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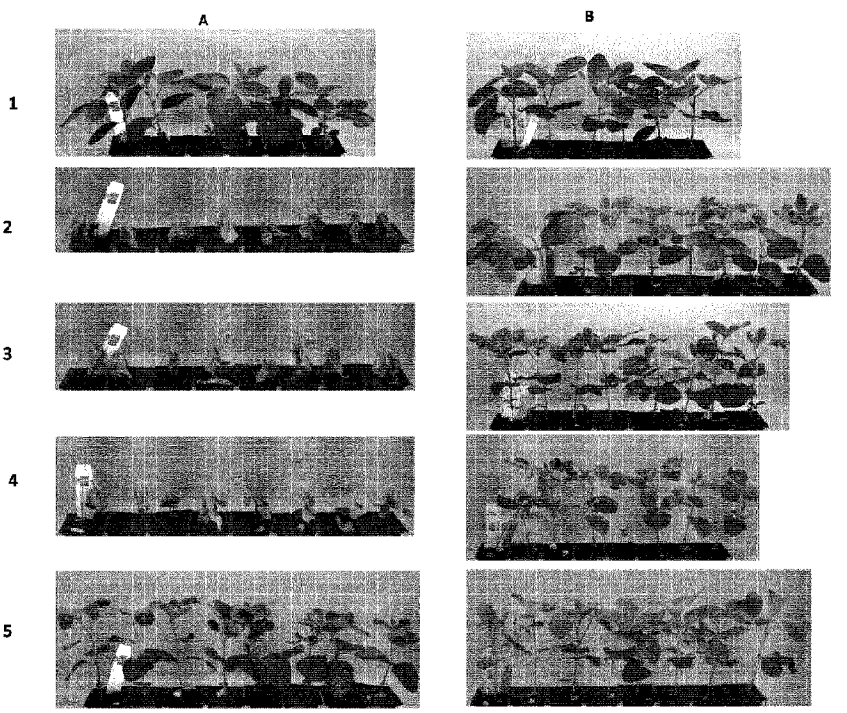
(54) **Titre : PLANTES POSSEDANT UNE TOLERANCE AMELIOREE AUX HERBICIDES INHIBANT LA PROTOPORPHYRINOGENE OXYDASE : NAPN-CRELN6**
(54) **Title: PLANTS HAVING INCREASED TOLERANCE TO PPO-INHIBITING HERBICIDES**



(57) **Abrégé/Abstract:**
The present invention refers to a method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of providing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a wild-type or a mutated protoporphyrinogen oxidase (PPO) which is resistant or tolerant to a PPO-inhibiting herbicide by applying to said site an effective amount of said herbicide. The invention further refers to plants comprising wild-type or mutated PPO enzymes, and methods of obtaining such plants.

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(54) Title: PLANTS HAVING INCREASED TOLERANCE TO HERBICIDES

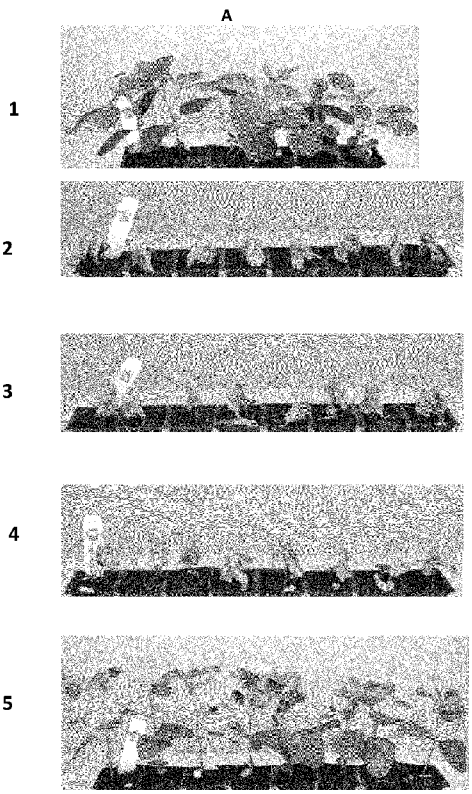

Figure 4

(57) Abstract: The present invention refers to a method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of providing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a wild-type or a mutated protoporphyrinogen oxidase (PPO) which is resistant or tolerant to a PPO-inhibiting herbicide by applying to said site an effective amount of said herbicide. The invention further refers to plants comprising wild-type or mutated PPO enzymes, and methods of obtaining such plants.

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PLANTS HAVING INCREASED TOLERANCE TO PPO-INHIBITING HERBICIDES

FIELD OF THE INVENTION

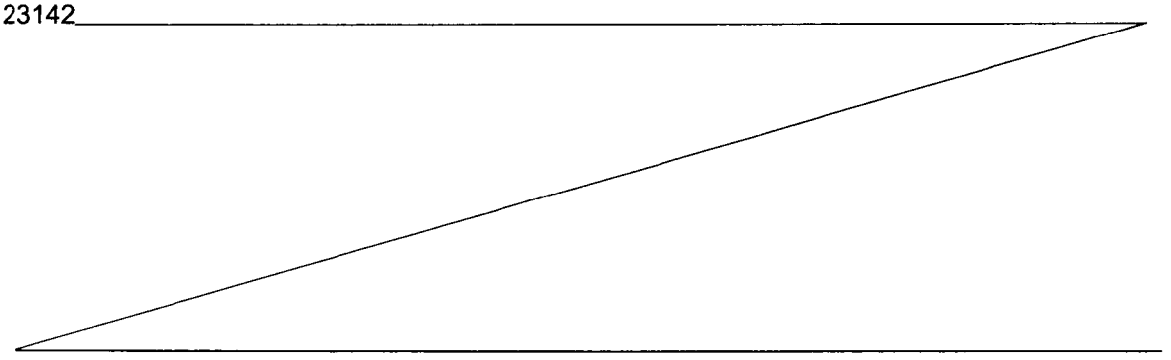
The present invention relates in general to methods for conferring on plants agricultural level tolerance to a herbicide. Particularly, the invention refers to plants having an increased tolerance to PPO-inhibiting herbicides. More specifically, the present invention relates to methods and plants obtained by mutagenesis and cross-breeding and transformation that have an increased tolerance to PPO-inhibiting herbicides.

BACKGROUND OF THE INVENTION

Herbicides that inhibit protoporphyrinogen oxidase (hereinafter referred to as Protox or PPO; EC:1.3.3.4), a key enzyme in the biosynthesis of protoporphyrin IX, have been used for selective weed control since the 1960s. PPO catalyzes the last common step in chlorophyll and heme biosynthesis which is the oxidation of protoporphyrinogen IX to protoporphyrin IX. (Matringe et al. 1989. Biochem. 1. 260: 231). PPO-inhibiting herbicides include many different structural classes of molecules (Duke et al. 1991. Weed Sci. 39: 465; Nandihalli et al. 1992. Pesticide Biochem. Physiol. 43: 193; Matringe et al. 1989. FEBS Lett. 245: 35; Yanase and Andoh. 1989. Pesticide Biochem. Physiol. 35: 70). These herbicidal compounds include the diphenylethers {e.g. lactofen, (+-)-2-ethoxy-1-methyl-2-oxoethyl 5-{2-chloro-4-(trifluoromethyl)phenoxy}-2-nitrobenzoate; acifluorfen, 5-{2-chloro-4-(trifluoromethyl)phenoxy}-2-nitrobenzoic acid; its methyl ester; or oxyfluorfen, 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluorobenzene)}, oxidiazoles, (e.g. oxidiazon, 3-{2,4-dichloro-5-(1-methylethoxy)phenyl}-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2-(3H)-one), cyclic imides (e.g. S-23142, N-(4-chloro-2-fluoro-5-propargyloxyphenyl)-3,4,5,6-tetrahydrophthalimide; chlorophthalim, N-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide), phenyl pyrazoles (e.g. TNPP-ethyl, ethyl 2-{1-(2,3,4-trichlorophenyl)-4-nitropyrazolyl-5-oxy}propionate; M&B 39279), pyridine derivatives (e.g. LS 82-556), and phenopylate and its O-phenylpyrrolidino- and piperidinocarbamate analogs. Many of these compounds competitively inhibit the normal reaction catalyzed by the enzyme, apparently acting as substrate analogs.

Application of PPO-inhibiting herbicides results in the accumulation of protoporphyrinogen IX in the chloroplast and mitochondria, which is believed to leak into the cytosol where it is oxidized by a peroxidase. When exposed to light, protoporphyrin IX causes formation of singlet oxygen in the cytosol and the formation of other reactive oxygen species, which can cause lipid peroxidation and membrane disruption leading to rapid cell death (Lee et al. 1993. Plant Physiol. 102: 881).

Not all PPO enzymes are sensitive to herbicides which inhibit plant PPO enzymes. Both the Escherichia coli and Bacillus subtilis PPO enzymes (Sasarmen et al. 1993. Can. J. Microbiol. 39: 1155; Dailey et al. 1994. J. Biol. Chem. 269: 813) are resistant to these herbicidal inhibitors. Mutants of the unicellular alga Chlamydomonas reinhardtii resistant to the phenylimide herbicide S-23142



have been reported (Kataoka et al. 1990. J. Pesticide Sci. 15: 449; Shibata et al. 1992. In Research in Photosynthesis, Vol. III, N. Murata, ed. Kluwer:Netherlands. pp. 567-70). At least one of these mutants appears to have an altered PPO activity that is resistant not only to the herbicidal inhibitor on which the mutant was selected, but also to other classes of protox inhibitors (Oshio et al. 1993. Z. Naturforsch. 48c: 339; Sato et al. 1994. In ACS Symposium on Porphyric Pesticides, S. Duke, ed. ACS Press: Washington, D.C.). A mutant tobacco cell line has also been reported that is resistant to the inhibitor S-21432 (Che et al. 1993. Z. Naturforsch. 48c: 350). Auxotrophic E. coli mutants have been used to confirm the herbicide resistance of cloned plant PPO-inhibting herbicides.

Three main strategies are available for making plants tolerant to herbicides, i.e. (1) detoxifying the herbicide with an enzyme which transforms the herbicide, or its active metabolite, into non-toxic products, such as, for example, the enzymes for tolerance to bromoxynil or to basta (EP242236, EP337899); (2) mutating the target enzyme into a functional enzyme which is less sensitive to the herbicide, or to its active metabolite, such as, for example, the enzymes for tolerance to glyphosate (EP293356, Padgette S. R. et al., J.Biol. Chem., 266, 33, 1991); or (3) overexpressing the sensitive enzyme so as to produce quantities of the target enzyme in the plant which are sufficient in relation to the herbicide, in view of the kinetic constants of this enzyme, so as to have enough of the functional enzyme available despite the presence of its inhibitor. The third strategy was described for successfully obtaining plants which were tolerant to PPO inhibitors (see e.g. US5,767,373 or US5,939,602, and patent family members thereof.). In addition, US 2010/0100988 and WO 2007/024739 discloses nucleotide sequences encoding amino acid sequences having enzymatic activity such that the amino acid sequences are resistant to PPO inhibitor herbicidal chemicals, in particular 3-phenyluracil inhibitor specific PPO mutants.

To date, the prior art has not described PPO-inhibiting herbicide tolerant plants containing at least one wild-type or mutated PPO nucleic acid according to the present invention. Nor has the prior art described PPO-inhibiting herbicide tolerant crop plants containing mutations on genomes other than the genome from which the PPO gene is derived. Therefore, what is needed in the art is the identification of PPO-inhibiting herbicide tolerance genes from additional genomes and species. What are also needed in the art are crop plants and crop plants having increased tolerance to herbicides such as PPO-inhibiting herbicide and containing at least one wildtype and/or mutated PPO nucleic acid. Also needed are methods for controlling weed growth in the vicinity of such crop plants or crop plants. These compositions and methods would allow for the use of spray over techniques when applying herbicides to areas containing crop plants or crop plants.

SUMMARY OF THE INVENTION

The problem is solved by the present invention which refers to a method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:

a) providing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a wild type protoporphyrinogen oxidase (PPO) or a mutated protoporphyrinogen oxidase (mut-PPO) which is resistant or tolerant to a PPO-inhibiting herbicide,

b) applying to said site an effective amount of said herbicide.

An object of the invention refers to a method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:

a) planting or sowing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a mutated protoporphyrinogen oxidase (mut-PPO) which is resistant or tolerant to a "PPO inhibiting herbicide" and
b) applying to said site an effective amount of said herbicide,
wherein the nucleotide sequence of a) comprises the sequence of SEQ ID NO: 1, 3, 5, 7 or a variant thereof, having at least 80% sequence identity to the nucleotide sequence SEQ ID NO: 1, 3, 5, or 7 and wherein a mut-PPO of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO:6 or SEQ ID NO: 8 comprises the following:
a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and
b) the amino acid corresponding to position 420 is Val or Met.

The present invention refers to a method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:

a) planting or sowing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a mutated protoporphyrinogen oxidase (mut-PPO) which is resistant or tolerant to a "PPO inhibiting herbicide" and
b) applying to said site an effective amount of said herbicide,
wherein the nucleotide sequence of a) comprises the sequence of SEQ ID NO: 1, 3, 5, 7 or a variant thereof, having at least 80% sequence identity over the full length of the nucleotide sequence SEQ ID NO: 1, 3, 5, or 7 and wherein a mut-PPO of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO:6 or SEQ ID NO: 8 comprises the following:
a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and
b) the amino acid corresponding to position 420 is Val or Met.

In addition, the present invention refers to a method for identifying a PPO-inhibiting herbicide by using a wild-type or mut-PPO of the present invention encoded by a nucleic acid which comprises the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, or a variant thereof.

Said method comprises the steps of:

a) generating a transgenic cell or plant comprising a nucleic acid encoding a mut-PPO of the present invention, wherein the mut-PPO of the present invention is expressed;
b) applying a PPO-inhibiting herbicide to the transgenic cell or plant of a) and to a control cell or plant of the same variety;
c) determining the growth or the viability of the transgenic cell or plant and the control cell or plant after application of said test compound, and
d) selecting test compounds which confer reduced growth to the control cell or plant as compared to the growth of the transgenic cell or plant.

Date Reçue/Date Received 2020-05-27

Another object refers to a method of identifying a nucleotide sequence encoding a mut-PPO which is resistant or tolerant to a PPO-inhibiting herbicide, the method comprising:

a) generating a library of mut-PPO-encoding nucleic acids,
b) screening a population of the resulting mut-PPO-encoding nucleic acids by expressing each of said nucleic acids in a cell or plant and treating said cell or plant with a PPO-inhibiting herbicide,
c) comparing the PPO-inhibiting herbicide-tolerance levels provided by said population of mut-PPO encoding nucleic acids with the PPO-inhibiting herbicide-tolerance level provided by a control PPO-encoding nucleic acid,
d) selecting at least one mut-PPO-encoding nucleic acid that provides a significantly increased level of tolerance to a PPO-inhibiting herbicide as compared to that provided by the control PPO-encoding nucleic acid.

In a preferred embodiment, the mut-PPO-encoding nucleic acid selected in step d) provides at least 2-fold as much tolerance to a PPO-inhibiting herbicide as compared to that provided by the control PPO-encoding nucleic acid.

The resistance or tolerance can be determined by generating a transgenic plant comprising a nucleic acid sequence of the library of step a) and comparing said transgenic plant with a control plant.

Another object refers to a method of identifying a plant or algae containing a nucleic acid encoding a mut-PPO which is resistant or tolerant to a PPO-inhibiting herbicide, the method comprising:

a) identifying an effective amount of a PPO-inhibiting herbicide in a culture of plant cells or green algae.
b) treating said plant cells or green algae with a mutagenizing agent,
c) contacting said mutagenized cells population with an effective amount of PPO-inhibiting herbicide, identified in a),
d) selecting at least one cell surviving these test conditions,
e) PCR-amplification and sequencing of PPO genes from cells selected in d) and comparing such sequences to wild-type PPO gene sequences, respectively.

In a preferred embodiment, the mutagenizing agent is ethylmethanesulfonate.

Another object refers to an isolated nucleic acid encoding a mut-PPO, the nucleic acid comprising the sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, or a variant thereof, as defined hereinafter.

Another object refers to an isolated mut-PPO polypeptide, the polypeptide comprising the sequence set forth in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46, a variant, derivative, orthologue, paralogue or homologue thereof, as defined hereinafter.

Date Reçue/Date Received 2020-05-27

In a preferred embodiment, the nucleic acid being identifiable by a method as defined above.

Another object refers to an expression cassette comprising an isolated nucleic acid encoding a mut-PPO polypeptide, wherein the nucleic acid has at least 80% sequence identity to the nucleotide sequence of SEQ ID NO: 1, 3, 5, or 7,
and wherein the encoded mut-PPO is a variant of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 in which the amino acid corresponding to position 420 is Met or Val, and the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, wherein said mut-PPO polypeptide confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

Another object refers to an expression cassette comprising an isolated nucleic acid encoding a mut-PPO polypeptide, wherein the nucleic acid has at least 80% sequence identity over the full length of the nucleotide sequence of SEQ ID NO: 1, 3, 5, or 7,
and wherein the encoded mut-PPO is a variant of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 in which the amino acid corresponding to position 420 is Met or Val, and the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, wherein said mut-PPO polypeptide confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

Another object refers to a mut-PPO polypeptide comprising a sequence which is at least 80% identical to SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8, in which the amino acid corresponding to position 420 is Met or Val, and the the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, wherein said mut-PPO polypeptide confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

Another object refers to a mut-PPO polypeptide comprising a sequence which is at least 80% identical over the full length of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8, in which the amino acid corresponding to position 420 is Met or Val, and the the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, wherein said mut-PPO polypeptide confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

In another embodiment, the invention refers to a plant cell transformed by and expressing a wild-type or a mut-PPO nucleic acid according to the present invention or a plant which has been mutated to obtain a plant expressing, preferably over-expressing a wild-type or a mut-PPO nucleic acid according to the present invention, wherein expression of said nucleic acid in the plant cell results in increased resistance or

Date Reçue/Date Received 2020-05-27

tolerance to a PPO-inhibiting herbicide as compared to a wild type variety of the plant cell.

In another embodiment, the invention refers to a transgenic plant cell transformed by a nucleic acid encoding a mut-PPO polypeptide which is at least 80% identical to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, wherein expression of the nucleic acid in the plant cell results in increased resistance or tolerance to a PPO inhibiting herbicide as compared to a wild type variety of the plant cell, and wherein the encoded mut-PPO is a variant of SEQ ID NO: 2 , SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, which includes: the amino acid corresponding to position 397 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, and the amino acid corresponding to position 420 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8 is Met or Val.

In another embodiment, the invention refers to a transgenic plant cell transformed by a nucleic acid encoding a mut-PPO polypeptide which is at least 80% identical over the full length of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, wherein expression of the nucleic acid in the plant cell results in increased resistance or tolerance to a PPO inhibiting herbicide as compared to a wild type variety of the plant cell, and wherein the encoded mut-PPO is a variant of SEQ ID NO: 2 , SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, which includes: the amino acid corresponding to position 397 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, and the amino acid corresponding to position 420 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8 is Met or Val.

In another embodiment, the invention refers to a plant comprising a plant cell according to the present invention, wherein expression of the nucleic acid in the plant results in the plant's increased resistance to PPO-inhibiting herbicide as compared to a wild type variety of the plant.

The plants of the present invention can be transgenic or non-transgenic.

Preferably, the expression of the nucleic acid of the invention in the plant results in the plant's increased resistance to PPO-inhibiting herbicides as compared to a wild type variety of the plant.

In another embodiment, the invention refers to a seed produced by a transgenic plant comprising a plant cell of the present invention, wherein the seed is true breeding for an increased resistance to a PPO-inhibiting herbicide as compared to a wild type variety of the seed.

In another embodiment, the invention refers to a seed cell produced by a transgenic

Date Reçue/Date Received 2020-05-27

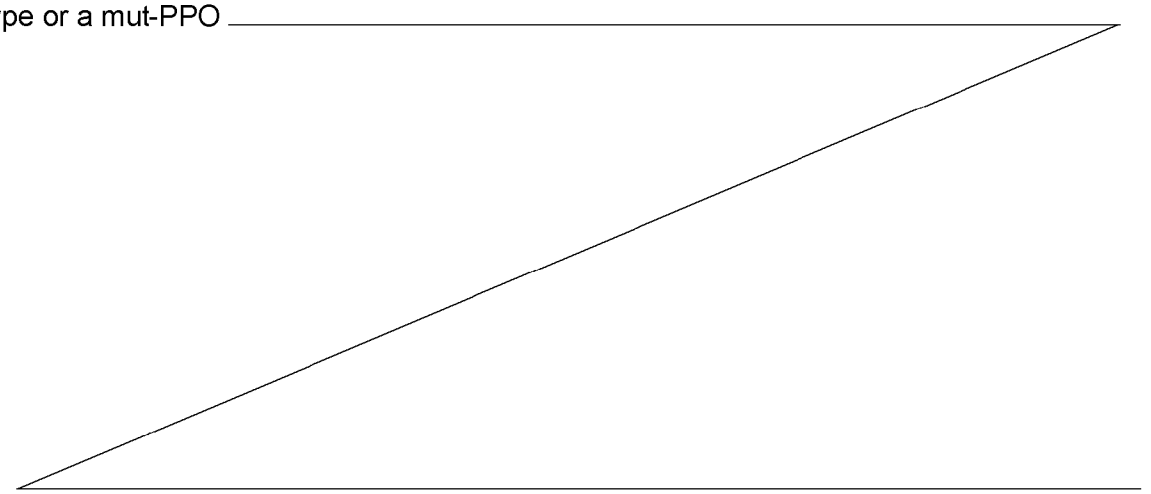
plant comprising the plant cell as defined in the present application, and which comprises the expression cassette as defined in the present application.

In another embodiment, the invention refers to a method of producing a transgenic plant cell with an increased resistance to a PPO-inhibiting herbicide as compared to a wild type variety of the plant cell comprising, transforming the plant cell with an expression cassette comprising a wild-type or a mut-PPO nucleic acid.

In another embodiment, the invention refers to a method of producing a transgenic plant comprising, (a) transforming a plant cell with an expression cassette comprising a nucleic acid encoding a mut-PPO polypeptide comprising a sequence which is at least 80% identical to SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8, in which the amino acid corresponding to position 420 is Val, or Met, and the amino acid corresponding to position 397 of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, and (b) generating a plant with an increased resistance to PPO inhibiting herbicide from the plant cell compared to a wild-type plant.

In another embodiment, the invention refers to a method of producing a transgenic plant comprising, (a) transforming a plant cell with an expression cassette comprising a nucleic acid encoding a mut-PPO polypeptide comprising a sequence which is at least 80% identical over the full length of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8, in which the amino acid corresponding to position 420 is Val, or Met, and the amino acid corresponding to position 397 of SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO: 6 or SEQ ID NO: 8 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln, and (b) generating a plant with an increased resistance to PPO inhibiting herbicide from the plant cell compared to a wild-type plant.

In another embodiment, the invention refers to a method of producing a transgenic plant comprising, (a) transforming a plant cell with an expression cassette comprising a wild-type or a mut-PPO



nucleic acid, and (b) generating a plant with an increased resistance to PPO-inhibiting herbicide from the plant cell.

Preferably, the expression cassette further comprises a transcription initiation regulatory region and a translation initiation regulatory region that are functional in the plant.

In another embodiment, the invention relates to using the mut-PPO of the invention as selectable marker. The invention provides a method of identifying or selecting a transformed plant cell, plant tissue, plant or part thereof comprising a) providing a transformed plant cell, plant tissue, plant or part thereof, wherein said transformed plant cell, plant tissue, plant or part thereof comprises an isolated nucleic acid encoding a mut-PPO polypeptide of the invention as described hereinafter, wherein the polypeptide is used as a selection marker, and wherein said transformed plant cell, plant tissue, plant or part thereof may optionally comprise a further isolated nucleic acid of interest; b) contacting the transformed plant cell, plant tissue, plant or part thereof with at least one PPO-inhibiting inhibiting compound; c) determining whether the plant cell, plant tissue, plant or part thereof is affected by the inhibitor or inhibiting compound; and d) identifying or selecting the transformed plant cell, plant tissue, plant or part thereof.

The invention is also embodied in purified mut-PPO proteins that contain the mutations described herein, which are useful in molecular modeling studies to design further improvements to herbicide tolerance. Methods of protein purification are well known, and can be readily accomplished using commercially available products or specially designed methods, as set forth for example, in Protein Biotechnology, Walsh and Headon (Wiley, 1994).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an amino acid sequence alignment of Amaranthus tuberculatus (A.tuberculatus), Amaranthus tuberculatus resistant (A.tuberculatus_R), Arabidopsis thaliana long (A.thaliana_2), Spinacia oleracea short (S.oleracea_2), Nicotiana tabacum short (N.tabacum_2), Glycine max (Glycine_max), Arabidopsis thaliana short (A.thaliana_1), Nicotiana tabacum long (N.tabacum_1), Chlamydomonas reinhardtii long (C.reinhardtii_1), Zea mays (Z.mays), Oryza sativa (O.sativa_1), Solanum tuberosum (S.tuberosum), Cucumis sativus (C.sativus), Cichorium intybus (C.intybus_1), Spinacia oleracea long (S.oleracea_1), Polytomella sp. Pringsheim 198.80 (Polytomella) PPO sequences. Conserved regions are indicated in light grey, grey and black.

Figure 2 shows the selection of Chlamydomonas reinhardtii strains resistant to PPO-inhibiting herbicide 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) . (A) Mutagenized cells plated on solid medium without a selecting agent. (B) Mutagenized cells plated on solid medium containing $1x10^{-7}$ M PPO-inhibiting herbicide 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4). Cells which are resistant to the PPO-inhibiting herbicide form colonies (circled and numbered 31 and 32), while susceptible cells do not grow. The higher number of colonies on plate A as compared to B, indicate that the colonies on plate B are resistant to PPO-inhibiting herbicide 1,5-

dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4).

Figure 3 shows growth-characteristics of selected Chlamydomonas reinhardtii strains as seen in Figure 2, resistant to PPO-inhibiting herbicide 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) . (A) Dose-reponse curve of Wild-type cells treated with PPO-inhibiting herbicide 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) with respective IC_{50}. (B) Dose-reponse curve of mutagenized cells (strain 17) treated with PPO-inhibiting herbicide 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) with respective IC_{50}.. Strain 17 (B), resistant to the PPO-inhibiting herbicide 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), shows much lower IC_{50}, compared to Wild-type cells.

Figure 4 shows wild type and transgenic T1 soybean plants treated with the indicated spray rate (g ai/ha) of PPO inhibiting herbicides with 1% MSO.
A means wild-type soybean plant
B means soybean plant transformed with a nucleic acid encoding a mut-PPO SEQ ID NO 2, wherein the Leucin at position 397 is substituted by Aspartic acid and the phenylalanin at position 420 is substituted by Valine.
1 means unsprayed
2 means 150 g Saflufenacil
3 means 100 g 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4)
4 means 150 g flumioxazin
5 means 600 g fomesafen

KEY TO SEQUENCE LISTING

Table 1

SEQ. ID NO:	Description	Organism	Gene	Accession No:
1	PPO nucleic acid	Amaranthus	PPX2L_WC	DQ386114
2	PPO amino acid	Amaranthus	ABD52326	
3	PPO nucleic acid	Amaranthus	PPX2L_AC	DQ386117
4	PPO amino acid	Amaranthus	ABD52329	
5	PPO nucleic acid	Amaranthus	PPX2L_CC_R	DQ386118
6	PPO amino acid	Amaranthus	ABD52330	
7	PPO nucleic acid	Amaranthus	PPX2L_AC_R	DQ386116
8	PPO amino acid	Amaranthus	ABD52328	
9	PPO nucleic acid	Arabidopsis	PPX	AB007650

10	PPO amino acid	Arabidopsis	BAB08301	
11	PPO nucleic acid	Nicotiana	ppxl	AF044128
12	PPO amino acid	Nicotiana	AAD02290	
13	PPO nucleic acid	Cichorium	PPX1	AF160961
14	PPO amino acid	Cichorium	AF160961_1	
15	PPO nucleic acid	Spinacia	SO-POX1	AB029492
16	PPO amino acid	Spinacia	BAA96808	
17	PPO nucleic acid	Spinacia	SO-POX2	AB046993
18	PPO amino acid	Spinacia	BAB60710	
19	PPO nucleic acid	Solanum	PPOX	AJ225107
20	PPO amino acid	Solanum	CAA12400	
21	PPO nucleic acid	Zea	ZM_BFc0091B03	BT063659
22	PPO amino acid	Zea	ACN28356	
23	PPO nucleic acid	Zea	prpo2	NM_001111534
24	PPO amino acid	Zea	NP_001105004	
25	PPO nucleic acid	Chlamydomonas	Ppx1	AF068635
26	PPO amino acid	Chlamydomonas	AAC79685	
27	PPO nucleic acid	Polytomella	PPO	AF332964
28	PPO amino acid	Polytomella	AF332964_1	
29	PPO nucleic acid	Sorghum	Hyp. Protein	XM_002446665
30	PPO amino acid	Sorghum	XP_002446710	
31	PPO nucleic acid	Chlorella		
32	PPO amino acid	Chlorella		51538
33	PPO nucleic acid	Oryza	PPOX1	AB057771
34	PPO amino acid	Oryza	BAB39760	
35	PPO nucleic acid	Amaranthus	PPX2	DQ386113
36	PPO amino acid	Amaranthus	ABD52325	
37	PPO nucleic acid	Arabidopsis	PPOX	NM_178952
38	PPO amino acid	Arabidopsis	NP_849283	
39	PPO nucleic acid	Nicotiana	ppxII	AF044129
40	PPO amino acid	Nicotiana	AAD02291	
41	PPO nucleic acid	Glycine	hemG	AB025102
42	PPO amino acid	Glycine	BAA76348	
43	PPO nucleic acid	Cucumis	CsPPO	AB512426
44	PPO amino acid	Cucumis	BAH84864.1	
45	PPO nucleic acid	Oryza	Hyp. Protein	AL606613
46	PPO amino acid	Oryza	CAE01661	

DETAILED DESCRIPTION

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

As used herein, the word "comprising," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

The present invention refers to a method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:

a) providing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a wild-type protoporphyrinogen oxidase or a mutated protoporphyrinogen oxidase (mut-PPO) which is resistant or tolerant to a PPO-inhibiting herbicide,
b) applying to said site an effective amount of said herbicide.

