; the citrus spiny whitefly *Aleurocanthus spiniferus*; the sweet potato whitefly *Bemesia tabaci*; the cabbage aphid *Brevicoryne brassicae*; the pear psylla *Cacopsylla pyricola*; the currant aphid *Cryptomyzus ribis*; the grape phylloxera *Daktulosphaira vitifoliae*; the citrus psylla *Diaphorina citri*; the potato leafhopper *Empoasca fabae*; the bean leafhopper *Empoasca Solana*; the vine leafhopper *Empoasca vitis*; the woolly aphid *Eriosoma lanigerum*; the European fruit scale *Eulecanium corni*; the mealy plum aphid *Hyalopterus arundinis*; the small brown planthopper *Laodelphax striatellus*; the potato aphid *Macrosiphum euphorbiae*; the green peach aphid *Myzus persicae*; the green rice leafhopper *Nephotettix cinticeps*; the brown planthopper *Nilaparvata lugens*; the hop aphid *Phorodon humuli*; the bird-cherry aphid *Rhopalosiphum padi*; the grain aphid *Sitobion avenae*; Lepidoptera such as *Adoxophyes orana* (summer fruit tortrix moth); *Archips podana* (fruit tree tortrix moth);

Bucculatrix pyrivorella (pear leafminer); *Bucculatrix thurberiella* (cotton leaf perforator); *Bupalus piniarius* (pine looper); *Carpocapsa pomonella* (codling moth); *Chilo suppressalis* (striped rice borer); *Choristoneura fumiferana* (eastern spruce budworm); *Cochylis hospes* (banded sunflower moth); *Diatraea grandiosella* (southwestern corn borer); *Eupoecilia ambiguella* (European grape berry moth); *Helicoverpa armigera* (cotton bollworm); *Helicoverpa zea* (cotton bollworm); *Heliothis virescens* (tobacco budworm), *Homeosoma electellum* (sunflower moth); *Homona magnanima* (oriental tea tree tortrix moth); *Lithocolletis blancardella* (spotted tentiform leafminer); *Lymantria dispar* (gypsy moth); *Malacosoma neustria* (tent caterpillar); *Mamestra brassicae* (cabbage armyworm); *Mamestra configurata* (Bertha armyworm); *Operophtera brumata* (winter moth); *Ostrinia nubilalis* (European corn borer), *Panolis flammea* (pine beauty moth), *Phyllocnistis citrella* (citrus leafminer); *Pieris brassicae* (cabbage white butterfly); *Rachiplusia ni* (soybean looper); *Spodoptera exigua* (beet armyworm); *Spodoptera littoralis* (cotton leafworm); *Sylepta derogata* (cotton leaf roller); *Trichoplusia ni* (cabbage looper); Orthoptera such as the common cricket *Acheta domesticus*, tree locusts (*Anacridium spp.*), the migratory locust *Locusta migratoria*, the twostriped grasshopper *Melanoplus bivittatus*, the differential grasshopper *Melanoplus differentialis*, the redlegged grasshopper *Melanoplus femurrubrum*, the migratory grasshopper *Melanoplus sanguinipes*, the northern mole cricket *Neocurtilla hexadectyla*, the red locust *Nomadacris septemfasciata*, the shortwinged mole cricket *Scapteriscus abbreviatus*, the southern mole cricket *Scapteriscus borellii*, the tawny mole cricket *Scapteriscus vicinus*, and the desert locust *Schistocerca gregaria*; Symphyla such as the garden symphylan *Scutigerella immaculata*; Thysanoptera such as the tobacco thrips *Frankliniella fusca*, the flower thrips *Frankliniella intonsa*, the western flower thrips *Frankliniella occidentalis*, the cotton bud thrips *Frankliniella schultzei*, the banded greenhouse thrips *Hercinothrips femoralis*, the soybean thrips *Neohydatothrips variabilis*, Kelly's citrus thrips *Pezothrips kellyanus*, the avocado thrips *Scirtothrips perseae*, the melon thrips *Thrips palmi*, and the onion thrips *Thrips tabaci*; and the like, and combinations comprising one or more of the foregoing organisms.

[0122] In some embodiments, expression of one or more protein toxins (*e.g.*, insecticidal proteins) in the saflufenacil-tolerant plants is effective for controlling flea beetles, *i.e.* members of the flea beetle tribe of family *Chrysomelidae*, preferably against *Phyllotreta*

spp., such as *Phyllotreta cruciferae* and/or *Phyllotreta triolata*. In other embodiments, expression of one or more protein toxins (*e.g.*, insecticidal proteins) in the saflufenacil-tolerant plants is effective for controlling cabbage seedpod weevil, the Bertha armyworm, Lygus bugs, or the diamondback moth.

[0123] Furthermore, in one embodiment, saflufenacil-tolerant plants are also covered which are, e.g. by the use of recombinant DNA techniques and/or by breeding and/or otherwise selected for such traits, rendered able to synthesize one or more proteins to increase the resistance or tolerance of those plants to bacterial, viral or fungal pathogens. The methods for producing such genetically modified plants are generally known to the person skilled in the art.

[0124] Furthermore, in another embodiment, saflufenacil-tolerant plants are also covered which are, e.g. by the use of recombinant DNA techniques and/or by breeding and/or otherwise selected for such traits, rendered able to synthesize one or more proteins to increase the productivity (*e.g.* oil content), tolerance to drought, salinity or other growth-limiting environmental factors or tolerance to pests and fungal, bacterial or viral pathogens of those plants.

[0125] Furthermore, in other embodiments, saflufenacil-tolerant plants are also covered which are, e.g. by the use of recombinant DNA techniques and/or by breeding and/or otherwise selected for such traits, altered to contain a modified amount of one or more substances or new substances, for example, to improve human or animal nutrition, *e.g.* oil crops that produce health-promoting long-chain omega-3 fatty acids or unsaturated omega-9 fatty acids (*e.g.* Nexera® rape, Dow Agro Sciences, Canada).

[0126] Furthermore, in some embodiments, saflufenacil-tolerant plants are also covered which are, *e.g.* by the use of recombinant DNA techniques and/or by breeding and/or otherwise selected for such traits, altered to contain increased amounts of vitamins and/or minerals, and/or improved profiles of nutraceutical compounds.

[0127] In one embodiment, saflufenacil-tolerant plants of the present invention, relative to a wild-type plant, comprise an increased amount of, or an improved profile of, a compound selected from the group consisting of: glucosinolates (*e.g.*, glucoraphanin (4-

methylsulfinylbutyl-glucosinolate), sulforaphane, 3-indolylmethyl-glucosinolate (glucobrassicin), 1-methoxy-3-indolylmethyl-glucosinolate (neoglucobrassicin)); phenolics (*e.g.*, flavonoids (*e.g.*, quercetin, kaempferol), hydroxycinnamoyl derivatives (*e.g.*, 1,2,2'-trisinapoylgentiobiose, 1,2-diferuloylgentiobiose, 1,2'-disinapoyl-2-feruloylgentiobiose, 3-O-caffeoyl-quinic (neochlorogenic acid)); and vitamins and minerals (*e.g.*, vitamin C, vitamin E, carotene, folic acid, niacin, riboflavin, thiamine, calcium, iron, magnesium, potassium, selenium, and zinc).

[0128] In another embodiment, saflufenacil-tolerant plants of the present invention, relative to a wild-type plant, comprise an increased amount of, or an improved profile of, a compound selected from the group consisting of: progoitrin; isothiocyanates; indoles (products of glucosinolate hydrolysis); glutathione; carotenoids such as beta-carotene, lycopene, and the xanthophyll carotenoids such as lutein and zeaxanthin; phenolics comprising the flavonoids such as the flavonols (*e.g.* quercetin, rutin), the flavans/tannins (such as the procyanidins comprising coumarin, proanthocyanidins, catechins, and anthocyanins); flavones; phytoestrogens such as coumestans, lignans, resveratrol, isoflavones *e.g.* genistein, daidzein, and glycitein; resorcyclic acid lactones; organosulphur compounds; phytosterols; terpenoids such as carnosol, rosmarinic acid, glycyrrhizin and saponins; chlorophyll; chlorphyllin, sugars, anthocyanins, and vanilla.

[0129] In other embodiments, saflufenacil-tolerant plants of the present invention, relative to a wild-type plant, comprise an increased amount of, or an improved profile of, a compound selected from the group consisting of: vincristine, vinblastine, taxanes (*e.g.*, taxol (paclitaxel), baccatin III, 10-desacetylbaccatin III, 10-desacetyl taxol, xylosyl taxol, 7-epitaxol, 7-epibaccatin III, 10-desacetylcephalomannine, 7-epicephalomannine, taxotere, cephalomannine, xylosyl cephalomannine, taxagifine, 8-benxoyloxy taxagifine, 9-acetyloxy taxusin, 9-hydroxy taxusin, taiwanxam, taxane Ia, taxane Ib, taxane Ic, taxane Id, GMP paclitaxel, 9-dihydro 13-acetylbaccatin III, 10-desacetyl-7-epitaxol, tetrahydrocannabinol (THC), cannabidiol (CBD), genistein, diadzein, codeine, morphine, quinine, shikonin, ajmalacine, serpentine, and the like.

[0130] In one embodiment, saflufenacil-tolerant plants of the invention also can be tolerant to herbicides that inhibit acetohydroxyacid synthase (AHAS). As used herein,

"herbicide tolerant AHASL" refers to the AHAS large subunit polypeptide expressed from one mutant AHASL allele of an AHASL gene in a plant cell and/or from either or both of two homologous alleles of the same mutant AHASL gene, *i.e.* in the same genome of, the plant cell, whereby that mutant AHASL can provide herbicide tolerance to an AHAS enzyme of the plant cell. A mutant AHASL gene can be recombinant, or can be obtained by application of a mutagenesis process, a breeding process, or other process known in the art. Such a gene can be hemizygous, heterozygous, or homozygous.

[0131] As used herein, "AHAS" and "AHASL" respectively refer to functional, plastidic AHAS enzymes and AHASL polypeptides thereof, *i.e.* which are functional in cells of the plants as described herein. Similarly, terms such as "gene" and "polynucleotide," when used in reference to those encoding such an "AHAS" and "AHASL," refer to functional genes therefor, *i.e.* genes that are expressible in such a cell.

[0132] As used herein, standard one letter abbreviations for amino acids will be used, for example, A indicates alanine, P indicates proline, W indicates tryptophan, X indicates any amino acid, etc. Mutations as compared to the wild-type sequence will be indicated by specifying the wild-type amino acid and position followed by the amino acid present in the mutant. For example, P197X will be used to indicate that the proline at position 197 can be substituted with any amino acid.

[0133] For ease of understanding, when referring to amino acid positions in an AHASL, the amino acid numbering system used herein may be the industry standard numbering used for the *Arabidopsis thaliana* (*At*) AHASL sequence, and can be denoted with an (*At*). For example, P197(*At*) can refer to the proline residue at the position in a plant AHASL that corresponds to the proline at position 197 of the *Arabidopsis thaliana* AHASL.

[0134] As used herein, an AHAS herbicide-tolerance-inducing mutation is an alteration in the amino acid sequence of an AHASL enzyme that confers tolerance to one or more herbicides (*i.e.*, sulfonylurea herbicides, imidazolinone herbicides, etc).

[0135] The following Table 11 provides a non-limiting list of possible sites for AHASL mutations, permissible substitutions, preferred substitutions, and more preferred substitutions. X indicates any amino acid.

[0136] Table 11. AHASL Mutations

w/t (*At*)	Permissible Sub.	Pref. Sub.	More. Pref.
G121	X	N SAD	
A122	X	TV DPY (or X)	TV
M124	X	E I	
R142	X	K	
V196	X	M	
P197	X	SAELQRSVWYIHCG	SLT
R199	X	AE	AE
T203	X	I	
A205	X	V CDERTWYN	V
F206	X	RAHWY	
K256	X	DENPTG	
M351	X	CKVGPQY	
H352	X	FMQ	
R373	X	F	
D375	X	NAE	
D376	X	EVN GPSWAC	
R377	X	K	
M570	X	ANC	
V571	X	ACNYIQSW	
W574	X	LMCSRGAFQY	L
F578	X	CGLNRDEIKPSW	
S653	X	N IFT	N
G654	X	QCED	E

[0137] In some embodiments, AHASL mutations can be selected from the group consisting of A122X, P197X, R199X, A205X, S653X, and G654X, and combinations thereof. In other embodiments, AHASL mutations can be selected from the group consisting of A122T, A122V, A122D, A122P, A122Y, P197S, P197L, P197T, R199A, R199E, A205V, A205C, A205D, A205E, A205R, A205T, A205W, A205Y, A205N, S653N, S653I, S653F, S653T, G654Q, G654C, W574L, W574M, W574C, W574S, W574R, W574G, W574A, W574F, W574Q, W574Y, G654E, G654D and combinations thereof. In some embodiments, AHASL mutations can be selected from the group consisting of A122T, A122V, R199A, R199E, A205V, S653N, G654E, and combinations thereof.

[0138] In various embodiments, saflufenacil-tolerant plants may further comprise an AHASL containing both a W574(*At*)X and a S653(*At*)X in plastidic AHASL polypeptides. These AHASL mutations can be present in different alleles, such as on different genomes,

with each containing a single mutation in the respective AHASL gene, or these two can be present in a single AHASL, as double-mutant allele. In various embodiments, these can be W574(*At*)L and S653(*At*)N: the former can be referred to as the "PM2" mutation and the latter as the "PM1" mutation.

[0139] In some embodiments, the saflufenacil-tolerant plants hereof can be inbred varieties, *e.g.*, open-pollinated varieties, or hybrids, *e.g.*, F1 hybrids.

[0140] In various embodiments, the AHAS trait or traits can be obtained by a process, excluding recombinant DNA techniques, and comprising mutagenesis, genoplasty, and/or isolation of spontaneous mutant plants. Many mutagenesis techniques are known in the art and these can involve application of a mutagenic chemical agent or radiation to seeds, plants parts, or cultured plant cells; alternatively, or in addition, the culturing of plant cells, or the conditions under which plant cells are cultured, can increase the rate of occurrence or accumulation of spontaneous mutations. Genoplasty techniques can include directed mutation-type strategies, such as methods comprising introduction, into the plant cell nucleus, of oligonucleotides that facilitate mismatch-repair-system-mediated nucleotide substitution.

[0141] In other aspects, the present invention provides saflufenacil-tolerant plants further comprising tolerance to at least one ACCase inhibitor herbicide at levels that would normally inhibit the growth of wild-type plant. In some embodiments, the saflufenacil-tolerant plant of the present invention expresses an ACCase in which the amino acid sequence differs from an amino acid sequence of an ACCase of a wild-type plant.

[0142] For ease of understanding, when referring to amino acid positions in an ACCase, the amino acid numbering system used herein may be the industry standard numbering system used for the ACCase from *Alopecurus myosuroides* [Huds.] (also referred to as black grass). The mRNA (cDNA) sequence encoding the *A. myosuroides* ACCase is available at GenBank Accession No. AJ310767 and the protein sequence is available at GenBank Accession No. CAC84161 both of which are specifically incorporated herein by reference. The number of the amino acid referred to will be followed with (*Am*) to indicate the amino acid in the *Alopecurus myosuroides* sequence to which the amino acid corresponds.

[0143] Table 12 shows the *Alopecurus myosuroides* ACCase amino acid sequence GenBank accession no. CAC84161. Amino acids that can be altered in the ACCase enzymes of the invention are indicted in bold double underline.

[0144] Table 12. *Alopecurus myosuroides* ACCase amino acid sequence GenBank accession no. CAC84161.

SEQ ID NO:27

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   1 MGSTHLPIVG FNASTTPSLS TLRQINSAAA AFQSSSPSRS SKKKSRRVKS IRDDGDGSVP
  61 DPAGHGQSIR QGLAGIIDLP KEGASAPDVD ISHGSEDHKA SYQMNGILNE SHNGRHASLS
 121 KVYEFCTELG GKTPIHSVLV ANNGMAAAKF MRSVRTWAND TFGSEKAIQL IAMATPEDMR
 181 INAEHIRIAD QFVEVPGGTN NNNYANVQLI VEIAERTGVS AVWPGWGHAS ENPELPDALT
 241 AKGIVFLGPP ASSMNALGDK VGSALIAQAA GVPTLAWSGS HVEIPLELCL DSIPEEMYRK
 301 ACVTTADEAV ASCQMIGYPA MIKASWGGGG KGIRKVNNDD EVKALFKQVQ GEVPGSPIFI
 361 MRLASQSRHL EVQLLCDEYG NVAALHSRDC SVQRRHQKII EEGPVTVAPR ETVKELEQAA
 421 RRLAKAVGYV GAATVEYLYS METGEYYFLE LNPRLQVEHP VTESIAEVNL PAAQVAVGMG
 481 IPLWQIPEIR RFYGMDNGGG YDIWRKTAAL ATPFNFDEVD SQWPKGHCVA VRITSENPDD
 541 GFKPTGGKVK EISFKSKPNV WGYFSVKSGG GIHEFADSQF GHVFAYGETR SAAITSMSLA
 601 LKEIQIRGEI HTNVDYTVDL LNAPDFRENT IHTGWLDTRI AMRVQAERPP WYISVVGGAL
 661 YKTITTNAET VSEYVSYLIK GQIPPKHISL VHSTISLNIE ESKYTIEIVR SGQGSYRLRL
 721 NGSLIEANVQ TLCDGGLLMQ LDGNSHVIYA EEEAGGTRLL IDGKTCLLQN DHDPSRLLAE
 781 TPCKLLRFLI ADGAHVDADV PYAEVEVMKM CMPLLSPAAG VINVLLSEGQ AMQAGDLIAR
 841 LDLDDPSAVK RAEPFEGSFP EMSLPIAASG QVHKRCAASL NAARMVLAGY DHAANKVVQD
 901 LVWCLDTPAL PFLQWEELMS VLATRLPRRL KSELEGKYNE YKLNVDHVKI KDFPTEMLRE
 961 TIEENLACVS EKEMVTIERL VDPLMSLLKS YEGGRESHAH FIVKSLFEEY LSVEELFSDG
1021 IQSDVIERLR LQYSKDLQKV VDIVLSHQGV RNKTKLILAL MEKLVYPNPA AYRDQLIRFS
1081 SLNHKRYYKL ALKASELLEQ TKLSELRTSI ARNLSALDMF TEEKADFSLQ DRKLAINESM
1141 GDLVTAPLPV EDALVSLFDC TDQTLQQRVI QTYISRLYQP QLVKDSIQLK YQDSGVIALW
1201 EFTEGNHEKR LGAMVILKSL ESVSTAIGAA LKDASHYASS AGNTVHIALL DADTQLNTTE
1261 DSGDNDQAQD KMDKLSFVLK QDVVMADLRA ADVKVVSCIV QRDGAIMPMR RTFLLSEEKL
1321 CYEEEPILRH VEPPLSALLE LDKLKVKGYN EMKYTPSRDR QWHIYTLRNT ENPKMLHRVF
1381 FRTLVRQPSA GNRFTSDHIT DVEVGHAEEP LSFTSSSILK SLKIAKEELE LHAIRTGHSH
1441 MYLCILKEQK LLDLVPVSGN TVVDVGQDEA TACSLLKEMA LKIHELVGAR MHHLSVCQWE
1501 VKLKLVSDGP ASGSWRVVTT NVTGHTCTVD IYREVEDTES QKLVYHSTAL SSGPLHGVAL
1561 NTSYQPLSVI DLKRCSARNN KTTYCYDFPL TFEAAVQKSW SNISSENNQC YVKATELVFA
1621 EKNGSWGTPI IPMQRAAGLN DIGMVAWILD MSTPEFPSGR QIIVIANDIT FRAGSFGPRE
1681 DAFFEAVTNL ACEKKLPLIY LAANSGARIG IADEVKSCFR VGWTDDSSPE RGFRYIYMTD
1741 EDHDRIGSSV IAHKMQLDSG EIRWVIDSVV GKEDGLGVEN IHGSAAIASA YSRAYEETFT
1801 LTFVTGRTVG IGAYLARLGI RCIQRIDQPI ILTGFSALNK LLGREVYSSH MQLGGPKIMA
1861 TNGVVHLTVP DDLEGVSNIL RWLSYVPANI GGPLPITKSL DPIDRPVAYI PENTCDPRAA
1921 ISGIDDSQGK WLGGMFDKDS FVETFEGWAK TVVTGRAKLG GIPVGVIAVE TQTMMQLVPA
1981 DPGQPDSHER SVPRAGQVWF PDSATKTAQA MLDFNREGLP LFILANWRGF SGGQRDLFEG
2041 ILQAGSTIVE NLRTYNQPAF VYIPKAAELR GGAWVVIDSK INPDRIECYA ERTAKGNVLE
2101 PQGLIEIKFR SEELKECMGR LDPELIDLKA RLQGANGSLS DGESLQKSIE ARKKQLLPLY
2161 TQIAVRFAEL HDTSLRMAAK GVIRKVVDWE DSRSFFYKRL RRRLSEDVLA KEIRGVIGEK
2221 FPHKSAIELI KKWYLASEAA AAGSTDWDDD DAFVAWRENP ENYKEYIKEL RAQRVSRLLS
2281 DVAGSSSDLQ ALPQGLSMLL DKMDPSKRAQ FIEEVMKVLK
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[0145] Examples of amino acid positions at which an ACCase of a saflufenacil-tolerant plant of the invention differs from the ACCase of the corresponding wild-type plant include, but are not limited to, one or more of the following positions: 1,781*(Am)*, 1,785*(Am)*, 1,786*(Am)*, 1,811*(Am)*, 1,824*(Am)*, 1,864*(Am)*, 1,999*(Am)*, 2,027*(Am)*, 2,039*(Am)*, 2,041*(Am)*, 2,049*(Am)*, 2,059*(Am)*, 2,074*(Am)*, 2,075*(Am)*, 2,078*(Am)*, 2,079*(Am)*, 2,080*(Am)*, 2,081*(Am)*, 2,088*(Am)*, 2,095*(Am)*, 2,096*(Am)*, or 2,098*(Am)*.

[0146] In other aspects, a method for treating a plant of the present invention is provided. In some embodiments, the method comprises contacting the plant with an agronomically acceptable composition. In one embodiment, the agronomically acceptable composition comprises an auxinic herbicide A.I.

[0147] In another aspect, the present invention provides a method for preparing a descendent seed. The method comprises planting a seed of or capable of producing a plant of the present invention. In one embodiment, the method further comprises growing a descendent plant from the seed; and harvesting a descendant seed from the descendent plant. In other embodiments, the method further comprises applying a saflufenacil herbicidal composition to the descendent plant.

[0148] In still further aspects, the present invention provides a method for producing a plant product. In some embodiments, the method comprises processing a plant or plant part thereof of the present invention. In some embodiments, the plant product is fodder, seed meal, oil, or seed-treatment-coated seeds. In other embodiments, the plant part is a seed.

[0149] Herbicides

[0150] In some embodiments, herbicidal compositions of the invention comprise saflufenacil and its agronomically acceptable salts and esters. As used herein "saflufenacil" includes the compound saflufenacil and its salts and esters, unless expressly stated otherwise. In one embodiment, the saflufenacil A.I. is, *e.g.*, IUPAC: 2-chloro-5-[3,6-dihydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)-1 (2*H*)-pyrimidinyl]-4-fluoro-*N*-[[methyl(1-methylethyl) amino] sulfonyl]benzamide (CAS: *N'*-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl] benzoyl}-*N*-isopropyl-*N*-methylsulfamide; Reg. No.: 372137-35-4); BAS-H800).

[0151] Post-emergent weed control methods useful in various embodiments hereof utilize about ≥0.3x application rates of saflufenacil; in some embodiments, this can be about, for example, ≥0.3x, ≥0.4x, ≥0.5x, ≥0.6x, ≥0.7x, ≥0.8x, ≥0.9x, or ≥1x of saflufenacil.

[0152] In one embodiment, saflufenacil-tolerant plants of the present invention have tolerance to a post-emergant application of a saflufenacil at an amount of about 25 to about 200 g ai/h.

[0153] In some embodiments, wherein the saflufenacil-tolerant plant is a dicot (*e.g.*, soy, cotton), the post-emergant application of the saflufenacil is at an amount of about 50 g i/h.

[0154] In another embodiment, wherein the saflufenacil-tolerant plant is a monocot (*e.g.*, maize, rice, sorghum), the post-emergant application of the saflufenacil is at an amount of about 200 g i/h.

[0155] In other embodiments, wherein the saflufenacil-tolerant plant is a *Brassica* (*e.g.*, canola), the post-emergant application of the saflufenacil is at an amount of about 25 g i/h.

[0156] In post-emergent weed control methods hereof, in some embodiments, the method can utilize saflufenacil application rates at about 7 to 10 days post-emergent.

[0157] In another embodiment, the application rate can exceed 1x saflufenacil; in some embodiments, the rate can be up to 4x saflufenacil, though more typically it will be about 2.5x or less, or about 2x or less, or about 1x or less.

[0158] In other embodiments, in addition to the one or more saflufenacil A.I.(s), the herbicidal compositions of the invention, optionally, can further comprise one or more agronomically acceptable A.I.(s) other than saflufenacil. As used herein, agronomically acceptable A.I.(s) include the A.I.s and their agronomically acceptable salts and esters. Additional classes of herbicides include, but are not limited to, AHAS inhibitors; bleaching herbicides such as hydroxyphenylpyruvate dioxygenase (HPPD) inhibitors or phytoene desaturase (PDS) inhibitors; enolpyruvyl shikimate 3-phosphate synthase (EPSPS) inhibitors such as glyphosate; glutamine synthetase (GS) inhibitors such as glufosinate; auxinic herbicides; lipid biosynthesis inhibitors such as ACCase inhibitors; or oxynil (*i.e.* bromoxynil or ioxynil) herbicides. AHAS-inhibitor herbicides include, *e.g.*, imidazolinone herbicides, one or more SU herbicides selected from the group consisting of amidosulfuron,

flupyrsulfuron, foramsulfuron, imazosulfuron, iodosulfuron, mesosulfuron, nicosulfuron, thifensulfuron, and tribenuron, agronomically acceptable salts and esters thereof, and combinations thereof. ACCase inhibitor herbicides include, *e.g.*, "dims" (*e.g.*, cycloxydim, sethoxydim, clethodim, or tepraloxydim), "fops" (*e.g.*, clodinafop, diclofop, fluazifop, haloxyfop, or quizalofop), and "dens" (such as pinoxaden). In addition to the one or more saflufenacils, the herbicide compositions of the invention, optionally, can further comprise one or more agronomically acceptable A.I.(s) of other classes, *e.g.*, agronomic fungicides, bactericides, algicides, nematicides, insecticides, and the like (*e.g.*, malathion, pyrethrins/pyrethrum, carbaryl, spinosad, permethrin, bifenthrin, and esfenvalerate).

[0159] The herbicidal compositions hereof comprising one or more saflufenacils, and optionally other agronomic A.I.(s) can be used in any agronomically acceptable format. For example, these can be formulated as ready-to-spray aqueous solutions, powders, suspensions; as concentrated or highly concentrated aqueous, oily or other solutions, suspensions or dispersions; as emulsions, oil dispersions, pastes, dusts, granules, or other broadcastable formats. The herbicide compositions can be applied by any method known in the art, including, for example, spraying, atomizing, dusting, spreading, watering, seed treatment, or co-planting in admixture with the seed. The use forms depend on the intended purpose; in any case, they should ensure the finest possible distribution of the A.I.s according to the invention. In some embodiments, an herbicidal composition hereof can comprise, e.g., a combination of: saflufenacil(s); AHAS-inhibitor(s), e.g., imidazolinone(s) and/or sulfonylurea(s); ACCase-inhibitor(s); EPSPS inhibitor(s), e.g., glyphosate; glutamine synthetase inhibitor(s), e.g.., glufosinate; auxinic herbicide(s), e.g., dicamba; fungicide(s), e.g., strobilurin fungicide(s) such as pyraclostrobin; and the like. In some embodiments, an herbicidal composition hereof can comprise, e.g., a combination of saflufenacil(s); and strobilurin fungicide(s) such as pyraclostrobin(s). An herbicidal composition will be selected according to the tolerances of a plant hereof, and the plant can be selected from among those having stacked tolerance traits.

[0160] In some embodiments, where the optional A.I. includes an AHAS-inhibitor, this can be selected from: (1) the imidazolinones, *e.g.* imazamox, imazethapyr, imazapyr, imazapic, imazaquin, and imazamethabenz, preferably from imazamox, imazethapyr,

imazapyr, and imazapic, preferably imazamox; (2) the SUs, *e.g.* amidosulfuron, flupyrsulfuron, foramsulfuron, imazosulfuron, iodosulfuron, mesosulfuron, nicosulfuron, thifensulfuron, and tribenuron; (2) the pyrimidinyloxy[thio]benzoates, *e.g.* including the pyrimidinyloxybenzoates (*e.g.*, bispyribac, pyriminobac, and pyribenzoxim) and the pyrimidinylthiobenzoates (*e.g.*, pyrithiobac and pyriftalid); and (3) the sulfonamides, *i.e.* including the sulfonylaminocarbonyltriazolinones (*e.g.*, flucarbazone and propoxycarbazone) and the triazolopyrimidines (*e.g.*, cloransulam, diclosulam, florasulam, flumetsulam, metosulam, and penoxsulam). The agronomically acceptable salts and esters of the foregoing are also included, as are combinations thereof.

[0161] In other embodiments, where the optional A.I. includes an herbicide from a different class to which the plant(s) hereof would normally be susceptible, the plant to be used is selected from among those that further comprise a trait of tolerance to such herbicide. Such further tolerance traits can be provided to the plant by any method known in the art, *e.g.*, including techniques of traditional breeding to obtain a tolerance trait gene by hybridization or introgression, of mutagenesis, and/or of transformation. Such plants can be described as having "stacked" traits.

[0162] Optional A.I.s of other herbicide classes include ACCase inhibitors, auxinic herbicides, EPSPS inhibitors, glutamine synthetase inhibitors, p-hydroxyphenylpyruvate dioxygenase (4-HPPD) inhibitors. Optional A.I.s of other types include, but are not limited to fungicides such as strobilurins, *e.g.*, pyraclostrobin, alone or in combination with, e.g., boscalid, epiconazole, metaconazole, tebuconazole, kresoxim-methyl, and the like; insecticides such as nematicides, lepidoptericides, coleoptericides; molluskicides), and others known in the art.

[0163] For example, suitable examples of herbicides that are ACCase inhibitors include, but are not limited to, cyclohexanedione herbicides (DIMs, also referred to as: cyclohexene oxime cyclohexanedione oxime; and CHD), aryloxyphenoxy propionate herbicides (also referred to as aryloxyphenoxy propanoate; aryloxyphenoxyalkanoate; oxyphenoxy; APP; AOPP; APA; APPA; FOP, note that these are sometime written with the suffix '-oic'), and phenylpyrazole herbicides (also known as DENs; and sometimes referred to under the more general class of Phenylpyrazole such as pinoxaden (*e.g.*, herbicides sold under the trade

names Axial and Traxos)). In some methods of controlling weeds and/or growing herbicide-tolerant plants, at least one herbicide is selected from the group consisting of sethoxydim, cycloxydim, tepraloxydim, haloxyfop, haloxyfop-P or a derivative of any of these herbicides. Table 13 is list of herbicides that interfere with ACCase activity.

[0164] Table 13. Examples of ACCase inhibitors.

ACCase Inhibitor	Class	Company	Examples of Synonyms and Trade Names
alloxydim	DIM	BASF	Fervin, Kusagard, NP-48Na, BAS 9021H, Carbodimedon, Zizalon
butroxydim	DIM	Syngenta	Falcon, ICI-A0500, Butroxydim
clethodim	DIM	Valent	Select, Prism, Centurion, RE-45601, Motsa
Clodinafop-propargyl	FOP	Syngenta	Discover, Topik, CGA 184 927
clofop	FOP		Fenofibric Acid, Alopex
cloproxydim	FOP		
chlorazifop	FOP		
cycloxydim	DIM	BASF	Focus, Laser, Stratos, BAS 517H
cyhalofop-butyl	FOP	Dow	Clincher, XDE 537, DEH 112, Barnstorm
diclofop-methyl	FOP	Bayer	Hoegrass, Hoelon, Illoxan, HOE 23408, Dichlorfop, Illoxan
fenoxaprop-P-ethyl	FOP	Bayer	Super Whip, Option Super, Exel Super, HOE-46360, Aclaim, Puma S, Fusion
fenthiaprop	FOP		Taifun; Joker
fluazifop-P-butyl	FOP	Syngenta	Fusilade, Fusilade 2000, Fusilade DX, ICI-A 0009, ICI-A 0005, SL-236, IH-773B, TF-1169, Fusion
haloxyfop-etotyl	FOP	Dow	Gallant, DOWCO 453EE
haloxyfop-methyl	FOP	Dow	Verdict, DOWCO 453ME
haloxyfop-P-methyl	FOP	Dow	Edge, DE 535
isoxapyrifop	FOP		
Metamifop	FOP	Dongbu	NA
pinoxaden	DEN	Syngenta	Axial
profoxydim	DIM	BASF	Aura, Tetris, BAS 625H, Clefoxydim
propaquizafop	FOP	Syngenta	Agil, Shogun, Ro 17-3664, Correct
quizalofop-P-ethyl	FOP	DuPont	Assure, Assure II, DPX-Y6202-3, Targa Super, NC-302, Quizafop
quizalofop-P-tefuryl	FOP	Uniroyal	Pantera, UBI C4874
sethoxydim	DIM	BASF	Poast, Poast Plus, NABU, Fervinal, NP-55, Sertin, BAS 562H, Cyethoxydim, Rezult
tepraloxydim	DIM	BASF	BAS 620H, Aramo, Caloxydim
tralkoxydim	DIM	Syngenta	Achieve, Splendor, ICI-A0604, Tralkoxydime, Tralkoxidym
trifop	FOP		

[0165] Examples of herbicides that are auxinic herbicides include, but are not limited to, the auxinic herbicides shown in Table 14.

[0166] Table 14. Auxinic herbicides.

Classification of Auxinic Herbicides (HRAC Group 'O'; WSSA Group '4')	
Subgroup	**Member Compound**
Phenoxy-carboxylic-acid Subgroup	Clomeprop
	cloprop ("3-CPA")
	4-chlorophenoxyacetic acid ("4-CPA")
	2-(4-chlorophenoxy)propionic acid ("4-CPP")
	2,4-dichlorophenoxy acetic acid ("2,4-D")
	(3,4-dichlorophenoxy)acetic acid ("3,4-DA")
	4-(2,4-dichlorophenoxy)butyric acid ("2,4-DB")
	2-(3,4-dichlorophenoxy)propionic acid ("3,4-DP")
	tris[2-(2,4-dichlorophenoxy)ethyl]phosphite ("2,4-DEP")
	dichlorprop ("2,4-DP")
	2,4,5-trichlorophenoxyacetic acid ("2,4,5-T")
	fenoprop ("2,4,5-TP")
	2-(4-chloro-2-methylphenoxy)acetic acid ("MCPA")
	4-(4-chloro-2-methylphenoxy)butyric acid ("MCPB")
	mecoprop ("MCPP")
Benzoic acid Subgroup	Chloramben
	Dicamba
	Tricamba
	2,3,6-trichlorobenzoic acid ("TBA")
Pyridine carboxylic acid Subgroup	Aminopyralid
	Clopyralid
	Fluroxypyr
	Picloram
	Triclopyr
Quinoline carboxylic acid Subgroup	Quinclorac
	Quinmerac
Other Subgroup	Benazolin

[0167] The herbicidal compositions comprising a saflufenacil, and optionally other agronomic A.I.(s) and/or their agriculturally suitable salts and esters can also comprise auxiliaries which are customary for the formulation of crop protection agents.

[0168] Examples of auxiliaries customary for the formulation of crop protection agents include inert auxiliaries, solid carriers, surfactants (such as dispersants, protective colloids, emulsifiers, wetting agents and tackifiers), organic and inorganic thickeners, penetrants (such as penentration-enhancing organosilicone surfactants or acidic sulfate chelates, *e.g.*, CT-301™ available from Cheltec, Inc.), safeners, bactericides, antifreeze agents, antifoams,

colorants, and adhesives. Formulations of the herbicide compositions useful herein can be prepared according to any method known useful therefor in the art

[0169] Examples of thickeners (*i.e.* compounds which impart to the formulation modified flow properties, *i.e.* high viscosity in the state of rest and low viscosity in motion) are polysaccharides, such as xanthan gum (Kelzan® from Kelco), Rhodopol® 23 (Rhone Poulenc) or Veegum® (from R.T. Vanderbilt), and also organic and inorganic sheet minerals, such as Attaclay® (from Engelhardt).

[0170] Examples of antifoams are silicone emulsions (such as, for example, Silikon® SRE, Wacker or Rhodorsil® from Rhodia), long-chain alcohols, fatty acids, salts of fatty acids, organofluorine compounds and mixtures thereof.

[0171] Bactericides can be added for stabilizing the aqueous herbicidal formulations. Examples of bactericides are bactericides based on diclorophen and benzyl alcohol hemiformal (Proxel® from ICI or Acticide® RS from Thor Chemie and Kathon® MK from Rohm & Haas), and also isothiazolinone derivates, such as alkylisothiazolinones and benzisothiazolinones (Acticide MBS from Thor Chemie).

[0172] Examples of antifreeze agents are ethylene glycol, propylene glycol, urea or glycerol.