The term “control of undesired vegetation” is to be understood as meaning 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. 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. Monocotyledonous weeds include, but are not limited to, weeds of 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. 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.

The term “plant” is used in its broadest sense as it pertains to organic material and is intended to encompass eukaryotic organisms that are members of the Kingdom Plantae, examples of which include but are not limited to vascular plants, vegetables, grains, flowers, trees, herbs, bushes, grasses, vines, ferns, mosses, fungi and algae, etc, as well as clones, offsets, and parts of plants used for asexual propagation (e.g. cuttings, pipings, shoots, rhizomes, underground stems, clumps, crowns, bulbs, corms, tubers, rhizomes, plants/tissues produced in tissue culture, etc.). The term “plant” further encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, leaves, roots (including tubers), flowers, florets, fruits, pedicles, peduncles, stamen, anther, stigma, style, ovary, petal, sepal, carpel, root tip, root cap, root hair, leaf hair, seed hair, pollen grain, microspore, cotyledon, hypocotyl, epicotyl, xylem, phloem, parenchyma, endosperm, a companion cell, a guard cell, and any other known organs, tissues, and cells of a plant, and tissues and organs, wherein each of the aforementioned comprise the gene/nucleic acid of interest. The term “plant” also encompasses plant cells, suspension cultures,

callus tissue, embryos, meristematic regions, gametophytes, sporophytes, pollen and microspores, again wherein each of the aforementioned comprises the gene/nucleic acid of interest.

Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantae, in particular monocotyledonous and dicotyledonous plants including fodder or forage legumes, ornamental plants, food crops, trees or shrubs selected from the list comprising Acer spp., Actinidia spp., Abelmoschus spp., Agave sisalana, Agropyron spp., Agrostis stolonifera, Allium spp., Amaranthus spp., Ammophila arenaria, Ananas comosus, Annona spp., Apium graveolens, Arachis spp, Artocarpus spp., Asparagus officinalis, Avena spp. (e.g. Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida), Averrhoa carambola, Bambusa sp., Benincasa hispida, Bertholletia excelsea, Beta vulgaris, Brassica spp. (e.g. Brassica napus, Brassica rapa ssp. [canola, oilseed rape, turnip rape]), Cadaba farinosa, Camellia sinensis, Canna indica, Cannabis sativa, Capsicum spp., Carex elata, Carica papaya, Carissa macrocarpa, Carya spp., Carthamus tinctorius, Castanea spp., Ceiba pentandra, Cichorium endivia, Cinnamomum spp., Citrullus lanatus, Citrus spp., Cocos spp., Coffea spp., Colocasia esculenta, Cola spp., Corchorus sp., Coriandrum sativum, Corylus spp., Crataegus spp., Crocus sativus, Cucurbita spp., Cucumis spp., Cynara spp., Daucus carota, Desmodium spp., Dimocarpus longan, Dioscorea spp., Diospyros spp., Echinochloa spp., Elaeis (e.g. Elaeis guineensis, Elaeis oleifera), Eleusine coracana, Eragrostis tef, Erianthus sp., Eriobotrya japonica, Eucalyptus sp., Eugenia uniflora, Fagopyrum spp., Fagus spp., Festuca arundinacea, Ficus carica, Fortunella spp., Fragaria spp., Ginkgo biloba, Glycine spp. (e.g. Glycine max, Soja hispida or Soja max), Gossypium hirsutum, Helianthus spp. (e.g. Helianthus annuus), Hemerocallis fulva, Hibiscus spp., Hordeum spp. (e.g. Hordeum vulgare), Ipomoea batatas, Juglans spp., Lactuca sativa, Lathyrus spp., Lens culinaris, Linum usitatissimum, Litchi chinensis, Lotus spp., Luffa acutangula, Lupinus spp., Luzula sylvatica, Lycopersicon spp. (e.g. Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme), Macrotyloma spp., Malus spp., Malpighia emarginata, Mammea americana, Mangifera indica, Manihot spp., Manilkara zapota, Medicago sativa, Melilotus spp., Mentha spp., Miscanthus sinensis, Momordica spp., Morus nigra, Musa spp., Nicotiana spp., Olea spp., Opuntia spp., Ornithopus spp., Oryza spp. (e.g. Oryza sativa, Oryza latifolia), Panicum miliaceum, Panicum virgatum, Passiflora edulis, Pastinaca sativa, Pennisetum sp., Persea spp., Petroselinum crispum, Phalaris arundinacea, Phaseolus spp., Phleum pratense, Phoenix spp., Phragmites australis, Physalis spp., Pinus spp., Pistacia vera, Pisum spp., Poa spp., Populus spp., Prosopis spp., Prunus spp., Psidium spp., Punica granatum, Pyrus communis, Quercus spp., Raphanus sativus, Rheum rhabarbarum, Ribes spp., Ricinus communis, Rubus spp., Saccharum spp., Salix sp., Sambucus spp., Secale cereale, Sesamum spp., Sinapis sp., Solanum spp. (e.g. Solanum tuberosum, Solanum integrifolium or Solanum lycopersicum), Sorghum bicolor, Spinacia spp., Syzygium spp., Tagetes spp., Tamarindus indica, Theobroma cacao, Trifolium spp., Tripsacum dactyloides, Triticosecale rimpaui, Triticum spp. (e.g. Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum, Triticum monococcum or Triticum vulgare), Tropaeolum minus, Tropaeolum majus, Vaccinium spp., Vicia spp., Vigna spp., Viola odorata, Vitis spp., Zea mays, Zizania palustris, Ziziphus spp., amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, strawberry, sugar beet, sugar cane, sunflower, tomato, squash, tea and algae, amongst others. According to a preferred embodiment of

the present invention, the plant is a crop plant. Examples of crop plants include inter alia soybean, sunflower, canola, alfalfa, rapeseed, cotton, tomato, potato or tobacco. Further preferebly, the plant is a monocotyledonous plant, such as sugarcane. Further preferably, the plant is a cereal, such as rice, maize, wheat, barley, millet, rye, sorghum or oats.

In a preferred embodiment, the plant has been previously produced by a process comprising recombinantly preparing a plant by introducing and over-expressing a wild-type or mut-PPO transgene according to the present invention, as described in greater detail hereinfter.

In another preferred embodiment, the plant has been previously produced by a process comprising in situ mutagenizing plant cells, to obtain plant cells which express a mut-PPO.

As disclosed herein, the nucleic acids of the invention find use in enhancing the herbicide tolerance of plants that comprise in their genomes a gene encoding a herbicide-tolerant wild-type or mut-PPO protein. Such a gene may be an endogenous gene or a transgene, as described hereinafter. Additionally, in certain embodiments, the nucleic acids of the present invention can be stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired phenotype. For example, the nucleic acids of the present invention may be stacked with any other polynucleotides encoding polypeptides having pesticidal and/or insecticidal activity, such as, for example, the Bacillus thuringiensis toxin proteins (described in U.S. Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; and Geiser et al (1986) Gene 48: 109), 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), Glyphosate acetyl transferase (GAT), cytochrome P450 monooxygenase, phosphinothricin acetyltransferase (PAT), Acetohydroxyacid synthase (AHAS; EC 4.1.3.18, also known as acetolactate synthase or ALS), hydroxyphenyl pyruvate dioxygenase (HPPD), Phytoene desaturase (PD) and dicamba degrading enzymes as disclosed in WO 02/068607, or phenoxyaceticacid- and phenoxypropionicacid-derivative degrading enzymes as disclosed in WO 2008141154 or WO 2005107437. The combinations generated can also include multiple copies of any one of the polynucleotides of interest.

Generally, the term “herbicide” is used herein to mean an active ingredient that kills, controls or otherwise adversely modifies the growth of plants. The preferred amount or concentration of the herbicide is an "effective amount" or "effective concentration." By "effective amount" and "effective concentration" is intended an amount and concentration, respectively, that is sufficient to kill or inhibit the growth of a similar, wild-type, plant, plant tissue, plant cell, or host cell, but that said amount does not kill or inhibit as severely the growth of the herbicide-resistant plants, plant tissues, plant cells, and host cells of the present invention. Typically, the effective amount of a herbicide is an amount that is routinely used in agricultural production systems to kill weeds of interest. Such an amount is known to those of ordinary skill in the art. Herbicidal activity is exhibited by herbicides useful for the the present invention when they are applied directly to the plant or to the locus of the plant at any stage of growth or before planting or emergence. The effect observed depends upon the plant species to be controlled, the stage of growth of the plant, the application parameters of dilution and spray drop size, the particle size of solid components, the environmental conditions at the time of use, the specific compound employed, the specific adjuvants and carriers employed, the soil type, and the like, as well as the amount of chemical applied. These and other factors can

be adjusted as is known in the art to promote non-selective or selective herbicidal action. Generally, it is preferred to apply the herbicide postemergence to relatively immature undesirable vegetation to achieve the maximum control of weeds.

By a "herbicide-tolerant" or "herbicide-resistant" plant, it is intended that a plant that is tolerant or resistant to at least one herbicide at a level that would normally kill, or inhibit the growth of, a normal or wild-type plant. By "herbicide-tolerant wildtype or mut-PPO protein" or "herbicide -resistant wildtype or mut-PPO protein", it is intended that such a PPO protein displays higher PPO activity, relative to the PPO activity of a wild-type PPO protein, when in the presence of at least one herbicide that is known to interfere with PPO activity and at a concentration or level of the herbicide that is known to inhibit the PPO activity of the wild-type mut-PPO protein. Furthermore, the PPO activity of such a herbicide-tolerant or herbicide-resistant mut-PPO protein may be referred to herein as "herbicide-tolerant" or "herbicide-resistant" PPO activity.

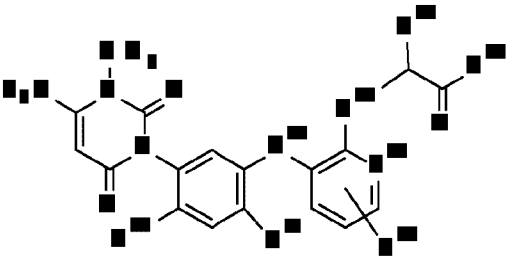
Generally, if the PPO-inhibiting herbicides (A) and/or the herbicidal compounds B as described herein, which can be employed in the context of the present invention are capable of forming geometrical isomers, for example E/Z isomers, it is possible to use both, the pure isomers and mixtures thereof, in the compositions according to the invention. If the PPO-inhibting herbicides A and/or the herbicidal compounds B as described herein 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. If the PPO-inhibting herbicides A and/or the herbicidal compounds B as described herein have ionizable functional groups, they can also be employed in the form of their agriculturally acceptable salts. Suitable are, in general, the salts of those cations and the acid addition salts of those acids whose cations and anions, respectively, have no adverse effect on the activity of the active compounds. Preferred cations are the ions of the alkali metals, preferably of lithium, sodium and potassium, of the alkaline earth metals, preferably of calcium and magnesium, and of the transition metals, preferably of manganese, copper, zinc and iron, further ammonium and substituted ammonium in which one to four hydrogen atoms are replaced by C_1-C_4-alkyl, hydroxy-C_1-C_4-alkyl, C_1-C_4-alkoxy-C_1-C_4-alkyl, hydroxy-C_1-C_4-alkoxy-C_1-C_4-alkyl, phenyl or benzyl, preferably ammonium, methylammonium, isopropylammonium, dimethylammonium, diisopropylammonium, trimethylammonium, heptylammonium, dodecylammonium, tetradecylammonium, tetramethylammonium, tetraethylammonium, tetrabutylammonium, 2-hydroxyethylammonium (olamine salt), 2-(2-hydroxyeth-1-oxy)eth-1-ylammonium (diglycolamine salt), di(2-hydroxyeth-1-yl)ammonium (diolamine salt), tris(2-hydroxyethyl)ammonium (trolamine salt), tris(2-hydroxypropyl)ammonium, benzyltrimethylammonium, benzyltriethylammonium, N,N,N-trimethylethanolammonium (choline salt), furthermore phosphonium ions, sulfonium ions, preferably tri(C_1-C_4-alkyl)sulfonium, such as trimethylsulfonium, and sulfoxonium ions, preferably tri(C_1-C_4-alkyl)sulfoxonium, and finally the salts of polybasic amines such as N,N-bis-(3-aminopropyl)methylamine and diethylenetriamine. Anions of useful acid addition salts are primarily chloride, bromide, fluoride, iodide, hydrogensulfate, methylsulfate, sulfate, dihydrogenphosphate, hydrogenphosphate, nitrate, bi-

carbonate, carbonate, hexafluorosilicate, hexafluorophosphate, benzoate and also the anions of C_1-C_4-alkanoic acids, preferably formate, acetate, propionate and butyrate.

The PPO-inhibting herbicides A and/or the herbicidal compounds B as described herein having a carboxyl group can be employed in the form of the acid, in the form of an agriculturally suitable salt as mentioned above or else in the form of an agriculturally acceptable derivative, for example as amides, such as mono- and di-C_1-C_6-alkylamides or arylamides, as esters, for example as allyl esters, propargyl esters, C_1-C_{10}-alkyl esters, alkoxyalkyl esters, tefuryl ((tetrahydrofuran-2-yl)methyl) esters and also as thioesters, for example as C_1-C_{10}-alkylthio esters. Preferred mono- and di-C_1-C_6-alkylamides are the methyl and the dimethylamides. Preferred arylamides are, for example, the anilides and the 2-chloroanilides. Preferred alkyl esters are, for example, the methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, mexyl (1-methylhexyl), meptyl (1-methylheptyl), heptyl, octyl or isooctyl (2-ethylhexyl) esters. Preferred C_1-C_4-alkoxy-C_1-C_4-alkyl esters are the straight-chain or branched C_1-C_4-alkoxy ethyl esters, for example the 2-methoxyethyl, 2-ethoxyethyl, 2-butoxyethyl (butotyl), 2-butoxypropyl or 3-butoxypropyl ester. An example of a straight-chain or branched C_1-C_{10}-alkylthio ester is the ethylthio ester.

Examples of PPO inhibiting herbicides which can be used according to the present invention are acifluorfen, acifluorfen-sodium, azafenidin, bencarbazone, benzfendizone, bifenox, butafenacil, carfentrazone, carfentrazone-ethyl, chlomethoxyfen, cinidon-ethyl, fluazolate, flufenpyr, flufenpyr-ethyl, flumiclorac, flumiclorac-pentyl, flumioxazin, fluoroglycofen, fluoroglycofen-ethyl, fluthiacet, fluthiacet-methyl, fomesafen, halosafen, lactofen, oxadiargyl, oxadiazon, oxyfluorfen, pentoxazone, profluazol, pyraclonil, pyraflufen, pyraflufen-ethyl, saflufenacil, sulfentrazone, thidiazimin, tiafenacil, chlornitrofen, flumipropyn, fluoronitrofen, flupropacil, furyloxyfen, nitrofluorfen, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), N-ethyl-3-2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9), N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9), N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7), N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione (CAS 451484-50-7), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione (CAS 1300118-96-0), 1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione, methyl (*E*)-4-[2-chloro-5-[4-chloro-5-(difluoromethoxy)-1*H*-methyl-pyrazol-3-yl]-4-fluoro-phenoxy]-3-methoxy-but-2-enoate [CAS 948893-00-3*]*, 3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4), and

uracils of formula III



III,

wherein

R^{30} and R^{31} independently of one another are F, Cl or CN;

R^{32} is O or S;

R^{33} is H, F, Cl, CH_3 or OCH_3;

R^{34} is CH or N;

R^{35} is O or S;

R^{36} is H, CN, CH_3, CF_3, OCH_3, OC_2H_5, SCH_3, SC_2H_5, $(CO)OC_2H_5$ or CH_2R^{38},

wherein R^{38} is F, Cl, OCH_3, SCH_3, SC_2H_5, CH_2F, CH_2Br or CH_2OH;

and

R^{37} is (C_1-C_6-alkyl)amino, (C_1-C_6-dialkyl)amino, $(NH)OR^{39}$, OH, OR^{40} or SR^{40}

wherein R^{39} is CH_3, C_2H_5 or phenyl; and

R^{40} is independently of one another C_1-C_6-alkyl, C_2-C_6-alkenyl, C_3-C_6-alkynyl, C_1-C_6-haloalkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkoxy-C_1-C_6-alkyl, C_2-C_6-cyanoalkyl, C_1-C_4-alkoxy-carbonyl-C_1-C_4-alkyl, C_1-C_4-alkyl-carbonyl-amino, C_1-C_6-alkylsulfinyl-C_1-C_6-alkyl, C_1-C_6-alkyl-sulfonyl-C_1-C_6-alkyl, C_1-C_6-dialkoxy-C_1-C_6-alkyl, C_1-C_6-alkyl-carbonyloxy-C_1-C_6-alkyl, phenyl-carbonyl-C_1-C_6-alkyl, tri(C_1-C_3-alkyl)-silyl-C_1-C_6-alkyl, tri(C_1-C_3-alkyl)-silyl-C_1-C_6-alkenyl, tri(C_1-C_3-alkyl)-silyl-C_1-C_6-alkynyl, tri(C_1-C_3-alkyl)-silyl-C_1-C_6-alkoxy-C_1-C_6-alkyl, dimethylamino, tetrahydropyranyl, tetrahydrofuranyl-C_1-C_3-alkyl, phenyl-C_1-C_6-alkoxy-C_1-C_6-alkyl, phenyl-C_1-C_3-alkyl, pyridyl-C_1-C_3-alkyl, pyridyl, phenyl,

which pyridyls and phenyls independently of one another are substituted by one to five substituents selected from the group consisting of halogen, C_1-C_3-alkyl or C_1-C_2-haloalkyl;

C_3-C_6-cycloalkyl or C_3-C_6-cycloalkyl-C_1-C_4-alkyl,

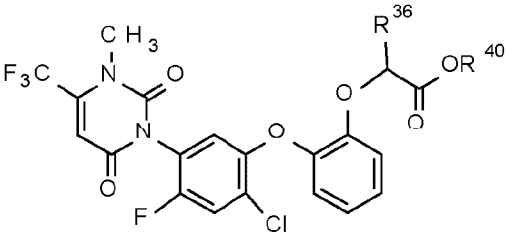
which cycloalkyls indenpently of one another are unsubstituted or substituted by one to five substituents selected from the group consisting of halogen, C_1-C_3-alkyl and C_1-C_2-haloalkyl;

including their agriculturally acceptable alkali metal salts or ammonium salts.

Preferred PPO-inhibiting herbicides that can be used according to the present invention are: Acifluorfen, acifluorfen-sodium, azafenidin, bencarbazone, benzfendizone, butafenacil, carfentrazone-ethyl, cinidon-ethyl, flufenpyr-ethyl, flumiclorac-pentyl, flumioxazin, fluoroglycofen-ethyl, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxadiazon, oxyfluorfen, pentoxazone, pyraflufen-

ethyl, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), N-ethyl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9), N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9), N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7), N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione (CAS 451484-50-7), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione (CAS 1300118-96-0);1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione (CAS 1304113-05-0), 3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4)

uracils of formula III.1 (corresponding to uracils of formula III, wherein R^{30} is F, R^{31} is Cl, R^{32} is O; R^{33} is H; R^{34} is CH; R^{35} is O and R^{37} is OR^{40})



III.1,

wherein

R^{36} is OCH_3, OC_2H_5, SCH_3 or SC_2H_5;

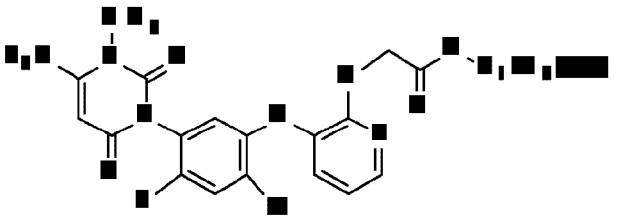
and

R^{40} is C_1-C_6-alkyl, C_2-C_6-alkenyl, C_3-C_6-alkynyl, C_1-C_6-haloalkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_3-cyanoalkyl, phenyl-C_1-C_3-alkyl, pyridyl-C_1-C_3-alkyl, C_3-C_6-cycloalkyl or C_3-C_6-cycloalkyl-C_1-C_4-alkyl,

which cycloalkyls are unsubstituted or substituted by one to five substituents selected from the group consisting of halogen, C_1-C_3-alkyl and C_1-C_2-haloalkyl;

and

uracils of formula III.2 (corresponding to uracils of formula III, wherein R^{30} is F; R^{31} is Cl; R^{32} is O; R^{33} is H; R^{34} is N; R^{35} is O and R^{37} is OR^{40} with R^{40} is C_1-C_6-alkyl)

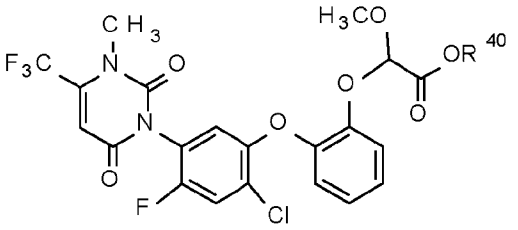


III.2,

Particularly preferred PPO-inhibiting herbicides that can be used according to the present invention are:

acifluorfen, acifluorfen-sodium, butafenacil, carfentrazone-ethyl, cinidon-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione (CAS 451484-50-7), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), and 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione (CAS 1300118-96-0), 1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione (CAS 1304113-05-0),

uracils of formula III.1.1 (corresponding to uracils of formula III, wherein R^{30} is F, R^{31} is Cl, R^{32} is O; R^{33} is H; R^{34} is CH; R^{35} is O, R^{36} is OCH_3 and R^{37} is OR^{40})



III.1.1,

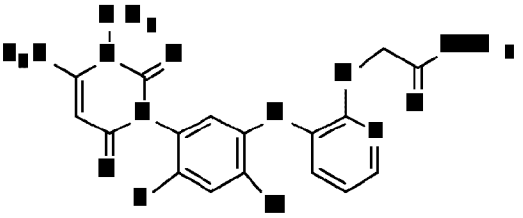
wherein

R^{40} is C_1-C_6-alkyl, C_2-C_6-alkenyl, C_3-C_6-alkynyl, C_1-C_6-haloalkyl, C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_6-alkoxy-C_1-C_6-alkoxy-C_1-C_6-alkyl, C_1-C_3-cyanoalkyl, phenyl-C_1-C_3-alkyl, pyridyl-C_1-C_3-alkyl, C_3-C_6-cycloalkyl or C_3-C_6-cycloalkyl-C_1-C_4-alkyl,

which cycloalkyls are unsubstituted or substituted by one to five substituents selected from the group consisting of halogen, C_1-C_3-alkyl and C_1-C_2-haloalkyl;

is preferably CH_3, $CH_2CH_2OC_2H_5$, CH_2CHF_2, cyclohexyl, (1-methylcyclopropyl)methyl or CH_2(pyridine-4-yl);

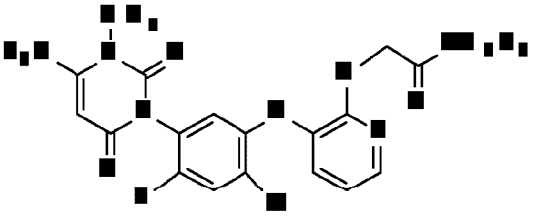
uracils of formula III.2.1 (corresponding to uracils of formula III, wherein R^{30} is F; R^{31} is Cl; R^{32} is O; R^{33} is H; R^{34} is N; R^{35} is O and R^{37} is OR^{40} with R^{40} is CH_3)



III.2.1,

and

uracils of formula III.2.2 (corresponding to uracils of formula III, wherein R^{30} is F; R^{31} is Cl; R^{32} is O; R^{33} is H; R^{34} is N; R^{35} is O and R^{37} is OR^{40} with R^{40} is C_2H_5)



III.2.2,

Especially preferred PPO-inhibiting herbicides are the PPO-inhibiting herbicides.1 to A.14 listed below in table A:

Table A

A.1	acifluorfen
A.2	butafenacil
A.3	carfentrazone-ethyl
A.4	cinidon-ethyl
A.5	flumioxazin
A.6	fluthiacet-methyl
A.7	fomesafen
A.8	lactofen
A.9	oxadiargyl
A.10	oxyfluorfen
A.11	saflufenacil
A.12	sulfentrazone
A.13	ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6)
A.14	1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4)

The PPO-inhibiting herbicides described above that are useful to carry out the present invention are often best applied in conjunction with one or more other herbicides to obtain control of a wider variety of undesirable vegetation. For example, PPO-inhibiting herbicides may further be used in

conjunction with additional herbicides to which the crop plant is naturally tolerant, or to which it is resistant via expression of one or more additional transgenes as mentioned supra. When used in conjunction with other targeting herbicides, the presently claimed compounds can be formulated with the other herbicide or herbicides, tank mixed with the other herbicide or herbicides, or applied sequentially with the other herbicide or herbicides.

Suitable components for mixtures are, for example, selected from the herbicides of class b1) to b15)

B) herbicides of class b1) to b15):

- b1) lipid biosynthesis inhibitors;
- b2) acetolactate synthase inhibitors (ALS inhibitors);
- b3) photosynthesis inhibitors;
- b4) protoporphyrinogen-IX oxidase inhibitors,
- b5) bleacher herbicides;
- b6) enolpyruvyl shikimate 3-phosphate synthase inhibitors (EPSP inhibitors);
- b7) glutamine synthetase inhibitors;
- b8) 7,8-dihydropteroate synthase inhibitors (DHP inhibitors);
- b9) mitosis inhibitors;
- b10) inhibitors of the synthesis of very long chain fatty acids (VLCFA inhibitors);
- b11) cellulose biosynthesis inhibitors;
- b12) decoupler herbicides;
- b13) auxinic herbicides;
- b14) auxin transport inhibitors; and
- b15) other herbicides selected from the group consisting of bromobutide, chlorflurenol, chlorflurenol-methyl, cinmethylin, cumyluron, dalapon, dazomet, difenzoquat, difenzoquat-metilsulfate, dimethipin, DSMA, dymron, endothal and its salts, etobenzanid, flamprop, flamprop-isopropyl, flamprop-methyl, flamprop-M-isopropyl, flamprop-M-methyl, flurenol, flurenol-butyl, flurprimidol, fosamine, fosamine-ammonium, indanofan, indaziflam, maleic hydrazide, mefluidide, metam, methiozolin (CAS 403640-27-7), methyl azide, methyl bromide, methyl-dymron, methyl iodide, MSMA, oleic acid, oxaziclomefone, pelargonic acid, pyributicarb, quinoclamine, triaziflam, tridiphane and 6-chloro-3-(2-cyclopropyl-6-methylphenoxy)-4-pyridazinol (CAS 499223-49-3) and its salts and esters;

including their agriculturally acceptable salts or derivatives.