[0173] Examples of colorants include members of colorant classes such as the sparingly water-soluble pigments and the water-soluble dyes. Some specific examples of these include the dyes known under the names Rhodamin B, C.I. Pigment Red 112 and C.I. Solvent Red 1, and also pigment blue 15:4, pigment blue 15:3, pigment blue 15:2, pigment blue 15:1, pigment blue 80, pigment yellow 1, pigment yellow 13, pigment red 112, pigment red 48:2, pigment red 48:1, pigment red 57:1, pigment red 53:1, pigment orange 43, pigment orange 34, pigment orange 5, pigment green 36, pigment green 7, pigment white 6, pigment brown 25, basic violet 10, basic violet 49, acid red 51, acid red 52, acid red 14, acid blue 9, acid yellow 23, basic red 10, basic red 108.

[0174] Examples of adhesives are polyvinylpyrrolidone, polyvinyl acetate, polyvinyl alcohol and tylose.

[0175] Suitable inert auxiliaries are, for example, the following: mineral oil fractions of medium to high boiling point, such as kerosene and diesel oil, furthermore coal tar oils and oils of vegetable or animal origin, aliphatic, cyclic and aromatic hydrocarbons, for example paraffin, tetrahydronaphthalene, alkylated naphthalenes and their derivatives, alkylated benzenes and their derivatives, alcohols such as methanol, ethanol, propanol, butanol and cyclohexanol, ketones such as cyclohexanone or strongly polar solvents, for example amines such as N-methylpyrrolidone, and water.

[0176] Suitable carriers include liquid and solid carriers.

[0177] Liquid carriers include *e.g.* non-aqueous solvents such as cyclic and aromatic hydrocarbons, *e.g.* paraffins, tetrahydronaphthalene, alkylated naphthalenes and their derivatives, alkylated benzenes and their derivatives, alcohols such as methanol, ethanol, propanol, butanol and cyc-lohexanol, ketones such as cyclohexanone, strongly polar solvents, *e.g.* amines such as N-methylpyrrolidone, and water as well as mixtures thereof.

[0178] Solid carriers include *e.g.* mineral earths such as silicas, silica gels, silicates, talc, kaolin, limestone, lime, chalk, bole, loess, clay, dolomite, diatomaceous earth, calcium sulfate, magnesium sulfate and magnesium oxide, ground synthetic materials, fertilizers such as ammonium sulfate, ammonium phosphate, ammonium nitrate and ureas, and products of vegetable origin, such as cereal meal, tree bark meal, wood meal and nutshell meal, cellulose powders, or other solid carriers.

[0179] Suitable surfactants (adjuvants, wetting agents, tackifiers, dispersants and also emulsifiers) are the alkali metal salts, alkaline earth metal salts and ammonium salts of aromatic sulfonic acids, for example lignosulfonic acids (*e.g.* Borrespers-types, Borregaard), phenolsulfonic acids, naphthalenesulfonic acids (Morwet types, Akzo Nobel) and dibutylnaphthalenesulfonic acid (Nekal types, BASF AG), and of fatty acids, alkyl- and alkylarylsulfonates, alkyl sulfates, lauryl ether sulfates and fatty alcohol sulfates, and salts of sulfated hexa-, hepta- and octadecanols, and also of fatty alcohol glycol ethers, condensates of sulfonated naphthalene and its derivatives with formaldehyde, condensates of naphthalene or of the naphthalenesulfonic acids with phenol and formaldehyde, polyoxyethylene octylphenol ether, ethoxylated isooctyl-, octyl- or nonylphenol, alkylphenyl or tributylphenyl polyglycol ether, alkylaryl polyether alcohols, isotridecyl alcohol, fatty alcohol/ethylene

oxide condensates, ethoxylated castor oil, polyoxyethylene alkyl ethers or polyoxypropylene alkyl ethers, lauryl alcohol polyglycol ether acetate, sorbitol esters, lignosulfite waste liquors and proteins, denatured proteins, polysaccharides (*e.g.* methylcellulose), hydrophobically modified starches, polyvinyl alcohol (Mowiol types, Clariant), polycarboxylates (BASF AG, Sokalan types), polyalkoxylates, polyvinylamine (BASF AG, Lupamine types), polyethyleneimine (BASF AG, Lupasol types), polyvinylpyrrolidone and copolymers thereof.

[0180] Powders, materials for broadcasting and dusts can be prepared by mixing or concomitant grinding the A.I.s together with a solid carrier.

[0181] Granules, for example coated granules, impregnated granules and homogeneous granules, can be prepared by binding the A.I.s to solid carriers.

[0182] Aqueous use forms can be prepared from emulsion concentrates, suspensions, pastes, wettable powders or water-dispersible granules by adding water.

[0183] To prepare emulsions, pastes or oil dispersions, the herbicidal compositions comprising an auxinic herbicide, and optionally other agronomic A.I.(s) and/or their agriculturally suitable salts and esters, either as such or dissolved in an oil or solvent, can be homogenized in water by means of a wetting agent, tackifier, dispersant or emulsifier. Alternatively, it is also possible to prepare concentrates comprising active compound, wetting agent, tackifier, dispersant or emulsifier and, if desired, solvent or oil, which are suitable for dilution with water.

[0184] The concentrations of the herbicides present in the herbicidal composition comprising an auxinic herbicide, and optionally other agronomic A.I.(s) and/or their agriculturally suitable salts and esters can be varied within wide ranges. In general, the formulations comprise approximately from 0.001 to 98% by weight, preferably 0.01 to 95% by weight of at least one active ingredient. The A.I.s are employed in a purity of from 90% to 100%, preferably 95% to 100% (according to NMR spectrum).

[0185] In the formulation, in some embodiments, the herbicides are present in suspended, emulsified or dissolved form. The formulation according to the invention can be in the form of aqueous solutions, powders, suspensions, also highly-concentrated aqueous, oily or other

suspensions or dispersions, aqueous emulsions, aqueous microemulsions, aqueous suspo-emulsions, oil dispersions, pastes, dusts, materials for spreading or granules.

[0186] In various embodiments, the herbicides can, for example, be formulated as follows:

[0187] 1. Products for dilution with water

[0188] A Water-soluble concentrates

[0189] 10 parts by weight of active compound are dissolved in 90 parts by weight of water or a water-soluble solvent. As an alternative, wetting agent(s) or other adjuvants are added. The active compound dissolves upon dilution with water. This gives a formulation with an active compound content of 10% by weight.

[0190] B Dispersible concentrates

[0191] 20 parts by weight of active compound are dissolved in 70 parts by weight of cyclohexanone with addition of 10 parts by weight of a dispersant, for example polyvinylpyrrolidone. Dilution with water gives a dispersion. The active compound content is 20% by weight.

[0192] C Emulsifiable concentrates

[0193] 15 parts by weight of active compound are dissolved in 75 parts by weight of an organic solvent (*e.g.* alkylaromatics) with addition of calcium dodecylbenzenesulfonate and castor oil ethoxylate (in each case 5 parts by weight). Dilution with water gives an emulsion. The formulation has an active compound content of 15% by weight.

[0194] D Emulsions

[0195] 25 parts by weight of active compound are dissolved in 35 parts by weight of an organic solvent (*e.g.* alkylaromatics) with addition of calcium dodecylbenzenesulfonate and castor oil ethoxylate (in each case 5 parts by weight). This mixture is introduced into 30 parts by weight of water by means of an emulsifier (Ultraturrax) and made into a homogeneous emulsion. Dilution with water gives an emulsion. The formulation has an active compound content of 25% by weight.

[0196] E Suspensions

[0197] In an agitated ball mill, 20 parts by weight of active compound are comminuted with addition of 10 parts by weight of dispersants and wetting agent(s) and 70 parts by weight of water or an organic solvent to give a fine active compound suspension. Dilution with water gives a stable suspension of the active compound. The active compound content in the formulation is 20% by weight.

[0198] F Water-dispersible granules and water-soluble granules

[0199] 50 parts by weight of active compound are ground finely with addition of 50 parts by weight of dispersants and wetting agent(s) and made into water-dispersible or water-soluble granules by means of technical appliances (for example extrusion, spray tower, fluidized bed). Dilution with water gives a stable dispersion or solution of the active compound. The formulation has an active compound content of 50% by weight.

[0200] G Water-dispersible powders and water-soluble powders

[0201] 75 parts by weight of active compound are ground in a rotor-stator mill with addition of 25 parts by weight of dispersants, wetting agent(s) and silica gel. Dilution with water gives a stable dispersion or solution of the active compound. The active compound content of the formulation is 75% by weight.

[0202] H Gel formulations

[0203] In a ball mill, 20 parts by weight of active compound, 10 parts by weight of dispersant, 1 part by weight of gelling agent and 70 parts by weight of water or of an organic solvent are mixed to give a fine suspension. Dilution with water gives a stable suspension with active compound content of 20% by weight.

[0204] 2. Products to be applied undiluted

[0205] I Dusts

[0206] 5 parts by weight of active compound are ground finely and mixed intimately with 95 parts by weight of finely divided kaolin. This gives a dusting powder with an active compound content of 5% by weight.

[0207] J Granules (GR, FG, GG, MG)

[0208] 0.5 parts by weight of active compound are ground finely and associated with 99.5 parts by weight of carriers. Current methods here are extrusion, spray-drying or the fluidized bed. This gives granules to be applied undiluted with an active compound content of 0.5% by weight.

[0209] K ULV solutions (UL)

[0210] 10 parts by weight of active compound are dissolved in 90 parts by weight of an organic solvent, for example xylene. This gives a product to be applied undiluted with an active compound content of 10% by weight.

[0211] Aqueous use forms can be prepared from emulsion concentrates, suspensions, pastes, wettable powders or water-dispersible granules by adding water.

[0212] The herbicides or the herbicidal compositions comprising them can be applied pre-, post-emergence or pre-plant, or together with the seed. It is also possible to apply the herbicidal composition or active compounds by applying seed, pretreated with the herbicidal compositions or active compounds, of a crop plant.

[0213] In a further embodiment, the herbicides or herbicidal compositions can be applied by treating seed. The treatment of seeds comprises essentially all procedures familiar to the person skilled in the art (*e.g.*, seed dressing, seed coating, seed dusting, seed soaking, seed film coating, seed multilayer coating, seed encrusting, seed dripping and seed pelleting). In some embodiments, the herbicidal compositions can be applied diluted or undiluted.

[0214] In one embodiment, the saflufenacil(s) and optionally other agronomic A.I.(s), can be mixed with a large number of representatives of other herbicidal or growth-regulating active ingredient groups and then applied concomitantly. Suitable components for mixtures are, for example, 1,2,4-thiadiazoles, 1,3,4-thiadiazoles, amides, aminophosphoric acid and its derivatives, aminotriazoles, anilides, (het)aryloxyalkanoic acids and their derivatives, benzoic acid and its derivatives, benzothiadiazinones, 2-aroyl-1,3-cyclohexanediones, 2-hetaroyl-1,3-cyclohexane-diones, hetaryl aryl ketones, benzylisoxazolidinones, meta-CF3-phenyl derivatives, carbamates, quinolinecarboxylic acid and its derivatives, chloroacetanilides, cyclohexenone oxime ether derivatives, diazines, dichloropropionic acid and its derivatives, dihydro-benzofurans, dihydrofuran-3-ones, dinitroanilines,

dinitrophenols, diphenyl ethers, dipyridyls, halocarboxylic acids and their derivatives, ureas, 3-phenyluracils, imidazoles, imidazolinones, N-phenyl-3,4,5,6-tetrahydrophthalimides, oxadiazoles, oxiranes, phenols, aryloxy- and hetaryloxyphenoxypropionic esters, phenylacetic acid and its derivatives, 2-phenylpropionic acid and its derivatives, pyrazoles, phenylpyrazoles, pyridazines, pyridinecarboxylic acid and its derivatives, pyrimidyl ethers, sulfonamides, sulfonylureas, triazines, triazinones, triazolinones, triazolecarboxamides, uracils, phenyl pyrazolines and isoxazolines and derivatives thereof. For the purposes of this paragraph, auxinic herbicide members are excluded from the following classes: benzoic acid and its derivatives; quinolinecarboxylic acid and its derivatives; 2-phenylpropionic acid and its derivatives; and pyridinecarboxylic acid and its derivatives.

[0215] It may furthermore be beneficial to apply the herbicides alone or in combination with other herbicides, or else in the form of a mixture with other crop protection agents, for example together with agents for controlling pests or phytopathogenic fungi or bacteria. Also of interest is the miscibility with mineral salt solutions, which are employed for treating nutritional and trace element deficiencies. Other additives such as non-phytotoxic oils and oil concentrates can also be added.

[0216] Moreover, it may be useful to apply the herbicides in combination with safeners. Safeners are chemical compounds which prevent or reduce herbicide-induced injury to useful plants without having a major impact on the herbicidal action of the herbicides. They can be applied either before sowings (*e.g.* on seed treatments, shoots or seedlings) or in the pre-emergence application or post-emergence application of the useful plant. The safeners and the herbicides can be applied simultaneously or in succession.

[0217] Suitable safeners are *e.g.* (quinolin-8-oxy)acetic acids, 1-phenyl-5-haloalkyl-1H-1,2,4-triazol-3-carboxylic acids, 1-phenyl-4,5-dihydro-5-alkyl-1H-pyrazol-3,5-dicarboxylic acids, 4,5-dihydro-5,5-diaryl-3-isoxazol carboxylic acids, dichloroacetamides, alpha-oximinophenylacetonitriles, acetophenonoximes, 4,6-dihalo-2-phenylpyrimidines, N-[[4-(aminocarbonyl)phenyl]sulfonyl]-2-benzoic amides, 1,8-naphthalic anhydride, 2-halo-4-(haloalkyl)-5-thiazol carboxylic acids, phosphorthiolates and N-alkyl-O-phenyl-carbamates and their agriculturally acceptable salts and their agriculturally acceptable derivatives such amides, esters, and thioesters, provided they have an acid group.

[0218] Examples of saferners are benoxacor, cloquintocet, cyometrinil, cyprosulfamide, dichlormid, dicyclonon, dietholate, fenchlorazole, fenclorim, flurazole, fluxofenim, furilazole, isoxadifen, mefenpyr, mephenate, naphthalic anhydride, oxabetrinil, 4-(dichloroacetyl)-1-oxa-4-azaspiro[4.5]decane (MON4660, CAS 71526-07-3) and 2,2,5-trimethyl-3-(dichloroacetyl)-1,3-oxazolidine (R-29148, CAS 52836-31-4).

[0219] Those skilled in the art will recognize that some of the above mentioned herbicides and/or safeners are capable of forming geometrical isomers, for example E/Z isomers. It is possible to use both, the pure isomers and mixtures thereof, in the compositions according to the invention. Furthermore, some of the above mentioned herbicides and/or safeners have one or more centers of chirality and, as a consequence, are present as enantiomers or diastereomers. It is possible to use both, the pure enantiomers and diastereomers and their mixtures, in the compositions according to the invention. In particular, some of the aryloxyphenoxy propionate herbicides are chiral, and some of them are commonly used in enantiomerically enriched or enantiopure form, e. g. clodinafop, cyhalofop, fenoxaprop-P, fluazifop-P, haloxyfop-P, metamifop, propaquizafop or quizalofop-P. As a further example, glufosinate may be used in enantiomerically enriched or enantiopure form, also known as glufosinate-P.

[0220] Those skilled in the art will recognize that any derivative of the above mentioned herbicides and/or safeners can be used in the practice of the invention, for example agriculturally suitable salts and esters.

[0221] Methods of controlling weeds

[0222] Herbicide-tolerant plants of the invention can be used in conjunction with an herbicide to which they are tolerant. Herbicides can be applied to the plants of the invention using any techniques known to those skilled in the art. Herbicides can be applied at any point in the plant cultivation process. For example, herbicides can be applied pre-planting, at planting, pre-emergence, post-emergence or combinations thereof. Herbicides may be applied to seeds and dried to form a layer on the seeds.

[0223] In some embodiments, seeds are treated with a safener, followed by a post-emergent application of a saflufenacil. In one embodiment, the post-emergent application of

the saflufenacil is about 7 to 10 days following planting of safener-treated seeds. In some embodiments, the safener is cloquintocet, dichlormid, fluxofenim, or combinations thereof.

[0224] In other aspects, the present invention provides a method for controlling weeds at a locus for growth of a plant or plant part thereof, the method comprising: applying a composition comprising a saflufenacil to the locus.

[0225] In some aspects, the present invention provides a method for controlling weeds at a locus for growth of a plant, the method comprising: applying an herbicide composition comprising saflufenacil to the locus; wherein said locus is: (a) a locus that contains: a plant or a seed capable of producing said plant; or (b) a locus that is to be after said applying is made to contain the plant or the seed; wherein the plant or the seed comprises in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to the saflufenacil.

[0226] Herbicide compositions hereof can be applied, *e.g.*, as foliar treatments, soil treatments, seed treatments, or soil drenches. Application can be made, *e.g.*, by spraying, dusting, broadcasting, or any other mode known useful in the art.

[0227] In one embodiment, herbicides can be used to control the growth of weeds that may be found growing in the vicinity of the herbicide-tolerant plants invention. In embodiments of this type, an herbicide can be applied to a plot in which herbicide-tolerant plants of the invention are growing in vicinity to weeds. An herbicide to which the herbicide-tolerant plant of the invention is tolerant can then be applied to the plot at a concentration sufficient to kill or inhibit the growth of the weed. Concentrations of herbicide sufficient to kill or inhibit the growth of weeds are known in the art and are disclosed above.

[0228] In other embodiments, the present invention provides a method for controlling weeds in the vicinity of a saflufenacil-tolerant plant of the invention. The method comprises applying an effective amount of a saflufenacil to the weeds and to the auxinic herbicide-tolerant plant, wherein the plant has increased tolerance to auxinic herbicide when compared to a wild-type plant. In some embodiments, the saflufenacil-tolerant plants of the invention

are preferably crop plants, including, but not limited to, sunflower, alfalfa, *Brassica sp.*, soybean, cotton, safflower, peanut, tobacco, tomato, potato, wheat, rice, maize, sorghum, barley, rye, millet, and sorghum.

[0229] In other aspects, herbicide(s) (*e.g.*, saflufenacil) can also be used as a seed treatment. In some embodiments, an effective concentration or an effective amount of herbicide(s), or a composition comprising an effective concentration or an effective amount of herbicide(s) can be applied directly to the seeds prior to or during the sowing of the seeds. Seed Treatment formulations may additionally comprise binders and optionally colorants.

[0230] Binders can be added to improve the adhesion of the active materials on the seeds after treatment. In one embodiments, suitable binders are block copolymers EO/PO surfactants but also polyvinylalcoholsl, polyvinylpyrrolidones, polyacrylates, polymethacrylates, polybutenes, polyisobutylenes, polystyrene, polyethyleneamines, polyethyleneamides, polyethyleneimines (Lupasol®, Polymin®), polyethers, polyurethans, polyvinylacetate, tylose and copolymers derived from these polymers. Optionally, also colorants can be included in the formulation. Suitable colorants or dyes for seed treatment formulations are Rhodamin B, C.I. Pigment Red 112, C.I. Solvent Red 1, pigment blue 15:4, pigment blue 15:3, pigment blue 15:2, pigment blue 15:1, pigment blue 80, pigment yellow 1, pigment yellow 13, pigment red 112, pigment red 48:2, pigment red 48:1, pigment red 57:1, pigment red 53:1, pigment orange 43, pigment orange 34, pigment orange 5, pigment green 36, pigment green 7, pigment white 6, pigment brown 25, basic violet 10, basic violet 49, acid red 51, acid red 52, acid red 14, acid blue 9, acid yellow 23, basic red 10, basic red 108.

[0231] The term seed treatment comprises all suitable seed treatment techniques known in the art, such as seed dressing, seed coating, seed dusting, seed soaking, and seed pelleting. In one embodiment, the present invention provides a method of treating soil by the application, in particular into the seed drill: either of a granular formulation containing the saflufenacil as a composition/formulation (*e.g.*, a granular formulation), with optionally one or more solid or liquid, agriculturally acceptable carriers and/or optionally with one or more agriculturally acceptable surfactants. This method is advantageously employed, for example, in seedbeds of cereals, maize, cotton, and sunflower.

[0232] The present invention also comprises seeds coated with or containing with a seed treatment formulation comprising saflufenacil and at least one other herbicide such as, *e.g.*, an AHAS-inhibitor selected from the group consisting of amidosulfuron, azimsulfuron, bensulfuron, chlorimuron, chlorsulfuron, cinosulfuron, cyclosulfamuron, ethametsulfuron, ethoxysulfuron, flazasulfuron, flupyrsulfuron, foramsulfuron, halosulfuron, imazosulfuron, iodosulfuron, mesosulfuron, metsulfuron, nicosulfuron, oxasulfuron, primisulfuron, prosulfuron, pyrazosulfuron, rimsulfuron, sulfometuron, sulfosulfuron, thifensulfuron, triasulfuron, tribenuron, trifloxysulfuron, triflusulfuron, tritosulfuron, imazamethabenz, imazamox, imazapic, imazapyr, imazaquin, imazethapyr, cloransulam, diclosulam, florasulam, flumetsulam, metosulam, penoxsulam, bispyribac, pyriminobac, propoxycarbazone, flucarbazone, pyribenzoxim, pyriftalid and pyrithiobac.

[0233] The term "coated with and/or containing" generally signifies that the active ingredient is for the most part on the surface of the propagation product at the time of application, although a greater or lesser part of the ingredient may penetrate into the propagation product, depending on the method of application. When the said propagation product is (re)planted, it may absorb the active ingredient.

[0234] In some embodiments, the seed treatment application with saflufenacil or with a formulation comprising the saflufenacil is carried out by spraying or dusting the seeds before sowing of the plants and before emergence of the plants.

[0235] In other embodiments, in the treatment of seeds, the corresponding formulations are applied by treating the seeds with an effective amount of saflufenacil or a formulation comprising the saflufenacil.

[0236] In other aspects, the present invention provides a method for combating undesired vegetation or controlling weeds comprising contacting the seeds of the saflufenacil-tolerant plants of the present invention before sowing and/or after pregermination with saflufenacil. The method can further comprise sowing the seeds, for example, in soil in a field or in a potting medium in greenhouse. The method finds particular use in combating undesired vegetation or controlling weeds in the immediate vicinity of the seed. The control of undesired vegetation is understood as the killing of weeds and/or otherwise retarding or

inhibiting the normal growth of the weeds. Weeds, in the broadest sense, are understood as meaning all those plants which grow in locations where they are undesired.

[0237] The weeds of the present invention include, for example, dicotyledonous and monocotyledonous weeds. Dicotyledonous weeds include, but are not limited to, weeds of the genera: *Sinapis, Lepidium, Galium, Stellaria, Matricaria, Anthemis, Galinsoga, Chenopodium, Urtica, Senecio, Amaranthus, Portulaca, Xanthium, Convolvulus, Ipomoea, Polygonum, Sesbania, Ambrosia, Cirsium, Carduus, Sonchus, Solanum, Rorippa, Rotala, Lindernia, Lamium, Veronica, Abutilon, Emex, Datura, Viola, Galeopsis, Papaver, Centaurea, Trifolium, Ranunculus, and Taraxacum.*

[0238] Monocotyledonous weeds include, but are not limited to, weeds of the genera: *Echinochloa, Setaria, Panicum, Digitaria, Phleum, Poa, Festuca, Eleusine, Brachiaria, Lolium, Bromus, Avena, Cyperus, Sorghum, Agropyron, Cynodon, Monochoria, Fimbristyslis, Sagittaria, Eleocharis, Scirpus, Paspalum, Ischaemum, Sphenoclea, Dactyloctenium, Agrostis, Alopecurus, and Apera.*

[0239] In addition, the weeds of the present invention can include, for example, crop plants that are growing in an undesired location. For example, a volunteer maize plant that is in a field that predominantly comprises soybean plants can be considered a weed, if the maize plant is undesired in the field of soybean plants.

[0240] In other embodiments, in the treatment of seeds, the corresponding formulations are applied by treating the seeds with an effective amount of saflufenacil or a formulation comprising the saflufenacil.

[0241] In still further aspects, treatment of loci, plants, plant parts, or seeds of the present invention comprises application of an agronomically acceptable composition that does not contain an A.I. In one embodiment, the treatment comprises application of an agronomically acceptable composition that does not contain a saflufenacil A.I. In some embodiments, the treatment comprises application of an agronomically acceptable composition that does not contain a saflufenacil A.I., wherein the composition comprises one or more of agronomically-acceptable carriers, diluents, excipients, plant growth regulators, and the like. In other embodiments, the treatment comprises application of an agronomically acceptable

composition that does not contain a saflufenacil A.I., wherein the composition comprises an adjuvant. In one embodiment, the adjuvant is a surfactant, a spreader, a sticker, a penetrant, a drift-control agent, a crop oil, an emulsifier, a compatibility agent, or combinations thereof.

[0242] It will be readily apparent to one of ordinary skill in the relevant arts that other suitable modifications and adaptations to the methods and applications described herein are obvious and can be made without departing from the scope of the invention or any embodiment thereof. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included herewith for purposes of illustration only and are not intended to be limiting of the invention.

[0243] While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims. All patents and publications cited herein are entirely incorporated herein by reference.

[0244] **EXAMPLES**

[0245] **Example 1**

[0246] **Expression analysis of safener and saflufenacil-induced monocot P450 genes**

[0247] Experiments were designed in rice, wheat, and maize to identify P450 genes induced by safener and/or saflufenacil application. Ten seeds were sown per 4 inch pot filled with Midwest soil and allowed to germinate for 4-5 days in standard greenhouse conditions. Once emergence from the soil was apparent, seedlings were sprayed according to the protocol shown in Table 15.

[0248] Table 15. Spray protocol for wheat, rice, and maize seedlings used for P450 expression analysis.

Sample	treatment	methylated seed oil adjuvant	safener (500 g ai/ha)	saflufenacil (g ai/ha)
wheat	1	0	0	0

	2	0.1% v/v	0	0
	3	0.1% v/v	cloquintocet	0
	4	0.1% v/v	dichlormid	0
	5	0.1% v/v	0	400
	6	0.1% v/v	0	800
	7	0.1% v/v	cloquintocet	400
	8	0.1% v/v	cloquintocet	800
	9	0.1% v/v	dichlormid	400
	10	0.1% v/v	dichlormid	800
rice	1	0	0	0
	2	0.1% v/v	0	0
	3	0.1% v/v	cloquintocet	0
	4	0.1% v/v	dichlormid	0
	5	0.1% v/v	0	400
	6	0.1% v/v	0	800
	7	0.1% v/v	cloquintocet	400
	8	0.1% v/v	cloquintocet	800
	9	0.1% v/v	dichlormid	400
	10	0.1% v/v	dichlormid	800
corn	1	0	0	0
	2	0.1% v/v	0	0
	3	0.1% v/v	dichlormid	0
	4	0.1% v/v	0	400
	5	0.1% v/v	0	800
	6	0.1% v/v	dichlormid	400
	7	0.1% v/v	dichlormid	800

[0249] There were 7-10 independent treatments per experiment, each performed in duplicate. Twenty-four hours after treatment, all tissue above the seed coat was collected and flash frozen in liquid Nitrogen. Tissue was subsequently ground while frozen and RNA was isolated using a modified Gentra Systems Versagene protocol. RNA was labeled and hybridized to wheat, rice, or maize gene chip arrays according to standard Affymetrix protocols. Data was RMA normalized, background subtracted, and quantile normalized.

[0250] Probesets corresponding to potential P450 genes were identified via sequence homology searches that compared the translated nucleotide sequence of the probeset to the amino acid sequences of known P450s in rice and Arabidopsis (blastx program). Sequences were considered potential P450s when the criteria were met of at least 50% identity within a length of at least 40 amino acids. A total of 349 wheat and 460 maize P450 target sequences

were identified. Genedata Expressionist software was used to generate ANOVA tables with gene annotation. Differential expression tests were conducted using SAS9.1.

[0251] For maize, 43 out of the 510 P450 probesets were differentially expressed at an FDR of $\leq$ 0.05 level. The probesets that were significantly differentially expressed and displayed a 4-10-fold induction upon treatment over unsprayed and/or adjuvant only controls were identified and targeted for subsequent cloning and transformation into various plant systems described below. Graphs indicating expression of the rice and maize P450 genes with the greatest fold induction are displayed in Figure 1. Based on these data, 9 rice genes and 4 maize genes were chosen for testing in rapid tolerance plant assays. Although several wheat transcripts were induced upon treatment, only one gene had known full-length sequence, TaCYP709C1. Its closest homolog in rice is OsjCYP72A1. For this reason, only this one gene from wheat was included in subsequent analyses. The amino acid sequences of the gene products identified in this screen were compared and a relational tree is indicated in Figure 2. A pairwise table of the sequences disclosed herein as conferring tolerance to saflufenacil is also provided in Table 16.

[0252] Table 16. Alignment table of P450 genes found to confer tolerance to saflufenacil as described in this document. Amino acid sequence identity is indicated as a percent of global alignment.

[0253] Table 16. Sequence identity table.

	NSF1	OsCYP72A15	OsCYP73A38	OsjCYP81A6
NSF1		13	23	47
OsCYP72A15			19	20
OsCYP73A38				30
OsjCYP81A6				

[0254] The selected monocot P450 genes were cloned into monocot and dicot plant transformation vectors using standard cloning procedures. P450 expression was driven by the constitutive Maize Ubiquitin promoter for monocot expression and the constitutive Parsley Ubiquitin promoter for dicot expression.

[0255] **Example 2**

[0256] Maize immature embryos (ies: JHAX(HiIIXA188) were isolated from surface sterilized cobs (9-10 days post pollination) and cultured for 1 week on M-MS-101 media (Table 17).

[0257] Table 17. Maize media.

Ingredients	Supplier	M-MS-101	M-LS-213	M-LS-513	M-LS-616	M-MS-710	R-N6-100	M-LS-002	M-LS-014
MS Salts M-5524	Phytotech	4.3 g/L	4.3 g/L	4.3 g/L	2.4 g/L	4.3 g/L		4.3 g/L	4.3 g/L
N6 Salts	Duchefa						3.96 g/L		
N6 vitamins 1000x soln	Duchefa						1x		
Sucrose	VWR	30 g/L	20 g/L	30 g/L	20 g/l	30 g/L		68.5 g/L	20 g/L
Glucose	VWR							36 g/L	10 g/L
Maltose	VWR						30 g/L		
Casamino Acid	BD						300 mg/L	1 g/L	
Nicotinic acid	Sigma		0.5 mg/L	0.5 mg/L	0.5 mg/L	0.5 mg/L		0.5 mg/L	0.5 mg/L
Pyridoxine HCl	Sigma		0.5 mg/L	0.5 mg/L	0.5 mg/L	0.5 mg/L		0.5 mg/L	0.5 mg/L
Thiamine HCl	Sigma		1 mg/L	1 mg/L	1 mg/L	1 mg/L		1 mg/L	1 mg/L
Myo-inositol	Sigma		100 mg/L	100 mg/L	100 mg/L	100 mg/L		100 mg/L	100 mg/L
L-Proline	Sigma	2.9 g/L	700 gm/L	700 gm/L		1.16 g/L	2.9 g/L		700 gm/L
MES	Sigma		500 mg/L	500 mg/L	500 mg/L	500 mg/L	500 mg/L		500 mg/L
Casein hydrolysate	Sigma	100 mg/L				1 g/L			
L-Asparagine monohydrate	Sigma					150 mg/L			
--> pH		5.8	5.8	5.8	5.8	5.7	5.8	5.2	5.8
Gelrite	Duchefa	2.0 g/L			2.0 g/L				
Purified Agar	Sigma		8.0 g/L	8.0 g/L		8.0 g/L			10 g/L

--> Autoclave (min)		20	20	20	20	20	20		20
2,4-D	Sigma	1.5 mg/L	0.5 mg/L			1 mg/L	2 mg/L	1.5	1.0 mg/L
MS Vitamins	Sigma	1.0mg/ mL							
Silver Nitrate	VWR	15µM							15 µM
Timentin	Bellamy DS	150mg/ L							
Picloram	Sigma		2 mg/L						
Kinetin	Sigma			0.5 mg/L					
Acetosyringone	Aldrich							200 µM	200 µM

[0258] Embryos with embryogenic callus were randomly distributed to 0, 50, 100, 250, 500, 750 or 1000 nM saflufenacil containing media (Modified M-LS-213: Table 17). Response was rated as stated above. Two plates were utilized/level/condition and 10 calli/plate. Growing callus was further transferred to regeneration media (modified M-LS-513: Table 17) with the above levels of saflufenacil. Following shoot formation, growing shoots were transferred to rooting boxes containing M-LS-616 (Table 17) with the above mentioned saflufenacil concentrations and additional concentrations of 2 and 3 µM.

[0259] **Example 3**

[0260] **Maize immature embryo transformation and response to herbicide**

[0261] Immature Embryo Transformation: Immature embryos were transformed according to the procedure outlined in WO2006/136596 to Peng *et al.* The exception was that a proportion of the embryos – 25 in most cases – were placed on media containing saflufenacil and kept in the light for direct selection. The remaining embryos in each experiment were selected on Pursuit as described. Plants were tested utilizing Taqman analysis with the target being the *nos* terminator which is present in all constructs. Healthy looking plants were sent to the greenhouse for hardening out and subsequent spray testing.

As with the BMS cells, RCB958 was utilized as a control both for transformation, saflufenacil selection, and for spray testing.

[0262] A total of 82 transgenic maize plants (2 constructs of interest and 1 control construct) were delivered to the greenhouse in tissue culture. The plants were individually transplanted into MetroMix 360 soil in 4" pots. Once they had been in the greenhouse (day/night cycle of 27°C /21°C with 14 hour day length supported by 600W high pressure sodium lights), they were allowed to grow for 14 days. They were then sprayed with a treatment of [200 g ai/ha] saflufenacil +1.0% v/v MSO. Herbicide injury evaluations were taken both 2 and 7 days Postspray to look for injury to new growth points and overall plant health. The top survivors were transplanted into gallon pots filled with MetroMix 360 for seed production.

[0263] T0 transgenic plants representing independent events indicated in Figure 3 were transplanted to the greenhouse and sprayed as indicated in the methods section. At just two days postspray most plants showed severe damage to their growth points, and major stress damage to leaf tissue where the herbicide made contact. However, several independent transgenic events expressing OsCYP72A15, OsjCYP81A6, and OsCYP73A38 showed increased tolerance as indicated by injury scores of 2 and 3.

[0264] **Example 4**

[0265] **Soybean transformation and root response to herbicide**

[0266] Soybean cv William 82 was used in all these experiments. Seeds were sterilized and germinated in seed germination medium for 7 d as described by Hong *et al.*, In Vitro Cell. Dev. Biol. Plant 2007: 43: 558-568 (2007). Epicotyls were completely removed from the seedlings, and hypocotyls 0.5 to 1 cm below the cot-node were cut. The cut surface of the hypocotyls was the target for *Agrobacterium* infection and root regeneration. Disarmed *Agrobacterium rhizogenes* SHA17 (Mankin *et al.*, In Vitro Cell. Dev. Biol. Plant, 43: 521-535 (2007)) harboring all the binary vectors such as RTP3137 was cultured on YEP growth medium containing 50 mg/L kanamycin for 2-4 d. Hypocotyl cut ends prepared as above were dipped onto the *Agrobacterium* cells on YEP plates, and then placed on 1% agar plate

(100 x 25 mm). Plates were sealed with parafilm or 3MM tape. The hypocotyl explants were co-cultured with *Agrobacterium* for 7 to 10 d under light. After 7 to 10 d co-cultivation, hypocotyl explants were transferred to root induction medium with arsenal (imazapyr) selection (root induction medium contained ½ MS salts and ½ B5 vitamins, 20 g/l sucrose, 7 g/l purified agar, 1 mg/l naphthaleneacetic acid (NAA), 400 mg/l timentin and 1 µM arsenal). After 3 d for root induction medium, hypocotyls were transferred to root growth medium without growth regulators containing 1/5 MS salts and 1/5 B5 vitamins, 20 g/l sucrose, 400 mg/l timemtin, 1 µM arsenal and 7 g/l gelrite for root elongation. Elongated roots were usually obtained after 12 to 20 d on this media.

[0267] Saflufenacil kill curves were determined in a preliminary experiment by culturing *Agrobacterium* infected hypocotyl explants on media supplemented with various amount of saflufenacil (0, 25, 50, 75, 200, 400, 600, 800, 1000 nM). In this experiment, SHA17 containing a control construct was used. Saflufenacil at 400 nM was chosen as the optimal concentration to test for tolerance.

[0268] *Root growth assay*: Hypocotyl explant containing transgenic roots (cultured on root growth medium for 10 to 12 d) were transferred to saflufenacil-containing medium (1/5 MS salts and 1/5 B5 vitamins, 20g/L sucrose, 200 mg/L timemtin, 7 g/L gelrite and 400 nM saflufenacil). The roots were pushed into the medium. After 2 to 3 week culture, the root growth was recorded and data were collected using the construct with AHAS II only as the control. If the roots further elongated and produced secondary roots, and root tips remained white or creamy yellow, the roots were considered as tolerant to saflufenacil.

[0269] *Root penetration assay*: Hypocotyl explant containing transgenic roots (cultured on root growth media for 20 d) were transferred to the same medium as above, but the roots were placed on the surface of the medium, instead of inside of the medium. Roots which penetrated into the media with white root tips and elongated for to minimum 0.5 cm in length were considered as tolerant to saflufenacil.