Examples of herbicides B which can be used in combination with the PPO-inhibiting herbicides according to the present invention are:

b1) from the group of the lipid biosynthesis inhibitors:
ACC-herbicides such as alloxydim, alloxydim-sodium, butroxydim, clethodim, clodinafop, clodinafop-propargyl, cycloxydim, cyhalofop, cyhalofop-butyl, diclofop, diclofop-methyl, fenoxaprop, fenoxaprop-ethyl, fenoxaprop-P, fenoxaprop-P-ethyl, fluazifop, fluazifop-butyl, fluazifop-P, fluazi-

fop-P-butyl, haloxyfop, haloxyfop-methyl, haloxyfop-P, haloxyfop-P-methyl, metamifop, pinoxaden, profoxydim, propaquizafop, quizalofop, quizalofop-ethyl, quizalofop-tefuryl, quizalofop-P, quizalofop-P-ethyl, quizalofop-P-tefuryl, sethoxydim, tepraloxydim, tralkoxydim,
4-(4'-Chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1312337-72-6); 4-(2',4'-Dichloro-4-cyclopropyl[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1312337-45-3); 4-(4'-Chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1033757-93-5); 4-(2',4'-Dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-2,2,6,6-tetramethyl-2H-pyran-3,5(4H,6H)-dione (CAS 1312340-84-3); 5-(Acetyloxy)-4-(4'-chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1312337-48-6); 5-(Acetyloxy)-4-(2´,4'-dichloro-4-cyclopropyl-[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one; 5-(Acetyloxy)-4-(4'-chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1312340-82-1); 5-(Acetyloxy)-4-(2',4'-dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1033760-55-2); 4-(4'-Chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1312337-51-1); 4-(2´,4'-Dichloro -4-cyclopropyl- [1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester; 4-(4'-Chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1312340-83-2); 4-(2',4'-Dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1033760-58-5); and non ACC herbicides such as benfuresate, butylate, cycloate, dalapon, dimepiperate, EPTC, esprocarb, ethofumesate, flupropanate, molinate, orbencarb, pebulate, prosulfocarb, TCA, thiobencarb, tiocarbazil, triallate and vernolate;

b2) from the group of the ALS inhibitors:
sulfonylureas such as amidosulfuron, azimsulfuron, bensulfuron, bensulfuron-methyl, chlorimuron, chlorimuron-ethyl, chlorsulfuron, cinosulfuron, cyclosulfamuron, ethametsulfuron, ethametsulfuron-methyl, ethoxysulfuron, flazasulfuron, flucetosulfuron, flupyrsulfuron, flupyrsulfuron-methyl-sodium, foramsulfuron, halosulfuron, halosulfuron-methyl, imazosulfuron, iodosulfuron, iodosulfuron-methyl-sodium, iofensulfuron, iofensulfuron-sodium, mesosulfuron, metazosulfuron, metsulfuron, metsulfuron-methyl, nicosulfuron, orthosulfamuron, oxasulfuron, primisulfuron, primisulfuron-methyl, propyrisulfuron, prosulfuron, pyrazosulfuron, pyrazosulfuron-ethyl, rimsulfuron, sulfometuron, sulfometuron-methyl, sulfosulfuron, thifensulfuron, thifensulfuron-methyl, triasulfuron, tribenuron, tribenuron-methyl, trifloxysulfuron, triflusulfuron, triflusulfuron-methyl and tritosulfuron,
imidazolinones such as imazamethabenz, imazamethabenz-methyl, imazamox, imazapic, imazapyr, imazaquin and imazethapyr, triazolopyrimidine herbicides and sulfonanilides such as cloransulam, cloransulam-methyl, diclosulam, flumetsulam, florasulam, metosulam, penoxsulam, pyrimisulfan and pyroxsulam,
pyrimidinylbenzoates such as bispyribac, bispyribac-sodium, pyribenzoxim, pyriftalid, pyriminobac, pyriminobac-methyl, pyrithiobac, pyrithiobac-sodium, 4-[[[2-[(4,6-dimethoxy-2-pyrimidinyl)oxy]phenyl]methyl]amino]-benzoic acid-1-methylethyl ester (CAS 420138-41-6), 4-[[[2-[(4,6-dimethoxy-2-pyrimidinyl)oxy]phenyl]methyl]amino]-benzoic acid propyl ester (CAS 420138-40-5), N-(4-bromophenyl)-2-[(4,6-dimethoxy-2-pyrimidinyl)oxy]benzenemethanamine (CAS

420138-01-8),
sulfonylaminocarbonyl-triazolinone herbicides such as flucarbazone, flucarbazone-sodium, propoxycarbazone, propoxycarbazone-sodium, thiencarbazone and thiencarbazone-methyl; and triafamone;
among these, a preferred embodiment of the invention relates to those compositions comprising at least one imidazolinone herbicide;

b3) from the group of the photosynthesis inhibitors:
amicarbazone, inhibitors of the photosystem II, e.g. triazine herbicides, including of chlorotriazine, triazinones, triazindiones, methylthiotriazines and pyridazinones such as ametryn, atrazine, chloridazone, cyanazine, desmetryn, dimethametryn,hexazinone, metribuzin, prometon, prometryn, propazine, simazine, simetryn, terbumeton, terbuthylazin, terbutryn and trietazin, aryl urea such as chlorobromuron, chlorotoluron, chloroxuron, dimefuron, diuron, fluometuron, isoproturon, isouron, linuron, metamitron, methabenzthiazuron, metobenzuron, metoxuron, monolinuron, neburon, siduron, tebuthiuron and thiadiazuron, phenyl carbamates such as desmedipham, karbutilat, phenmedipham, phenmedipham-ethyl, nitrile herbicides such as bromofenoxim, bromoxynil and its salts and esters, ioxynil and its salts and esters, uraciles such as bromacil, lenacil and terbacil, and bentazon and bentazon-sodium, pyridate, pyridafol, pentanochlor and propanil and inhibitors of the photosystem I such as diquat, diquat-dibromide, paraquat, paraquat-dichloride and paraquat-dimetilsulfate. Among these, a preferred embodiment of the invention relates to those compositions comprising at least one aryl urea herbicide. Among these, likewise a preferred embodiment of the invention relates to those compositions comprising at least one triazine herbicide. Among these, likewise a preferred embodiment of the invention relates to those compositions comprising at least one nitrile herbicide;

b4) from the group of the protoporphyrinogen-IX oxidase inhibitors:
acifluorfen, acifluorfen-sodium, azafenidin, bencarbazone, benzfendizone, bifenox, butafenacil, carfentrazone, carfentrazone-ethyl, chlomethoxyfen, cinidon-ethyl, fluazolate, flufenpyr, flufenpyr-ethyl, flumiclorac, flumiclorac-pentyl, flumioxazin, fluoroglycofen, fluoroglycofen-ethyl, fluthiacet, fluthiacet-methyl, fomesafen, halosafen, lactofen, oxadiargyl, oxadiazon, oxyfluorfen, pentoxazone, profluazol, pyraclonil, pyraflufen, pyraflufen-ethyl, saflufenacil, sulfentrazone, thidiazimin, tiafenacil, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100, N-ethyl-3-(2,6-dichloro-4-trifluoro-methylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9), N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9), N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7), N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-

benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione, 1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione (CAS 1304113-05-0), methyl (*E*)-4-[2-chloro-5-[4-chloro-5-(difluoromethoxy)-1*H*-methyl-pyrazol-3-yl]-4-fluoro-phenoxy]-3-methoxy-but-2-enoate [CAS 948893-00-3*]*, and 3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4);

b5) from the group of the bleacher herbicides:
PDS inhibitors: beflubutamid, diflufenican, fluridone, flurochloridone, flurtamone, norflurazon, picolinafen, and 4-(3-trifluoromethylphenoxy)-2-(4-trifluoromethylphenyl)pyrimidine (CAS 180608-33-7), HPPD inhibitors: benzobicyclon, benzofenap, clomazone, isoxaflutole, mesotrione, pyrasulfotole, pyrazolynate, pyrazoxyfen, sulcotrione, tefuryltrione, tembotrione, topramezone and bicyclopyrone, bleacher, unknown target: aclonifen, amitrole and flumeturon;

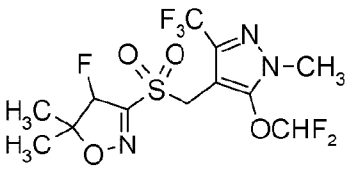
b6) from the group of the EPSP synthase inhibitors:
glyphosate, glyphosate-isopropylammonium, glyposate-potassium and glyphosate-trimesium (sulfosate);

b7) from the group of the glutamine synthase inhibitors:
bilanaphos (bialaphos), bilanaphos-sodium, glufosinate, glufosinate-P and glufosinate-ammonium;

b8) from the group of the DHP synthase inhibitors:
asulam;

b9) from the group of the mitosis inhibitors:
compounds of group K1: dinitroanilines such as benfluralin, butralin, dinitramine, ethalfluralin, fluchloralin, oryzalin, pendimethalin, prodiamine and trifluralin, phosphoramidates such as amiprophos, amiprophos-methyl, and butamiphos, benzoic acid herbicides such as chlorthal, chlorthal-dimethyl, pyridines such as dithiopyr and thiazopyr, benzamides such as propyzamide and tebutam; compounds of group K2: chlorpropham, propham and carbetamide, among these, compounds of group K1, in particular dinitroanilines are preferred;

b10) from the group of the VLCFA inhibitors:
chloroacetamides such as acetochlor, alachlor, butachlor, dimethachlor, dimethenamid, dimethenamid-P, metazachlor, metolachlor, metolachlor-S, pethoxamid, pretilachlor, propachlor, propisochlor and thenylchlor, oxyacetanilides such as flufenacet and mefenacet, acetanilides such as diphenamid, naproanilide and napropamide, tetrazolinones such fentrazamide, and other herbicides such as anilofos, cafenstrole, fenoxasulfone, ipfencarbazone, piperophos, pyroxasulfone and isoxazoline compounds of the formulae II.1, II.2, II.3, II.4, II.5, II.6, II.7, II.8 and II.9



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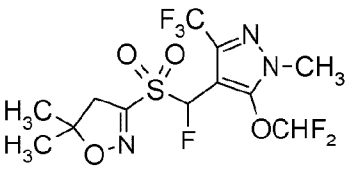

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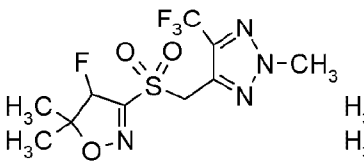

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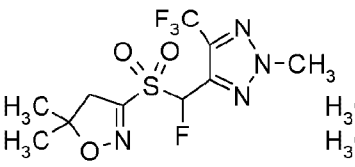

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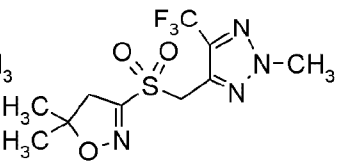

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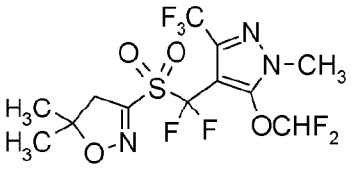

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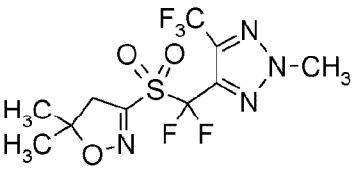

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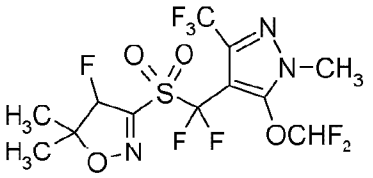

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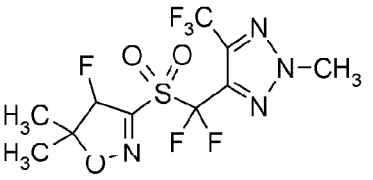


II.9

the isoxazoline compounds of the formula (I)I are known in the art, e.g. from WO 2006/024820, WO 2006/037945, WO 2007/071900 and WO 2007/096576;

among the VLCFA inhibitors, preference is given to chloroacetamides and oxyacetamides;

b11) from the group of the cellulose biosynthesis inhibitors:
chlorthiamid, dichlobenil, flupoxam, indaziflam, triaziflam, isoxaben and 1-Cyclohexyl-5-pentafluorphenyloxy-14-[1,2,4,6]thiatriazin-3-ylamine;

b12) from the group of the decoupler herbicides:
dinoseb, dinoterb and DNOC and its salts;

b13) from the group of the auxinic herbicides:
2,4-D and its salts and esters such as clacyfos, 2,4-DB and its salts and esters, aminocyclopyrachlor and its salts and esters, aminopyralid and its salts such as aminopyralid-tris(2-hydroxypropyl)ammonium and its esters, benazolin, benazolin-ethyl, chloramben and its salts and

esters, clomeprop, clopyralid and its salts and esters, dicamba and its salts and esters, dichlorprop and its salts and esters, dichlorprop-P and its salts and esters, fluroxypyr, fluroxypyr-butometyl, fluroxypyr-meptyl, halauxifen and its salts and esters (CAS 943832-60-8); MCPA and its salts and esters, MCPA-thioethyl, MCPB and its salts and esters, mecoprop and its salts and esters, mecoprop-P and its salts and esters, picloram and its salts and esters, quinclorac, quinmerac, TBA (2,3,6) and its salts and esters and triclopyr and its salts and esters;

b14) from the group of the auxin transport inhibitors: diflufenzopyr, diflufenzopyr-sodium, naptalam and naptalam-sodium;

b15) from the group of the other herbicides: bromobutide, chlorflurenol, chlorflurenol-methyl, cinmethylin, cumyluron, cyclopyrimorate (CAS 499223-49-3) and its salts and esters, dalapon, dazomet, difenzoquat, difenzoquat-metilsulfate, dimethipin, DSMA, dymron, endothal and its salts, etobenzanid, flamprop, flamprop-isopropyl, flamprop-methyl, flamprop-M-isopropyl, flamprop-M-methyl, flurenol, flurenol-butyl, flurprimidol, fosamine, fosamine-ammonium, indanofan, indaziflam, maleic hydrazide, mefluidide, metam, methiozolin (CAS 403640-27-7), methyl azide, methyl bromide, methyl-dymron, methyl iodide, MSMA, oleic acid, oxaziclomefone, pelargonic acid, pyributicarb, quinoclamine, triaziflam and tridiphane..

Preferred herbicides B that can be used in combination with the PPO-inhibiting herbicides according to the present invention are:

b1) from the group of the lipid biosynthesis inhibitors:
clethodim, clodinafop-propargyl, cycloxydim, cyhalofop-butyl, diclofop-methyl, fenoxaprop-P-ethyl, fluazifop-P-butyl, haloxyfop-P-methyl, metamifop, pinoxaden, profoxydim, propaquizafop, quizalofop-P-ethyl, quizalofop-P-tefuryl, sethoxydim, tepraloxydim, tralkoxydim, 4-(4'-Chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1312337-72-6); 4-(2',4'-Dichloro-4-cyclopropyl[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1312337-45-3); 4-(4'-Chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1033757-93-5); 4-(2',4'-Dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-2,2,6,6-tetramethyl-2H-pyran-3,5(4H,6H)-dione (CAS 1312340-84-3); 5-(Acetyloxy)-4-(4'-chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1312337-48-6); 5-(Acetyloxy)-4-(2´,4'-dichloro-4-cyclopropyl- [1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one; 5-(Acetyloxy)-4-(4'-chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1312340-82-1); 5-(Acetyloxy)-4-(2',4'-dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1033760-55-2); 4-(4'-Chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1312337-51-1); 4-(2´,4'-Dichloro -4-cyclopropyl- [1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester; 4-(4'-Chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1312340-83-2); 4-(2',4'-Dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1033760-58-5); benfuresate, dimepiperate, EPTC, esprocarb, ethofumesate, moli-

nate, orbencarb, prosulfocarb, thiobencarb and triallate;

b2) from the group of the ALS inhibitors:
amidosulfuron, azimsulfuron, bensulfuron-methyl, bispyribac-sodium, chlorimuron-ethyl, chlorsulfuron, cloransulam-methyl, cyclosulfamuron, diclosulam, ethametsulfuron-methyl, ethoxysulfuron, flazasulfuron, florasulam, flucarbazone-sodium, flucetosulfuron, flumetsulam, flupyrsulfuron-methyl-sodium, foramsulfuron, halosulfuron-methyl, imazamethabenz-methyl, imazamox, imazapic, imazapyr, imazaquin, imazethapyr, imazosulfuron, iodosulfuron, iodosulfuron-methyl-sodium, iofensulfuron, iofensulfuron-sodium, mesosulfuron, metazosulfuron, metosulam, metsulfuron-methyl, nicosulfuron, orthosulfamuron, oxasulfuron, penoxsulam, primisulfuron-methyl, propoxycarbazon-sodium, propyrisulfuron, prosulfuron, pyrazosulfuron-ethyl, pyribenzoxim, pyrimisulfan, pyriftalid, pyriminobac-methyl, pyrithiobac-sodium, pyroxsulam, rimsulfuron, sulfometuron-methyl, sulfosulfuron, thiencarbazone-methyl, thifensulfuron-methyl, triasulfuron, tribenuron-methyl, trifloxysulfuron, triflusulfuron-methyl, tritosulfuron and triafamone;

b3) from the group of the photosynthesis inhibitors:
ametryn, amicarbazone, atrazine, bentazone, bentazone-sodium, bromoxynil and its salts and esters, chloridazone, chlorotoluron, cyanazine, desmedipham, diquat-dibromide, diuron, fluometuron, hexazinone, ioxynil and its salts and esters, isoproturon, lenacil, linuron, metamitron, methabenzthiazuron, metribuzin, paraquat, paraquat-dichloride, phenmedipham, propanil, pyridate, simazine, terbutryn, terbuthylazine and thidiazuron;

b4) from the group of the protoporphyrinogen-IX oxidase inhibitors:
acifluorfen, acifluorfen-sodium, azafenidin, bencarbazone, benzfendizone, butafenacil, carfentrazone-ethyl, cinidon-ethyl, flufenpyr-ethyl, flumiclorac-pentyl, flumioxazin, fluoroglycofen-ethyl, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxadiazon, oxyfluorfen, pentoxazone, pyraflufen-ethyl, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), N-ethyl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9), N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9), N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7), N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione ;1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione, and 3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4);

b5) from the group of the bleacher herbicides:
aclonifen, beflubutamid, benzobicyclon, clomazone, diflufenican, flurochloridone, flurtamone, isoxaflutole, mesotrione, norflurazon, picolinafen, pyrasulfotole, pyrazolynate, sulcotrione, tefuryltrione, tembotrione, topramezone, bicyclopyrone, 4-(3-trifluoromethylphenoxy)-2-(4-trifluoromethylphenyl)pyrimidine (CAS 180608-33-7), amitrole and flumeturon;

b6) from the group of the EPSP synthase inhibitors:
glyphosate, glyphosate-isopropylammonium, glyphosate-potassium and glyphosate-trimesium (sulfosate);

b7) from the group of the glutamine synthase inhibitors:
glufosinate, glufosinate-P, glufosinate-ammonium;

b8) from the group of the DHP synthase inhibitors: asulam;

b9) from the group of the mitosis inhibitors:
benfluralin, dithiopyr, ethalfluralin, oryzalin, pendimethalin, thiazopyr and trifluralin;

b10) from the group of the VLCFA inhibitors:
acetochlor, alachlor, anilofos, butachlor, cafenstrole, dimethenamid, dimethenamid-P, fentrazamide, flufenacet, mefenacet, metazachlor, metolachlor, S-metolachlor, naproanilide, napropamide, pretilachlor, fenoxasulfone, ipfencarbazone, pyroxasulfone thenylchlor and isoxazoline-compounds of the formulae II.1, II.2, II.3, II.4, II.5, II.6, II.7, II.8 and II.9 as mentioned above;

b11) from the group of the cellulose biosynthesis inhibitors: dichlobenil, flupoxam, isoxaben and 1-Cyclohexyl-5-pentafluorphenyloxy-1^4-[1,2,4,6]thiatriazin-3-ylamine;

b13) from the group of the auxinic herbicides:
2,4-D and its salts and esters, aminocyclopyrachlor and its salts and esters, aminopyralid and its salts such as aminopyralid-tris(2-hydroxypropyl)ammonium and its esters, clopyralid and its salts and esters, dicamba and its salts and esters, dichlorprop-P and its salts and esters, fluroxypyr-meptyl, halauxifen and its salts and esters (CAS 943832-60-8), MCPA and its salts and esters, MCPB and its salts and esters, mecoprop-P and its salts and esters, picloram and its salts and esters, quinclorac, quinmerac and triclopyr and its salts and esters;

b14) from the group of the auxin transport inhibitors: diflufenzopyr and diflufenzopyr-sodium;

b15) from the group of the other herbicides: bromobutide, cinmethylin, cumyluron, cyclopyrimorate (CAS 499223-49-3) and its salts and esters, dalapon, difenzoquat, difenzoquat-metilsulfate, DSMA, dymron (= daimuron), flamprop, flamprop-isopropyl, flamprop-methyl, flamprop-M-isopropyl, flamprop-M-methyl, indanofan, indaziflam, metam, methylbromide, MSMA, oxaziclomefone, pyributicarb, triaziflam and tridiphane.

Particularly preferred herbicides B that can be used in combination with the PPO-inhibiting herbicides according to the present invention are:

b1) from the group of the lipid biosynthesis inhibitors: clodinafop-propargyl, cycloxydim, cyhalofop-butyl, fenoxaprop-P-ethyl, pinoxaden, profoxydim, tepraloxydim, tralkoxydim, 4-(4'-Chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1312337-72-6); 4-(2',4'-Dichloro-4-cyclopropyl[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1312337-45-3); 4-(4'-Chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5-hydroxy-2,2,6,6-tetramethyl-2H-pyran-3(6H)-one (CAS 1033757-93-5); 4-(2',4'-Dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-2,2,6,6-tetramethyl-2H-pyran-3,5(4H,6H)-dione (CAS 1312340-84-3); 5-(Acetyloxy)-4-(4'-chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1312337-48-6); 5-(Acetyloxy)-4-(2´,4'-dichloro-4-cyclopropyl- [1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one; 5-(Acetyloxy)-4-(4'-chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1312340-82-1); 5-(Acetyloxy)-4-(2',4'-dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-3,6-dihydro-2,2,6,6-tetramethyl-2H-pyran-3-one (CAS 1033760-55-2); 4-(4'-Chloro-4-cyclopropyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1312337-51-1); 4-(2´,4'-Dichloro -4-cyclopropyl- [1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester; 4-(4'-Chloro-4-ethyl-2'-fluoro[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1312340-83-2); 4-(2',4'-Dichloro-4-ethyl[1,1'-biphenyl]-3-yl)-5,6-dihydro-2,2,6,6-tetramethyl-5-oxo-2H-pyran-3-yl carbonic acid methyl ester (CAS 1033760-58-5); esprocarb, prosulfocarb, thiobencarb and triallate;

b2) from the group of the ALS inhibitors: bensulfuron-methyl, bispyribac-sodium, cyclosulfamuron, diclosulam, flumetsulam, flupyrsulfuron-methyl-sodium, foramsulfuron, imazamox, imazapic, imazapyr, imazaquin, imazethapyr, imazosulfuron, iodosulfuron, iodosulfuron-methyl-sodium, iofensulfuron, iofensulfuron-sodium, mesosulfuron, metazosulfuron, nicosulfuron, penoxsulam, propoxycarbazon-sodium, propyrisulfuron, pyrazosulfuron-ethyl, pyroxsulam, rimsulfuron, sulfosulfuron, thiencarbazon-methyl, tritosulfuron and triafamone;

b3) from the group of the photosynthesis inhibitors: ametryn, atrazine, diuron, fluometuron, hexazinone, isoproturon, linuron, metribuzin, paraquat, paraquat-dichloride, propanil, terbutryn and terbuthylazine;

b4) from the group of the protoporphyrinogen-IX oxidase inhibitors: acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), and 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-

tetrahydro-isoindole-1,3-dione, and 1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione;

b5) from the group of the bleacher herbicides: clomazone, diflufenican, flurochloridone, isoxaflutole, mesotrione, picolinafen, sulcotrione, tefuryltrione, tembotrione, topramezone, bicyclopyrone, amitrole and flumeturon;

b6) from the group of the EPSP synthase inhibitors: glyphosate, glyphosate-isopropylammonium and glyphosate-trimesium (sulfosate);

b7) from the group of the glutamine synthase inhibitors: glufosinate, glufosinate-P and glufosinate-ammonium;

b9) from the group of the mitosis inhibitors: pendimethalin and trifluralin;

b10) from the group of the VLCFA inhibitors: acetochlor, cafenstrole, dimethenamid-P, fentrazamide, flufenacet, mefenacet, metazachlor, metolachlor, S-metolachlor, fenoxasulfone, ipfencarbazone and pyroxasulfone; likewise, preference is given to isoxazoline compounds of the formulae II.1, II.2, II.3, II.4, II.5, II.6, II.7, II.8 and II.9 as mentioned above;

b11) from the group of the cellulose biosynthesis inhibitors: isoxaben;

b13) from the group of the auxinic herbicides: 2,4-D and its salts and esters such as clacyfos, and aminocyclopyrachlor and its salts and esters, aminopyralid and its salts and its esters, clopyralid and its salts and esters, dicamba and its salts and esters, fluroxypyr-meptyl, quinclorac and quinmerac;

b14) from the group of the auxin transport inhibitors: diflufenzopyr and diflufenzopyr-sodium,

b15) from the group of the other herbicides: dymron (= daimuron), indanofan, indaziflam, oxaziclomefone and triaziflam.

Moreover, it may be useful to apply the PPO-inhibiting herbicides, when used in combination with a compound B described SUPRA, in combination with safeners. Safeners are chemical compounds which prevent or reduce damage on useful plants without having a major impact on the herbicidal action of herbicides towards unwanted plants. 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.

Furthermore, the safeners C, the PPO-inhibiting herbicides and/or the herbicides B can be applied simultaneously or in succession.

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-phenylcarbamates and their agriculturally acceptable salts and their agriculturally acceptable derivatives such amides, esters, and thioesters, provided they have an acid group.

Examples of preferred safeners C 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).

Especially preferred safeners C are benoxacor, cloquintocet, cyprosulfamide, dichlormid, fenchlorazole, fenclorim, flurazole, fluxofenim, furilazole, isoxadifen, mefenpyr, 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).

Particularly preferred safeners C are benoxacor, cloquintocet, cyprosulfamide, dichlormid, fenchlorazole, fenclorim, furilazole, isoxadifen, mefenpyr, naphtalic anhydride, 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).

Also preferred safeners C are benoxacor, cloquintocet, cyprosulfamide, dichlormid, fenchlorazole, fenclorim, furilazole, isoxadifen, mefenpyr, 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)..

Particularly preferred safeners C, which, as component C, are constituent of the composition according to the invention are the safeners C as defined above; in particular the safeners C.1 - C.12 listed below in table C:

Table C

	Safener C
	C.1 benoxacor
	C.2 cloquintocet
	C.3 cyprosulfamide
	C.4 dichlormid
	C.5 fenchlorazole
	C.6 fenclorim
	C.7 furilazole

	C.8 isoxadifen
	C.9 mefenpyr
	C.10 naphtalic acid anhydride
	C.11 4-(dichloroacetyl)-1-oxa-4-azaspiro[4.5]decane (MON4660, CAS 71526-07-3)
	C.12 2,2,5-trimethyl-3-(dichloro-acetyl)-1,3-oxazolidine (R-29148, CAS 52836-31-4)

The PPO-inhibiting herbicides and the active compounds B of groups b1) to b15) and the active compounds C are known herbicides and safeners, see, for example, The Compendium of Pesticide Common Names; Farm Chemicals Handbook 2000 volume 86, Meister Publishing Company, 2000; B. Hock, C. Fedtke, R. R. Schmidt, Herbizide [Herbicides], Georg Thieme Verlag, Stuttgart 1995; W. H. Ahrens, Herbicide Handbook, 7th edition, Weed Science Society of America, 1994; and K. K. Hatzios, Herbicide Handbook, Supplement for the 7th edition, Weed Science Society of America, 1998. 2,2,5-Trimethyl-3-(dichloroacetyl)-1,3-oxazolidine [CAS No. 52836-31-4] is also referred to as R-29148. 4-(Dichloroacetyl)-1-oxa-4-azaspiro[4.5]decane [CAS No. 71526-07-3] is also referred to as AD-67 and MON 4660.

The assignment of the active compounds to the respective mechanisms of action is based on current knowledge. If several mechanisms of action apply to one active compound, this substance was only assigned to one mechanism of action.

Active compounds B and C having a carboxyl group can be employed in the form of the acid, in the form of an agriculturally suitable salt as mentioned above or else in the form of an agriculturally acceptable derivative in the compositions according to the invention.

In the case of dicamba, suitable salts include those, where the counterion is an agriculturally acceptable cation. For example, suitable salts of dicamba are dicamba-sodium, dicamba-potassium, dicamba-methylammonium, dicamba-dimethylammonium, dicamba-isopropylammonium, dicamba-diglycolamine, dicamba-olamine, dicamba-diolamine, dicamba-trolamine, dicamba-N,N-bis-(3-aminopropyl)methylamine and dicamba-diethylenetriamine. Examples of a suitable ester are dicamba-methyl and dicamba-butotyl.
Suitable salts of 2,4-D are 2,4-D-ammonium, 2,4-D-dimethylammonium, 2,4-D-diethylammonium, 2,4-D-diethanolammonium (2,4-D-diolamine), 2,4-D-triethanolammonium, 2,4-D-isopropylammonium, 2,4-D-triisopropanolammonium, 2,4-D-heptylammonium, 2,4-D-dodecylammonium, 2,4-D-tetradecylammonium, 2,4-D-triethylammonium, 2,4-D-tris(2-hydroxypropyl)ammonium, 2,4-D-tris(isopropyl)ammonium, 2,4-D-trolamine, 2,4-D-lithium, 2,4-D-sodium. Examples of suitable esters of 2,4-D are 2,4-D-butotyl, 2,4-D-2-butoxypropyl, 2,4-D-3-butoxypropyl, 2,4-D-butyl, 2,4-D-ethyl, 2,4-D-ethylhexyl, 2,4-D-isobutyl, 2,4-D-isooctyl, 2,4-D-isopropyl, 2,4-D-meptyl, 2,4-D-methyl, 2,4-D-octyl, 2,4-D-pentyl, 2,4-D-propyl, 2,4-D-tefuryl and clacyfos.
Suitable salts of 2,4-DB are for example 2,4-DB-sodium, 2,4-DB-potassium and 2,4-DB-dimethylammonium. Suitable esters of 2,4-DB are for example 2,4-DB-butyl and 2,4-DB-isoctyl.
Suitable salts of dichlorprop are for example dichlorprop-sodium, dichlorprop-potassium and di-

chlorprop-dimethylammonium. Examples of suitable esters of dichlorprop are dichlorprop-butotyl and dichlorprop-isoctyl.

Suitable salts and esters of MCPA include MCPA-butotyl, MCPA-butyl, MCPA-dimethylammonium, MCPA-diolamine, MCPA-ethyl, MCPA-thioethyl, MCPA-2-ethylhexyl, MCPA-isobutyl, MCPA-isoctyl, MCPA-isopropyl, MCPA-isopropylammonium, MCPA-methyl, MCPA-olamine, MCPA-potassium, MCPA-sodium and MCPA-trolamine.

A suitable salt of MCPB is MCPB sodium. A suitable ester of MCPB is MCPB-ethyl.

Suitable salts of clopyralid are clopyralid-potassium, clopyralid-olamine and clopyralid-tris-(2-hydroxypropyl)ammonium. Example of suitable esters of clopyralid is clopyralid-methyl.

Examples of a suitable ester of fluroxypyr are fluroxypyr-meptyl and fluroxypyr-2-butoxy-1-methylethyl, wherein fluroxypyr-meptyl is preferred.

Suitable salts of picloram are picloram-dimethylammonium, picloram-potassium, picloram-triisopropanolammonium, picloram-triisopropylammonium and picloram-trolamine. A suitable ester of picloram is picloram-isoctyl.

A suitable salt of triclopyr is triclopyr-triethylammonium. Suitable esters of triclopyr are for example triclopyr-ethyl and triclopyr-butotyl.

Suitable salts and esters of chloramben include chloramben-ammonium, chloramben-diolamine, chloramben-methyl, chloramben-methylammonium and chloramben-sodium. Suitable salts and esters of 2,3,6-TBA include 2,3,6-TBA-dimethylammonium, 2,3,6-TBA-lithium, 2,3,6-TBA-potassium and 2,3,6-TBA-sodium.