[0270] To date 18 constructs containing various P450 GOIs were assayed using the two methods as described above, roots containing construct RTP3173 (GOI: ZmNSF1) showed tolerance to saflufenacil based on both root growth and root penetration ability. Roots

containing construct RTP 3155 (GOI: OsjCYP72A1) showed slight tolerance compared to the control (Table 18).

[0271] Table 18. Summary of saflufenacil tolerance experiments using soy TRAP roots.

Constructs	Explants	Explants with strong roots	Strong root penetrated to media	Tolerance rate
	number			
Experiment 1				
NH4-5 (ck)	15	0	0	0
RTP3173	15	4	3	+++
RTP3034	15	1	0	0
RTP3155	15	3	1	+
RTP3435	15	0	0	0
RTP3432	15	0	0	0
Experiment 2				
NH4-5	20	0	0	0
RTP3237	20	0	0	0
RTP3235	20	0	0	0
RTP3233	20	0	0	0
RTP3157	20	0	0	0
RTP2681	20	0	0	0
Experiment 3				
NH4-5	30	0	0	0
RTP3155	30	2	0	+
RTP3173	30	6	5	+++
RTP2549	30	0	0	0
Experiment 4				
NH4-5	30	0	0	0
RTP3036	30	0	0	0
RTP3239	30	0	0	0
RTP3438	30	0	0	0

RTP3035	30	0	0	0

[0272] The association of vector names and expression cassettes are shown in Table 19.

[0273] Table 19. Association of vector names and P450 expression cassettes.

Vector name	Promoter	P450 GOI
RTP3034	p-PcUBI4-2	OsjCYP72A15
RTP3035	p-PcUBI4-2	OsjCYP81A6
RTP3036	p-PcUBI4-2	OsjCYP73A38
RTP3155	p-PcUBI4-2	OsjCYP72A1
RTP3156	p-PcUBI4-2	TaCYP709C1
RTP3157	p-PcUBI4-2	Osj07g0635500
RTP3167	p-ZmUBI+I	OsjCYP72A15
RTP3168	p-ZmUBI+I	OsjCYP81A6
RTP3169	p-ZmUBI+I	OsjCYP73A38
RTP3170	p-ZmUBI+I	OsjCYP72A1
RTP3171	p-ZmUBI+I	TaCYP709C1
RTP3172	p-ZmUBI+I	Osj07g0635500
RTP3173	p-PcUBI4-2	ZmNSF1
RTP3174	p-ZmUBI+I	ZmNSF1
RTP3232	p-ZmUBI+I	Os02g0467600
RTP3233	p-PcUBI4-2	Os02g0467600
RTP3234	p-ZmUBI+I	Os06g0569500
RTP3235	p-PcUBI4-2	Os06g0569500
RTP3236	p-ZmUBI+I	Os08g0508000
RTP3237	p-PcUBI4-2	Os08g0508000
RTP3238	p-ZmUBI+I	Os12g0150200
RTP3239	p-PcUBI4-2	Os12g0150200
RTP3431	p-ZmUBI+I	ZM1s60596158
RTP3432	p-PcUBI4-2	ZM1s60596158
RTP3434	p-ZmUBI+I	ZM4s40785
RTP3435	p-PcUBI4-2	ZM4s40785
RTP3437	p-ZmUBI+I	ZM1s57311919
RTP3438	p-PcUBI4-2	ZM1s57311919
RTP3545	p-ZmUBI+I	OsICYP81A6
RTP3546	p-PcUBI4-2	OsICYP81A6

[0274] None of the other 16 constructs exhibited tolerance in this bioassay using this transgenic soy root assay.

[0275] References

[0276] Didierjean *et al.*, Plant Physiol. 130:179-189 (2002).

[0277] Siminszky et al., Phytochem. Rev. 5:445-458 (2006).

[0278] Nelson et al., Phytochem. Rev. 5:193-204 (2006).

[0279] Williams *et al.*, "Map-based cloning of the nsf1 gene of maize," In: Program and Abstracts of the 48th Maize Genetic Conference, Pacific Grove, California, USA (2006).

[0280] WO 2007/103567 to Dam *et al.*

[0281] Riechers *et al.*, Personal communication: Biochemical mechanisms for tolerance to BAS-800H in corn and soybeans. BASF AP Kixor™ Metabolism meeting, RTP NC USA (2008).

[0282] Bell *et al.*, Biochem. Soc. Trans. 31:558-562 (2003).

[0283] Abecassis *et al.*, Biocatal. Biotrans. 21:55-66 (2003).

[0284] Siminszky *et al.*, Pestic. Biochem. Physiol. 77:35-43 (2003).

[0285] Hiei and Komari, Nat Protoc. 3: 824-34 (2008).

[0286] WO2006/136596 to Peng *et al.*

[0287] **Example 6**

[0288] **CYP450 amino acid and nucleic acid sequences**

[0289] Examples of CYP450 amino acid and nucleic acid sequences are shown in Table 20.

[0290] Table 20. CYP450 sequences.

OsICYP81A6 (SEQ ID NO:28)

atggataacgcctacattattgccattctctctgtagctatcctcttcttgctccactactacctcctcggccgcgg
caatggcggggcggcgcggctgccgccgggtccaccggccgtcccgatcctgggacacctccacctcgtcaagaagc
cgatgcacgccaccatgtcccgcctcgccgagcggtacgggccggtgttctcgctgcgcctcgggtcgcggcgtgcc
gtggtggtgtcgtcgccggggtgcgccagggagtgcttcaccgagcacgacgtgaccttcgcgaaccggcccaggtt
cgagtcgcagctgctggtctcgttcaacggcgccgcgctcgccacggcgagctacggcgcgcactggcgcaacctcc
gccggatcgtcgccgtgcagctgctctccgcgcaccgcgtcggcctcatgtcggggctcatcgccggcgaggtccgc
gccatggtgcggaggatgtaccgcgccgcggccgcgtcccccgccggcgccgcgcgcatccagctgaagcggaggct
gttcgaggtctccctcagcgtgctcatggagaccatcgcccacaccaaggcgacccgccccgagacggacccggaca
ccgacatgtccgtggaagcccaggagtttaagcaggtcgtcgacgagatcatcccgcacatcggcgcggccaacctg
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tgatcgccgtgctgctcactctgcagaagacagagccggaggtgtacaccgataacatgatcacagctctaacggcg

aacttgttcggagcaggaacagagacaacctcgacgacatcagaatgggcgatgtcgctactgctgaaccaccccga
cacactcaagaaagcgcaagccgagatcgacgcatccgtcggcaactctcgcctgatcaccgccgacgacgtgactc
gcctcggctacctccagtgcatcgtcagggagacgctccgcctgtaccccgccgcgccgatgctcctcccgcacgag
tcctccgccgactgcaaggtcggcggctacaacatcccgcgcgggtcgatgttgctcatcaacgcgtacgccatcca
ccgtgacccggcggtgtgggaggagccggagaagttcatgccggagaggttcgaggacggcgggtgcgacggcaatc
tcttgatgccgttcgggatggggaggcggaggtgccccggcgagacgctggcgctgcgcacagtggggttggtgctg
ggcacgctgatccagtgcttcgactgggagagggtcgacggcgtggaggtcgacatgactgaaggtggcgggctcac
catccccaaggtcgtgccgttggaggccatgtgcaggccgcgcgacgccatgggtggtgttcttcgcgagctcgtct
ga

OsICYP81A6(SEQ ID NO:29)

Mdnayiiailsvailfllhyyllgrgnggaarlppgppavpilghlhlvkkpmhatmsrlaerygpvfslrlgsrra
vvvsspgcarecftehdvtfanrprfesqllvsfngaalatasygahwrnlrrivavqllsahrvglmsgliagevr
amvrrmyraaaaspagaariqlkrrlfevslsvlmetiahtkatrpetdpdtdmsveaqefkqvvdeiiphigaanl
wdylpalrwfdvfgvrrkilaavsrrdaflrrlidaerrrlddgdegekksmiavlltlqktepevytdnmitalta
nlfgagtettsttsewamslllnhpdtlkkaqaeidasvgnsrlitaddvtrlgylqcivretlrlypaapmllphe
ssadckvggyniprgsmllinayaihrdpavweepekfmperfedggcdgnllmpfgmgrrrcpgetlalrtvglvl
gtliqcfdwervdgvevdmtegggltipkvvpleamcrprdamggvlrelv

OsjCYP81A6 (SEQ ID NO:30)

atggcgttcttgggatgggcggtcgacatcgcccgcgactccggcgcgtcgagctccgtcgtgctcacctgcgacgg
ctacggctcggcgctctacttctcgccgtgggacagtgtcccgcttccggcgacagcttcccccgacgacggcttcc
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tccgcctgtaccccgccgcgccgatgctcctcccgcacgagtcctccgccgactgcaaggtcggcggctacaacatc
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catgccggagaggttcgaggacggcgggtgcgacggcaatctcttgatgccgttcgggatggggaggcggaggtgcc
ccggcgagacgctggcgctgcgcacagtggggttggtgctgggcacgctgatccagtgcttcgactgggagagggtc
gacggcgtggaggtcgacatgactgaaggtggcgggctcaccatccccaaggtcgtgccgttggaggccatgtgcag
gccgcgcgacgccatgggtggtgttcttcgcgagctcgtctga

Sb01g007420.1 (codon optimized for yeast expression) (SEQ ID NO:31)

atggataaggcctatgtcgccgttttatccttcgccttcttattcgtattacattatttggttggaagagcaggcgg
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tcttaggacatttgcatttagtaaagactccttttcatgaggccttagcaggcttagcagctaggcatggcccagta
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accgatactatgataatggctttgtgcggtaatttatttggagccggaactgaaaccacatcttctactactgagtg
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ccagcagcacctttgttgttgccacacgagagtgctgctgattgcaaggtaggtggctacgatgtaccaagaggcac
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ggtttgaagatggtaaggctgaaggtagattgttgatgccttttggtatgggtagaagaaagtgccctggtgaaaca
ttggcattaaggacagtgggtttagttttaggtacattgattcaatgtatcgactgggatagagtggatggtttgga
aattgatatgacagctggtggtggtttgaccatgcctagggctgtgcccttggaagctacctgtaaacccagggcag
ctatgagggatgtgttgatggaattgtaa

Sb01g007420.1(SEQ ID NO:32)

MDKAYVAVLSFAFLFVLHYLVGRAGGNGRKGNNGKGNAAQQRLPPSPPAVPFLGHLHLVKTPFHEALAGLAARHGPV
FSMRMGSRGAVVVSSPECAKECFTEHDVAFANRPRFATQELVSFGGAALATASYGPYWRNLRRVAAVQLLSAHRVAC
MSSVISAEVRAMVRRMSRAAAAAPDGAARVQLKRRLFEVSLSVLMETIAQTKTSRTEADADTDMSPEAHEFKQIVDE
IVPHLGTANLWDYLPVLQWFDVFGVRNKIMAAVSRRDAFLRRLIDAERRRMDDGGDSDKKSMIAVLLSLQKSEPELY
TDTMIMALCGNLFGAGTETTSSTTEWAMSLLLNHPEALKKAQAEIDAVVGNSRLITAEDVPRLGYLQCVINETLRMY
PAAPLLLPHESAADCKVGGYDVPRGTLLIVNAYAIHRDPAVWEDPAEFRPERFEDGKAEGRLLMPFGMGRRKCPGET
LALRTVGLVLGTLIQCIDWDRVDGLEIDMTAGGGLTMPRAVPLEATCKPRAAMRDVLMEL

OsCYP73A38(SEQ ID NO:33)

atggacgccctcctcgtggagaaggtcctcctgggcctgttcgtggcggcggtgctggccctagtggtggccaagct
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tcggccgccgcagctgccccgggatcatcctcgcgctgcccatcatcgggatcacgctcggccgcctcgtccagagc
ttcgacctgctgccgccgcccgggatggacaaggtggacaccaccgagaagcccggccagttcagcaaccagatcct
caagcacgccaccgtcgtctgcaagcccatcgacgcctag

Rice: Q94HA3_ORYSJ (SEQ ID NO:34)

MVKAYIAIFSIAVLLLIHFLFRRRGRSNGMPLPPSPPAIPFFGHLHLIDKPFHAALSRLAERHGPVFSLRLGSRNAV

VVSSPECARECFTDNDVCFANRPRFPSQMLATFNGTSLGSANYGPHWRNLRRIATVHLLSSHRVSGMSGIISGQARH

MVRRMYRAATASAAGVARVQLNRRLFELSLSVLMEAIAQSKTTRREAPDADTDMSMEAQELRHVLDELNPLIGAANL

WDYLPALRWFDVFGVKRKIVAAVNRRNAFMRRLIDAERQRMDNNDVDGGDDGEKKSMISVLLTLQKTQPEVYTDTLI

MTLCAPLFGAGTETTSTTIEWAMSLLLNHPEILKKAQAEIDMSVGNSRLISVVDVHRLGYLQCIINETLRMYPAAPL

LLPHESSADCKVGGYHIPSGAMLLVNVAAIQRDPVIWKEPSEFKPERFENGRFEGLFMIPFGMGRRRCPGEMLALQT

IGLVLGTMIQCFDWGRVDDAMVDMTQSNGLTSLKVIPLEAMCKPREAMCDVLRKFM

Rice: A2XM72_ORYSI(SEQ ID NO:35)

MDKAYIAVFSIVILFLLVDYLRRLRGGGTSNGKNKGMRLPPGLPAVPIIGHLHLVKKPMHATLSRLAARHGPVFSLR

LGSRRAVVVSSPGCARECFTEHDVAFANRPRFESQLLMSFDGTALAMASYGPHWRNLRRVAAVQLLSARRVGLMSGL

IAGESKATRPETTDTDTDMSMEAQEYKQVVEEILERIGTGNLCDYLPALRWFDVFGVRNRILAAVSRRDAFLRRLIY

AARWRMDDGEKKSMIAVLLTLQKTQPEVYTDNMITALCSNLLGAGTETTSTTIEWAMSLLLNHPETLKKAQAEIDAS

VGNSRLITADDVPRITYLQCIVRETLRLYPAAPMLIPHESSADCEVGGYSVPRGTMLLVNAYAIHRDPAAWEEPERF

VPERFEGGGCDGNLSMPFGMGRRRCPGETLALHTVGLVLGTLIQCFDWERVDGVEVDMAEGGGLTMPKVVPLEAVCR

PRDAMGGVLREL

Rice: Q94HA4_ORYSJ(SEQ ID NO:36)

MDKAYIAVFSIAILFLLVDYFRCRRRRGSGSNNGENKGMLQLPPSPPAIPFFGHLHLIDKPLHAALSRLAERHGPVF
SLRLGSRNAVVVSSPECARECFTDNDVCFANRPQFPSQMPATFYGAGFGFANYGAHWRNLRRIATVHLLSAHRVRGM
AGVVSGEIRPMVQRMYRAAAAAGVGVARVQLKRRLFELSLSVLMEAIAQTKTTRPEADDADTDMSVEAQEFKNVLDE
LNPLLGAANLWDYLPALRVFDVLGVKRKIATLANRRDAFVRRLIDAERQRMDNGVDGGDDGEKKSVISVLLSLQKTE
PEVYKDIVIVNLCAALFAAGTETTAMTIEWAMSLLLNHPKILKKAKAEIDASVGNSRLINGDDMPHLSYLQCIINET
LRLYPVAPLLIPHESSADCKVNGYHIPSGTMLLVNVIAIQRDPMVWKEPNEFKPERFENGESEGLFMIPFGMGRRKC
PGETMALQTIGLVLGALIQCFDWDRVDGAEVDMTQGSGLTNPRAVPLEAMCKPREAMSDVFRELL

Rice: B9F5T6_ORYSJ(SEQ ID NO:37)

MKYSTSVTMDKAYIAVFSIVILFLLVDYLRRLRGGGTSNGKNKGMRLPPGLPAVPIIGHLHLVKKPMHATLSRLAAR
HGPVFSLRLGSRRAVVVSSPGCARECFTEHDVAFANRPRFESQLLMSFDGTALAMASYGPHWRNLRRVAAVQLLSAR
RVGLMSGLIAGEVRAMVRSLCRRPAAAAPVQLKRRLFELSLSVLMETIAQSKATRPETTDTDTDMSMEAQEYKQVVE
EILERIGTGNLCDYLPALRWFDVFGVRNRILAAVSRRDAFLRRLIYAARWRMDDGEKKSMIAVLLTLQKTQPEVYTD
NMITALCSNLLGAGTETTSTTIEWAMSLLLNHPETLKKAQAEIDASVGNSRLITADDVPRITYLQCIVRETLRLYPA
APMLIPHESSADCEVGGYSVPRGTMLLVNAYAIHRDPAAWEEPERFVPERFEGGGCDGNLSMPFGMGRRRCPGETLA
LHTVGLVLGTLIQCFDWERVDGVEVDMAEGGGLTMPKVVPLEAVCRPRDAMGGVLREL

Rice: Q94HA6_ORYSJ(SEQ ID NO:38)

MDKAYIAVFSIVILFLLVDYLRRLRGGGTSNGKNKGMRLPPGLPAVPIIGHLHLVKKPMHATLSRLAARHGPVFSLR
LGSRRAVVVSSPGCARECFTEHDVAFANRPRFESQLLMSFDGTALAMASYGPHWRNLRRVAAVQLLSARRVGLMSGL
IAGEVRAMVRSLCRRPAAAAPVQLKRRLFELSLSVLMETIAQSKATRPETTDTDTDMSMEAQEYKQVVEEILERIGT
GNLCDYLPALRWFDVFGVRNRILAAVSRRDAFLRRLIYAARWRMDDGEKKSMIAVLLTLQKTQPEVYTDNMITALCS
NLLGAGTETTSTTIEWAMSLLLNHPETLKKAQAEIDASVGNSRLITADDVPRITYLQCIVRETLRLYPAAPMLIPHE
SSADCEVGGYSVPRGTMLLVNAYAIHRDPAAWEEPERFVPERFEGGGCDGNLSMPFGMGRRRCPGETLALHTVGLVL
GTLIQCFDWERVDGVEVDMAEGGGLTMPKVVPLEAVCRPRDAMGGVLREL

Sorghum: C5X058_Sb01g007400(SEQ ID NO:39)

MDKAYIAVLSGAFLFLLHYLVGRAGSGGKGKGKGSQQLPPSPPAIPFLGHLYLVKAPFHAALARLAARHGPVFSLRM
GSRRAVVVSSPECAKECFTEHDLNFANRPLFPSLRLVSFNGAMFSVASYGPYWRNLRRVAATQLLSAHRVACMTPTI
AGEVRAMVQRMDHAAAAAPGGAARIQLKRRLFELSLSVLMETIAQTKTSRTEANADTDMSPEAHEFKQIIDELVPYL
GTANRWDYLPVLRWFDVFGVRNKILDAVSRRDAFLRRLIDGERRRLDDGSESEVKSMIAVLLTMQKSEPEVYTDTVI
IALCANLFLAGTETTSTTTEWAMSLLLNHPEVLKKAQAEIDAAVGTSRLVTADDVSRLTYLQCIINETLRLHPAAPL
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Sorghum: C5X059_Sb01g007410(SEQ ID NO:40)

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VVVSSPECAKACFTEYDQSFANRPHFQSMRLVSFDGAMLSVSSYGPYWRNLRRVAAVQLLSAHRVACMSPVIAAEVR
AMVRRMNRLAATSPGGAARVQLRRRLFELSLSVLMETIAQTKTSRSEAYADTDISPEANELTQISQEIMPYLGTANL
WDYLPFLRWFDVFGVRNKLMAAVRWRDAFLRRLIDAERRRLDDAGDSEKKSMLAVLLSLQKSEPELYTDTMIMALCG
DMFGAGTETTSSTTEWAMSLLLNHPEALNKARAEIDAVVGSSRLITPDDVPRLGYLHCVINETLRMYPAAPLLLPHE
SSADCNVGGYDVPRGTLLIVNAYAIHRDPAVWEDPAEFRPERFEDGKAEGRLLMPFGMGRRKCPGETLALRTVGLVL
GTLIQCFDWDRVDGVEIDMTEAGGLTMPRAVPLEATCKPRAAVSDVLKQL

Sorghum: C5X060_Sb01g007420(SEQ ID NO:41)

MDKAYVAVLSFAFLFVLHYLVGRAGGNGRKGNNGKGNAAQQRLPPSPPAVPFLGHLHLVKTPFHEALAGLAARHGPV
FSMRMGSRGAVVVSSPECAKECFTEHDVAFANRPRFATQELVSFGGAALATASYGPYWRNLRRVAAVQLLSAHRVAC
MSSVISAEVRAMVRRMSRAAAAAPDGAARVQLKRRLFEVSLSVLMETIAQTKTSRTEADADTDMSPEAHEFKQIVDE
IVPHLGTANLWDYLPVLQWFDVFGVRNKIMAAVSRRDAFLRRLIDAERRRMDDGGDSDKKSMIAVLLSLQKSEPELY
TDTMIMALCGNLFGAGTETTSSTTEWAMSLLLNHPEALKKAQAEIDAVVGNSRLITAEDVPRLGYLQCVINETLRMY
PAAPLLLPHESAADCKVGGYDVPRGTLLIVNAYAIHRDPAVWEDPAEFRPERFEDGKAEGRLLMPFGMGRRKCPGET
LALRTVGLVLGTLIQCIDWDRVDGLEIDMTAGGGLTMPRAVPLEATCKPRAAMRDVLMEL

Sorghum: C5X061_Sb01g007430(SEQ ID NO:42)

MEPAYVAILSFVFLFLLHRLFGRHRRRINGKNNRAQLPPSPPAIPVLGHLHLLGKKPIHAALARLAERYGPVFSLRL
GSREAVVVSSAACATECFTENDVCFANRPRFPTLLLVSFGGATLPMCRYGPYWRSIRRVATVHLLSAHRVSCMLPVI
SAEVRAMARRMYRSAAGGGAARVELKRRLFELSLSALMETIARTKMSRAVADDDTDMSPEAQEFMKALDVLLRLLSA
ANSWDYLPVLRWLDMFGVRNKILAAVSARDAFLRRLIDAERRRLEEGEGGENDEKKSMIGVLLSLQKSEPEVYTDTT
IMALCSSMFAGGSETTATTAEWAMSLLLSHPDVLKKAQAEIDASVGHSRLLGADDVPRLGYLQCIVTETLRLYPVVP
TLVPHESTADCTVGGHHVPSGTMLLVNVYAIHRDPATWADPAAFRPERFEDGGRAQGLFMMPFGMGRRKCPGEALAL
RTLGLVLGTLIQCFDWETVGGAEVDMAEGVGITLPRAVPLEAICKPRHAMLEVLKGL

Maize: B6SYC0_ZM_CYP81A3v2(SEQ ID NO:43)

MDKAYVAVLSVAFLFLVHYLVGRAAAGGGKGRKRLPPSPLAIPFLGHLHLVKTPFHSALGRLAERHGPVFSLRMGCR
RAVVVSSPECARACFTEHDQSFANRPRFESMRLVSFDGAMLSVSSYGPYWRTLRRVAAVQLLSAHRVACMSPVICAE
VRAMVRRMARLAAGGAARVQLRRRLFELSLGVLMETIARTKTSRSEACAADTDVSPEASELTRISEEIMPYLGTANL
WDYLPFLRWFDVFGVRKKLMAAVRWRDAFLRRLIDAERRRMDGDGDGEKKSMIAVLLSLQKSEPELYTETMIMALCG
DLFGAGTETTSVTTEWAMSLLLNHPEALKKAQAEIDAVVGNSRRLITADDVPRLGYLHCVINETLRMYPAAPLLLPH
ESAADCKVGGYDVPRGTLLIVNAYAIHRDPAVWEDPGRFVPERFEDGKAEGRLLMPFGMGRRKCPGETLALRTVGLV
LATLLQCFDWDTVDGAQVDMTESGGLTMPRAVPLEAMCKPRAAMCDVLREL

Maize: B6SSF2_ZM_CYP81A16(SEQ ID NO:44) MDKAYIAALSAAALFLLHYLLGRRAGGEGKTKGSQRRLPPSPPAIPFLGHLHLVKAPFHAALARLAARHGPVFSMRL GTRRAVVVSSPDCARECFTEHDVNFANRPLFPSMRLASFDGAMLSVSSYGPYWRNLRRVAAVQLLSAHRVACMAPAI EAQVRAMVRRMDRAAAAGGGGAARVQLKRRLFELSLSVLMETIAHTKTSRAEADADSDMSPEAHEFKQIVDELVPYI GTANRWDYLPVLRWFDVFGVRNKILDAVGRRDAFLRRLIDGERRRLDAGDDSESKSMIAVLLTLQKSEPEVYTDTVI TALCANLFGAGTETTSTTTEWAMSLLLNHREALKKAQAEIDAAVGTSRLVTADDVPHLTYLQCIVDETLRLHPAAPL LLPHESAADCTVGGYDVPRGTMLLVNVHAVHRDPAVWDDPDRFVPERFEGGKAEGRLLMPFGMGRRKCPGETLALRT VGLVLGTLLQCFDWDTVDGAQVDMKASGGLTMPRAVPLEAMCRPRTAMRDVLKRL
Brachypodium distachyon: Bradi_1g07930.1(SEQ ID NO:45) MDNAYIAALSLAFVFLLHYLLKGKRSNGGNLPPSPPAIPILGHLHLVEKPLHAALWRLAGRLGPVFSLRLGSRPVVV VSSPELAKECFTEHDVTFADRPQFPSQLLVSFGGTALATASYGPHWRNLRRVAAVHLLSAHRVAAMSSGVISAEVRA MARRLFRASADGSGGARVQLKRRLFELSLSVLMEAIAQTKATRPDDADGDDTDMSVEAQEFKKVVDEIIPHLGTANL WDYLPVLRWFDVFGVRNKILAAVRRRDAFLGRLIEAERRRLEEEGGGEGDQQGEKTSMIAVLLTLQKTEPEVYTDTM ITALCANLFGAGTETTSTTTEWAMSLLLNHPEVLKKAQAEMDASVGTSRLVTADDVAHRLPYLQHIVSETLRLYPAA PMLLPHQSSADCKIGGYTVPRGTMLLVNAYAIHRDPAAWGPAPEEFRPERFEDASNKGEELPLMLPFGMGRRKCPGE TLALRTVGMVLGTLVQCFEWERVGGVEVDMTQGTGLTMPKAVPLEAVCRPRAAMRDVLQKL
Brachypodium distachyon: Bradi_1g14900.1(SEQ ID NO:46) MDKAYIAVLSLAFLFLVHYLLGKINGNKQKTSKLQLPPSPPAIPFLGHLHLVETPFHLALRRLAARHGPVFYLRLGS RRAVVVSSAACARECFTEHDVTFANRPQFPSQQLVSFDGAGLAQSSYGPHWRNLRRVAAVQLLSAHRVACMSGVISA EVRAMARRLFRASASAPARVQLKRRLFELSLSVLMETIARTKGTRPEADADVDMSVEAQEFKKLVDEIVPHLGTANL WDYLPLLRWFDVMGVRNKILKLVRRRDVFLGRLIDAERRRLDEGGDGDDKKSMISVMLTLQKTEPELYTDTMIKSLC ANLFGAGTETTSTTTEWAMSLLLNHPEVLKKAQAEMDSCVGTSRLVSFDDVPRLAYLQCVLSETLRLYPAAPLLLPH HSSADTKVGGYDVPADTMLIVNAYAIHREPAGAWGERPEEFRPERFEDGKAEGAFMIPFGMGRRRCPGETLALRTVG MVLATLVQCFDWERVDGLEVDMAEGGGLTMPKVVPLEAVCTPRGTMLRVLREL

[0291] **Example 7**

[0292] **Saflufenacil Spray Tests**

[0293] *Maize whole plant transformation and saflufenacil tolerance testing:* Immature embryos were transformed according to the procedure outlined in Peng *et al. supra*. Plants were tested for the presence of the T-DNA by Taqman analysis with the target being the *nos* terminator which is present in all constructs. Healthy looking plants were sent to the greenhouse for hardening out and subsequent spray testing.

[0294] The plants were individually transplanted into MetroMix 360 soil in 4" pots. Once they had been in the greenhouse (day/night cycle of 27°C /21°C with 14 hour day length supported by 600W high pressure sodium lights), they were allowed to grow for 14 days. They were then sprayed with a treatment of [200 g ai/ha] saflufenacil +1.0% v/v methylated seed oil (MSO). Herbicide injury evaluations were taken both 2 and 7 days Postspray to look for injury to new growth points and overall plant health. The top survivors were transplanted into gallon pots filled with MetroMix 360 for seed production.

[0295] *Soybean transformation and saflufenacil tolerance testing:* Soybean cv Williams 82 was transformed as previously described by Siminszky *et al.*, Phytochem Rev. 5:445-458 (2006). After regeneration, transformants were transplanted to soil in small pots, placed in growth chambers (16 hr day/ 8 hr night; 25°C day/ 23°C night; 65% relative humidity; 130-150 $\mu E\ m^{-2}\ s^{-1}$) and subsequently tested for the presence of the T-DNA via Taqman analysis. After a few weeks, healthy, transgenic positive, single copy events were transplanted to larger pots and allowed to grow in the growth chamber. An optimal shoot for cutting was about 3-4" tall, with at least two nodes present. Each cutting was taken from the original transformant (mother plant) and dipped into rooting hormone powder (indole-3-butyric acid, IBA). The cutting was then placed in oasis wedges inside a bio-domc. The mother plant was taken to maturity in the greenhouse and harvested for seed. Wild type cuttings were also taken simultaneously to serve as negative controls. The cuttings were kept in the bio-dome for 5-7 days and then transplanted to 3" pots and then acclimated in the growth chamber for two more days. Subsequently, the cuttings were transferred to the greenhouse, acclimated for approximately 4 days, and then subjected to spray tests containing 0, 25, and 50 g ai/ha saflufenacil plus 1% MSO. Herbicide injury evaluations were taken at 7 days after treatment.

[0296] *Yeast expression system and analysis of saflufenacil metabolism:* Rice cytochrome P450 81A6 was cloned into pESC-His yeast expression vector (Stratagene) using BamH1 and Sal1. Constructs were transformed into YPH500 yeast strain (Stratagene) using a Yeast Maker Transformation System from Clontech. Positive clones were selected on SD-agar plates (0.7% yeast nitrogen base without amino acids, 2% glucose, 0.13% amino acid drop-out without histidine; plates solidified with 2% agar). Bioconversion of substrates was directly assayed in yeast cells expressing the respective cytochrome P450 gene. Cells were

induced in SG-His medium (same composition as SD but with galactose instead of glucose) for 18-24 h (Pompon *et al.*, Methods in Enzymology 272:51-64 (1996); Urban *et al.*, Eur. J. Biochem. 222:853-850 (1994)). Afterwards, the BAS800H substrate was added and samples were incubated for an additional 24h. Samples were analyzed by LC/MS/MS.

[0297] The association of vector names and the genes expressed therefrom is shown in Table 21.

[0298] Table 21. Association of vector names and the genes expressed therefrom.

gene	corn vector	soy vector	yeast vector
NSF1	RTP3174	RTP3173	RTP5743
OsICYP81A6	RTP3545	RTP3546	RTP5737
sorghum 81A6			RTP5789
OsCYP72A1	RTP3170	RTP3155	RTP5745
OsjCYP81A6	RTP3168	RTP3035	None

[0299] *Maize whole plant transformation and saflufenacil tolerance testing:*

[0300] Sixteen vectors harboring different P450 genes were tested for the ability to increase tolerance to saflufenacil in T0 maize plants. Figure 3 indicates the injury scores of those transformants treated with 200 g ai/ha saflufenacil + 1% MSO. OsCYP72A15 and OsCYP73A38 performed the best in maize with several events having injury scores of 3 or less. T1 seed was harvested from selected tolerant T0 plants and replanted and sprayed with 240 g ai/ha saflufenacil + 1% MSO at the 2-4 leaf stage (2 weeks post-sowing). Injury rating was evaluated 7 and 14 days after application. Figure 4 indicates the number of plants from each segregating event that were evaluated and scored for herbicidal injury. OsjCYP81A6 and OsICYP81A both conferred increased tolerance to the maize plants.as indicated by the reduced injury scores as compared to the J553 wild type germplasm alone.

[0301] *Soybean transformation and saflufenacil tolerance testing:*

[0302] Four vectors harboring different monocot P450s genes were tested for the ability to confer tolerance to saflufenacil in T0 soybean cuttings. Figures 5-7 indicate the level of

injury of those plants treated with 0, 25, or 50 g ai/ha saflufenacil + 1% MSO. In these assays, ZmNSF1 and OsjCYP81A6 conferred the greatest level of tolerance.

[0303] *Yeast expression system and analysis of saflufenacil metabolism:*

[0304] OsICYP81A6 and Sb01g007420.1 catalyzed degradation of BAS800H. After 24h incubation, 10% of the initial content of BAS800H was converted into several metabolites with majority (5%) being found as the inactive BAS800H02.

What is claimed is:

1. A plant or plant part comprising a recombinant polynucleotide encoding a cytochrome P450 polypeptide, the expression of which confers to the plant or plant part tolerance to saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

2. The plant or plant part of claim 1, wherein the CYP72A15 polypeptide is a rice polypeptide or an ortholog thereof, wherein the rice polypeptide has the amino acid sequence set forth in SEQ ID NO:7.

3. The plant or plant part of claim 1, wherein the isoform of CYP81A is a rice CYP81A6 polypeptide or an ortholog thereof, wherein the rice CYP81A6 polypeptide has the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:29.

4. The plant or plant part of claim 1, wherein the CYP73 polypeptide is a rice CYP73A38 polypeptide or an ortholog thereof, wherein the rice CYP73A38 polypeptide has the amino acid sequence set forth in SEQ ID NO:5.

5. A seed capable of germination into a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in said plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

6. A plant cell of or capable of regenerating a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the plant cell comprises the recombinant polynucleotide operably linked to a promoter, wherein the

polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

7. A plant cell comprising a recombinant polynucleotide operably linked to a promoter operable in the cell, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the cell tolerance to saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

8. A plant product prepared from a plant or plant part comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

9. A progeny or descendant plant derived from a plant comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to saflufenacil, wherein the progeny or descendant plant comprises in at least some of its cells the recombinant polynucleotide operably linked to the regulatory sequence, the expression of the polypeptide in the progeny or descendant plant conferring to the progeny or descendant plant tolerance to the saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

10. A method for controlling weeds at a locus for growth of a plant, the method comprising:

applying an herbicide composition comprising saflufenacil to the locus; wherein said locus is:

(a) a locus that contains: a plant or a seed capable of producing said plant; or

(b) a locus that is to be after said applying is made to contain the plant or the seed;

wherein the plant or the seed comprises in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant tolerance to the saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

11. The method of claim 10, wherein step (a) occurs before, after, or concurrently with step (b).

12. The method of claim 10, wherein herbicide composition is applied to the weeds and to the plant produced by the seed.

13. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises an herbicidally-effective imidazolinone.

14. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises imazethapyr.

15. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises imazapic.

16. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises imazaquin.

17. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises imazamox.

18. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises imazapyr.

19. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises a herbicidally-effective sulfonylurea.

20. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises nicosulfuron.

21. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises metsulfuron methyl.

22. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises thifensulfuron methyl.

23. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises tribenuron methyl.

24. The method of Claim 10, wherein the herbicide composition that is applied to the locus further comprises pyrithiobac sodium.

25. A method of producing a plant having tolerance to saflufenacil, the method comprising regenerating a plant from a plant cell transformed with a recombinant polynucleotide operably linked to a promoter operable in the cell, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide, conferring to the plant tolerance to the saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

26. A method of producing a progeny plant having tolerance to saflufenacil, the method comprising:

crossing a first saflufenacil-tolerant plant with a second plant to produce a saflufenacil-tolerant progeny plant, wherein the first plant and the progeny plant comprise in at least some of their cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring tolerance to saflufenacil, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

27. A plant or plant part comprising in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil, wherein the plant or plant part further exhibits a second herbicide-tolerant charasterictic, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

28. The plant of claim 27, wherein the second herbicide-tolerant characteristic is imidazolinone herbicide-tolerance.

29. The seed of claim 5, wherein the seed has, disposed on a surface thereof, a composition comprising at least one agronomically acceptable active ingredient (A.I.).

30. The seed according to claim 5, wherein the seed has, disposed on a surface thereof, a composition comprising at least one agronomically acceptable fungicidal, nematicidal, or insecticidal A.I. or a combination thereof.

31. The seed according to claim 5, wherein the seed has, disposed on a surface thereof, a composition comprising at least one agronomically acceptable herbicidal, fungicidal, nematicidal, or insecticidal A.I. or a combination thereof, with the proviso that the herbicidal A.I. is not a saflufenacil A.I.

32. A method for preparing a treated seed comprising providing the seed of claim 5 and applying thereto a seed treatment composition.

33. A method for treating the plant of claim 1 with an agronomically acceptable composition, the method comprising contacting the agronomically acceptable composition with the plant, wherein the composition comprises saflufenacil.

34. A method for treating the plant of claim 1 with an agronomically acceptable composition, the method comprising contacting the agronomically acceptable composition with the plant, with the proviso that the composition does not contain a saflufenacil A.I.