Suitable salts and esters of aminopyralid include aminopyralid-potassium and aminopyralid-tris(2-hydroxypropyl)ammonium.

Suitable salts of glyphosate are for example glyphosate-ammonium, glyphosate-diammonium, glyphoste-dimethylammonium, glyphosate-isopropylammonium, glyphosate-potassium, glyphosate-sodium, glyphosate-trimesium as well as the ethanolamine and diethanolamine salts, preferably glyphosate-diammonium, glyphosate-isopropylammonium and glyphosate-trimesium (sulfosate).

A suitable salt of glufosinate is for example glufosinate-ammonium.

A suitable salt of glufosinate-P is for example glufosinate-P-ammonium.

Suitable salts and esters of bromoxynil are for example bromoxynil-butyrate, bromoxynil-heptanoate, bromoxynil-octanoate, bromoxynil-potassium and bromoxynil-sodium.

Suitable salts and esters of ioxonil are for example ioxonil-octanoate, ioxonil-potassium and ioxonil-sodium.

Suitable salts and esters of mecoprop include mecoprop-butotyl, mecoprop-dimethylammonium, mecoprop-diolamine, mecoprop-ethadyl, mecoprop-2-ethylhexyl, mecoprop-isoctyl, mecoprop-methyl, mecoprop-potassium, mecoprop-sodium and mecoprop-trolamine.

Suitable salts of mecoprop-P are for example mecoprop-P-butotyl, mecoprop-P-dimethylammonium, mecoprop-P-2-ethylhexyl, mecoprop-P-isobutyl, mecoprop-P-potassium and mecoprop-P-sodium.

A suitable salt of diflufenzopyr is for example diflufenzopyr-sodium.

A suitable salt of naptalam is for example naptalam-sodium.

Suitable salts and esters of aminocyclopyrachlor are for example aminocyclopyrachlor-dimethylammonium, aminocyclopyrachlor-methyl, aminocyclopyrachlor-triisopropanolammonium, aminocyclopyrachlor-sodium and aminocyclopyrachlor-potassium.

A suitable salt of quinclorac is for example quinclorac-dimethylammonium.
A suitable salt of quinmerac is for example quinclorac-dimethylammonium.
A suitable salt of imazamox is for example imazamox-ammonium.
Suitable salts of imazapic are for example imazapic-ammonium and imazapic-isopropylammonium.
Suitable salts of imazapyr are for example imazapyr-ammonium and imazapyr-isopropylammonium.
A suitable salt of imazaquin is for example imazaquin-ammonium.
Suitable salts of imazethapyr are for example imazethapyr-ammonium and imazethapyr-isopropylammonium.
A suitable salt of topramezone is for example topramezone-sodium.

The preferred embodiments of the invention mentioned herein below have to be understood as being preferred either independently from each other or in combination with one another.

According to a preferred embodiment of the invention, the composition comprises as component B at least one, preferably exactly one herbicide B.

According to another preferred embodiment of the invention, the composition comprises at least two, preferably exactly two, herbicides B different from each other.

According to another preferred embodiment of the invention, the composition comprises at least three, preferably exactly three, herbicides B different from each other.

According to another preferred embodiment of the invention, the composition comprises as component A at least one, preferably exactly one PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100;, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), and as component B at least one, preferably exactly one, herbicide B.

According to another preferred embodiment of the invention, the composition comprises as component A at least one, preferably exactly preferably exactly one PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), and

at least two, preferably exactly two, herbicides B different from each other.

According to another preferred embodiment of the invention, the composition comprises as component A at least one, preferably exactly preferably exactly one PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) and at least three, preferably exactly three, herbicides B different from each other.

According to another preferred embodiment of the invention, the composition comprises, in addition to a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b1), in particular selected from the group consisting of clethodim, clodinafop-propargyl, cycloxydim, cyhalofop-butyl, fenoxaprop-P-ethyl, fluazifop, pinoxaden, profoxydim, quizalofop, sethoxydim, tepraloxydim, tralkoxydim, esprocarb, prosulfocarb, thiobencarb and triallate.

According to another preferred embodiment of the invention, the composition comprises, in addition to a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b2), in particular selected from the group consisting of bensulfuron-methyl, bispyribac-sodium, cloransulam-methyl, cyclosulfamuron, diclosulam, flumetsulam, flupyrsulfuron-methyl-sodium, foramsulfuron, halosulfuron-methyl, imazamox, imazapic, imazapyr, imazaquin, imazethapyr, imazosulfuron, iodosulfuron, iodosulfuron-methyl-sodium, mesosulfuron-methyl, met-

azosulfuron, nicosulfuron, penoxsulam, propoxycarbazon-sodium, pyrazosulfuron-ethyl, pyrithiobac-sodium, pyroxsulam, rimsulfuron, sulfosulfuron, thiencarbazon-methyl, thifensulfuron-methyl, trifloxysulfuron and tritosulfuron.

According to another preferred embodiment of the invention, the composition comprises, in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b3), in particular selected from the group consisting of ametryn, atrazine, bentazon, bromoxynil, diuron, fluometuron, hexazinone, isoproturon, linuron, metribuzin, paraquat, paraquat-dichloride, prometryne, propanil, terbutryn and terbuthylazine.

According to another preferred embodiment of the invention, the composition comprises, in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b4), in particular selected from the group consisting of acifluorfen, acifluorfen-sodium, azafenidin, bencarbazone, benzfendizone, bifenox, butafenacil, carfentrazone, carfentrazone-ethyl, chlomethoxyfen, cinidon-ethyl, fluazolate, flufenpyr, flufenpyr-ethyl, flumiclorac, flumiclorac-pentyl, flumioxazin, fluoroglycofen, fluoroglycofen-ethyl, fluthiacet, fluthiacet-methyl, fomesafen, halosafen, lactofen, oxadiargyl, oxadiazon, oxyfluorfen, pentoxazone, profluazol, pyraclonil, pyraflufen, pyraflufen-ethyl, saflufenacil, sulfentrazone, thidiazimin, tiafenacil, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), N-ethyl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9), N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9), N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7), N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7), 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-

dione (CAS 1258836-72-4), 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione, 1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione, methyl (*E*)-4-[2-chloro-5-[4-chloro-5-(difluoromethoxy)-1*H*-methyl-pyrazol-3-yl]-4-fluoro-phenoxy]-3-methoxy-but-2-enoate [CAS 948893-00-3*]*, 3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4).

According to another preferred embodiment of the invention, the composition comprises, in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b5), in particular selected from the group consisting of clomazone, diflufenican, flurochloridone, isoxaflutole, mesotrione, picolinafen, sulcotrione, tefuryltrione, tembotrione, topramezone, bicyclopyrone, amitrole and flumeturon.

According to another preferred embodiment of the invention, the composition comprises, in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b6), in particular selected from the group consisting of glyphosate, glyphosate-isopropylammonium and glyphosate-trimesium (sulfosate).

According to another preferred embodiment of the invention, the composition comprises, in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active com-

pound from group b7), in particular selected from the group consisting of glufosinate, glufosinate-P and glufosinate-ammonium.

According to another preferred embodiment of the invention, the composition comprises, in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4) especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b9), in particular selected from the group consisting of pendimethalin and trifluralin.

According to another preferred embodiment of the invention, the composition comprises, in addition to a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4)*)*, especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b10), in particular selected from the group consisting of acetochlor, cafenstrole, dimethenamid-P, fentrazamide, flufenacet, mefenacet, metazachlor, metolachlor, S-metolachlor, fenoxasulfone and pyroxasulfone. Likewise, preference is given to compositions comprising in addition to a a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b10), in particular selected from the group consisting of isoxazoline compounds of the formulae II.1, II.2, II.3, II.4, II.5, II.6, II.7, II.8 and II.9, as defined above.

According to another preferred embodiment of the invention, the composition comprises, in addition to a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-

tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b13), in particular selected from the group consisting of 2,4-D and its salts and esters, aminocyclopyrachlor and its salts and esters, aminopyralid and its salts such as aminopyralid-tris(2-hydroxypropyl)ammonium and its esters, clopyralid and its salts and esters, dicamba and its salts and esters, fluroxypyr-meptyl, quinclorac and quinmerac.

According to another preferred embodiment of the invention, the composition comprises, in addition to a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b14), in particular selected from the group consisting of diflufenzopyr and diflufenzopyr-sodium.

According to another preferred embodiment of the invention, the composition comprises, in addition to a PPO A, preferably acifluorfen, acifluorfen-sodium, butafenacil, cinidon-ethyl, carfentrazone-ethyl, flumioxazin, fluthiacet-methyl, fomesafen, lactofen, oxadiargyl, oxyfluorfen, saflufenacil, sulfentrazone, ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100), 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), especially preferred saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4), at least one and especially exactly one herbicidally active compound from group b15), in particular selected from the group consisting of dymron (= daimuron), indanofan, indaziflam, oxaziclomefone and triaziflam.

Here and below, the term “binary compositions” includes compositions comprising one or more, for example 1, 2 or 3, active compounds of the PPO A and either one or more, for example 1, 2 or 3, herbicides B.

In binary compositions comprising at least one PPO A as component A and at least one herbicide B, the weight ratio of the active compounds A:B is generally in the range of from 1:1000 to 1000:1,

preferably in the range of from 1:500 to 500:1, in particular in the range of from 1:250 to 250:1 and particularly preferably in the range of from 1:75 to 75:1.

Particularly preferred herbicides B are the herbicides B as defined above; in particular the herbicides B.1 - B.229 listed below in table B:

Table B:

	Herbicide B
B.1	clethodim
B.2	clodinafop-propargyl
B.3	cycloxydim
B.4	cyhalofop-butyl
B.5	fenoxaprop-ethyl
B.6	fenoxaprop-P-ethyl
B.7	fluazifop
B.8	metamifop
B.9	pinoxaden
B.10	profoxydim
B.11	quizalofop
B.12	sethoxydim
B.13	tepraloxydim
B.14	tralkoxydim
B.15	esprocarb
B.16	ethofumesate
B.17	molinate
B.18	prosulfocarb
B.19	thiobencarb
B.20	triallate
B.21	bensulfuron-methyl
B.22	bispyribac-sodium
B.23	cloransulam-methyl
B.24	chlorsulfuron
B.25	clorimuron
B.26	cyclosulfamuron
B.27	diclosulam
B.28	florasulam
B.29	flumetsulam
B.30	flupyrsulfuron-methyl-sodium
B.31	foramsulfuron
B.32	halosulfuron-methyl
B.33	imazamox

	Herbicide B
B.34	imazamox-ammonium
B.35	imazapic
B.36	imazapic-ammonium
B.37	imazapic-isopropylammonium
B.38	imazapyr
B.39	imazapyr-ammonium
B.40	imazapyr-isopropylammonium
B.41	imazaquin
B.42	imazaquin-ammonium
B.43	imazethapyr
B.44	imazethapyr-ammonium
B.45	imazethapyr-isopropylammonium
B.46	imazosulfuron
B.47	iodosulfuron-methyl-sodium
B.48	iofensulfuron
B.49	iofensulfuron-sodium
B.50	mesosulfuron-methyl
B.51	metazosulfuron
B.52	metsulfuron-methyl
B.53	metosulam
B.54	nicosulfuron
B.55	penoxsulam
B.56	propoxycarbazon-sodium
B.57	pyrazosulfuron-ethyl
B.58	pyribenzoxim
B.59	pyriftalid
B.60	pyrithiobac-sodium
B.61	pyroxsulam
B.62	propyrisulfuron
B.63	rimsulfuron
B.64	sulfosulfuron
B.65	thiencarbazone-methyl

	Herbicide B
B.66	thifensulfuron-methyl
B.67	tribenuron-methyl
B.68	trifloxysulfuron
B.69	tritosulfuron
B.70	triafamone
B.71	ametryne
B.72	atrazine
B.73	bentazon
B.74	bromoxynil
B.75	bromoxynil-octanoate
B.76	bromoxynil-heptanoate
B.77	bromoxynil-potassium
B.78	diuron
B.79	fluometuron
B.80	hexazinone
B.81	isoproturon
B.82	linuron
B.83	metamitron
B.84	metribuzin
B.85	prometryne
B.86	propanil
B.87	simazin
B.88	terbuthylazine
B.89	terbutryn
B.90	paraquat-dichloride
B.91	acifluorfen
B.92	acifluorfen-sodium
B.93	azafenidin
B.94	bencarbazone
B.95	benzfendizone
B.96	bifenox
B.97	butafenacil
B.98	carfentrazone
B.99	carfentrazone-ethyl
B.100	chlomethoxyfen
B.101	cinidon-ethyl
B.102	fluazolate
B.103	flufenpyr
B.104	flufenpyr-ethyl

	Herbicide B
B.105	flumiclorac
B.106	flumiclorac-pentyl
B.107	flumioxazin
B.108	fluoroglycofen
B.109	fluoroglycofen-ethyl
B.110	fluthiacet
B.111	fluthiacet-methyl
B.112	fomesafen
B.113	halosafen
B.114	lactofen
B.115	oxadiargyl
B.116	oxadiazon
B.117	oxyfluorfen
B.118	pentoxazone
B.119	profluazol
B.120	pyraclonil
B.121	pyraflufen
B.122	pyraflufen-ethyl
B.123	saflufenacil
B.124	sulfentrazone
B.125	thidiazimin
B.126	tiafenacil
B.127	ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyl-oxy]acetate (CAS 353292-31-6)
B.128	1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]-oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4)
B.129	N-ethyl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9)

	Herbicide B
B.130	N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9)
B.131	N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7)
B.132	N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoro-methylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7)
B.133	3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione
B.134	2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione
B.135	1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione
B.136	methyl (*E*)-4-[2-chloro-5-[4-chloro-5-(difluoromethoxy)-1*H*-methyl-pyrazol-3-yl]-4-fluoro-phenoxy]-3-methoxy-but-2-enoate [CAS 948893-00-3]
B.137	3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4)

	Herbicide B
B.138	benzobicyclon
B.139	clomazone
B.140	diflufenican
B.141	flurochloridone
B.142	isoxaflutole
B.143	mesotrione
B.144	norflurazone
B.145	picolinafen
B.146	sulcotrione
B.147	tefuryltrione
B.148	tembotrione
B.149	topramezone
B.150	topramezone-sodium
B.151	bicyclopyrone
B.152	amitrole
B.153	fluometuron
B.154	glyphosate
B.155	glyphosate-ammonium
B.156	glyphosate-dimethylammonium
B.157	glyphosate-isopropylammonium
B.158	glyphosate-trimesium (sulfosate)
B.159	glyphosate-potassium
B.160	glufosinate
B.161	glufosinate-ammonium
B.162	glufosinate-P
B.163	glufosinate-P-ammonium
B.164	pendimethalin
B.165	trifluralin
B.166	acetochlor
B.167	butachlor
B.168	cafenstrole
B.169	dimethenamid-P
B.170	fentrazamide
B.171	flufenacet
B.172	mefenacet
B.173	metazachlor
B.174	metolachlor
B.175	S-metolachlor

	Herbicide B
B.176	pretilachlor
B.177	fenoxasulfone
B.178	isoxaben
B.179	ipfencarbazone
B.180	pyroxasulfone
B.181	2,4-D
B.182	2,4-D-isobutyl
B.183	2,4-D-dimethylammonium
B.184	2,4-D-N,N,N-trimethylethanolammonium
B.185	aminopyralid
B.186	aminopyralid-methyl
B.187	aminopyralid-tris(2-hydroxypropyl)ammonium
B.188	clopyralid
B.189	clopyralid-methyl
B.190	clopyralid-olamine
B.191	dicamba
B.192	dicamba-butotyl
B.193	dicamba-diglycolamine
B.194	dicamba-dimethylammonium
B.195	dicamba-diolamine
B.196	dicamba-isopropylammonium
B.197	dicamba-potassium
B.198	dicamba-sodium
B.199	dicamba-trolamine
B.200	dicamba-N,N-bis-(3-aminopropyl)methylamine
B.201	dicamba-diethylenetriamine

	Herbicide B
B.202	fluroxypyr
B.203	fluroxypyr-meptyl
B.204	MCPA
B.205	MCPA-2-ethylhexyl
B.206	MCPA-dimethylammonium
B.207	quinclorac
B.208	quinclorac-dimethylammonium
B.209	quinmerac
B.210	quinmerac-dimethylammonium
B.211	aminocyclopyrachlor
B.212	aminocyclopyrachlor-potassium
B.213	aminocyclopyrachlor-methyl
B.214	diflufenzopyr
B.215	diflufenzopyr-sodium
B.216	dymron
B.217	indanofan
B.218	indaziflam
B.219	oxaziclomefone
B.220	triaziflam
B.221	II.1
B.222	II.2
B.223	II.3
B.224	II.4
B.225	II.5
B.226	II.6
B.227	II.7
B.228	II.8
B.229	II.9

Particularly preferred are compositions 1.1 to 1.229, comprising acifluorfen and the substance(s) as defined in the respective row of table B-1:

Table B-1(compositions 1.1 to 1.229):

comp. no.	herbicide B
1.1	B.1
1.2	B.2
1.3	B.3
1.4	B.4
1.5	B.5
1.6	B.6
1.7	B.7
1.8	B.8
1.9	B.9
1.10	B.10
1.11	B.11
1.12	B.12
1.13	B.13
1.14	B.14
1.15	B.15
1.16	B.16
1.17	B.17
1.18	B.18
1.19	B.19
1.20	B.20
1.21	B.21
1.22	B.22
1.23	B.23
1.24	B.24
1.25	B.25
1.26	B.26
1.27	B.27
1.28	B.28
1.29	B.29
1.30	B.30
1.31	B.31
1.32	B.32
1.33	B.33
1.34	B.34
1.35	B.35
1.36	B.36
1.37	B.37
1.38	B.38
1.39	B.39
1.40	B.40
1.41	B.41
1.42	B.42
1.43	B.43
1.44	B.44
1.45	B.45
1.46	B.46
1.47	B.47
1.48	B.48
1.49	B.49
1.50	B.50
1.51	B.51
1.52	B.52
1.53	B.53
1.54	B.54
1.55	B.55
1.56	B.56
1.57	B.57
1.58	B.58.
1.59	B.59
1.60	B.60
1.61	B.61
1.62	B.62
1.63	B.63
1.64	B.64
1.65	B.65
1.66	B.66
1.67	B.67
1.68	B.68
1.69	B.69
1.70	B.70
1.71	B.71
1.72	B.72
1.73	B.73
1.74	B.74
1.75	B.75
1.76	B.76
1.77	B.77
1.78	B.78
1.79	B.79
1.80	B.80
1.81	B.81
1.82	B.82
1.83	B.83
1.84	B.84
1.85	B.85
1.86	B.86
1.87	B.87
1.88	B.88
1.89	B.89
1.90	B.90
1.91	B.91
1.92	B.92
1.93	B.93
1.94	B.94
1.95	B.95
1.96	B.96
1.97	B.97
1.98	B.98
1.99	B.99
1.100	B.100
1.101	B.101
1.102	B.102
1.103	B.103
1.104	B.104
1.105	B.105

1.106	B.106
1.107	B.107
1.108	B.108
1.109	B.109
1.110	B.110
1.111	B.111
1.112	B.112
1.113	B.113
1.114	B.114
1.115	B.115
1.116	B.116
1.117	B.117
1.118	B.118
1.119	B.119
1.120	B.120
1.121	B.121
1.122	B.122
1.123	B.123
1.124	B.124
1.125	B.125
1.126	B.126
1.127	B.127
1.128	B.128
1.129	B.129
1.130	B.130
1.131	B.131
1.132	B.132
1.133	B.133
1.134	B.134
1.135	B.135
1.136	B.136
1.137	B.137
1.138	B.138
1.139	B.139
1.140	B.140
1.141	B.141
1.142	B.142
1.143	B.143
1.144	B.144
1.145	B.145
1.146	B.146
1.147	B.147

1.148	B.148
1.149	B.149
1.150	B.150
1.151	B.151
1.152	B.152
1.153	B.153
1.154	B.154
1.155	B.155
1.156	B.156
1.157	B.157
1.158	B.158
1.159	B.159
1.160	B.160
1.161	B.161
1.162	B.162
1.163	B.163
1.164	B.164
1.165	B.165
1.166	B.166
1.167	B.167
1.168	B.168
1.169	B.169
1.170	B.170
1.171	B.171
1.172	B.172
1.173	B.173
1.174	B.174
1.175	B.175
1.176	B.176
1.177	B.177
1.178	B.178
1.179	B.179
1.180	B.180
1.181	B.181
1.182	B.182
1.183	B.183
1.184	B.184
1.185	B.185
1.186	B.186
1.187	B.187
1.188	B.188
1.189	B.189

1.190	B.190
1.191	B.191
1.192	B.192
1.193	B.193
1.194	B.194
1.195	B.195
1.196	B.196
1.197	B.197
1.198	B.198
1.199	B.199
1.200	B.200
1.201	B.201
1.202	B.202
1.203	B.203
1.204	B.204
1.205	B.205
1.206	B.206
1.207	B.207
1.208	B.208
1.209	B.209
1.210	B.210
1.211	B.211
1.212	B.212
1.213	B.213
1.214	B.214
1.215	B.215
1.216	B.216
1.217	B.217
1.218	B.218
1.219	B.219
1.220	B.220
1.221	B.221
1.222	B.222
1.223	B.223
1.224	B.224
1.225	B.225
1.226	B.226
1.227	B.227
1.228	B.228
1.229	B.229

Also especially preferred are compositions 2.1. to 2.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A acifluorfen-sodium.

Also especially preferred are compositions 3.1. to 3.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A azafenidin.

Also especially preferred are compositions 4.1. to 4.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A bencarbazone.

Also especially preferred are compositions 5.1. to 5.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A benzfendizone.

Also especially preferred are compositions 6.1. to 6.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A bifenox.

Also especially preferred are compositions 7.1. to 7.229 which differ from the corresponding compositions 1.1 to 1.227 only in that they comprise as component A butafenacil.

Also especially preferred are compositions 8.1. to 8.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A carfentrazone.

Also especially preferred are compositions 9.1. to 9.229which differ from the corresponding compositions 1.1 to 1. 229 only in that they comprise as component A carfentrazone-ethyl.

Also especially preferred are compositions 10.1. to 10.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A chlomethoxyfen.

Also especially preferred are compositions 11.1. to 11.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A cinidon-ethyl.

Also especially preferred are compositions 12.1. to 12.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A fluazolate.

Also especially preferred are compositions 13.1. to 13.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A flufenpyr.

Also especially preferred are compositions 14.1. to 14.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A flufenpyr-ethyl.

Also especially preferred are compositions 15.1. to 15.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A flumiclorac.

Also especially preferred are compositions 16.1. to 16.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A flumiclorac-pentyl.

Also especially preferred are compositions 17.1. to 17.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A flumioxazin.

Also especially preferred are compositions 18.1. to 18.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A fluoroglycofen.

Also especially preferred are compositions 19.1. to 19.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A fluoroglycofen-ethyl.

Also especially preferred are compositions 20.1. to 20.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A fluthiacet.

Also especially preferred are compositions 21.1. to 21.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A fluthiacet-methyl.

Also especially preferred are compositions 22.1. to 22.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A fomesafen.

Also especially preferred are compositions 23.1. to 23.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A halosafen.

Also especially preferred are compositions 24.1. to 24.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A lactofen.

Also especially preferred are compositions 25.1. to 25.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A oxadiargyl.

Also especially preferred are compositions 26.1. to 26.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A oxadiazon.

Also especially preferred are compositions 27.1. to 27.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A oxyfluorfen.

Also especially preferred are compositions 28.1. to 28.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A pentoxazone.

Also especially preferred are compositions 29.1. to 29.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A profluazol.

Also especially preferred are compositions 30.1. to 30.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A pyraclonil.

Also especially preferred are compositions 31.1. to 31.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A pyraflufen.

Also especially preferred are compositions 32.1. to 32.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A pyraflufen-ethyl.

Also especially preferred are compositions 33.1. to 33.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A saflufenacil.

Also especially preferred are compositions 34.1. to 34.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A sulfentrazone.

Also especially preferred are compositions 35.1. to 35.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A thidiazimin.

Also especially preferred are compositions 36.1. to 36.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A tiafenacil.

Also especially preferred are compositions 37.1. to 37.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A ethyl [3-[2-chloro-4-fluoro-5-(1-methyl-6-trifluoromethyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-3-yl)phenoxy]-2-pyridyloxy]acetate (CAS 353292-31-6; S-3100).

Also especially preferred are compositions 38.1. to 38.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (CAS 1258836-72-4)

Also especially preferred are compositions 39.1. to 39.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A N-ethyl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452098-92-9).

Also especially preferred are compositions 40.1. to 40.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A N-tetrahydrofurfuryl-3-(2,6-dichloro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 915396-43-9).

Also especially preferred are compositions 41.1. to 41.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A

N-ethyl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452099-05-7).

Also especially preferred are compositions 42.1. to 42.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A N-tetrahydrofurfuryl-3-(2-chloro-6-fluoro-4-trifluoromethylphenoxy)-5-methyl-1*H*-pyrazole-1-carboxamide (CAS 452100-03-7).

Also especially preferred are compositions 43.1. to 43.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A 3-[7-fluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl]-1,5-dimethyl-6-thioxo-[1,3,5]triazinan-2,4-dione.

Also especially preferred are compositions 44.1. to 44.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A methyl (*E*)-4-[2-chloro-5-[4-chloro-5-(difluoromethoxy)-1*H*-methyl-pyrazol-3-yl]-4-fluoro-phenoxy]-3-methoxy-but-2-enoate (CAS 948893-00-3).

Also especially preferred are compositions 45.1. to 45.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A 3-[7-Chloro-5-fluoro-2-(trifluoromethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)-1H-pyrimidine-2,4-dione (CAS 212754-02-4).

Also especially preferred are compositions 46.1. to 46.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A 2-(2,2,7-Trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-4,5,6,7-tetrahydro-isoindole-1,3-dione.

Also especially preferred are compositions 47.1. to 47.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they comprise as component A 1-Methyl-6-trifluoromethyl-3-(2,2,7-trifluoro-3-oxo-4-prop-2-ynyl-3,4-dihydro-2H-benzo[1,4]oxazin-6-yl)-1H-pyrimidine-2,4-dione

Also especially preferred are compositions 48.1. to 48.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise benoxacor as safener C.

Also especially preferred are compositions 49.1. to 49.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise cloquintocet as safener C.

Also especially preferred are compositions 50.1. to 50.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise cyprosulfamide as safener C.

Also especially preferred are compositions 51.1. to 51.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise dichlormid as safener C.

Also especially preferred are compositions 52.1. to 52.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise fenchlorazole as safener C.

Also especially preferred are compositions 53.1. to 53.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise fenclorim as safener C.

Also especially preferred are compositions 54.1. to 54.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise furilazole as safener C.

Also especially preferred are compositions 55.1. to 55.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise isoxadifen as safener C.

Also especially preferred are compositions 56.1. to 56.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise mefenpyr as safener C.

Also especially preferred are compositions 57.1. to 57.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise 4-(dichloroacetyl)-1-oxa-4-azaspiro[4.5]decane (MON4660, CAS 71526-07-3) as safener C.

Also especially preferred are compositions 58.1. to 58.229 which differ from the corresponding compositions 1.1 to 1.229 only in that they additionally comprise 2,2,5-trimethyl-3-(dichloroacetyl)-1,3-oxazolidine (R-29148, CAS 52836-31-4) as safener C.

It is generally preferred to use the compounds of the invention in combination with herbicides that are selective for the crop being treated and which complement the spectrum of weeds controlled by these compounds at the application rate employed. It is further generally preferred to apply the compounds of the invention and other complementary herbicides at the same time, either as a combination formulation or as a tank mix.

It is recognized that the polynucleotide molecules and polypeptides of the invention encompass polynucleotide molecules and polypeptides comprising a nucleotide or an amino acid sequence that is sufficiently identical to nucleotide sequences set forth in SEQ ID Nos: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, or to the amino acid sequences set forth in SEQ ID Nos: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46. The term "sufficiently identical" is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity.

Generally, "sequence identity" refers to the extent to which two optimally aligned DNA or amino

acid sequences are invariant throughout a window of alignment of components, e.g., nucleotides or amino acids. An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components that are shared by the two aligned sequences divided by the total number of components in reference sequence segment, i.e., the entire reference sequence or a smaller defined part of the reference sequence. "Percent identity" is the identity fraction times 100. Optimal alignment of sequences for aligning a comparison window are well known to those skilled in the art and may be conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and preferably by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the GCG. Wisconsin Package. (Accelrys Inc. Burlington, Mass.)

Polynucleotides and Oligonucleotides

By an "isolated polynucleotide", including DNA, RNA, or a combination of these, single or double stranded, in the sense or antisense orientation or a combination of both, dsRNA or otherwise, we mean a polynucleotide which is at least partially separated from the polynucleotide sequences with which it is associated or linked in its native state. Preferably, the isolated polynucleotide is at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated. As the skilled addressee would be aware, an isolated polynucleotide can be an exogenous polynucleotide present in, for example, a transgenic organism which does not naturally comprise the polynucleotide. Furthermore, the terms "polynucleotide(s)", "nucleic acid sequence(s)", "nucleotide sequence(s)", “nucleic acid(s)”, “nucleic acid molecule” are used interchangeably herein and refer to nucleotides, either ribonucleotides or deoxyribonucleotides or a combination of both, in a polymeric unbranched form of any length.

The term “mut-PPO nucleic acid” refers to a PPO nucleic acid having a sequence that is mutated from a wild-type PPO nucleic acid and that confers increased PPO-inhibiting herbicide tolerance to a plant in which it is expressed. Furthermore, the term “mutated protoporphyrinogen oxidase (mut-PPO)” refers to the replacement of an amino acid of the wild-type primary sequences SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46, or a variant, a derivative, a homologue, an orthologue, or paralogue thereof, with another amino acid. The expression "mutated amino acid" will be used below to designate the amino acid which is replaced by another amino acid, thereby designating the site of the mutation in the primary sequence of the protein.

In a preferred embodiment, the PPO nucleotide sequence comprises the sequence of SEQ ID NO: 1, 25, 37 or 39 or a variant or derivative thereof.

Furthermore, it will be understood by the person skilled in the art that the PPO nucleotide sequences encompasse homologues, paralogues and and orthologues of SEQ ID NO: 1, 25, 37 or 39 as defined hereinafter.

The term "variant" with respect to a sequence (e.g., a polypeptide or nucleic acid sequence such

as – for example – a transcription regulating nucleotide sequence of the invention) is intended to mean substantially similar sequences. For nucleotide sequences comprising an open reading frame, variants include those sequences that, because of the degeneracy of the genetic code, encode the identical amino acid sequence of the native protein. Naturally occurring allelic variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis and for open reading frames, encode the native protein, as well as those that encode a polypeptide having amino acid substitutions relative to the native protein. Generally, nucleotide sequence variants of the invention will have at least 30, 40, 50, 60, to 70%, e.g., preferably 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, to 79%, generally at least 80%, e.g., 81%-84%, at least 85%, e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, to 98% and 99% nucleotide "sequence identity" to the nucleotide sequence of SEQ ID NO: SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45. The % identity of a polynucleotide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. Unless stated otherwise, the query sequence is at least 45 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 45 nucleotides. Preferably, the query sequence is at least 150 nucleotides in length, and the GAP analysis aligns the two sequences over a region of at least 150 nucleotides. More preferably, the query sequence is at least 300 nucleotides in length and the GAP analysis aligns the two sequences over a region of at least 300 nucleotides. Even more preferably, the GAP analysis aligns the two sequences over their entire length.

Polypeptides

By "substantially purified polypeptide" or "purified" a polypeptide is meant that has been separated from one or more lipids, nucleic acids, other polypeptides, or other contaminating molecules with which it is associated in its native state. It is preferred that the substantially purified polypeptide is at least 60% free, more preferably at least 75% free, and more preferably at least 90% free from other components with which it is naturally associated. As the skilled addressee will appreciate, the purified polypeptide can be a recombinantly produced polypeptide. The terms "polypeptide" and "protein" are generally used interchangeably and refer to a single polypeptide chain which may or may not be modified by addition of non-amino acid groups. It would be understood that such polypeptide chains may associate with other polypeptides or proteins or other molecules such as co-factors. The terms "proteins" and "polypeptides" as used herein also include variants, mutants, modifications, analogous and/or derivatives of the polypeptides of the invention as described herein.

The % identity of a polypeptide is determined by GAP (Needleman and Wunsch, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 25 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 25 amino acids. More preferably, the query sequence is at least 50 amino acids in length, and the GAP analysis aligns the two sequences over a region of at least 50 amino acids.

More preferably, the query sequence is at least 100 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 100 amino acids. Even more preferably, the query sequence is at least 250 amino acids in length and the GAP analysis aligns the two sequences over a region of at least 250 amino acids. Even more preferably, the GAP analysis aligns the two sequences over their entire length.

With regard to a defined polypeptide, it will be appreciated that % identity figures higher than those provided above will encompass preferred embodiments. Thus, where applicable, in light of the minimum % identity figures, it is preferred that the PPO polypeptide of the invention comprises an amino acid sequence which is at least 40%, more preferably at least 45%, more preferably at least 50%, more preferably at least 55%, more preferably at least 60%, more preferably at least 65%, more preferably at least 70%, more preferably at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 91%, more preferably at least 92%, more preferably at least 93%, more preferably at least 94%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98%, more preferably at least 99%, more preferably at least 99.1%, more preferably at least 99.2%, more preferably at least 99.3%, more preferably at least 99.4%, more preferably at least 99.5%, more preferably at least 99.6%, more preferably at least 99.7%, more preferably at least 99.8%, and even more preferably at least 99.9% identical to SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46.

By "variant" polypeptide is intended a polypeptide derived from the protein of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46 by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Such variants may result from, for example, genetic polymorphism or from human manipulation. Methods for such manipulations are generally known in the art.

"Derivatives" of a protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived.

"Homologues" of a protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived.

A deletion refers to removal of one or more amino acids from a protein.

An insertion refers to one or more amino acid residues being introduced into a predetermined site in a protein. Insertions may comprise N-terminal and/or C-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid

sequence will be smaller than N- or C-terminal fusions, of the order of about 1 to 10 residues. Examples of N- or C-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)-6-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

A substitution refers to replacement of amino acids of the protein with other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide and may range from 1 to 10 amino acids; insertions will usually be of the order of about 1 to 10 amino acid residues. The amino acid substitutions are preferably conservative amino acid substitutions. Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company (Eds).

Table 2: Examples of conserved amino acid substitutions

Residue	Conservative Substitutions	Residue	Conservative Substitutions
Ala	Ser	Leu	Ile; Val
Arg	Lys	Lys	Arg; Gln
Asn	Gln; His	Met	Leu; Ile
Asp	Glu	Phe	Met; Leu; Tyr
Gln	Asn	Ser	Thr; Gly
Cys	Ser	Thr	Ser; Val
Glu	Asp	Trp	Tyr
Gly	Pro	Tyr	Trp; Phe
His	Asn; Gln	Val	Ile; Leu
Ile	Leu, Val		

Amino acid substitutions, deletions and/or insertions may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulation. Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen in vitro mutagenesis (USB, Cleveland, OH), Quick-Change Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

"Derivatives" further include peptides, oligopeptides, polypeptides which may, compared to the amino acid sequence of the naturally-occurring form of the protein, such as the protein of interest, comprise substitutions of amino acids with non-naturally occurring amino acid residues, or additions of non-naturally occurring amino acid residues. "Derivatives" of a protein also encompass peptides, oligopeptides, polypeptides which comprise naturally occurring altered (glycosylated,

acylated, prenylated, phosphorylated, myristoylated, sulphated etc.) or non-naturally altered amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents or additions compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence, such as a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein. Furthermore, "derivatives" also include fusions of the naturally-occurring form of the protein with tagging peptides such as FLAG, HIS6 or thioredoxin (for a review of tagging peptides, see Terpe, Appl. Microbiol. Biotechnol. 60, 523-533, 2003).

"Orthologues" and "paralogues" encompass evolutionary concepts used to describe the ancestral relationships of genes. Paralogues are genes within the same species that have originated through duplication of an ancestral gene; orthologues are genes from different organisms that have originated through speciation, and are also derived from a common ancestral gene. A non-limiting list of examples of such orthologues are shown in Table 1.

It is well-known in the art that paralogues and orthologues may share distinct domains harboring suitable amino acid residues at given sites, such as binding pockets for particular substrates or binding motifs for interaction with other proteins.

The term "domain" refers to a set of amino acids conserved at specific positions along an alignment of sequences of evolutionarily related proteins. While amino acids at other positions can vary between homologues, amino acids that are highly conserved at specific positions indicate amino acids that are likely essential in the structure, stability or function of a protein. Identified by their high degree of conservation in aligned sequences of a family of protein homologues, they can be used as identifiers to determine if any polypeptide in question belongs to a previously identified polypeptide family.

The term "motif" or "consensus sequence" refers to a short conserved region in the sequence of evolutionarily related proteins. Motifs are frequently highly conserved parts of domains, but may also include only part of the domain, or be located outside of conserved domain (if all of the amino acids of the motif fall outside of a defined domain).

Specialist databases exist for the identification of domains, for example, SMART (Schultz et al. (1998) Proc. Natl. Acad. Sci. USA 95, 5857-5864; Letunic et al. (2002) Nucleic Acids Res 30, 242-244), InterPro (Mulder et al., (2003) Nucl. Acids. Res. 31, 315-318), Prosite (Bucher and Bairoch (1994), A generalized profile syntax for biomolecular sequences motifs and its function in automatic sequence interpretation. (In) ISMB-94; Proceedings 2nd International Conference on Intelligent Systems for Molecular Biology. Altman R., Brutlag D., Karp P., Lathrop R., Searls D., Eds., pp53-61, AAAI Press, Menlo Park; Hulo et al., Nucl. Acids. Res. 32:D134-D137, (2004)), or Pfam (Bateman et al., Nucleic Acids Research 30(1): 276-280 (2002)). A set of tools for in silico analysis of protein sequences is available on the ExPASy proteomics server (Swiss Institute of Bioinformatics (Gasteiger et al., ExPASy: the proteomics server for in-depth protein knowledge and analysis, Nu-

cleic Acids Res. 31:3784-3788(2003)). Domains or motifs may also be identified using routine techniques, such as by sequence alignment.

Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch ((1970) J Mol Biol 48: 443-453) to find the global (i.e. spanning the complete sequences) alignment of two sequences that maximizes the number of matches and minimizes the number of gaps. The BLAST algorithm (Altschul et al. (1990) J Mol Biol 215: 403-10) calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (NCBI). Homologues may readily be identified using, for example, the ClustalW multiple sequence alignment algorithm (version 1.83), with the default pairwise alignment parameters, and a scoring method in percentage. Global percentages of similarity and identity may also be determined using one of the methods available in the MatGAT software package (Campanella et al., BMC Bioinformatics. 2003 Jul 10;4:29. MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences.). Minor manual editing may be performed to optimise alignment between conserved motifs, as would be apparent to a person skilled in the art. Furthermore, instead of using full-length sequences for the identification of homologues, specific domains may also be used. The sequence identity values may be determined over the entire nucleic acid or amino acid sequence or over selected domains or conserved motif(s), using the programs mentioned above using the default parameters. For local alignments, the Smith-Waterman algorithm is particularly useful (Smith TF, Waterman MS (1981) J. Mol. Biol 147(1);195-7).

The Inventors of the present invention have found that by substituting one or more of the key amino acid residues the herbicide tolerance or resistance could be remarkably increased as compared to the activity of the wild type PPO enzymes with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46. Preferred substitutions of mut-PPO are those that increase the herbicide tolerance of the plant, but leave the biological activitiy of the oxidase activity substantially unaffected.

Accordingly, in another object of the present invention the key amino acid residues of a PPO enzyme, a variant, derivative, orthologue, paralogue or homologue thereof, is substituted by any other amino acid.

In a preferred embodiment, the key amino acid residues of a PPO enzyme, a variant, derivative, orthologue, paralogue or homologue thereof, is substituted by a conserved amino acid as depicted in Table 2.

It will be understood by the person skilled in the art that amino acids located in a close proximity to the positions of amino acids mentioned below may also be substituted. Thus, in another embodiment the variant of SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46, a variant, derivative, orthologue, paralogue or homologue thereof comprises a mut-PPO, wherein an amino acid ±3, ±2 or ±1 amino acid positions from a key amino acid is sub-

stituted by any other amino acid.

Based on techniques well-known in the art, a highly characteristic sequence pattern can be developed, by means of which further of mut-PPO candidates with the desired activity may be searched.

Searching for further mut-PPO candidates by applying a suitable sequence pattern would also be encompassed by the present invention. It will be understood by a skilled reader that the present sequence pattern is not limited by the exact distances between two adjacent amino acid residues of said pattern. Each of the distances between two neighbours in the above patterns may, for example, vary independently of each other by up to ±10, ± 5, ±3, ±2 or ±1 amino acid positions without substantially affecting the desired activity.

Furthermore, by applying the method of site directed mutagenesis, the inventors of the present invention have identified specific combinations of mutations, which combination refers to a substitution of the Phenylalanine residue at position 420 in SEQ ID NO:2 or 4, combined with a second substitution of the Leucin at position 397 in SEQ ID NO:2 or 4.

Thus, in a particularly preferred embodiment, the variant or derivative of the mut-PPO of SEQ ID NO: 2 or SEQ ID NO: 4 is selected from the combined amino acid substitutions of the following Table 3a.

Table 3a: SEQ ID NO: 2 or SEQ ID NO:4 (combined amino acid substitutions obtained by site directed mutagenesis.)

Combination Number	SEQ ID NO:	Key amino acid position combination	Preferred Substitution
1	2 or 4	Leu397	Gly
		Phe420	Met
2	2 or 4	Leu397	Ala
		Phe420	Met
3	2 or 4	Leu397	Ser
		Phe420	Met
4	2 or 4	Leu397	Thr
		Phe420	Met
5	2 or 4	Leu397	Cys
		Phe420	Met
6	2 or 4	Leu397	Val
		Phe420	Met
7	2 or 4	Leu397	Ile
		Phe420	Met
8	2 or 4	Leu397	Met
		Phe420	Met
9	2 or 4	Leu397	Pro
		Phe420	Met

10	2 or 4	Leu397	Phe
		Phe420	Met
11	2 or 4	Leu397	Tyr
		Phe420	Met
12	2 or 4	Leu397	Trp
		Phe420	Met
13	2 or 4	Leu397	Asp
		Phe420	Met
14	2 or 4	Leu397	Glu
		Phe420	Met
15	2 or 4	Leu397	Asn
		Phe420	Met
16	2 or 4	Leu397	Gln
		Phe420	Met
17	2 or 4	Leu397	His
		Phe420	Met
18	2 or 4	Leu397	Lys
		Phe420	Met
19	2 or 4	Leu397	Arg
		Phe420	Met
20	2 or 4	Leu397	Gly
		Phe420	Val
21	2 or 4	Leu397	Ala
		Phe420	Val
22	2 or 4	Leu397	Ser
		Phe420	Val
23	2 or 4	Leu397	Thr
		Phe420	Val
24	2 or 4	Leu397	Cys
		Phe420	Val
25	2 or 4	Leu397	Val
		Phe420	Val
26	2 or 4	Leu397	Ile
		Phe420	Val
27	2 or 4	Leu397	Met
		Phe420	Val
28	2 or 4	Leu397	Pro
		Phe420	Val
29	2 or 4	Leu397	Phe
		Phe420	Val
30	2 or 4	Leu397	Tyr
		Phe420	Val
31	2 or 4	Leu397	Trp

		Phe420	Val
32	2 or 4	Leu397	Asp
		Phe420	Val
33	2 or 4	Leu397	Glu
		Phe420	Val
34	2 or 4	Leu397	Asn
		Phe420	Val
35	2 or 4	Leu397	Gln
		Phe420	Val
36	2 or 4	Leu397	His
		Phe420	Val
37	2 or 4	Leu397	Lys
		Phe420	Val
38	2 or 4	Leu397	Arg
		Phe420	Val

In a further particularly preferred embodiment, the variant or derivative of the mut-PPO of SEQ ID NO: 2 or SEQ ID NO: 4 is selected from the combined amino acid substitutions of the following Table 3b.

Table 3b: SEQ ID NO: 2 or SEQ ID NO:4 (combined amino acid substitutions)

Combination Number	SEQ ID NO:	Key amino acid position combination	Preferred Substitution
39	2 or 4	Leu397	Asp, Glu, Gln, Asn
		Leu400	Ala, Ile, Val, Met
40	2 or 4	Leu397	Asp, Glu, Gln, Asn
		Phe457	Met, Ala, Leu, Ile, Val
41	2 or 4	Phe204	Ala, Leu, Ile, Val
		Leu397	Asp, Glu, Gln, Asn
42	2 or 4	Thr208	Ser
		Leu400	Ala, Ile, Val, Met
43	2 or 4	Leu400	Ala
		Phe457	Met, Ala, Leu, Ile, Val
44	2 or 4	Phe204	Ile
		Leu400	Ala, Ile, Val, Met
45	2 or 4	The208	Ser
		Phe457	Met, Ala, Leu, Ile, Val
46	2 or 4	Phe204	Ile
		Thr208	Ser
47	2 or 4	Phe204	Ile
		Phe457	Met, Ala, Leu, Ile, Val
48	2 or 4	Leu400	Ala, Ile, Val, Met

		Phe420	Val, Met, Ala, Ile, Leu
49	2 or 4	Phe204	Ile
		Phe420	Val, Met, Ala, Ile, Leu
50	2 or 4	Phe420	Val, Met, Ala, Ile, Leu
		Phe457	Met, Ala, Leu, Ile, Val
51	2 or 4	Arg128	Ala, Leu, Ile, Val
		Leu397	Asp, Glu, Gln, Asn
52	2 or 4	Thr208	Ser
		Leu397	Asp, Glu, Gln, Asn
		Phe457	Met, Ala, Leu, Ile, Val
53	2 or 4	Phe204	Ile
		The208	Ser
		Leu397	Asp, Glu, Gln, Asn
54	2 or 4	Phe204	Ile
		Leu397	Asp, Glu, Gln, Asn
		Phe457	Met, Ala, Leu, Ile, Val
55	2 or 4	Thr208	Ser
		Leu400	Ala, Ile, Val, Met
		Phe457	Met, Ala, Leu, Ile, Val
56	2 or 4	Phe204	Ile
		Thr208	Ser
		Leu400	Ala, Ile, Val, Met
57	2 or 4	Phe204	Ile
		Leu400	Ala, Ile, Val, Met
		Phe457	Met, Ala, Leu, Ile, Val
58	2 or 4	Thr208	Ser
		Leu397	Asp, Glu, Gln, Asn
		Leu400	Ala, Ile, Val, Met
59	2 or 4	Leu397	Asp, Glu, Gln, Asn
		Leu400	Ala, Ile, Val, Met
		Phe457	Met, Ala, Leu, Ile, Val
60	2 or 4	Phe204	Ile
		Leu397	Asp, Glu, Gln, Asn
		Leu400	Ala, Ile, Val, Met
61	2 or 4	Phe204	Ile
		Thr208	Ser
		Phe457	Met, Ala, Leu, Ile, Val
62	2 or 4	Leu397	Asp, Glu, Gln, Asn
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
63	2 or 4	Leu397	Asp, Glu, Gln, Asn
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val

64	2 or 4	Phe204	Ile
		Leu397	Asp, Glu, Gln, Asn
		Phe420	Met, Ala, Leu, Ile, Val
65	2 or 4	Phe204	Ile
		Thr208	Ser
		Phe420	Met, Ala, Leu, Ile, Val
66	2 or 4	Thr208	Ser
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
67	2 or 4	Phe204	Ile
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
68	2 or 4	Arg128	Ala, Leu, Ile, Val
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
69	2 or 4	Arg128	Ala, Leu, Ile, Val
		Leu397	Asp, Glu, Gln, Asn
		Phe420	Met, Ala, Leu, Ile, Val
70	2 or 4	Arg128	Ala, Leu, Ile, Val
		Phe204	Ile
		Phe420	Met, Ala, Leu, Ile, Val
71	2 or 4	Arg128	Ala, Leu, Ile, Val
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
72	2 or 4	Thr208	Ser
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
73	2 or 4	Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
74	2 or 4	Phe204	Ile
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
75	2 or 4	Phe204	Ile
		Thr208	Ser
		Leu400	Ala, Ile, Val, Met
		Phe457	Met, Ala, Leu, Ile, Val
76	2 or 4	Phe204	Ile
		Thr208	Ser
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
77	2 or 4	Arg128	Ala, Leu, Ile, Val
		Phe204	Ile

		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
78	2 or 4	Phe204	Ile
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
79	2 or 4	Phe204	Ile
		Thr208	Ser
		Leu397	Asp, Glu, Gln, Asn
		Leu400	Ala, Ile, Val, Met
80	2 or 4	Phe204	Ile
		Thr208	Ser
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val
81	2 or 4	Arg128	Ala, Leu, Ile, Val
		Phe204	Ile
		Leu400	Ala, Ile, Val, Met
		Phe420	Met, Ala, Leu, Ile, Val
		Phe457	Met, Ala, Leu, Ile, Val

It is to be understood that any amino acid besides the ones mentioned in the above tables 3 could be used as a substitutent. Assays to test for the functionality of such mutants are readily available in the art, and respectively, described in the Example section of the present invention.

In a preferred embodiment, the amino acid sequence differs from an amino acid sequence of a PPO of SEQ ID NO: 2 or SEQ ID NO: 4 at one or more of the following positions: 128, 204, 208, 397, 400, 420, 457.

Examples of differences at these amino acid positions include, but are not limited to, one or more of the following:
the amino acid at position 128 is other than Arginine;
the amino acid at position 204 is other than Phenylalanine;
the amino acid at position 208 is other than Threonine;
the amino acid at position 397 is other than Leucine,
the amino acid at position 400 is other than Leucine,
the amino acid at position 420 is other than Phenylalanine,
the amino acid at position 457 is other than Phenylalanine.

In some embodiments, the mut-PPO enzyme of SEQ ID NO: 2 or SEQ ID NO: 4 comprises one or more of the following:

the amino acid at position 128 is Leu, Ala, Val, or Ile;
the amino acid at position 204 is Ala, Leu, Ile, or Val;

the amino acid at position 208 is Ser;
the amino acid at position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln;
the amino acid at position 400 is Ala, Ile, Val, or Met;
the amino acid at position 420 is Val, Met, Ala, Ile, or Leu;
the amino acid at position 457 is Met, Ala, Leu, Ile, Val;

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Tyr, Trp, Asp, Glu, Asn, Gln, His, Lys, or Arg, and the amino acid at position 420 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, Glu, Gln, Asn, and the amino acid at position 400 is Ala, Ile, Val, Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 397 is Asp, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, Glu, Gln, Asn, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 397 is Gln, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, Val, and the amino acid at position 397 is Asp, Glu, Gln, Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 204 is Ala, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Ala, Ile, Val, Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Ala, Ile, Val, Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 208 is Ser, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 208 is Ser.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 208 is Ser.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 420 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 420 is Met, Ala, Leu, Ile, Val, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, Leu, Ile, Val, and the amino acid at position 397 is Asp, Glu, Gln, Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Tyr, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg, and the amino acid at position 420 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gly, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ala, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ser, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Thr, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Cys, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Val, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Ile, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Met, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Pro, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Tyr, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Trp, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is His, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Lys, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Arg, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, Glu, Gln, Asn, and the amino acid at position 400 is Ala, Ile, Val, Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, Glu, Gln, Asn, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 397 is Gln, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asn, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, Val, and the amino acid at position 397 is Asp, Glu, Gln, Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Leu, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Val, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Ala, Ile, Val, Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 400 is Ala, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Ala, Ile, Val, Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 400 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 208 is Ser.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 208 is Ser.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, Ile, Val, Met, and the amino acid at position 420 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ile, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Val, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 400 is Met, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Met, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ile, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, Ala, Leu, Ile, Val, and the amino acid at position 457 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 420 is Met, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Met, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ala, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Leu, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Ile, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 420 is Val, and the amino acid at position 457 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 420 is Val, and the amino acid at position 457 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, Leu, Ile, Val, and the amino acid at position 397 is Asp, Glu, Gln, Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ala, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Ile, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Asp.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Glu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Val, and the amino acid at position 397 is Asn.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Tyr, Trp, Asp, Glu, Asn, Gln, His, Lys, Arg, and the amino acid at position 392 is Met, Ala, Leu, Ile, Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gly, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gly, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gly, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gly, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gly, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ala, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ala, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ala, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ala, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ala, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ser, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 369 is Ser, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ser, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ser, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ser, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Thr, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Thr, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Thr, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Thr, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Thr, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Cys, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Cys, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Cys, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Cys, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Cys, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Val, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Val, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Val, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Val, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Val, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ile, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ile, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ile, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ile, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Ile, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Met, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Met, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Met, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Met, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Met, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Pro, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Pro, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Pro, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Pro, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Pro, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a

variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Tyr, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Tyr, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Tyr, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Tyr, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Tyr, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Trp, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Trp, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Trp, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Trp, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Trp, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asp, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 369 is Asp, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asp, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asp, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asp, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Glu, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Glu, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Glu, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Glu, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Glu, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asn, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asn, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asn, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asn, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Asn, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gln, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gln, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gln, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gln, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Gln, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is His, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is His, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is His, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is His, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is His, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Lys, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Lys, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Lys, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Lys, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Lys, and the amino acid at position 392 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Arg, and the amino acid at position 392 is Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Arg, and the amino acid at position 392 is Ala.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Arg, and the amino acid at position 392 is Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Arg, and the amino acid at position 392 is Ile.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 40, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 369 is Arg, and the amino acid at position 392 is Val.

In a particularly preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Val.

In a particularly preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Glu, and the amino acid at position 420 is Met.

In a particularly preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Val.

In a particularly preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Gln, and the amino acid at position 420 is Met.

In a particularly preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or 4, a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 397 is Asp, and the amino acid at position 420 is Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, and the amino acid at position 397 is Asn, Asp, Glu, or Gln.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, and the amino acid at position 400 is Ala, Ile, Val, or Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 208 is Ser, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 400 is Ala, Ile, Val, or Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 397 is Asn, Asp, Glu, or Gln, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 400 is Ala, Ile, Val, or Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 397 is Asn, Asp, Glu, or Gln, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 397 is Asn, Asp, Glu, or Gln, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which: the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, and the

amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, Ala, Val, or Ile, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, Ala, Val, or Ile, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, Ala, Val, or Ile, the amino acid at position 204 is Ala, Leu, Ile, or Val, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, Ala, Val, or Ile, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 208 is Ser, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 400 is Ala, Ile, Val, or Met, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:

the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 420 is Val, Met, Ala, Ile, or Leu.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, the amino acid at position 400 is Ala, Ile, Val, or Met, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, Ala, Val, or Ile, the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 400 is Ala, Ile, Val, or Met, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, the amino acid at position 397 is Asn, Asp, Glu, or Gln, and the amino acid at position 400 is Ala, Ile, Val, or Met.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 208 is Ser, the amino acid at position 400 is Ala, Ile, Val, or Met, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

In another preferred embodiment, the mut-PPO comprises a sequence of SEQ ID NO: 2 or SEQ ID NO:4 a variant, derivative, orthologue, paralogue or homologue thereof, in which:
the amino acid at position 128 is Leu, Ala, Val, or Ile, the amino acid at position 204 is Ala, Leu, Ile, or Val, the amino acid at position 400 is Ala, Ile, Val, or Met, the amino acid at position 420 is Val, Met, Ala, Ile, or Leu, and the amino acid at position 457 is Met, Ala, Leu, Ile, or Val.

It will be within the knowledge of the skilled artisan to identify conserved regions and motifs shared between the homologues, orthologues and paralogues encoded by SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, such as those depicted in Table 1. Having identified such conserved regions that may represent suitable binding motifs, amino acids corresponding to the amino acids listed in Table 3a and 3b, can be chosen to be subsituted by any other amino acid, preferably by conserved amino acids as shown in table 2, and more preferably by the amino acids of tables 3a and 3b.

In addition, the present invention refers to a method for identifying a PPO-inhibiting herbicide by using a mut-PPO encoded by a nucleic acid which comprises the nucleotide sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, or a variant or derivative thereof.

Said method comprises the steps of:

a) generating a transgenic cell or plant comprising a nucleic acid encoding a mut-PPO, wherein the mut-PPO is expressed;
b) applying a PPO-inhibiting herbicide to the transgenic cell or plant of a) and to a control cell or plant of the same variety;
c) determining the growth or the viability of the transgenic cell or plant and the control cell or plant after application of said PPO-inhibiting herbicide, and
d) selecting "PPO-inhibiting herbicides" which confer reduced growth to the control cell or plant as compared to the growth of the transgenic cell or plant.

By "control cell" or "similar, wild-type, plant, plant tissue, plant cell or host cell" is intended a plant, plant tissue, plant cell, or host cell, respectively, that lacks the herbicide-resistance characteristics and/or particular polynucleotide of the invention that are disclosed herein. The use of the term "wild-type" is not, therefore, intended to imply that a plant, plant tissue, plant cell, or other host cell lacks recombinant DNA in its genome, and/or does not possess herbicide-resistant characteristics that are different from those disclosed herein.

Another object refers to a method of identifying a nucleotide sequence encoding a mut-PPO which is resistant or tolerant to a PPO-inhibiting herbicide, the method comprising:

a) generating a library of mut-PPO-encoding nucleic acids,
b) screening a population of the resulting mut-PPO-encoding nucleic acids by expressing each of said nucleic acids in a cell or plant and treating said cell or plant with a PPO-inhibiting herbicide,
c) comparing the PPO-inhibiting herbicide-tolerance levels provided by said population of mut-PPO encoding nucleic acids with the PPO-inhibiting herbicide-tolerance level provided by a control PPO-encoding nucleic acid,
d) selecting at least one mut-PPO-encoding nucleic acid that provides a significantly increased level of tolerance to a PPO-inhibiting herbicide as compared to that provided by the control PPO-encoding nucleic acid.

In a preferred embodiment, the mut-PPO-encoding nucleic acid selected in step d) provides at least 2-fold as much resistance or tolerance of a cell or plant to a PPO-inhibiting herbicide as compared to that provided by the control PPO-encoding nucleic acid.

In a further preferred embodiment, the mut-PPO-encoding nucleic acid selected in step d) provides at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 500-fold, as much resistance or tolerance of a cell or plant to a PPO-inhibiting herbicide as compared to that provided by the control PPO-encoding nucleic acid.

The resistance or tolerance can be determined by generating a transgenic plant or host cell, preferably a plant cell, comprising a nucleic acid sequence of the library of step a) and comparing said transgenic plant with a control plant or host cell, preferably a plant cell.

Another object refers to a method of identifying a plant or algae containing a nucleic acid comprising a nucleotide sequence encoding a wild-type or mut-PPO which is resistant or tolerant to a PPO-inhibiting herbicide, the method comprising:

a) identifying an effective amount of a PPO-inhibiting herbicide in a culture of plant cells or green algae that leads to death of said cells.
b) treating said plant cells or green algae with a mutagenizing agent,
c) contacting said mutagenized cells population with an effective amount of PPO-inhibiting herbicide, identified in a),
d) selecting at least one cell surviving these test conditions,
e) PCR-amplification and sequencing of PPO genes from cells selected in d) and comparing such sequences to wild-type PPO gene sequences, respectively.

In a preferred embodiment, said mutagenizing agent is ethylmethanesulfonate (EMS).