35. The method of claim 33, wherein the composition further comprises a fungicide, an insecticide, or a nematicide.

36. A method for treating the seed of claim 1 with an agronomically acceptable composition, the method comprising contacting the agronomically acceptable composition with the seed, wherein the composition comprises saflufenacil.

37. The method of claim 36, wherein the composition further comprises a fungicide, an insecticide, or a nematicide.

38. A method for preparing a descendant seed, the method comprising planting a seed of or capable of producing the plant of claim 1.

39. The method of claim 38 further comprising growing a descendent plant from the seed.

40. The method of claim 39 further comprising harvesting a descendant seed from the descendent plant.

41. The method of claim 39 further comprising applying an auxinic herbicidal composition to the descendent plant.

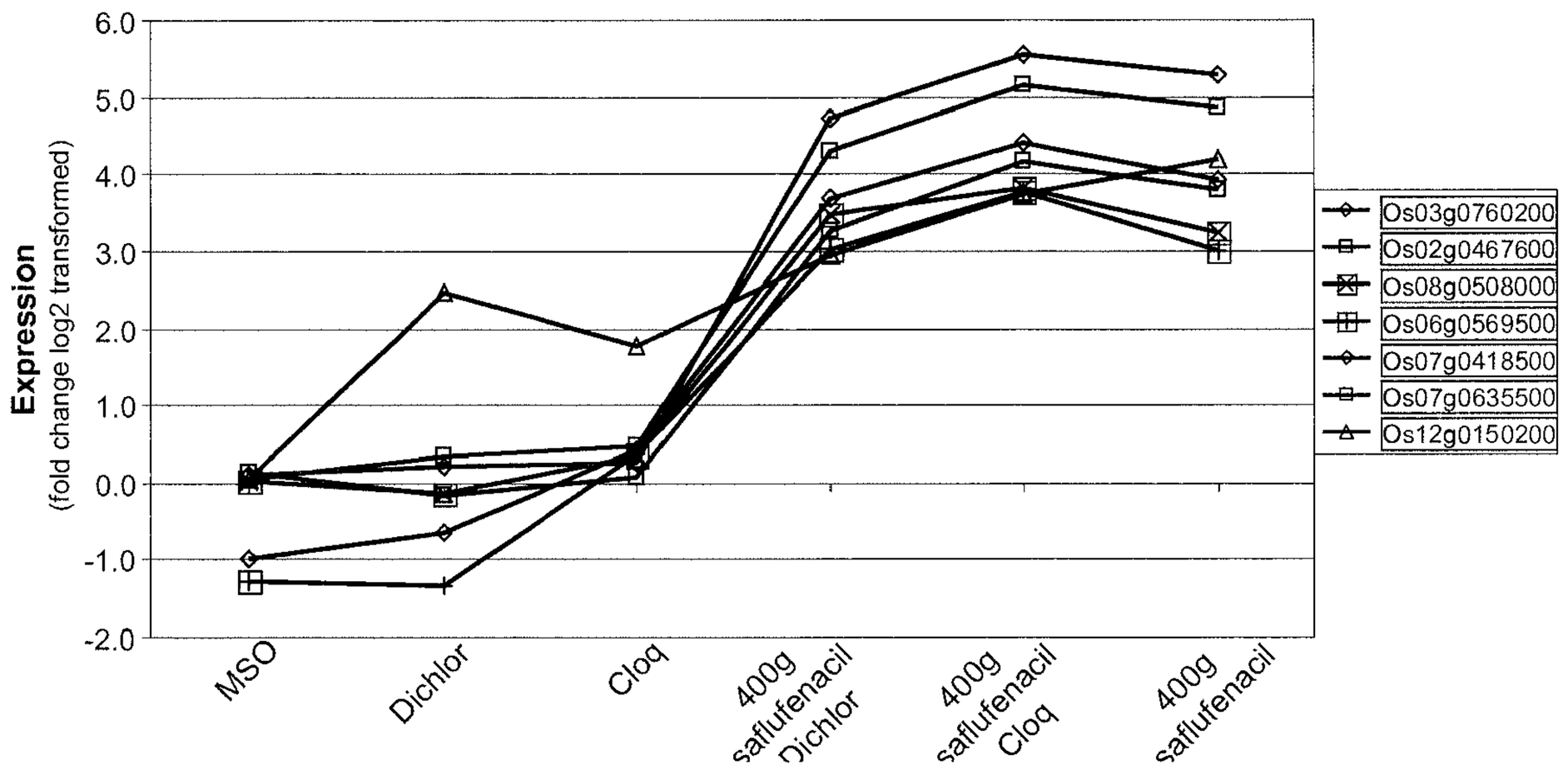
42. A method for producing a plant product from the plant of claim 1, the method comprising processing the plant or a plant part thereof to obtain the plant product.

43. The method of claim 42, wherein the plant product is fodder, seed meal, oil, or seed-treatment-coated seeds.

44. The method of claim 42, wherein the plant part is a seed.

45. A plant product obtained from a plant or plant part thereof, wherein the plant or plant part comprises in at least some of its cells a recombinant polynucleotide operably linked to a promoter operable in plant cells, the promoter capable of expressing a cytochrome P450 polypeptide encoded by the polynucleotide, the expression of the polypeptide conferring to the plant or plant part tolerance to saflufenacil, wherein the plant or plant part further exhibits a second herbicide-tolerant charasterictic, wherein the polypeptide is a CYP72A15 polypeptide or an isoform of CYP81A or CYP73A, with the proviso that the isoform of CYP81A is not a maize NSF1 polypeptide.

46. The plant product of claim 45, wherein the product is fodder, seed meal, oil, or seed-treatment-coated seed.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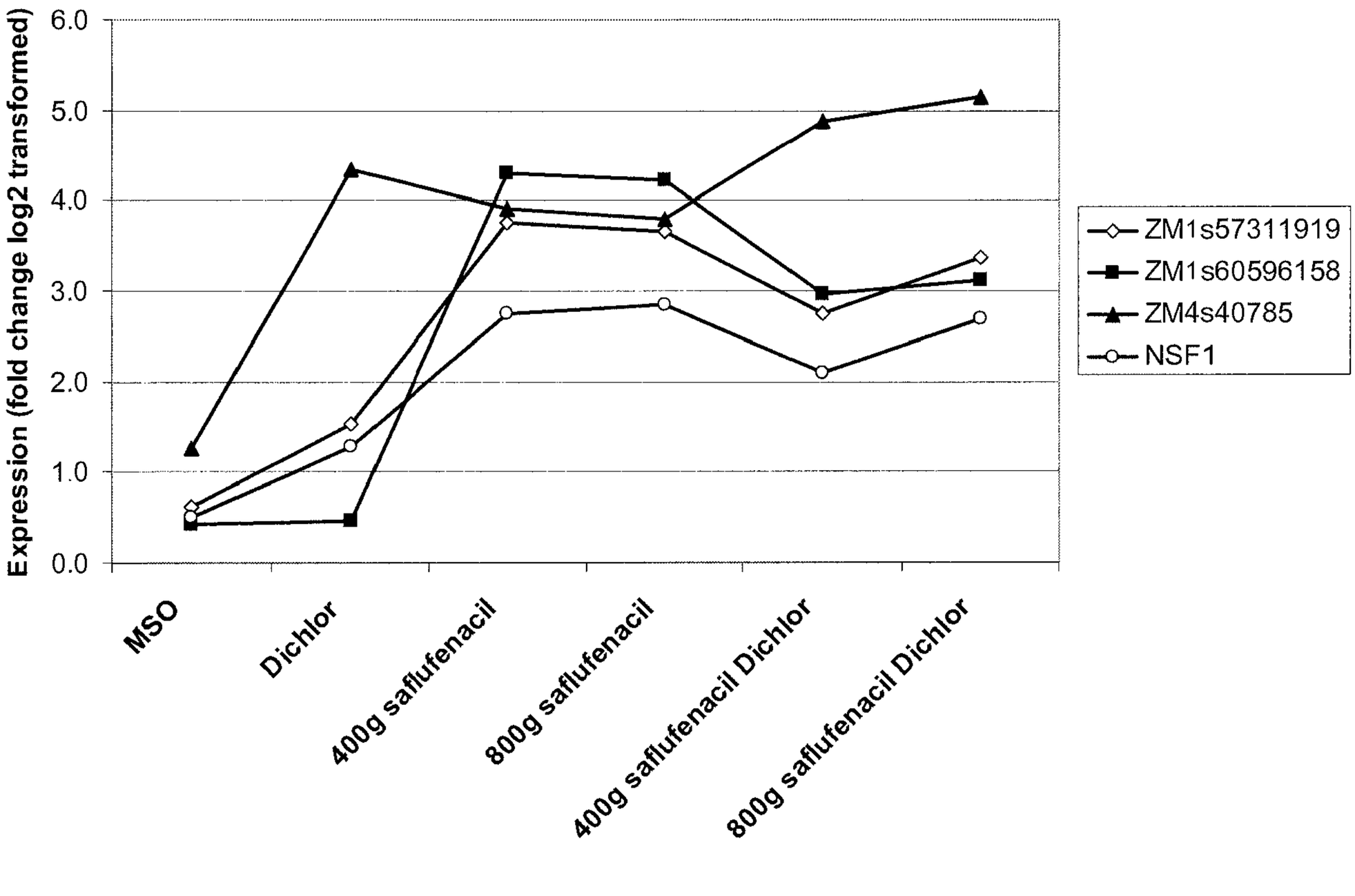
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FIG.1

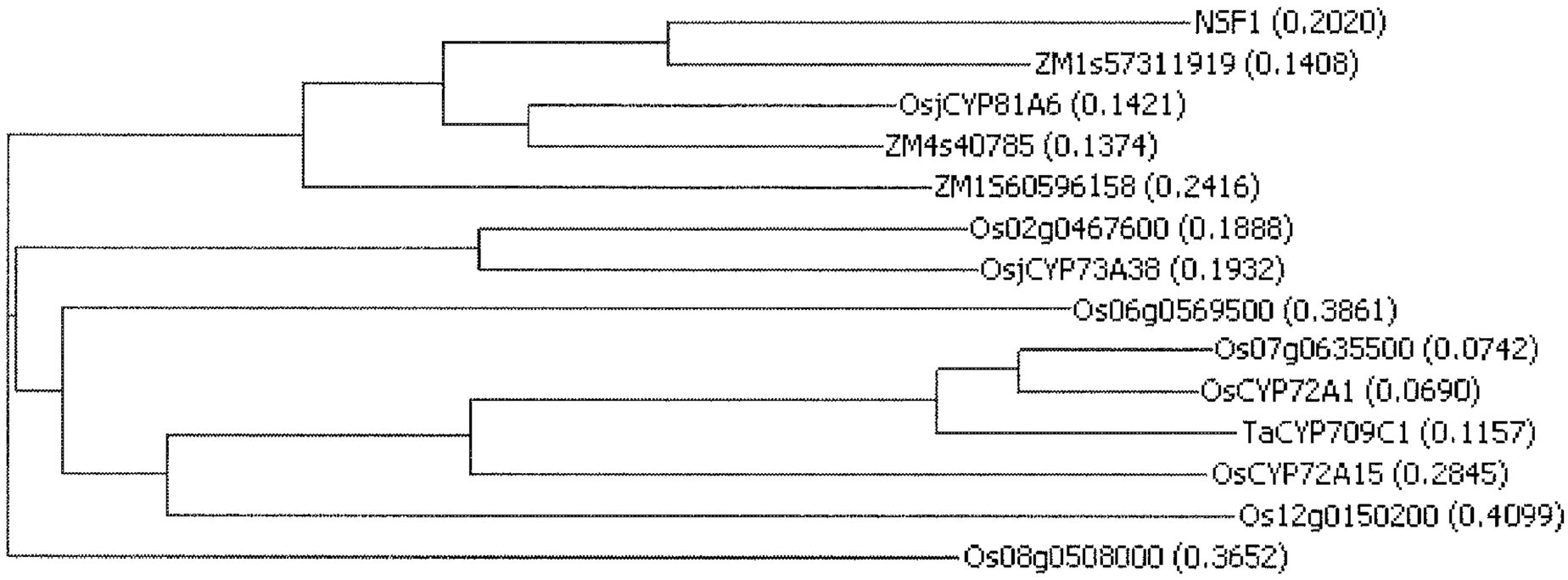
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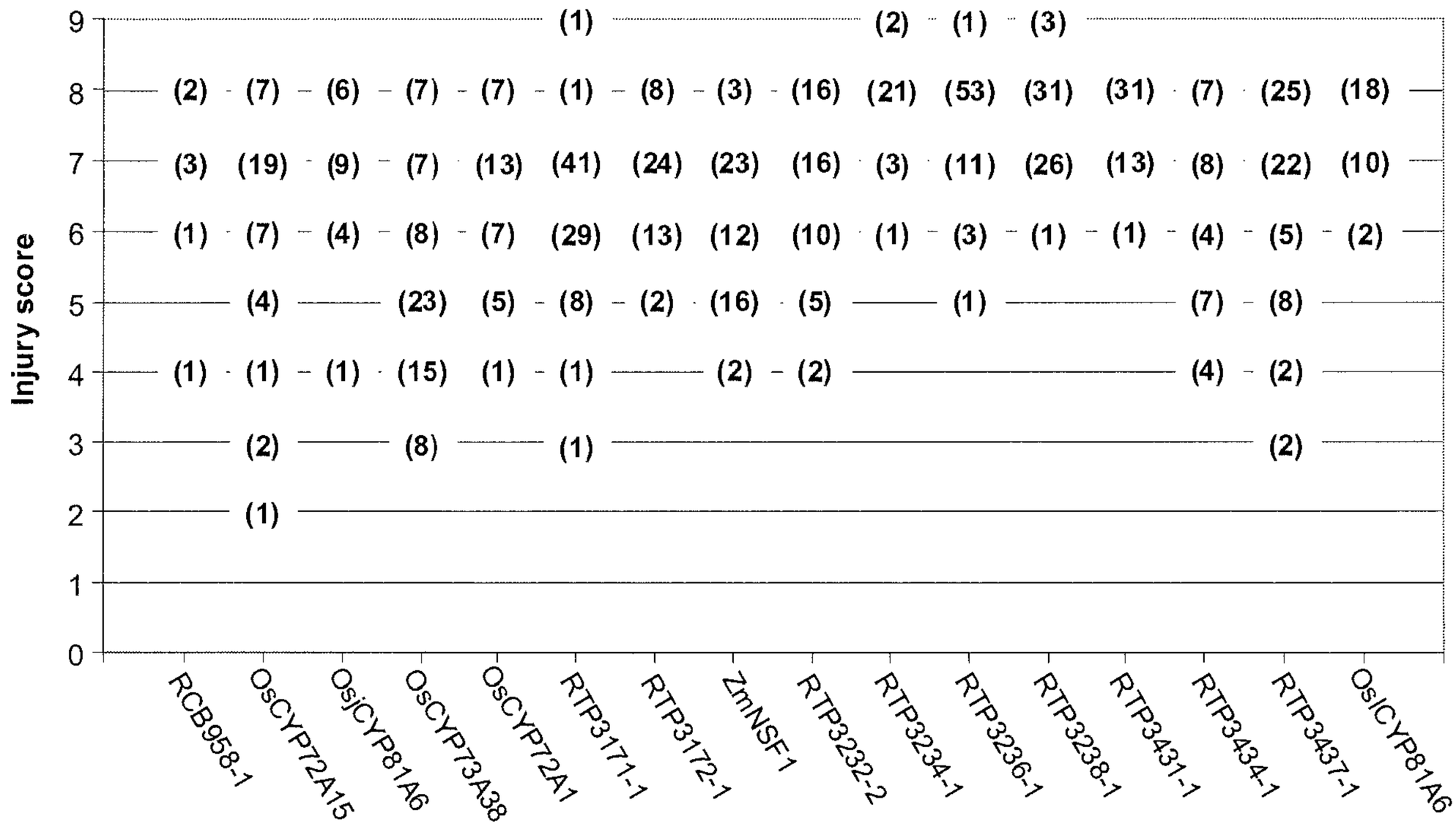
FIG. 2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0
(1) (2) (1) (3)
(2) (7) (6) (7) (7) (1) (8) (3) (16) (21) (53) (31) (31) (7) (25) (18)
(3) (19) (9) (7) (13) (41) (24) (23) (16) (3) (11) (26) (13) (8) (22) (10)
(1) (7) (4) (8) (7) (29) (13) (12) (10) (1) (3) (1) (1) (4) (5) (2)
(4) (23) (5) (8) (2) (16) (5) (1) (7) (8)
(1) (1) (1) (15) (1) (1) (2) (2) (4) (2)
(2) (8) (1) (2)
(1)
RCB958-1
OsCYP72A15
OsjCYP81A6
OsCYP73A38
OsCYP72A1
RTP3171-1
RTP3172-1
ZmNSF1
RTP3232-2
RTP3234-1
RTP3236-1
RTP3238-1
RTP3431-1
RTP3434-1
RTP3437-1
OsjCYP81A6