Many methods well known to the skilled artisan are available for obtaining suitable candidate nucleic acids for identifying a nucleotide sequence encoding a mut-PPO from a variety of different potential source organisms including microbes, plants, fungi, algae, mixed cultures etc. as well as environmental sources of DNA such as soil. These methods include inter alia the preparation of cDNA or genomic DNA libraries, the use of suitably degenerate oligonucleotide primers, the use of probes based upon known sequences or complementation assays (for example, for growth upon tyrosine) as well as the use of mutagenesis and shuffling in order to provide recombined or shuffled mut-PPO-encoding sequences.

Nucleic acids comprising candidate and control PPO encoding sequences can be expressed in yeast, in a bacterial host strain, in an alga or in a higher plant such as tobacco or Arabidopsis and the relative levels of inherent tolerance of the PPO encoding sequences screened according to a visible indicator phenotype of the transformed strain or plant in the presence of different concentrations of the selected PPO-inhibiting herbicide. Dose responses and relative shifts in dose responses associated with these indicator phenotypes (formation of brown color, growth inhibition, herbicidal effect etc) are conveniently expressed in terms, for example, of GR50 (concentration for 50% reduction of growth) or MIC (minimum inhibitory concentration) values where increases in values

correspond to increases in inherent tolerance of the expressed PPO. For example, in a relatively rapid assay system based upon transformation of a bacterium such as E. coli, each mut-PPO encoding sequence may be expressed, for example, as a DNA sequence under expression control of a controllable promoter such as the lacZ promoter and taking suitable account, for example by the use of synthetic DNA, of such issues as codon usage in order to obtain as comparable a level of expression as possible of different PPO sequences. Such strains expressing nucleic acids comprising alternative candidate PPO sequences may be plated out on different concentrations of the selected PPO-inhibiting herbicide in, optionally, a tyrosine supplemented medium and the relative levels of inherent tolerance of the expressed PPO enzymes estimated on the basis of the extent and MIC for inhibition of the formation of the brown, ochronotic pigment.

In another embodiment, candidate nucleic acids are transformed into plant material to generate a transgenic plant, regenerated into morphologically normal fertile plants which are then measured for differential tolerance to selected PPO-inhibiting herbicides. Many suitable methods for transformation using suitable selection markers such as kanamycin, binary vectors such as from Agrobacterium and plant regeneration as, for example, from tobacco leaf discs are well known in the art. Optionally, a control population of plants is likewise transformed with a nucleic acid expressing the control PPO. Alternatively, an untransformed dicot plant such as Arabidopsis or Tobacco can be used as a control since this, in any case, expresses its own endogenous PPO. The average, and distribution, of herbicide tolerance levels of a range of primary plant transformation events or their progeny to PPO-inhibiting herbicides described supra are evaluated in the normal manner based upon plant damage, meristematic bleaching symptoms etc. at a range of different concentrations of herbicides. These data can be expressed in terms of, for example, GR50 values derived from dose/response curves having "dose" plotted on the x-axis and "percentage kill", "herbicidal effect", "numbers of emerging green plants" etc. plotted on the y-axis where increased GR50 values correspond to increased levels of inherent tolerance of the expressed PPO. Herbicides can suitably be applied pre-emergence or post-emergence.

Another object refers to an isolated nucleic acid encoding a mut-PPO, wherein the nucleic acid is identifiable by a method as defined above.

In another embodiment, the invention refers to a plant cell transformed by a wild-type or a mut-PPO nucleic acid or or a plant cell which has been mutated to obtain a plant expressing a wild-type or a mut-PPO nucleic acid, wherein expression of the nucleic acid in the plant cell results in increased resistance or tolerance to a PPO-inhibiting herbicide as compared to a wild type variety of the plant cell.

The term “expression/expressing” or “gene expression” means the transcription of a specific gene or specific genes or specific genetic construct. The term “expression” or “gene expression” in particular means the transcription of a gene or genes or genetic construct into structural RNA (rRNA, tRNA) or mRNA with or without subsequent translation of the latter into a protein. The process includes transcription of DNA and processing of the resulting mRNA product.

To obtain the desired effect, i.e. plants that are tolerant or resistant to the PPO-inhibiting herbicide

derivative herbicide of the present invention, it will be understood that the at least one nucleic acid is "over-expressed" by methods and means known to the person skilled in the art.

The term "increased expression" or "overexpression" as used herein means any form of expression that is additional to the original wild-type expression level. Methods for increasing expression of genes or gene products are well documented in the art and include, for example, overexpression driven by appropriate promoters, the use of transcription enhancers or translation enhancers. Isolated nucleic acids which serve as promoter or enhancer elements may be introduced in an appropriate position (typically upstream) of a non-heterologous form of a polynucleotide so as to upregulate expression of a nucleic acid encoding the polypeptide of interest. For example, endogenous promoters may be altered in vivo by mutation, deletion, and/or substitution (see, Kmiec, US 5,565,350; Zarling et al., WO9322443), or isolated promoters may be introduced into a plant cell in the proper orientation and distance from a gene of the present invention so as to control the expression of the gene.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added may be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

An intron sequence may also be added to the 5' untranslated region (UTR) or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold (Buchman and Berg (1988) Mol. Cell biol. 8: 4395-4405; Callis et al. (1987) Genes Dev 1:1183-1200). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of the maize introns Adh1-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. For general information see: The Maize Handbook, Chapter 116, Freeling and Walbot, Eds., Springer, N.Y. (1994)

The term "introduction" or "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated there from. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell may then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

The transfer of foreign genes into the genome of a plant is called transformation. Transformation of plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. The methods described for the transformation and regeneration of plants from plant tissues or plant cells may be utilized for transient or for stable transformation. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., (1982) Nature 296, 72-74; Negrutiu I et al. (1987) Plant Mol Biol 8: 363-373); electroporation of protoplasts (Shillito R.D. et al. (1985) Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A et al., (1986) Mol. Gen Genet 202: 179-185); DNA or RNA-coated particle bombardment (Klein TM et al., (1987) Nature 327: 70) infection with (non-integrative) viruses and the like. Transgenic plants, including transgenic crop plants, are preferably produced via Agrobacterium-mediated transformation. An advantageous transformation method is the transformation in planta. To this end, it is possible, for example, to allow the agrobacteria to act on plant seeds or to inoculate the plant meristem with agrobacteria. It has proved particularly expedient in accordance with the invention to allow a suspension of transformed agrobacteria to act on the intact plant or at least on the flower primordia. The plant is subsequently grown on until the seeds of the treated plant are obtained (Clough and Bent, Plant J. (1998) 16, 735–743). Methods for Agrobacterium-mediated transformation of rice include well known methods for rice transformation, such as those described in any of the following: European patent application EP 1198985 A1, Aldemita and Hodges (Planta 199: 612-617, 1996); Chan et al. (Plant Mol Biol 22 (3): 491-506, 1993), Hiei et al. (Plant J 6 (2): 271-282, 1994). In the case of corn transformation, the preferred method is as described in either Ishida et al. (Nat. Biotechnol 14(6): 745-50, 1996) or Frame et al. (Plant Physiol 129(1): 13-22, 2002). Said methods are further described by way of example in B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press (1993) 128-143 and in Potrykus Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 205-225). The nucleic acids or the construct to be expressed is preferably cloned into a vector, which is suitable for transforming Agrobacterium tumefaciens, for example pBin19 (Bevan et al., Nucl. Acids Res. 12 (1984) 8711). Agrobacteria transformed by such a vector can then be used in known manner for the transformation of plants, such as plants used as a model, like Arabidopsis (Arabidopsis thaliana is within the scope of the present invention not considered as a crop plant), or crop plants such as, by way of example, tobacco plants, for example by immersing bruised leaves or chopped leaves in an agrobacterial solution and then culturing them in suitable media. The transformation of plants by means of Agrobacterium tumefaciens is described, for example, by Höfgen and Willmitzer in Nucl. Acid Res. (1988) 16, 9877 or is known inter alia from F.F. White, Vectors for Gene Transfer in Higher Plants; in Transgenic Plants, Vol. 1, Engineering and Utilization, eds. S.D. Kung and R. Wu, Academic Press, 1993, pp. 15-38.

In addition to the transformation of somatic cells, which then have to be regenerated into intact plants, it is also possible to transform the cells of plant meristems and in particular those cells

which develop into gametes. In this case, the transformed gametes follow the natural plant development, giving rise to transgenic plants. Thus, for example, seeds of Arabidopsis are treated with agrobacteria and seeds are obtained from the developing plants of which a certain proportion is transformed and thus transgenic [Feldman, KA and Marks MD (1987). Mol Gen Genet 208:274-289; Feldmann K (1992). In: C Koncz, N-H Chua and J Shell, eds, Methods in Arabidopsis Research. Word Scientific, Singapore, pp. 274-289]. Alternative methods are based on the repeated removal of the inflorescences and incubation of the excision site in the center of the rosette with transformed agrobacteria, whereby transformed seeds can likewise be obtained at a later point in time (Chang (1994). Plant J. 5: 551-558; Katavic (1994). Mol Gen Genet, 245: 363-370). However, an especially effective method is the vacuum infiltration method with its modifications such as the "floral dip" method. In the case of vacuum infiltration of Arabidopsis, intact plants under reduced pressure are treated with an agrobacterial suspension [Bechthold, N (1993). C R Acad Sci Paris Life Sci, 316: 1194-1199], while in the case of the "floral dip" method the developing floral tissue is incubated briefly with a surfactant-treated agrobacterial suspension [Clough, SJ and Bent AF (1998) The Plant J. 16, 735-743]. A certain proportion of transgenic seeds are harvested in both cases, and these seeds can be distinguished from non-transgenic seeds by growing under the above-described selective conditions. In addition the stable transformation of plastids is of advantages because plastids are inherited maternally is most crops reducing or eliminating the risk of transgene flow through pollen. The transformation of the chloroplast genome is generally achieved by a process which has been schematically displayed in Klaus et al., 2004 [Nature Biotechnology 22 (2), 225-229]. Briefly the sequences to be transformed are cloned together with a selectable marker gene between flanking sequences homologous to the chloroplast genome. These homologous flanking sequences direct site specific integration into the plastome. Plastidal transformation has been described for many different plant species and an overview is given in Bock (2001) Transgenic plastids in basic research and plant biotechnology. J Mol Biol. 2001 Sep 21; 312 (3):425-38 or Maliga, P (2003) Progress towards commercialization of plastid transformation technology. Trends Biotechnol. 21, 20-28. Further biotechnological progress has recently been reported in form of marker free plastid transformants, which can be produced by a transient co-integrated maker gene (Klaus et al., 2004, Nature Biotechnology 22(2), 225-229). The genetically modified plant cells can be regenerated via all methods with which the skilled worker is familiar. Suitable methods can be found in the abovementioned publications by S.D. Kung and R. Wu, Potrykus or Höfgen and Willmitzer.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant. To select transformed plants, the plant material obtained in the transformation is, as a rule, subjected to selective conditions so that transformed plants can be distinguished from untransformed plants. For example, the seeds obtained in the above-described manner can be planted and, after an initial growing period, subjected to a suitable selection by spraying. A further possibility consists in growing the seeds, if appropriate after sterilization, on agar plates using a suitable selection agent so that only the transformed seeds can grow into plants. Alternatively, the transformed plants are screened for the presence of a selectable marker such as the ones described above.

Following DNA transfer and regeneration, putatively transformed plants may also be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed and homozygous second-generation (or T2) transformants selected, and the T2 plants may then further be propagated through classical breeding techniques. The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

Preferably, the wild-type or mut-PPO nucleic acid comprises a polynucleotide sequence selected from the group consisting of : a) a polynucleotide as shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, or a variant or derivative thereof; b) a polynucleotide encoding a polypeptide as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46, or a variant or derivative thereof; c) a polynucleotide comprising at least 60 consecutive nucleotides of any of a) or b); and d) a polynucleotide complementary to the polynucleotide of any of a) through c).

Preferably, the expression of the nucleic acid in the plant results in the plant's increased resistance to PPO-inhibiting herbicide as compared to a wild type variety of the plant.

In another embodiment, the invention refers to a plant, preferably a transgenic plant, comprising a plant cell according to the present invention, wherein expression of the nucleic acid in the plant results in the plant's increased resistance to PPO-inhibiting herbicide as compared to a wild type variety of the plant.

The plants described herein can be either transgenic crop plants or non-transgenic plants.

For the purposes of the invention, "transgenic", “transgene” or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette, gene construct or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

(a) the nucleic acid sequences encoding proteins useful in the methods of the invention, or

(b) genetic control sequence(s) which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or

(c) a) and b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition,

deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original plant or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding nucleic acid sequence encoding a polypeptide useful in the methods of the present invention, as defined above – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

A transgenic plant for the purposes of the invention is thus understood as meaning, as above, that the nucleic acids used in the method of the invention are not at their natural locus in the genome of said plant, it being possible for the nucleic acids to be expressed homologously or heterologously. However, as mentioned, transgenic also means that, while the nucleic acids according to the invention or used in the inventive method are at their natural position in the genome of a plant, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acids takes place. Preferred transgenic plants are mentioned herein. Furthermore, the term “transgenic” refers to any plant, plant cell, callus, plant tissue, or plant part, that contains all or part of at least one recombinant polynucleotide. In many cases, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations. For the purposes of the invention, the term “recombinant polynucleotide” refers to a polynucleotide that has been altered, rearranged, or modified by genetic engineering. Examples include any cloned polynucleotide, or polynucleotides, that are linked or joined to heterologous sequences. The term “recombinant” does not refer to alterations of polynucleotides that result from naturally occurring events, such as spontaneous mutations, or from non-spontaneous mutagenesis followed by selective breeding.

Plants containing mutations arising due to non-spontaneous mutagenesis and selective breeding are referred to herein as non-transgenic plants and are included in the present invention. In embodiments wherein the plant is transgenic and comprises multiple mut-PPO nucleic acids, the nucleic acids can be derived from different genomes or from the same genome. Alternatively, in embodiments wherein the plant is non-transgenic and comprises multiple mut-PPO nucleic acids, the nucleic acids are located on different genomes or on the same genome.

In certain embodiments, the present invention involves herbidicide-resistant plants that are produced by mutation breeding. Such plants comprise a polynucleotide encoding a mut-PPO and are tolerant to one or more PPO-inhibiting herbicides. Such methods can involve, for example, exposing the plants or seeds to a mutagen, particularly a chemical mutagen such as, for example, ethyl

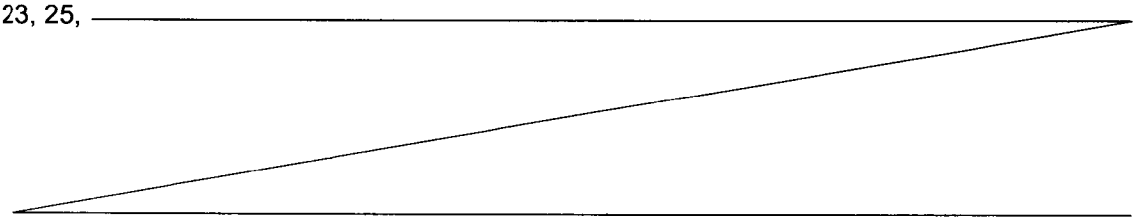
methanesulfonate (EMS) and selecting for plants that have enhanced tolerance to at least one or more PPO-inhibiting herbicide.

However, the present invention is not limited to herbicide-tolerant plants that are produced by a mutagenesis method involving the chemical mutagen EMS. Any mutagenesis method known in the art may be used to produce the herbicide-resistant plants of the present invention. Such mutagenesis methods can involve, for example, the use of any one or more of the following mutagens: radiation, such as X-rays, Gamma rays (e.g., cobalt 60 or cesium 137), neutrons, (e.g., product of nuclear fission by uranium 235 in an atomic reactor), Beta radiation (e.g., emitted from radioisotopes such as phosphorus 32 or carbon 14), and ultraviolet radiation (preferably from 2500 to 2900 nm), and chemical mutagens such as base analogues (e.g., 5-bromo-uracil), related compounds (e.g., 8-ethoxy caffeine), antibiotics (e.g., streptonigrin), alkylating agents (e.g., sulfur mustards, nitrogen mustards, epoxides, ethylenamines, sulfates, sulfonates, sulfones, lactones), azide, hydroxylamine, nitrous acid, or acridines. Herbicide-resistant plants can also be produced by using tissue culture methods to select for plant cells comprising herbicide-resistance mutations and then regenerating herbicide-resistant plants therefrom. See, for example, U.S. Patent Nos. 5,773,702 and 5,859,348. Further details of mutation breeding can be found in "Principals of Cultivar Development" Fehr, 1993 Macmillan Publishing Company.

In addition to the definition above, the term "plant" is intended to encompass crop plants at any stage of maturity or development, as well as any tissues or organs (plant parts) taken or derived from any such plant unless otherwise clearly indicated by context. Plant parts include, but are not limited to, stems, roots, flowers, ovules, stamens, leaves, embryos, meristematic regions, callus tissue, anther cultures, gametophytes, sporophytes, pollen, microspores, protoplasts, and the like.

The plant of the present invention comprises at least one mut-PPO nucleic acid or over-expressed wild-type PPO nucleic acid, and has increased tolerance to a PPO-inhibiting herbicide as compared to a wild-type variety of the plant. It is possible for the plants of the present invention to have multiple wild-type or mut-PPO nucleic acids from different genomes since these plants can contain more than one genome. For example, a plant contains two genomes, usually referred to as the A and B genomes. Because PPO is a required metabolic enzyme, it is assumed that each genome has at least one gene coding for the PPO enzyme (i.e. at least one PPO gene). As used herein, the term "PPO gene locus" refers to the position of an PPO gene on a genome, and the terms "PPO gene" and "PPO nucleic acid" refer to a nucleic acid encoding the PPO enzyme. The PPO nucleic acid on each genome differs in its nucleotide sequence from an PPO nucleic acid on another genome. One of skill in the art can determine the genome of origin of each PPO nucleic acid through genetic crossing and/or either sequencing methods or exonuclease digestion methods known to those of skill in the art.

The present invention includes plants comprising one, two, three, or more mut-PPO alleles, wherein the plant has increased tolerance to a PPO-inhibiting herbicide as compared to a wild-type variety of the plant. The mut-PPO alleles can comprise a nucleotide sequence selected from the group consisting of a polynucleotide as defined in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25,



27, 29, 31, 33, 35, 37, 39, 41, 43, or 45,, or a variant or derivative thereof, a polynucleotide encoding a polypeptide as defined in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46,, or a variant or derivative, homologue, orthologue, paralogue thereof, a polynucleotide comprising at least 60 consecutive nucleotides of any of the aforementioned polynucleotides; and a polynucleotide complementary to any of the aforementioned polynucleotides.

"Alleles" or "allelic variants" are alternative forms of a given gene, located at the same chromosomal position. Allelic variants encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp. SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms

The term "variety" refers to a group of plants within a species defined by the sharing of a common set of characteristics or traits accepted by those skilled in the art as sufficient to distinguish one cultivar or variety from another cultivar or variety. There is no implication in either term that all plants of any given cultivar or variety will be genetically identical at either the whole gene or molecular level or that any given plant will be homozygous at all loci. A cultivar or variety is considered "true breeding" for a particular trait if, when the true-breeding cultivar or variety is self-pollinated, all of the progeny contain the trait. The terms "breeding line" or "line" refer to a group of plants within a cultivar defined by the sharing of a common set of characteristics or traits accepted by those skilled in the art as sufficient to distinguish one breeding line or line from another breeding line or line. There is no implication in either term that all plants of any given breeding line or line will be genetically identical at either the whole gene or molecular level or that any given plant will be homozygous at all loci. A breeding line or line is considered "true breeding" for a particular trait if, when the true-breeding line or breeding line is self-pollinated, all of the progeny contain the trait. In the present invention, the trait arises from a mutation in a PPO gene of the plant or seed.

The herbicide-resistant plants of the invention that comprise polynucleotides encoding mut-PPO polypeptides also find use in methods for increasing the herbicide-resistance of a plant through conventional plant breeding involving sexual reproduction. The methods comprise crossing a first plant that is a herbicide-resistant plant of the invention to a second plant that may or may not be resistant to the same herbicide or herbicides as the first plant or may be resistant to different herbicide or herbicides than the first plant. The second plant can be any plant that is capable of producing viable progeny plants (i.e., seeds) when crossed with the first plant. Typically, but not necessarily, the first and second plants are of the same species. The methods can optionally involve selecting for progeny plants that comprise the mut-PPO polypeptides of the first plant and the herbicide resistance characteristics of the second plant. The progeny plants produced by this method of the present invention have increased resistance to a herbicide when compared to either the first or second plant or both. When the first and second plants are resistant to different herbicides, the progeny plants will have the combined herbicide tolerance characteristics of the first and second plants. The methods of the invention can further involve one or more generations of backcrossing the progeny plants of the first cross to a plant of the same line or genotype as either the first or second plant. Alternatively, the progeny of the first cross or any subsequent cross can be crossed to a third plant that is of a different line or genotype than either the first or second plant. The pre-

sent invention also provides plants, plant organs, plant tissues, plant cells, seeds, and non-human host cells that are transformed with the at least one polynucleotide molecule, expression cassette, or transformation vector of the invention. Such transformed plants, plant organs, plant tissues, plant cells, seeds, and non-human host cells have enhanced tolerance or resistance to at least one herbicide, at levels of the herbicide that kill or inhibit the growth of an untransformed plant, plant tissue, plant cell, or non-human host cell, respectively. Preferably, the transformed plants, plant tissues, plant cells, and seeds of the invention are Arabidopsis thaliana and crop plants.

It is to be understood that the plant of the present invention can comprise a wild type PPO nucleic acid in addition to a mut-PPO nucleic acid. It is contemplated that the PPO-inhibiting herbicide tolerant lines may contain a mutation in only one of multiple PPO isoenzymes. Therefore, the present invention includes a plant comprising one or more mut-PPO nucleic acids in addition to one or more wild type PPO nucleic acids.

In another embodiment, the invention refers to a seed produced by a transgenic plant comprising a plant cell of the present invention, wherein the seed is true breeding for an increased resistance to a PPO-inhibiting herbicide as compared to a wild type variety of the seed.

In another embodiment, the invention refers to a method of producing a transgenic plant cell with an increased resistance to a PPO-inhibiting herbicide as compared to a wild type variety of the plant cell comprising, transforming the plant cell with an expression cassette comprising a mut-PPO nucleic acid.

In another embodiment, the invention refers to a method of producing a transgenic plant comprising, (a) transforming a plant cell with an expression cassette comprising a mut-PPO nucleic acid, and (b) generating a plant with an increased resistance to PPO-inhibiting herbicide from the plant cell.

Consequently, mut-PPO nucleic acids of the invention are provided in expression cassettes for expression in the plant of interest. The cassette will include regulatory sequences operably linked to a mut-PPO nucleic acid sequence of the invention. The term "regulatory element" as used herein refers to a polynucleotide that is capable of regulating the transcription of an operably linked polynucleotide. It includes, but not limited to, promoters, enhancers, introns, 5' UTRs, and 3' UTRs. By "operably linked" is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the mut-PPO nucleic acid sequence to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The expression cassette of the present invention will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), a mut-PPO nucleic acid sequence of the invention, and a transcriptional and translational termination region (i.e., termination region) functional in plants. The promoter may be native or analogous, or foreign or heterologous, to the plant host and/or to the mut-PPO nucleic acid sequence of the invention. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. Where the promoter is "foreign" or "heterologous" to the plant host, it is intended that the promoter is not found in the native plant into which the promoter is introduced. Where the promoter is "foreign" or "heterologous" to the mut-PPO nucleic acid sequence of the invention, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked mut-PPO nucleic acid sequence of the invention. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

While it may be preferable to express the mut-PPO nucleic acids of the invention using heterologous promoters, the native promoter sequences may be used. Such constructs would change expression levels of the mut-PPO protein in the plant or plant cell. Thus, the phenotype of the plant or plant cell is altered.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked mut-PPO sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the mut-PPO nucleic acid sequence of interest, the plant host, or any combination thereof). Convenient termination regions are available from the Ti-plasmid of A. tumefaciens , such as the octopine synthase and nopaline synthase termination regions. See also Guerineau et al. (1991) Mol. Gen. Genet. 262: 141-144; Proudfoot (1991) Cell 64:671-674; Sanfacon et al. (1991) Genes Dev. 5: 141-149; Mogen et al. (1990) Plant Cell 2: 1261-1272; Munroe et al. (1990) Gene 91: 151-158; Ballas t al. (1989) Nucleic Acids Res. 17:7891-7903; and Joshi et al. (1987) Nucleic Acid Res. 15:9627-9639. Where appropriate, the gene(s) may be optimized for increased expression in the transformed plant. That is, the genes can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) Plant Physiol. 92: 1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing 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.

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. When possible, the sequence is 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 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 Mol. 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 mut-PPO gene expression, the plant expression vectors of the invention may also 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.

The expression cassettes of the present invention may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein et al. (1989) Proc. Natl. Acad. ScL USA 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. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, in vitro mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and trans versions, may be involved.

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. Such 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 Mol. Biol. 12:619-632 and Christensen et al. (1992) Plant Mol. 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.

Tissue-preferred promoters can be utilized to target enhanced mut-PPO 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. Tissue-preferred promoters include 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) Mol. 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 et al. (1993) Plant Mol Biol. 23(6): 1129-1138; Matsuoka e/ [alpha]/. (1993) Proc Natl. Acad. Sci. USA 90(20):9586-9590; and Guevara-Garcia et al. (1993) Plant J. 4(3):495-505. Such promoters can be modified, if necessary, for weak expression. In one embodiment, the nucleic acids of interest are targeted to the chloroplast for expression. In this manner, where the nucleic acid of interest is not directly inserted into the chloroplast, the expression cassette 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 mut-PPO nucleic acid 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 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. While the mut-PPO proteins of the invention include a native chloroplast transit peptide, any chloroplast transit peptide known in the art can be fused to the amino acid sequence of a mature mut-PPO protein of the invention by operably linking a choloroplast-targeting sequence to the 5'-end of a nucleotide sequence encoding a mature mut-PPO protein of the invention. 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); 5 -(enolpyruvyl)shikimate-3 -phosphate synthase (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.

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. 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.

In a preferred embodiment, the mut-PPO nucleic acid comprises a polynucleotide sequence selected from the group consisting of: a) a polynucleotide as shown in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, or 45, or a variant or derivative thereof; b) a polynucleotide encoding a polypeptide as shown in SEQ ID NO: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, or 46, or a variant or derivative thereof; c) a polynucleotide comprising at least 60 consecutive nucleotides of any of a) or c); and d) a polynucleotide complementary to the polynucleotide of any of a) through c)

Preferably, the expression cassette of the present invention further comprises a transcription initiation regulatory region and a translation initiation regulatory region that are functional in the plant.

While the polynucleotides of the invention find use as selectable marker genes for plant transformation, the expression cassettes of the invention can include another selectable marker gene for the selection of transformed cells. Selectable marker genes, including those of the present invention, are utilized for the selection of transformed cells or tissues. Marker genes include, but are not limited to, genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). See generally, Yarranton (1992) Curr. Opin. Biotech. 3 :506-511 ; Christophers on et al (1992) Proc. Natl. Acad. ScL USA 89:6314-6318; Yao et al. (1992) Cell 71:63-72; Reznikoff (1992) Mol Microbiol 6:2419-2422; Barkley et al (1980) in The Operon, pp. 177-220; Hu et al (1987) Cell 48:555-566; Brown et al (1987) Cell 49:603-612; Figge et al (1988) Cell 52:713-722; Deuschle et al (1989) Proc. Natl Acad. AcL USA 86:5400-5404; Fuerst et al (1989) Proc. Natl Acad. ScL USA 86:2549-2553; Deuschle et al (1990) Science 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines et al (1993) Proc. Natl Acad. ScL USA 90: 1917-1921; Labow et al (1990) Mol Cell Biol 10:3343-3356; Zambretti et al (1992) Proc. Natl Acad. ScL USA 89:3952-3956; Bairn et al (1991) Proc. Natl Acad. ScL USA 88:5072-5076; Wyborski et al (1991) Nucleic Acids Res. 19:4647-4653; Hillenand-Wissman (1989) Topics Mol Struc. Biol 10: 143- 162; Degenkolb et al (1991) Antimicrob. Agents Chemother. 35: 1591-1595; Kleinschnidt et al (1988) Biochemistry 27: 1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen et al (1992) Proc. Natl Acad. ScL USA 89:5547- 5551; Oliva et al (1992) Antimicrob. Agents Chemother. 36:913-919; Hlavka et al (1985) Handbook of Experimental Pharmacology, Vol. 78 (Springer-Verlag, Berlin); Gill et al (1988) Nature 334:721-724. The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

The invention further provides an isolated recombinant expression vector comprising the expres-

sion cassette containing a mut-PPO nucleic acid as described above, wherein expression of the vector in a host cell results in increased tolerance to a PPO-inhibiting herbicide as compared to a wild type variety of the host cell. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses, and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operably linked to the nucleic acid sequence to be expressed. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides or peptides, encoded by nucleic acids as described herein (e.g., mut-PPO polypeptides, fusion polypeptides, etc.).

In a preferred embodiment of the present invention, the mut-PPO polypeptides are expressed in plants and plants cells such as unicellular plant cells (such as algae) (See Falciatore et al., 1999, Marine Biotechnology 1(3):239-251 and references therein) and plant cells from higher plants (e.g., the spermatophytes, such as crop plants). A mut-PPO polynucleotide may be "introduced" into a plant cell by any means, including transfection, transformation or transduction, electroporation, particle bombardment, agroinfection, biolistics, and the like.

Suitable methods for transforming or transfecting host cells including plant cells can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, ed: Gartland and Davey, Humana Press, Totowa, New Jersey. As increased tolerance to PPO-inhibiting herbicides is a general trait wished to be inherited into a wide variety of plants like maize, wheat,

rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed and canola, manihot, pepper, sunflower and tagetes, solanaceous plants like potato, tobacco, eggplant, and tomato, Vicia species, pea, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut), perennial grasses, and forage crops, these crop plants are also preferred target plants for a genetic engineering as one further embodiment of the present invention. In a preferred embodiment, the plant is a crop plant. Forage crops include, but are not limited to, Wheatgrass, Canarygrass, Bromegrass, Wildrye Grass, Bluegrass, Orchardgrass, Alfalfa, Salfoin, Birdsfoot Trefoil, Alsike Clover, Red Clover, and Sweet Clover.

In one embodiment of the present invention, transfection of a mut-PPO polynucleotide into a plant is achieved by Agrobacterium mediated gene transfer. One transformation method known to those of skill in the art is the dipping of a flowering plant into an Agrobacteria solution, wherein the Agrobacteria contains the mut-PPO nucleic acid, followed by breeding of the transformed gametes. Agrobacterium mediated plant transformation can be performed using for example the GV3101(pMP90) (Koncz and Schell, 1986, Mol. Gen. Genet. 204:383-396) or LBA4404 (Clontech) Agrobacterium tumefaciens strain. Transformation can be performed by standard transformation and regeneration techniques (Deblaere et al., 1994, Nucl. Acids. Res. 13:4777-4788; Gelvin, Stanton B. and Schilperoort, Robert A, Plant Molecular Biology Manual, 2nd Ed. - Dordrecht : Kluwer Academic Publ., 1995. - in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, Bernard R. and Thompson, John E., Methods in Plant Molecular Biology and Biotechnology, Boca Raton : CRC Press, 1993 360 S., ISBN 0-8493-5164-2). For example, rapeseed can be transformed via cotyledon or hypocotyl transformation (Moloney et al., 1989, Plant Cell Report 8:238-242; De Block et al., 1989, Plant Physiol. 91:694-701). Use of antibiotics for Agrobacterium and plant selection depends on the binary vector and the Agrobacterium strain used for transformation. Rapeseed selection is normally performed using kanamycin as selectable plant marker. Agrobacterium mediated gene transfer to flax can be performed using, for example, a technique described by Mlynarova et al., 1994, Plant Cell Report 13:282-285. Additionally, transformation of soybean can be performed using for example a technique described in European Patent No. 0424 047, U.S. Patent No. 5,322,783, European Patent No. 0397 687, U.S. Patent No. 5,376,543, or U.S. Patent No. 5,169,770. Transformation of maize can be achieved by particle bombardment, polyethylene glycol mediated DNA uptake, or via the silicon carbide fiber technique. (See, for example, Freeling and Walbot "The maize handbook" Springer Verlag: New York (1993) ISBN 3-540-97826-7). A specific example of maize transformation is found in U.S. Patent No. 5,990,387, and a specific example of wheat transformation can be found in PCT Application No. WO 93/07256.

According to the present invention, the introduced mut-PPO polynucleotide may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced mut-PPO polynucleotide may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active. In one embodiment, a homologous recombinant microorganism can be created wherein the mut-PPO polynucleotide is integrated into a chromosome, a vector is prepared which contains at least a portion of an PPO gene into which a deletion, addition, or substitution has been introduced to thereby alter, e.g., functionally disrupt, the endogenous PPO gene and to create a mut-PPO gene. To create a point mutation via homologous recombination, DNA-RNA hybrids can be used in a

technique known as chimeraplasty (Cole-Strauss et al., 1999, Nucleic Acids Research 27(5):1323-1330 and Kmiec, 1999, Gene therapy American Scientist 87(3):240-247). Other homologous recombination procedures in Triticum species are also well known in the art and are contemplated for use herein.

In the homologous recombination vector, the mut-PPO gene can be flanked at its 5' and 3' ends by an additional nucleic acid molecule of the PPO gene to allow for homologous recombination to occur between the exogenous mut-PPO gene carried by the vector and an endogenous PPO gene, in a microorganism or plant. The additional flanking PPO nucleic acid molecule is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several hundreds of base pairs up to kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector (see e.g., Thomas, K. R., and Capecchi, M. R., 1987, Cell 51:503 for a description of homologous recombination vectors or Strepp et al., 1998, PNAS, 95(8):4368-4373 for cDNA based recombination in Physcomitrella patens). However, since the mut-PPO gene normally differs from the PPO gene at very few amino acids, a flanking sequence is not always necessary. The homologous recombination vector is introduced into a microorganism or plant cell (e.g., via polyethylene glycol mediated DNA), and cells in which the introduced mut-PPO gene has homologously recombined with the endogenous PPO gene are selected using art-known techniques.

In another embodiment, recombinant microorganisms can be produced that contain selected systems that allow for regulated expression of the introduced gene. For example, inclusion of a mut-PPO gene on a vector placing it under control of the lac operon permits expression of the mut-PPO gene only in the presence of IPTG. Such regulatory systems are well known in the art.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but they also apply to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic or eukaryotic cell. For example, a mut-PPO polynucleotide can be expressed in bacterial cells such as C. glutamicum, insect cells, fungal cells, or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells), algae, ciliates, plant cells, fungi or other microorganisms like C. glutamicum. Other suitable host cells are known to those skilled in the art.

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a mut-PPO polynucleotide. Accordingly, the invention further provides methods for producing mut-PPO polypeptides using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a mut-PPO polypeptide has been introduced, or into which genome has been introduced a gene encoding a wild-type or mut-PPO polypeptide) in a suitable medium until mut-PPO polypeptide is produced. In another embodiment, the method further comprises isolating mut-PPO polypeptides from the medium or the host cell. Another aspect of the invention pertains to

isolated mut-PPO polypeptides, and biologically active portions thereof. An "isolated" or "purified" polypeptide or biologically active portion thereof is free of some of the cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of mut-PPO polypeptide in which the polypeptide is separated from some of the cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a mut-PPO polypeptide having less than about 30% (by dry weight) of non-mut-PPO material (also referred to herein as a "contaminating polypeptide"), more preferably less than about 20% of non-mut-PPO material, still more preferably less than about 10% of non-mut-PPO material, and most preferably less than about 5% non-mut-PPO material.

When the mut-PPO polypeptide, or biologically active portion thereof, is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the polypeptide preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of mut-PPO polypeptide in which the polypeptide is separated from chemical precursors or other chemicals that are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of a mut-PPO polypeptide having less than about 30% (by dry weight) of chemical precursors or non-mut-PPO chemicals, more preferably less than about 20% chemical precursors or non-mut-PPO chemicals, still more preferably less than about 10% chemical precursors or non-mut-PPO chemicals, and most preferably less than about 5% chemical precursors or non-mut-PPO chemicals. In preferred embodiments, isolated polypeptides, or biologically active portions thereof, lack contaminating polypeptides from the same organism from which the mut-PPO polypeptide is derived. Typically, such polypeptides are produced by recombinant expression of, for example, a mut-PPO polypeptide in plants other than, or in microorganisms such as C. glutamicum, ciliates, algae, or fungi.

As described above, the present invention teaches compositions and methods for increasing the PPO-inhibiting tolerance of a crop plant or seed as compared to a wild-type variety of the plant or seed. In a preferred embodiment, the PPO-inhibiting tolerance of a crop plant or seed is increased such that the plant or seed can withstand a PPO-inhibiting herbicide application of preferably approximately 1-1000 g ai ha^{-1}, more preferably 1-200 g ai ha^{-1}, even more preferably 5-150 g ai ha^{-1}, and most preferably 10-100 g ai ha^{-1}. As used herein, to "withstand" a PPO-inhibiting herbicide application means that the plant is either not killed or not, or only moderately injured by such application. It will be understood by the person skilled in the art that the application rates may vary, depending on the environmental conditions such as temperature or humidity, and depending on the chosen kind of herbicide (active ingredient ai).

Furthermore, the present invention provides methods that involve the use of at least one PPO-inhibiting herbicide, optionally in combination with one or more herbicidal compounds B, and, optionally, a safener C, as described in detail supra.

In these methods, the PPO-inhibiting herbicide can be applied by any method known in the art including, but not limited to, seed treatment, soil treatment, and foliar treatment. Prior to application, the PPO-inhibiting herbicide can be converted into the customary formulations, for example solutions, emulsions, suspensions, dusts, powders, pastes and granules. The use form depends on the particular intended purpose; in each case, it should ensure a fine and even distribution of the compound according to the invention.

By providing plants having increased tolerance to PPO-inhibiting herbicide, a wide variety of formulations can be employed for protecting plants from weeds, so as to enhance plant growth and reduce competition for nutrients. A PPO-inhibiting herbicide can be used by itself for pre-emergence, post-emergence, pre-planting, and at-planting control of weeds in areas surrounding the crop plants described herein, or a PPO-inhibiting herbicide formulation can be used that contains other additives. The PPO-inhibiting herbicide can also be used as a seed treatment. Additives found in a PPO-inhibiting herbicide formulation include other herbicides, detergents, adjuvants, spreading agents, sticking agents, stabilizing agents, or the like. The PPO-inhibiting herbicide formulation can be a wet or dry preparation and can include, but is not limited to, flowable powders, emulsifiable concentrates, and liquid concentrates. The PPO-inhibiting herbicide and herbicide formulations can be applied in accordance with conventional methods, for example, by spraying, irrigation, dusting, or the like.

Suitable formulations are described in detail in PCT/EP2009/063387 and PCT/EP2009/063386.

It should also be understood that the foregoing relates to preferred embodiments of the present invention and that numerous changes may be made therein without departing from the scope of the invention. The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof, which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLES

EXAMPLE 1: Site-directed mutagenesis of Amaranthus PPO

Cloning of Aramanthus PPO

The Amaranthus tuberculatus coding sequence for PPO-susceptible and –resistant isoforms, and all mutant combinations and multiple mutations, (SEQ ID No: 1, 3, 5, 7) were synthesized and cloned by Geneart (Geneart AG, Regensburg, Germany).

Plasmids were isolated from E. coli TOP10 by performing a plasmid minpreparation and confirmed by DNA sequencing.

Expression and purification of recombinant wildtype and mutant PPO

(Taken from: Franck E. Dayan, Pankaj R. Daga, Stephen O. Duke, Ryan M. Lee, Patrick J. Tranel, Robert J. Doerksen. Biochemical and structural consequences of a glycine deletion in the α-8 helix of protoporphyrinogen oxidase. Biochimica et Biophysica Acta 1804 (2010), 1548-56)

Clones in pRSET vector were transformed into BL21(DE3)-pLysS strain of E. coli. Cells were grown in 250 mL of LB with 100 μgmL−1 of carbenicillin, shaking overnight at 37 °C. Cultures were diluted in 1 L of LB with antibiotic and grown at 37 °C shaking for 2 h, induced with 1 mM IPTG and grown at 25 °C shaking for 5 more hours. The cells were harvested by centrifugation at 1600×g, washed with 0.09% NaCl, and stored at −80 °C.

Cells were lysed using a French press at 140 MPa in 50 mM sodium phosphate pH 7.5, 1 M NaCl, 5 mM imidazole, 5% glycerol, and 1 μg mL−1 leupeptin. Following lysis, 0.5 U of benzonase (Novagen, EMD Chemicals, Inc., Gibbstown, NJ) and PMSF (final concentration of 1 mM) were added. Cell debris was removed by centrifugation at 3000×g. His-tagged PPO proteins were purified on a nickel activated Hitrap Chelating HP column (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) equilibrated with 20 mM sodium phosphate pH 8.0, 50 mM NaCl, 5 mM imidazole, 5 mM MgCl2, 0.1mM EDTA, and 17% glycerol.

PPO eluted with 250 mM imidazole. The active proteinwas desalted on a PD-10 column (GE Healthcare Bio-Sciences Corp., Piscataway, NJ) equilibrated with a 20 mM sodium phosphate buffer, pH 7.5, 5 mM MgCl2, 1 mM EDTA and 17% glycerol. Each liter of culture provided approximately 10 mg of pure PPO, which was stored at −20 °C until being used in assays.

PPO Activity Assay

PPO Enzyme Assay (non-recombinant). PPO protein (EC 1.3.3.4) was extracted from coleoptiles or shoots (150 g fresh weight) of dark-grown corn, black nightshade, morning glory, and velvetleaf seedlings as described previously (Grossmann et al. 2010). Before harvesting, the seedlings were allowed to green for 2 hours in the light in order to achieve the highest specific enzyme activities in the thylakoid fractions at low chlorophyll concentrations. At high chlorophyll concentrations significant quenching of fluorescence occurs, which limits the amount of green thylakoids that can be used in the test. Plant materials were homogenized in the cold with a Braun blender using a fresh-weight-to-volume ratio of 1:4. Homogenization buffer consisted of tris(hydroxymethyl)aminomethane (Tris)-HCl (50 mM; pH 7.3), sucrose (0.5 M), magnesium chloride (1 mM), ethylenediaminetetraacetic acid (EDTA) (1 mM) and bovine serum albumin (2 g L^{-1}). After filtration through four layers of Miracloth, crude plastid preparations were obtained after centrifugation at 10 000 x g for 5 min and resuspension in homogenization buffer before centrifugation at 150 x g for 2 min to remove crude cell debris. The supernatant was centrifuged at 4000 x g for 15 min and the pellet fraction was resuspended in 1 ml of a buffer containing Tris-HCl (50 mM; pH 7.3), EDTA (2 mM), leupeptin (2 μM), pepstatin (2 μM) and glycerol (200 ml L^{-1}) and stored at -80°C until use. Protein was determined in the enzyme extract with bovine serum albumin as a standard. PPO activity was assayed fluorometrically by monitoring the rate of Proto formation from chemically reduced protoporphyrinogen IX under initial velocity conditions. The assay mixture con-

sisted of Tris-HCl (100 mM; pH 7.3), EDTA (1 mM), dithiothreitol (5 mM), Tween 80 (0.085%), protoporphyrinogen IX (2 µM), and 40 µg extracted protein in a total volume of 200 µl. The reaction was initiated by addition of substrate protoporphyrinogen IX at 22°C. saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione, flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control, were prepared in dimethyl sulfoxide (DMSO) solution (0.1 mM concentration of DMSO in the assay) and added to the assay mixture in concentrations of 0.005 pM to 5 µM before incubation. Fluorescence was monitored directly from the assay mixture using a POLARstar Optima / Galaxy (BMG) with excitation at 405 nm and emission monitored at 630 nm. Non-enzymatic activity in the presence of heat-inactivated extract was negligible. Inhibition of enzyme activity induced by the herbicide was expressed as percentage inhibition relative to untreated controls. Molar concentrations of compound required for 50% enzyme inhibition (IC_{50} values) were calculated by fitting the values to the dose-response equation using non-linear regression analysis.

PPO Enzyme Assay (recombinant). Proto was purchased from Sigma-Aldrich (Milwaukee,WI). Protogen was prepared according to Jacobs and Jacobs (N.J. Jacobs, J.M. Jacobs, Assay for enzymatic protoporphyrinogen oxidation, a late step in heme synthesis, Enzyme 28 (1982) 206–219) . Assays were conducted in 100 mM sodium phosphate pH 7.4 with 0.1 mM EDTA, 0.1% Tween 20, 5 µM FAD, and 500mM imidazole. Dose–response curves with the PPO inhibitors saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione, flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control, and MC-15608 were obtained in the presence of 150 µM Protogen. The excitation and emission bandwidths were set at 1.5 and 30 nm, respectively. All assays were made in duplicates or triplicates and measured using a POLARstar Optima / Galaxy (BMG) with excitation at 405 nm and emission monitored at 630 nm. Molar concentrations of compound required for 50% enzyme inhibition (IC_{50} values) were calculated by fitting the values to the dose-response equation using non-linear regression analysis.

The dose response (IC_{50}) values for the substituted PPO enzymes are greater than the IC_{50} value for the wild type (non-substituted) PPO enzyme (Table 4a). This indicates that these substituted PPO enzymes have an inherent resistance to the PPO-inhibiting herbicides tested. The substituted PPO enzyme dG210 and R128L are known substituted PPO enzymes found within Amaranthus tuberculatus and *Ambrosia artemisiifolia* , respectively, and are shown to be responsible for *in planta* PPO resistance to a variety of PPO herbicides (Dayan et al., 2010, Biochimica et Biophysica Acta, 1804:1548). This indicates that the other substituted PPO enzymes listed, also with a higher IC_{50} value than dG210 or R128L, are also substituted PPO enzymes that are responsible for *in planta* resistance against a variety of PPO herbicides, (Table 4a). All substituted PPO enzymes show comparable enzyme activity, fluorescence unit change per minute (FU/min) as compared to the wild type PPO enzyme (Table 4a). In addition, all activity values for substituted PPO enzymes are larger than substituted PPO enzyme dG210 or R128L. Substituted PPO enzyme dG210 and R128L are sufficiently active for *in planta* function as already shown. This indicates that all other substituted PPO enzymes indicated are also sufficiently active for *in planta* function.

Table 4: IC_{50} (M) values for wild type and amino acid substituted PPO enzyme, for the inhibitors saflufenacil and 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione.

Amino Acid Substitution	SEQ. ID NO.	Relative Ezyme Activity (FU/min)	Saflufenacil	1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione
			IC50 (M)	
PPO herbicide sensitive PPO2 WC	2	1000	1,86E-09	5,17E-10
PPO herbicide sensitive PPO2 AC	4	800	1,78E-10	5,96E-11
dG210	6 & 8	80	1,60E-06	2,12E-09
R128L	2 & 4	700	2,22E-07	7,73E-10
R128A, L397D	2 & 4	100	1,00E-05	5,90E-09
R128L, L397D	2 & 4	ND	ND	
F204I, T208S	2 & 4	745	5,89E-11	1,29E-10
F204I, L397D	2 & 4	ND	ND	
F204I, L400A	2 & 4	150		4,57E-11
F204I, F420V	2 & 4	265		4,69E-09
F204I, F457M	2 & 4	200	1,89E-11	7,52E-11
T208S, L397D	2 & 4	150	4,08E-07	1,25E-10
T208S, L400A	2 & 4	ND	ND	
T208S, F420V	2 & 4	520	8,48E-07	2,34E-09
T208S, F457M	2 & 4	550	1,02E-10	1,95E-10
L397D, L400A	2 & 4	ND	ND	
L397R, F420M	2 & 4	ND	ND	
L397N, F420M	2 & 4	90		1,63E-08
L397D, F420M	2 & 4	120	>0,00001	2,95E-08
L397A, F420V	2 & 4	ND	ND	
L397R, F420V	2 & 4	ND	ND	
L397N, F420V	2 & 4	ND	ND	
L397Q, F420V	2 & 4	90	>0,00001	1,01E-07
L397K, F420V	2 & 4	ND	ND	
L397F, F420V	2 & 4	ND	ND	
L397P, F420V	2 & 4	ND	ND	
L397W, F420V	2 & 4	ND	ND	
L397V, F420V	2 & 4	150		1,21E-08
L397H, F420V	2 & 4	ND	ND	
L397I, F420M	2 & 4	410		1,98E-10
L397M, F420K	2 & 4	ND	ND	
L397M, F420M	2 & 4	250		2,32E-10

L397F, F420M	2 & 4	ND	ND	
L397S, F420M	2 & 4	210		3,33E-09
L397W, F420M	2 & 4	ND	ND	
L397Y, F420M	2 & 4	ND	ND	
L397I, F420V	2 & 4	100		4,09E-09
L397A, F420M	2 & 4	150		4,53E-09
L397C, F420M	2 & 4	370		1,79E-09
L397D, F420V	2 & 4	60	>0,00001	1,16E-06
L397C, F420V	2 & 4	150		5,54E-08
L397E, F420V	2 & 4	105	>0,00001	1,41E-07
L397G, F420V	2 & 4	ND	ND	
L397H, F420V	2 & 4	ND	ND	
L397M, F420V	2 & 4	140		8,79E-09
L397S, F420V	2 & 4	110		4,26E-08
L397T, F420V	2 & 4	150		1,31E-08
L397Q, F420M	2 & 4	110	1,00E-06	5,41E-09
L397E, F420M	2 & 4	340	1,00E-06	6,03E-09
L397G, F420M	2 & 4	80		6,06E-08
L397P, F420M	2 & 4	ND	ND	
L397T, F420M	2 & 4	ND	ND	
L397V, F420M	2 & 4	400		1,05E-09
L397D, F457M	2 & 4	ND	ND	
L400A, F420V	2 & 4	ND	ND	
L400A, F457M	2 & 4	160		1,35E-11
F420V, F457M	2 & 4	105		1,02E-09
R128A, F204I, F420V	2 & 4	ND	ND	
R128A, T208S, F420V	2 & 4	200	>0,00001	1,25E-08
R128A, L397D, F420V	2 & 4	ND	ND	
R128A, L400A, F420V	2 & 4	ND	ND	
R128A, F420V, F457M	2 & 4	ND	ND	
F204I, T208S, L397D	2 & 4	100		5,52E-11
F204I, T208S, L400A	2 & 4	105		2,64E-11
F204I, T208S, F420V	2 & 4	80		3,87E-09
F204I, T208S, F457M	2 & 4	200		4,21E-11
F204I, T208S, F457M	2 & 4	470	5,11E-11	1,70E-10
F204I, L397D, L400A	2 & 4	ND	ND	
F204I, L397D, F420V	2 & 4	ND	ND	
F204I, L397D, F457M	2 & 4	ND	ND	
F204I, L400A, F420V	2 & 4	100		8,23E-08
F204I, L400A, F457M	2 & 4	ND	ND	
F204I, F420V, F457M	2 & 4	80		2,10E-09
T208S, L397D, L400A	2 & 4	ND	ND	
T208S, L397D, F420V	2 & 4	ND	ND	
T208S, L397D, F457M	2 & 4	ND	ND	
T208S, L400A, F420V	2 & 4	ND	ND	

T208S, L400A, F457M	2 & 4	60		9,68E-12
T208S, F420V, F457M	2 & 4	90		3,41E-09
L397D, L400A, F420V	2 & 4	ND	ND	
L397D, L400A, F457M	2 & 4	ND	ND	
L397D, F420V, F457M	2 & 4	ND	ND	
L400A, F420V, F457M	2 & 4	ND	ND	
R128A, F204I, T208S, F420V	2 & 4	ND	ND	
R128A, F204I, F420V, F457M	2 & 4	80		4,63E-08
R128A, F204I, L397D, F420V	2 & 4	ND	ND	
R128A, F204I, L400A, F420V	2 & 4	ND	ND	
R128A, T208S, L397D, F420V	2 & 4	ND	ND	
R128A, T208S, L400A, F420V	2 & 4	ND	ND	
R128A, T208S, F420V, F457M	2 & 4	ND	ND	
R128A, L397D, F420V, F457M	2 & 4	ND	ND	
R128A, L400A, F420V, F457M	2 & 4	ND	ND	
F204I, T208S, L397D, L400A	2 & 4	80		2,38E-09
F204I, T208S, L397D, F420V	2 & 4	ND	ND	
F204I, T208S, L397D, F457M	2 & 4	ND	ND	
F204I, T208S, L400A, F420V	2 & 4	ND	ND	
F204I, T208S, L400A, F457M	2 & 4	100		2,88E-11
F204I, T208S, F420V, F457M	2 & 4	200		4,72E-09
F204I, L397D, L400A, F420V	2 & 4	ND	ND	
F204I, L397D, L400A, F457M	2 & 4	ND	ND	
F204I, L397D, F420V, F457M	2 & 4	ND	ND	
F204I, L400A, F420A, F457M	2 & 4	60		3,69E-08
T208S, L397D, L400A, F420V	2 & 4	ND	ND	
T208S, L397D, L400A, F457M	2 & 4	ND	ND	
T208S, L397D, F420V, F457M	2 & 4	ND	ND	
T208S, L400A, F420V, F457M	2 & 4	ND	ND	
L397D, L400A, F420V, F457M	2 & 4	ND	ND	
R128A, F204I, T208S, L400A, F420V	2 & 4	ND	ND	
R128A, F204I, T208S, L397D, F420V	2 & 4	ND	ND	
R128A, F204I, T208S, F420V, F457M	2 & 4	ND	ND	
R128A, F204I, L397D, F420V, F457M	2 & 4	ND	ND	
R128A, T208S, L397D, F420V, F457M	2 & 4	ND	ND	
R128A, T208S, L400A, F420V, F457M	2 & 4	ND	ND	
F204I, T208S, L397D, L400A, F420V	2 & 4	ND	ND	
F204I, T208S, L397D, L400A, F457M	2 & 4	ND	ND	

F204I, T208S, L397D, F420V, F457M	2 & 4	ND	ND	
F204I, T208S, L400A, F420V, F457M	2 & 4	60		9,24E-08
F204I, L397D, L400A, F420V, F457M	2 & 4	ND	ND	
T208S, L397D, L400A, F420V, F457M	2 & 4	ND	ND	
R128A, F204I, L400A, F420V, F457M	2 & 4	50		4,05E-07
R128A, F204I, T208S, L397D, F420V, F457M	2 & 4	ND	ND	
R128A, F204I, T208S, L400A, F420V, F457M	2 & 4	ND	ND	
F204I, T208S, L397D, L400A, F420V, F457M	2 & 4	ND	ND	

EXAMPLE 2. Engineering PPO-inhibiting herbicide tolerant plants having wildtype or mutated PPO sequences.

PPO-derivative herbicide tolerant soybean (Glycine max) or corn (Zea mays) plants are produced by a method as described by Olhoft et al. (US patent 2009/0049567). For transformation of soybean or Arabidopsis thaliana, Wildtype or Mutated PPO sequences are cloned with standard cloning techniques as described in Sambrook et al. (Molecular cloning (2001) Cold Spring Harbor Laboratory Press) in a binary vector containing resistance marker gene cassette (AHAS) and mutated PPO sequence (marked as GOI) in between ubiquitin promoter (PcUbi) and nopaline synthase terminator (NOS) sequence. For corn transformation, Wildtype or Mutated PPO sequences are cloned with standard cloning techniques as described in Sambrook et al. (Molecular cloning (2001) Cold Spring Harbor Laboratory Press) in a binary vector containing resistance marker gene cassette (AHAS) and mutated PPO sequence (marked as GOI) in between corn ubiquitin promoter (ZmUbi) and nopaline synthase terminator (NOS) sequence. Binary plasmids are introduced to Agrobacterium tumefaciens for plant transformation. Plasmid constructs are introduced into soybean's axillary meristem cells at the primary node of seedling explants via Agrobacterium-mediated transformation. After inoculation and co-cultivation with Agrobacteria, the explants are transferred to shoot introduction media without selection for one week. The explants were subsequently transferred to a shoot induction medium with 1-3 µM imazapyr (Arsenal) for 3 weeks to select for transformed cells. Explants with healthy callus/shoot pads at the primary node are then transferred to shoot elongation medium containing 1-3 µM imazapyr until a shoot elongated or the explant died. Transgenic plantlets are rooted, subjected to TaqMan analysis for the presence of the transgene, transferred to soil and grown to maturity in greenhouse. Transformation of corn plants are done by a method described by McElver and Singh (WO 2008/124495). Plant transformation vector constructs containing mutated PPO sequences are introduced into maize immature embryos via Agrobacterium-mediated transformation.

Transformed cells were selected in selection media supplemented with 0.5-1.5 µM imazethapyr for 3-4 weeks. Transgenic plantlets were regenerated on plant regeneration media and rooted after-

wards. Transgenic plantlets are subjected to TaqMan analysis for the presence of the transgene before being transplanted to potting mixture and grown to maturity in greenhouse. Arabidopsis thaliana are transformed with wildtype or mutated PPO sequences by floral dip method as decribed by McElver and Singh (WO 2008/124495). Transgenic Arabidopsis plants were subjected to TaqMan analysis for analysis of the number of integration loci. Transformation of Oryza sativa (rice) are done by protoplast transformation as decribed by Peng et al. (US 6653529) T0 or T1 transgenic plant of soybean, corn, and rice containing mutated PPO sequences are tested for improved tolerance to PPO-derived herbicides in greenhouse studies and mini-plot studies with the following PPO-inhibiting herbicides: saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control. .

Transgenic Arabidopsis thaliana plants were assayed for improved tolerance to saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control, in 48-well plates. Therefore, T2 seeds are surface sterilized by stirring for 5 min in ethanol + water (70+30 by volume), rinsing one time with ethanol + water (70+30 by volume) and two times with sterile, deionized water. The seeds are resuspended in 0.1% agar dissolved in water (w/v) Four to five seeds per well are plated on solid nutrient medium consisting of half-strength murashige skoog nutrient solution, pH 5.8 (Murashige and Skoog (1962) Physiologia Plantarum 15: 473-497). Compounds are dissolved in dimethylsulfoxid (DMSO) and added to the medium prior solidification (final DMSO concentration 0.1%). Multi well plates are incubated in a growth chamber at 22°C, 75% relative humidity and 110 µmol Phot * m^{-2} * s^{-1} with 14 : 10 h light : dark photoperiod. Growth inhibition is evaluated seven to ten days after seeding in comparison to wild type plants. Additionally, transgenic T1 Arabidopsis plants were tested for improved tolerance to PPO-inhibiting herbicides in greenhouse studies with the following PPO-inhibiting herbicides: saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione, flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control. The results are shown in Table 5.

Table 5

				Injury Rating 0 - 100%		
				(0 = no injury, 100 = total control)		
				300	150	75
Line	Assesment DAT (DAT = Days After Treatment)	SEQ_ID	Substitution	1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione g/Ha + 1%MSO		
1	7	2 & 4	L397D, F420V	100	30	98
1	19	2 & 4	L397D, F420V	98	65	95

2	7	2 & 4	L397D, F420V	30	98	100
2	19	2 & 4	L397D, F420V	60	100	100
3	7	2 & 4	L397D, F420V	35	98	80
3	19	2 & 4	L397D, F420V	80	100	95

EXAMPLE 3. Tissue Culture Conditions.