FIG. 3

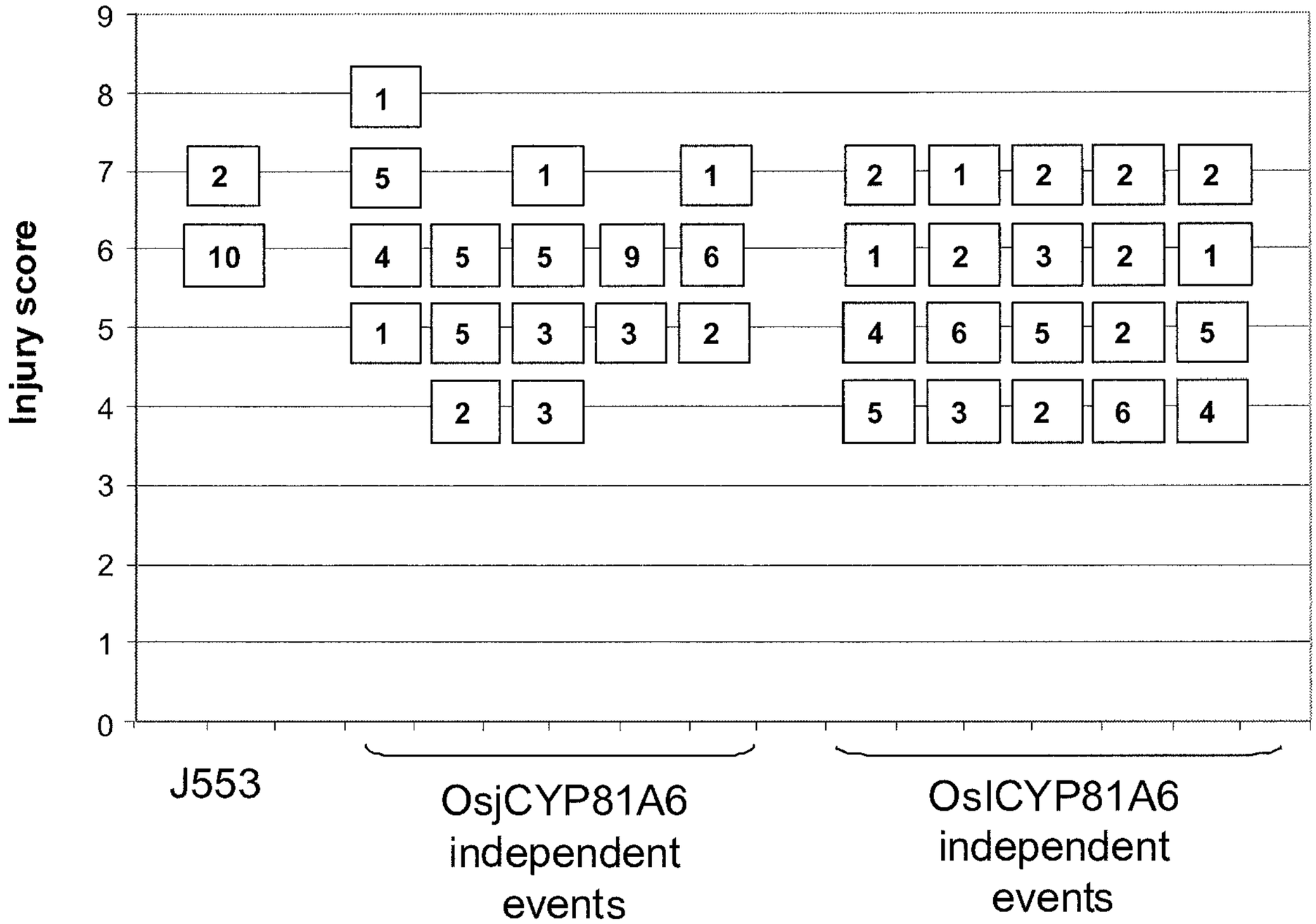
Injury score
9
8
7
6
5
4
3
2
1
0
1
2
5
1
1
2
1
2
2
2
10
4
5
5
9
6
1
2
3
2
1
1
5
3
3
2
4
6
5
2
5
2
3
5
3
2
6
4
J553
OsjCYP81A6 independent events
OsICYP81A6 independent events

FIG.4

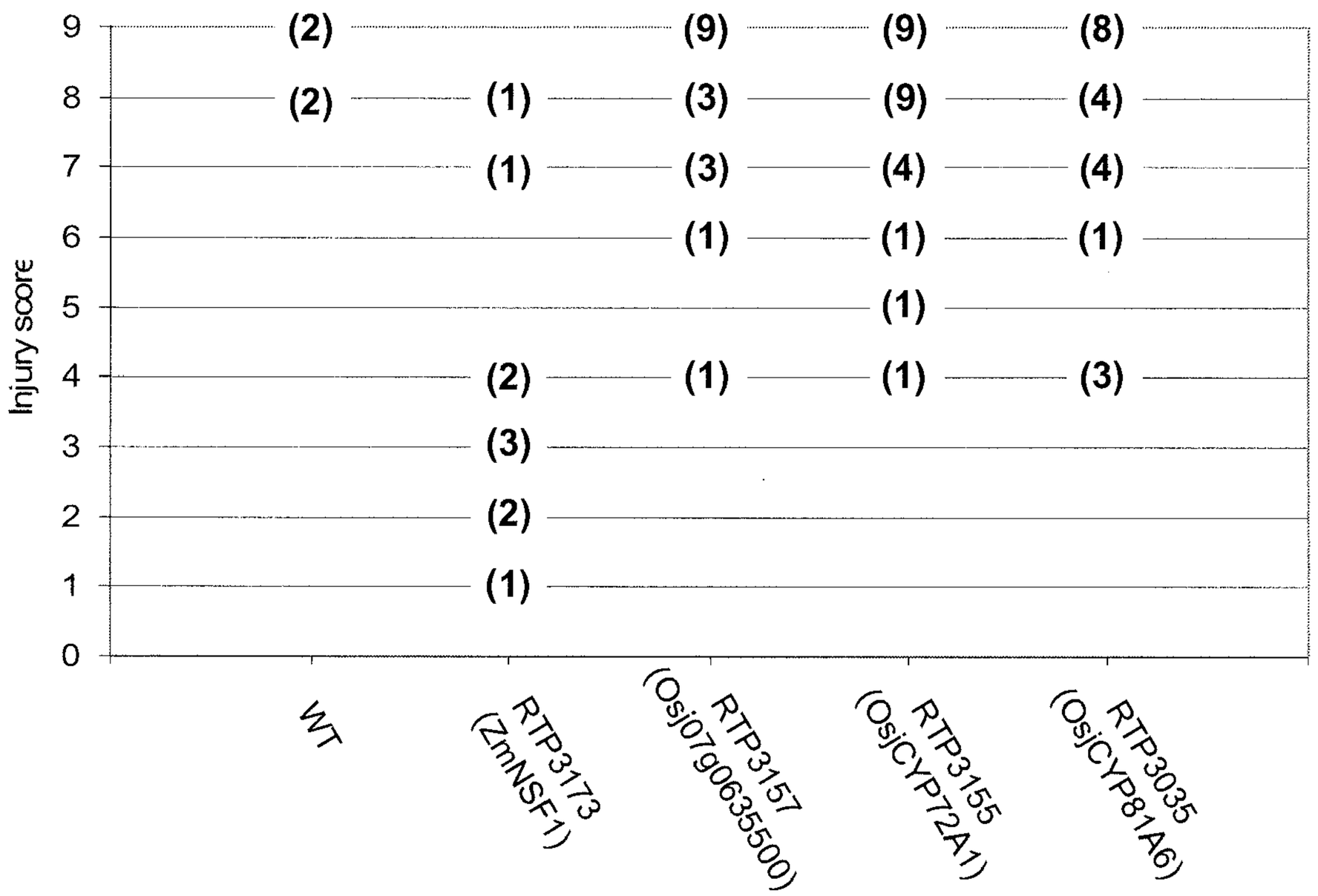
Injury score
9
8
7
6
5
4
3
2
1
0
(2)
(9)
(9)
(8)
(2)
(1)
(3)
(9)
(4)
(1)
(3)
(4)
(4)
(1)
(1)
(1)
(1)
(2)
(1)
(1)
(3)
(3)
(2)
(1)
WT
RTP3173 (ZmNSF1)
RTP3157 (Osj0790635500)
RTP3155 (OsjCYP72A1)
RTP3035 (OsjCYP81A6)

FIG. 5

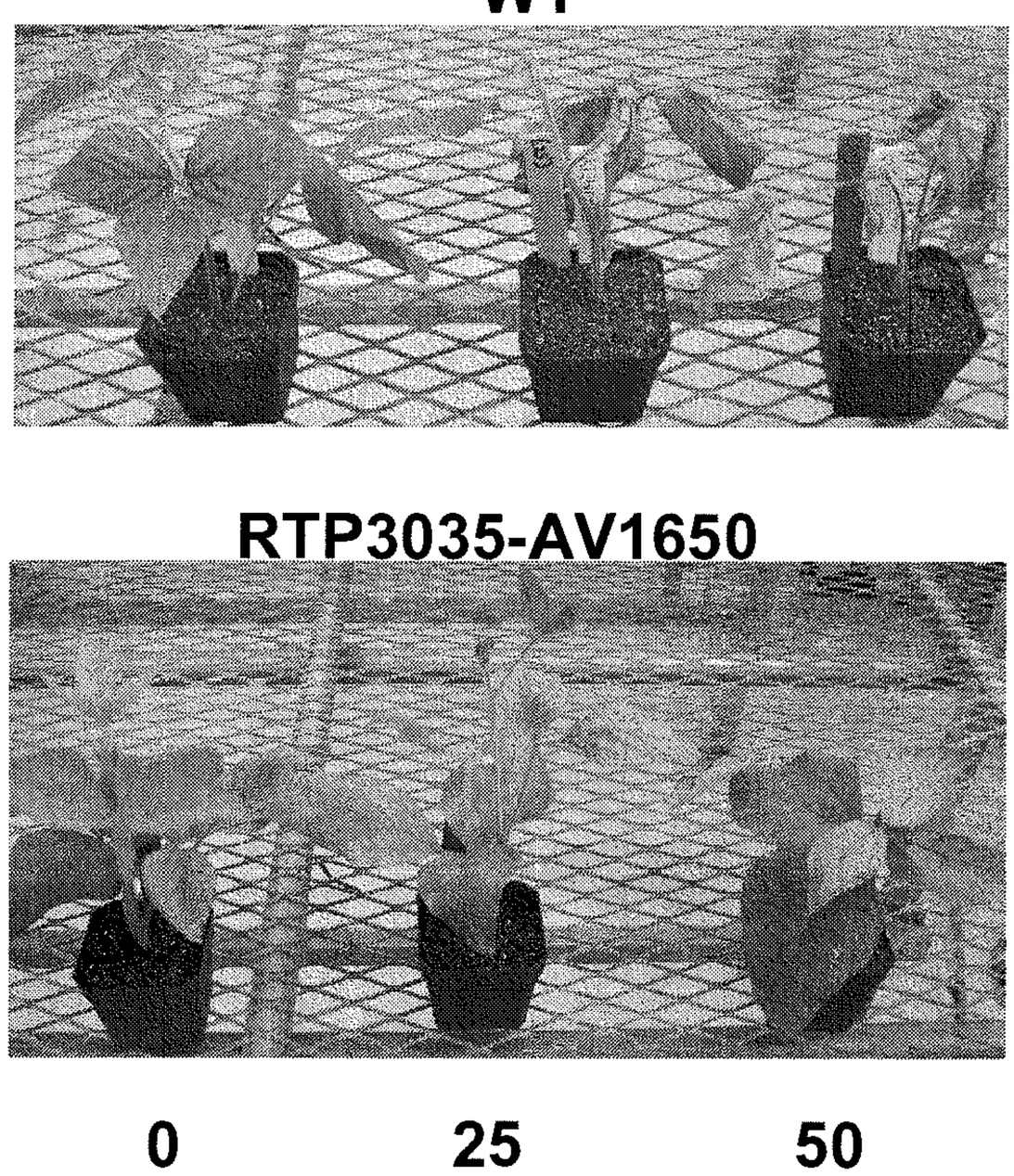

FIG. 6

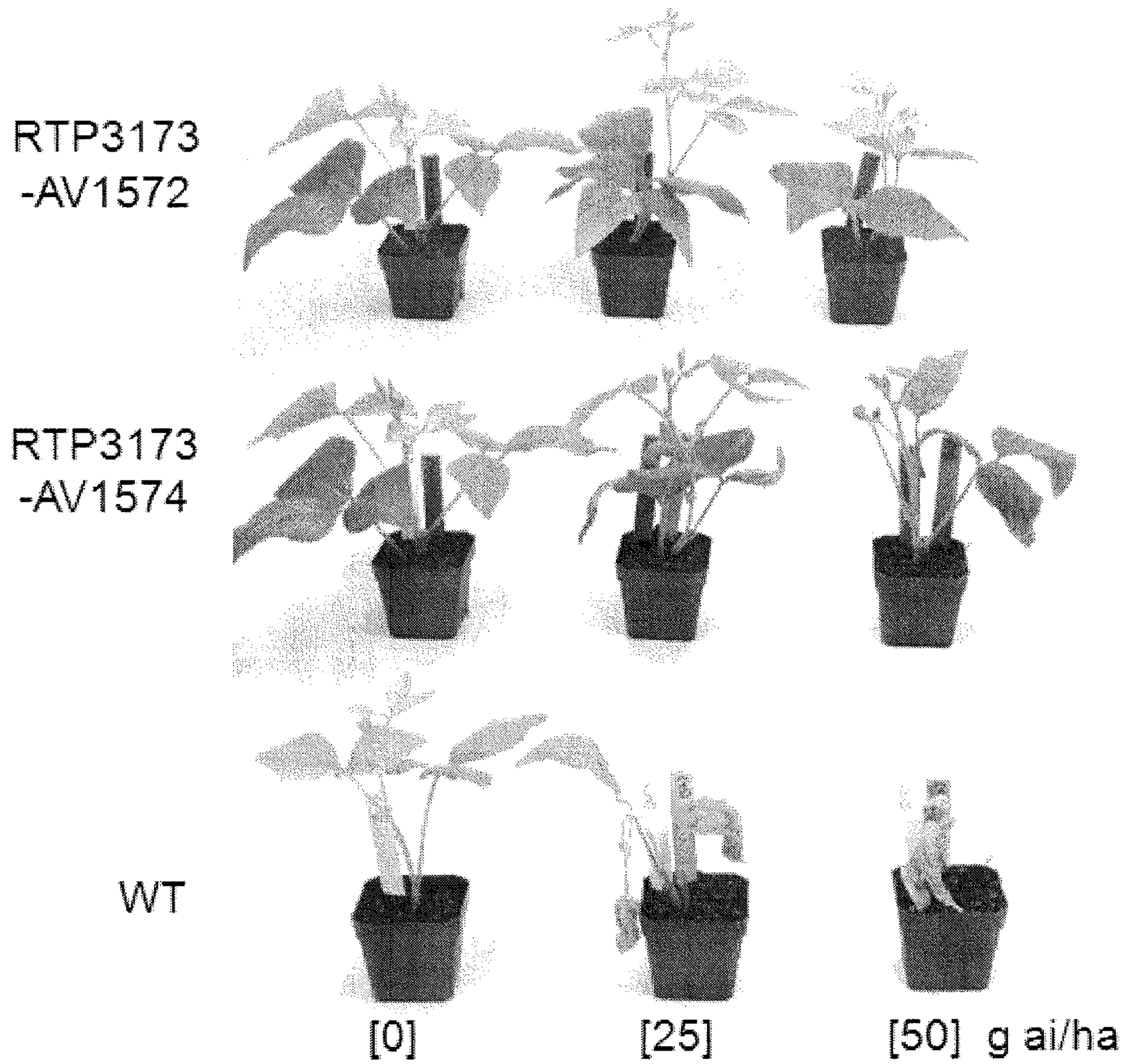

FIG. 7

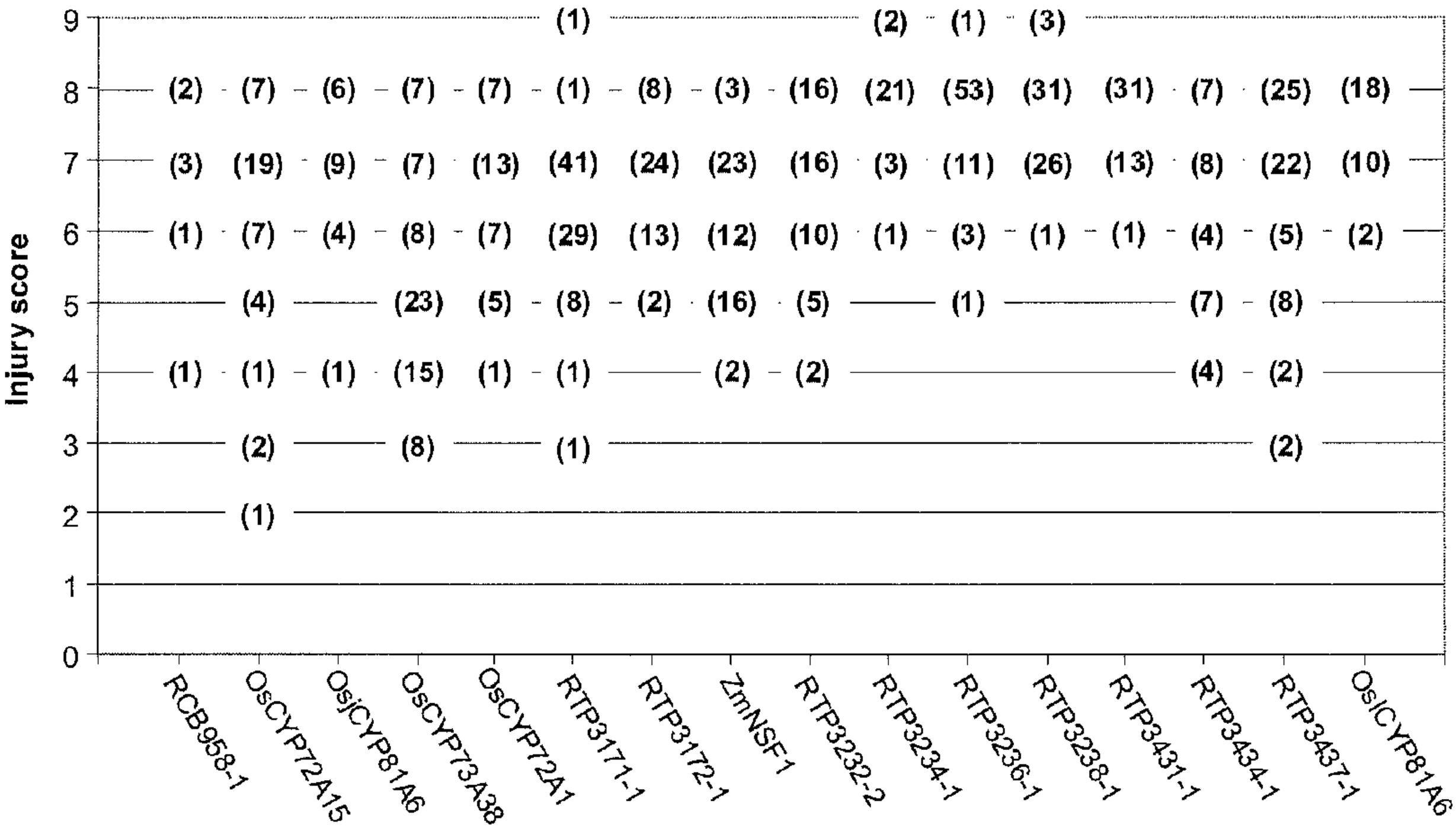
Injury score
9
8
7
6
5
4
3
2
1
0
(1)
(2) (1) (3)
(2) (7) (6) (7) (7) (1) (8) (3) (16) (21) (53) (31) (31) (7) (25) (18)
(3) (19) (9) (7) (13) (41) (24) (23) (16) (3) (11) (26) (13) (8) (22) (10)
(1) (7) (4) (8) (7) (29) (13) (12) (10) (1) (3) (1) (1) (4) (5) (2)
(4) (23) (5) (8) (2) (16) (5) (1) (7) (8)
(1) (1) (1) (15) (1) (1) (2) (2) (4) (2)
(2) (8) (1) (2)
(1)
RCB958-1
OsCYP72A15
OsjCYP81A6
OsCYP73A38
OsCYP72A1
RTP3171-1
RTP3172-1
ZmNSF1
RTP3232-2
RTP3234-1
RTP3236-1
RTP3238-1
RTP3431-1
RTP3434-1
RTP3437-1
OsjCYP81A6

FIG. 3