An in vitro tissue culture mutagenesis assay has been developed to isolate and characterize plant tissue (e.g., maize, rice tissue) that is tolerant to protoporphyrinogen oxidase (PPO) inhibiting herbicides, (e.g. saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione, flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone). The assay utilizes the somaclonal variation that is found in in vitro tissue culture. Spontaneous mutations derived from somaclonal variation can be enhanced by chemical mutagenesis and subsequent selection in a stepwise manner, on increasing concentrations of herbicide.

The present invention provides tissue culture conditions for encouraging growth of friable, embryogenic maize or rice callus that is regenerable. Calli were initiated from 4 different maize or rice cultivars encompassing Zea mays and Japonica (Taipei 309, Nipponbare, Koshihikari) and Indica (Indica 1) varieties, respectively. Seeds were surface sterilized in 70% ethanol for approximately 1 min followed by 20% commercial Clorox bleach for 20 minutes. Seeds were rinsed with sterile water and plated on callus induction media. Various callus induction media were tested. The ingredient lists for the media tested are presented in Table 6.

Table 6

Ingredient	Supplier	R001M	R025M	R026M	R327M	R008M	MS711R
B5 Vitamins	Sigma					1.0 X	
MS salts	Sigma			1.0 X	1.0 X	1.0 X	1.0 X
MS Vitamins	Sigma			1.0 X	1.0 X		
N6 salts	Phytotech	4.0 g/L	4.0g/L				
N6 vitamins	Phytotech	1.0 X	1.0 X				
L-Proline	Sigma	2.9 g/L	0.5 g/L				1.2 g/L
Casamino Acids	BD	0.3 g/L	0.3 g/L	2 g/L			
Casein Hydrolysate	Sigma						1.0 g/L
L-Asp Monohydrate	Phytotech						150 mg/L
Nicotinic Acid	Sigma						0.5 mg/L
Pyridoxine HCl	Sigma						0.5 mg/L
Thiamine HCl	Sigma						1.0 mg/L
Myo-inositol	Sigma						100 mg/L

Ingredient	Supplier	R001M	R025M	R026M	R327M	R008M	MS711R
MES	Sigma	500 mg/L	500 mg/L	500 mg/L	500 mg/L	500 mg/L	500 mg/L
Maltose	VWR	30 g/L	30 g/L	30 g/L	30 g/L		
Sorbitol	Duchefa			30 g/L			
Sucrose	VWR					10 g/L	30 g/L
NAA	Duchefa					50 µg/L	
2,4-D	Sigma	2.0 mg/L					1.0 mg/L
$MgCl_2 \cdot 6H_2O$	VWR					750 mg/L	
→pH		5.8	5.8	5.8	5.8	5.8	5.7
Gelrite	Duchefa	4.0 g/L				2.5 g/L	
Agarose Type1	Sigma		7.0 g/L	10 g/L	10 g/L		
→Autoclave		15 min	15 min	15 min	15 min	15 min	20 min
Kinetin	Sigma		2.0 mg/L	2.0 mg/L			
NAA	Duchefa		1.0 mg/L	1.0 mg/L			
ABA	Sigma		5.0 mg/L				
Cefotaxime	Duchefa		0.1 g/L	0.1 g/L	0.1 g/L		
Vancomycin	Duchefa		0.1 g/L	0.1 g/L	0.1 g/L		
G418 Disulfate	Sigma		20 mg/L	20 mg/L	20 mg/L		

R001M callus induction media was selected after testing numerous variations. Cultures were kept in the dark at 30°C. Embryogenic callus was subcultured to fresh media after 10-14 days.

EXAMPLE 4. Selection of Herbicide-tolerant Calli.

Once tissue culture conditions were determined, further establishment of selection conditions were established through the analysis of tissue survival in kill curves with saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione, flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control. . Careful consideration of accumulation of the herbicide in the tissue, as well as its persistence and stability in the cells and the culture media was performed. Through these experiments, a sub-lethal dose has been established for the initial selection of mutated material.

After the establishment of the starting dose of saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone, plus photosynthesis inhibitor diuron, which was used as negative control, in selection media, the tissues were selected in a step-wise fashion by increasing the concentration of the PPO inhibitor with each transfer until cells are recovered that grew vigorously in the presence of toxic doses. The resulting calli were further subcultured every 3-4 weeks to R001M with selective agent. Over 26,000 calli were subjected to selection for 4-5 subcultures until the selective pressure was above toxic levels as determined by kill curves and observations of continued culture.

Alternatively, liquid cultures initiated from calli in MS711R with slow shaking and weekly subcultures. Once liquid cultures were established, selection agent was added directly to the flask at each subculture. Following 2-4 rounds of liquid selection, cultures were transferred to filters on solid R001M media for further growth.

EXAMPLE 5. Regeneration of Plants.

Tolerant tissue was regenerated and characterized molecularly for PPO gene sequence mutations and/or biochemically for altered PPO activity in the presence of the selective agent. In addition, genes involved directly and/or indirectly in tetrapyrrole biosynthesis and/or metabolism pathways were also sequenced to characterize mutations. Finally, enzymes that change the fate (e.g. metabolism, translocation, transportation) were also sequence to characterized mutations.

Following herbicide selection, calli were regenerated using a media regime of R025M for 10 – 14 days, R026M for ca. 2 weeks, R327M until well formed shoots were developed, and R008S until shoots were well rooted for transfer to the greenhouse. Regeneration was carried out in the light. No selection agent was included during regeneration.

Once strong roots were established, M0 regenerants were transplant to the greenhouse in square or round pots. Transplants were maintained under a clear plastic cup until they were adapted to greenhouse conditions. The greenhouse was set to a day/night cycle of 27°C/21°C (80°F/70°F) with 600W high pressure sodium lights supplementing light to maintain a 14 hour day length. Plants were watered according to need, depending in the weather and fertilized daily.

EXAMPLE 6. Sequence Analysis.

Leaf tissue was collected from clonal plants separated for transplanting and analyzed as individuals. Genomic DNA was extracted using a Wizard® 96 Magnetic DNA Plant System kit (Promega, US Patent Nos. 6,027,945 & 6,368,800) as directed by the manufacturer. Isolated DNA was PCR amplified using the appropriate forward and reverse primer.

PCR amplification was performed using Hotstar Taq DNA Polymerase (Qiagen) using touchdown thermocycling program as follows: 96°C for 15 min, followed by 35 cycles (96°C, 30 sec; 58°C – 0.2 °C per cycle, 30 sec; 72°C, 3 min and 30 sec), 10 min at 72°C.

PCR products were verified for concentration and fragment size via agarose gel electrophoresis. Dephosphorylated PCR products were analyzed by direct sequence using the PCR primers (DNA Landmarks, or Entelechon). Chromatogram trace files (.scf) were analyzed for mutation relative to the wild-type gene using Vector NTI Advance 10™ (Invitrogen). Based on sequence information, mutations were identified in several individuals. Sequence analysis was performed on the representative chromatograms and corresponding AlignX alignment with default settings and edited to call secondary peaks.

EXAMPLE 7. Demonstration of Herbicide-tolerance.

Selected mutants and escapes were transferred to small pots. Wild-type cultivars were germinated

from seed to serve as controls.

After ca. 3 weeks post-transplant, M0 regenerants were sprayed using a track sprayer with saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone (plus diuron) supplemented with 0.1% methylated seed oil. After the plants had adapted to greenhouse conditions, a subset were sprayed with additional saflufenacil or 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione . Once sprayed, plants were kept on drought conditions for 24 hours before being watered and fertilized again. Sprayed plants were photographed and rated for herbicide injury at 1 and 3 weeks after treatment. No or low injury levels were observed on plants containing the heterozygous mutation while control plants and tissue culture escapes (regenerated plants negative for the sequenced mutations) were heavily damaged after treatment.

Tolerance rates for corn and soybean areshown in Tables 7 a-c.

Table 7 a) Corn

Amino acid substitution	SEQ ID NO	Event	Saflufenacil (g ai/ha) 0	25	50	100	1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (g ai/ha) 50
none	none	1	0	*	*	*	*
		2	3	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	*	*	7	*	*
		9	*	*	7	*	*
		10	*	*	*	7	*
		11	*	*	*	7	*
		12	*	*	*	8	*
		13	*	*	*	7	*
		14	*	*	*	8	*
		15	*	*	*	*	6
		16	*	*	*	*	7
		17	*	*	*	*	6
		18	*	*	*	*	6
		19	*	*	*	*	7

		20	*	*	*	*	6
none	40	1	*	7	*	*	*
		2	*	7	*	*	*
		3	*	*	*	*	*
		4	*	8	*	*	*
		5	*	7	*	*	*
		6	*	*	8	*	*
		7	*	*	8	*	*
		8	*	*	7	*	*
		9	*	*	8	*	*
		1	*	*	8	*	*
		2	*	*	8	*	*
		3	*	*	*	7	*
		4	*	*	*	8	*
		5	*	*	*	8	*
		6	*	*	*	8	*
		7	*	*	*	8	*
		8	*	7	*	*	*
		9	*	7	*	*	*
		10	*	7	*	*	*
		11	*	4	*	*	*
		12	*	5	*	*	*
		13	*	4	*	*	*
		14	*	7	*	*	*
		15	*	*	7	*	*
		16	*	*	7	*	*
		17	*	*	7	*	*
		18	*	*	6	*	*
		19	*	*	7	*	*
		20	*	*	7	*	*
		21	*	*	7	*	*
		22	*	*	7	*	*
		23	*	*	7	*	*
		24	*	*	7	*	*
		25	*	*	6	*	*
		26	*	*	7	*	*
		27	*	*	7	*	*
		28	*	*	*	7	*
		29	*	*	*	7	*
		30	*	*	*	6	*
		31	*	*	*	6	*
		32	*	*	*	7	*
		33	*	*	*	8	*

		34	*	*	*	7	*
		35	*	*	*	7	*
		36	*	*	*	6	*
		37	*	*	*	8	*
		38	*	*	*	7	*
L369D, F392V	40	1	*	7	*	*	*
		2	*	4	*	*	*
		3	*	6	*	*	*
		4	*	6	*	*	*
		5	*	7	*	*	*
		6	*	6	*	*	*
		7	*	7	*	*	*
		8	*	*	7	*	*
		9	*	*	6	*	*
		10	*	*	7	*	*
		11	*	*	6	*	*
		12	*	*	8	*	*
		13	*	*	7	*	*
		14	*	*	7	*	*
		15	*	*	8	*	*
		16	*	*	6	*	*
		17	*	*	7	*	*
		18	*	*	7	*	*
		19	*	*	7	*	*
		20	*	*	7	*	*
		21	*	*	8	*	*
		22	*	*	9	*	*
		23	*	*	7	*	*
		24	*	*	4	*	*
		25	*	*	6	*	*
		26	*	*	7	*	*
		27	*	*	7	*	*
		28	*	*	8	*	*
		29	*	*	5	*	*
		30	*	*	*	8	*
		31	*	*	*	8	*
		32	*	*	*	7	*
		33	*	*	*	7	*
		34	*	*	*	7	*
		35	*	*	*	8	*
		36	*	*	*	7	*
		37	*	*	*	6	*

		38	*	*	*	7	*
		39	*	*	*	7	*
		40	*	*	*	8	*
		41	*	*	*	8	*
		42	*	*	*	8	*
		43	*	*	*	7	*
		44	*	*	*	8	*
		45	*	*	*	7	*
		46	*	*	*	7	*
		47	*	*	*	8	*
none	2	1	*	7	*	*	*
		2	*	7	*	*	*
		3	*	8	*	*	*
		4	*	6	*	*	*
		5	*	6	*	*	*
		6	*	7	*	*	*
		7	*	7	*	*	*
		8	*	7	*	*	*
		9	*	7	*	*	*
		10	*	6	*	*	*
		11	*	*	6	*	*
		12	*	*	6	*	*
		13	*	*	7	*	*
		14	*	*	8	*	*
		15	*	*	7	*	*
		16	*	*	6	*	*
		17	*	*	7	*	*
		18	*	*	6	*	*
		19	*	*	7	*	*
		20	*	*	7	*	*
		21	*	*	8	*	*
		22	*	*	8	*	*
		23	*	*	7	*	*
		24	*	*	8	*	*
		25	*	*	7	*	*
		26	*	*	8	*	*
		27	*	*	*	8	*
		28	*	*	*	7	*
		29	*	*	*	8	*
		30	*	*	*	7	*
		31	*	*	*	7	*
		32	*	*	*	8	*
		33	*	*	*	8	*

		34	*	*	*	7	*
		35	*	*	*	8	*
		36	*	*	*	7	*
		37	*	*	*	7	*
		38	*	*	*	8	*
		39	*	*	*	6	*
		40	*	*	*	7	*
		41	*	*	*	8	*
		42	*	*	*	7	*
F420V	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	*	6	*	*	*
		4	*	7	*	*	*
		5	*	6	*	*	*
		6	*	7	*	*	*
		7	*	6	*	*	*
		8	*	4	*	*	*
		9	*	6	*	*	*
		10	*	6	*	*	*
		11	*	6	*	*	*
		12	*	7	*	*	*
		13	*	7	*	*	*
		14	*	7	*	*	*
		15	*	*	7	*	*
		16	*	*	8	*	*
		17	*	*	7	*	*
		18	*	*	7	*	*
		19	*	*	7	*	*
		20	*	*	7	*	*
		21	*	*	7	*	*
		22	*	*	7	*	*
		23	*	*	7	*	*
		24	*	*	6	*	*
		25	*	*	7	*	*
		26	*	*	8	*	*
		27	*	*	7	*	*
		28	*	*	6	*	*
		29	*	*	7	*	*
		30	*	*	7	*	*
		31	*	*	7	*	*
		32	*	*	7	*	*
		33	*	*	*	7	*
		34	*	*	*	7	*

		35	*	*	*	7	*
		36	*	*	*	7	*
		37	*	*	*	7	*
		38	*	*	*	7	*
		39	*	*	*	7	*
		40	*	*	*	8	*
		41	*	*	*	7	*
		42	*	*	*	8	*
		43	*	*	*	7	*
		44	*	*	*	7	*
		45	*	*	*	9	*
		46	*	*	*	8	*
		47	*	*	*	7	*
		48	*	*	*	7	*
		49	*	*	*	8	*
		50	*	*	*	7	*
		51	*	*	*	7	*
L397D	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	0	*	*	*	*
		9	0	*	*	*	*
		10	0	*	*	*	*
		11	0	*	*	*	*
		12	0	*	*	*	*
		13	1	*	*	*	*
		14	0	*	*	*	*
		15	0	*	*	*	*
		16	0	*	*	*	*
		17	0	*	*	*	*
		18	0	*	*	*	*
		19	0	*	*	*	*
		20	0	*	*	*	*
		21	0	*	*	*	*
		22	0	*	*	*	*
		23	0	*	*	*	*
		24	0	*	*	*	*
		25	0	*	*	*	*
		26	0	*	*	*	*

27	0	*	*	*	*
28	0	*	*	*	*
29	0	*	*	*	*
30	0	*	*	*	*
31	0	*	*	*	*
32	0	*	*	*	*
33	0	*	*	*	*
34	0	*	*	*	*
35	1	*	*	*	*
36	0	*	*	*	*
37	*	6	*	*	*
38	*	6	*	*	*
39	*	6	*	*	*
40	*	6	*	*	*
41	*	6	*	*	*
42	*	7	*	*	*
43	*	6	*	*	*
44	*	5	*	*	*
45	*	6	*	*	*
46	*	8	*	*	*
47	*	7	*	*	*
48	*	7	*	*	*
49	*	6	*	*	*
50	*	4	*	*	*
51	*	7	*	*	*
52	*	4	*	*	*
53	*	4	*	*	*
54	*	*	6	*	*
55	*	*	6	*	*
56	*	*	6	*	*
57	*	*	6	*	*
58	*	*	6	*	*
59	*	*	7	*	*
60	*	*	6	*	*
61	*	*	6	*	*
62	*	*	4	*	*
63	*	*	7	*	*
64	*	*	7	*	*
65	*	*	7	*	*
66	*	*	7	*	*
67	*	*	6	*	*
68	*	*	6	*	*
69	*	*	5	*	*

		70	*	*	4	*	*
		71	*	*	7	*	*
		72	*	*	7	*	*
		73	*	*	6	*	*
		74	*	*	7	*	*
		75	*	*	6	*	*
		76	*	*	7	*	*
		77	*	*	7	*	*
		78	*	*	7	*	*
		79	*	*	7	*	*
		80	*	*	7	*	*
		81	*	*	5	*	*
L397D, F420V	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	0	*	*	*	*
		9	0	*	*	*	*
		10	0	*	*	*	*
		11	0	*	*	*	*
		12	0	*	*	*	*
		13	0	*	*	*	*
		14	0	*	*	*	*
		15	0	*	*	*	*
		16	0	*	*	*	*
		17	0	*	*	*	*
		18	0	*	*	*	*
		19	0	*	*	*	*
		20	0	*	*	*	*
		21	0	*	*	*	*
		22	0	*	*	*	*
		23	0	*	*	*	*
		24	0	*	*	*	*
		25	0	*	*	*	*
		26	*	6	*	*	*
		27	*	5	*	*	*
		28	*	6	*	*	*
		29	*	7	*	*	*
		30	*	7	*	*	*

		31	*	6	*	*	*
		32	*	6	*	*	*
		33	*	*	4	*	*
		34	*	*	6	*	*
		35	*	*	6	*	*
		36	*	*	6	*	*
		37	*	*	6	*	*
		38	*	*	6	*	*
		39	*	*	7	*	*
		40	*	*	6	*	*
		41	*	*	7	*	*
		42	*	*	6	*	*
		43	*	*	7	*	*
		44	*	*	7	*	*
		45	*	*	7	*	*
		46	*	*	7	*	*
		47	*	*	7	*	*
		48	*	*	7	*	*
		49	*	*	7	*	*
		50	*	*	6	*	*
F420L	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	*	6	*	*	*
		6	*	7	*	*	*
		7	*	6	*	*	*
		8	*	7	*	*	*
		9	*	7	*	*	*
		10	*	6	*	*	*
		11	*	7	*	*	*
		12	*	6	*	*	*
		13	*	7	*	*	*
		14	*	7	*	*	*
		15	*	6	*	*	*
		16	*	7	*	*	*
		17	*	*	7	*	*
		18	*	*	8	*	*
		19	*	*	7	*	*
		20	*	*	7	*	*
		21	*	*	7	*	*
		22	*	*	7	*	*
		23	*	*	7	*	*

		24	*	*	7	*	*
		25	*	*	7	*	*
		26	*	*	7	*	*
		27	*	*	7	*	*
		28	*	*	7	*	*
		29	*	*	8	*	*
		30	*	*	7	*	*
		31	*	*	6	*	*
		32	*	*	7	*	*
		33	*	*	7	*	*
		34	*	*	7	*	*
		35	*	*	6	*	*
		36	*	*	7	*	*
		37	*	*	7	*	*
		38	*	*	7	*	*
		39	*	*	7	*	*
		40	*	*	*	8	*
		41	*	*	*	7	*
		42	*	*	*	7	*
		43	*	*	*	7	*
		44	*	*	*	7	*
		45	*	*	*	7	*
		46	*	*	*	7	*
		47	*	*	*	7	*
		48	*	*	*	7	*
		49	*	*	*	7	*
		50	*	*	*	7	*
		51	*	*	*	7	*
		52	*	*	*	7	*
		53	*	*	*	7	*
		54	*	*	*	7	*
		55	*	*	*	6	*
		56	*	*	*	8	*
		57	*	*	*	7	*
		58	*	*	*	8	*
		59	*	*	*	7	*
		60	*	*	*	7	*
F420M	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	1	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*

		7	0	*	*	*	*
		8	0	*	*	*	*
		9	0	*	*	*	*
		10	0	*	*	*	*
		11	0	*	*	*	*
		12	0	*	*	*	*
		13	0	*	*	*	*
		14	0	*	*	*	*
		15	0	*	*	*	*
		16	*	4	*	*	*
		17	*	6	*	*	*
		18	*	8	*	*	*
		19	*	7	*	*	*
		20	*	7	*	*	*
		21	*	5	*	*	*
		22	*	5	*	*	*
		23	*	6	*	*	*
		24	*	8	*	*	*
		25	*	*	7	*	*
		26	*	*	7	*	*
		27	*	*	6	*	*
		28	*	*	8	*	*
		29	*	*	8	*	*
		30	*	*	7	*	*
		31	*	*	7	*	*
		32	*	*	7	*	*
		33	*	*	7	*	*
		34	*	*	7	*	*
		35	*	*	8	*	*
		36	*	*	8	*	*
		37	*	*	8	*	*
		38	*	*	7	*	*
		39	*	*	7	*	*
L397E, F420V	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	1	*	*	*	*
		9	0	*	*	*	*

10	2	*	*	*	*
11	0	*	*	*	*
12	1	*	*	*	*
13	0	*	*	*	*
14	0	*	*	*	*
15	0	*	*	*	*
16	0	*	*	*	*
17	0	*	*	*	*
18	2	*	*	*	*
19	1	*	*	*	*
20	0	*	*	*	*
21	1	*	*	*	*
22	2	*	*	*	*
23	1	*	*	*	*
24	2	*	*	*	*
25	2	*	*	*	*
26	1	*	*	*	*
27	2	*	*	*	*
28	1	*	*	*	*
29	*	1	*	*	*
30	*	0	*	*	*
31	*	0	*	*	*
32	*	3	*	*	*
33	*	1	*	*	*
34	*	0	*	*	*
35	*	1	*	*	*
36	*	*	1	*	*
37	*	*	1	*	*
38	*	*	0	*	*
39	*	*	2	*	*
40	*	*	0	*	*
41	*	*	1	*	*
42	*	*	3	*	*
43	*	*	2	*	*
44	*	*	0	*	*
45	*	*	2	*	*
46	*	*	1	*	*
47	*	*	2	*	*
48	*	*	2	*	*
49	*	*	3	*	*
50	*	*	2	*	*
51	*	*	*	0	*
52	*	*	*	0	*

		53	*	*	*	1	*
		54	*	*	*	3	*
		55	*	*	*	2	*
		56	*	*	*	2	*
		57	*	*	*	2	*
		58	*	*	*	1	*
		59	*	*	*	1	*
		60	*	*	*	*	3
		61	*	*	*	*	1
		62	*	*	*	*	2
		63	*	*	*	*	2
		64	*	*	*	*	3
L397Q, F420V	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	1	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	0	*	*	*	*
		9	0	*	*	*	*
		10	*	*	*	4	*
		11	*	*	*	2	*
		12	*	*	*	3	*
		13	*	*	*	2	*
		14	*	*	*	7	*
		15	*	*	*	3	*
		16	*	*	*	3	*
		17	*	*	*	3	*
		18	*	*	*	5	*
		19	*	*	*	*	3
		20	*	*	*	*	2
		21	*	*	*	*	3
		22	*	*	*	*	3
		23	*	*	*	*	2
L397E, F420M	2	1	1	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*

		7	0	*	*	*	*
		8	0	*	*	*	*
		9	*	*	*	1	*
		10	*	*	*	0	*
		11	*	*	*	1	*
		12	*	*	*	0	*
		13	*	*	*	7	*
		14	*	*	*	1	*
		15	*	*	*	0	*
		16	*	*	*	1	*
		17	*	*	*	*	0
		18	*	*	*	*	1
L397Q, F420M	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	1	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	0	*	*	*	*
		9	1	*	*	*	*
		10	*	*	*	0	*
		11	*	*	*	0	*
		12	*	*	*	1	*
		13	*	*	*	1	*
		14	*	*	*	1	*
		15	*	*	*	1	*
		16	*	*	*	1	*
		17	*	*	*	*	3
		18	*	*	*	*	3
L397D, F420M	2	1	0	*	*	*	*
		2	0	*	*	*	*
		3	0	*	*	*	*
		4	0	*	*	*	*
		5	0	*	*	*	*
		6	0	*	*	*	*
		7	0	*	*	*	*
		8	0	*	*	*	*
		9	0	*	*	*	*
		10	0	*	*	*	*
		11	1	*	*	*	*

		12	1	*	*	*	*
		13	0	*	*	*	*
		14	0	*	*	*	*
		15	2	*	*	*	*
		16	1	*	*	*	*
		17	1	*	*	*	*
		18	1	*	*	*	*
		19	0	*	*	*	*
		20	1	*	*	*	*
		21	*	2	*	*	*
		22	*	2	*	*	*
		23	*	2	*	*	*
		24	*	3	*	*	*
		25	*	1	*	*	*
		26	*	3	*	*	*
		27	*	3	*	*	*
		28	*	3	*	*	*
		29	*	3	*	*	*
		30	*	3	*	*	*
		31	*	3	*	*	*
		32	*	*	1	*	*
		33	*	*	1	*	*
		34	*	*	2	*	*
		35	*	*	1	*	*
		36	*	*	1	*	*
		37	*	*	3	*	*
		38	*	*	3	*	*
		39	*	*	2	*	*
		40	*	*	0	*	*
		41	*	*	3	*	*
		42	*	*	3	*	*
		43	*	*	2	*	*
		44	*	*	1	*	*
		45	*	*	2	*	*
		46	*	*	2	*	*
		47	*	*	*	7	*
		48	*	*	*	3	*
		49	*	*	*	3	*
		50	*	*	*	3	*
		51	*	*	*	*	3
L397Q	2	1	0	*	*	*	*
		2	1	*	*	*	*
		3	0	*	*	*	*

4	1	*	*	*	*
5	0	*	*	*	*
6	0	*	*	*	*
7	*	*	*	4	*
8	*	*	*	4	*
9	*	*	*	4	*
10	*	*	*	4	*
11	*	*	*	4	*
12	*	*	*	5	*
13	*	*	*	5	*
14	*	*	*	5	*
15	*	*	*	*	6
16	*	*	*	*	6
17	*	*	*	*	5
18	*	*	*	*	7
19	*	*	*	*	6

Table 7 b) Soy

Amino acid substitution	SEQ ID NO	saflufenacil (g ai/ha)						1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione (g ai/ha)			
		0	12,5	25	50	100	200	12,5	25	50	75
none	none	0	9	9	9	9	9	7	8	9	9
none	40	1	8	9	8	*	*	8	8	*	*
		1	9	9	*	*	*	8	9	*	*
		0	3	6	9	*	*	6	6	*	*
		2	4	6	8	*	*	6	7	*	*
		1	9	9	9	*	*	7	7	*	*
		0	9	9	9	*	*	8	8	*	*
		0	8	9	8	*	*	8	*	*	*
		0	8	9	9	*	*	7	9	*	*
		0	7	9	9	*	*	8	9	*	*
		0	9	9	9	*	*	8	9	*	*
		0	9	9	9	*	*	9	9	*	*
		0	8	9	8	*	*	7	8	*	*
		0	9	9	9	*	*	8	9	*	*
L369D, F392V	40	0	9	9	9	*	*	8	9	*	*
		0	7	9	9	*	*	8	8	*	*
		0	4	5	6	*	*	5	6	*	*
		0	5	6	7	*	*	8	8	*	*
		0	9	9	9	*	*	7	9	*	*

		0	6	7	7	*	*	8	7	*	*
		0	4	4	5	*	*	5	4	*	*
		2	5	6	6	*	*	6	7	*	*
		*	4	5	6	*	*	4	6	*	*
		0	5	6	6	*	*	5	7	*	*
		0	4	5	5	*	*	4	6	*	*
		0	*	6	9	9	*	*	8	8	*
		*	*	5	6	6	*	*	6	7	*
none	2	0	2	4	7	*	*	3	5	*	*
		0	4	3	7	*	*	7	7	*	*
		0	4	7	8	*	*	6	6	*	*
		0	7	9	9	*	*	6	8	*	*
		1	7	8	9	*	*	7	8	*	*
		0	8	7	9	*	*	5	6	*	*
		0	5	8	9	*	*	7	*	*	*
		5	7	8	9	*	*	7	*	*	*
		0	5	7	6	*	*	6	7	*	*
L397D	2	2	*	7	9	9	*	*	8	9	*
		1	*	2	4	4	*	*	4	5	*
		1	*	9	9	9	*	*	8	8	*
		1	*	2	3	4	*	*	4	4	*
		0	*	5	6	6	*	*	5	5	*
		0	*	3	5	5	*	*	4	5	*
		*	*	2	4	6	*	*	5	6	*
L397E	2	0	5	3	3	*	*	5	5	*	*
		1	4	6	6	*	*	7	8	*	*
		0	4	4	4	*	*	6	8	*	*
		*	4	5	6	*	*	4	6	*	*
		0	4	6	6	*	*	6	7	*	*
		1	4	6	6	*	*	6	8	*	*
		0	0	1	3	*	*	8	6	*	*
		0	5	4	4	*	*	5	8	*	*
		0	3	6	7	*	*	6	8	*	*
L397Q	2	0	4	3	8	*	*	7	9	*	*
F420L	2	1	*	9	9	9	*	*	9	*	*
		0	1	0	0	*	*	2	1	*	*
		1	0	2	2	*	*	*	3	*	*
		1	3	1	1	*	*	1	*	*	*
		0	*	2	3	4	*	*	4	6	*
		0	*	2	5	6	*	*	5	6	*
		0	*	2	3	5	*	*	5	6	*
F420M	2	1	*	0	3	3	*	*	4	*	*
		0	*	4	6	5	*	*	*	3	*
		0	*	0	2	3	*	*	1	4	*
		0	*	4	3	3	*	*	4	*	*

	0	*	0	3	2	*	*	*	2	*
	0	*	0	3	3	*	*	1	2	*
	2	*	5	6	6	*	*	5	5	*
	0	*		9	9	9	*	8	9	*
L397D, F420V	2 0	*	3	6	6	*	*	5	4	*
	1	1	1	2	*	*	2	*	*	*
	1	0	0	1	*	*	3	1	*	*
	2	*	4	6	6	*	*	6	6	*
	0	*	6	7	7	*	*	6	6	*
	0	*	5	4	5	*	*	5	5	*
	0	*	5	4	4	*	*	6	*	*
	0	*	5	5	5	*	*	6	*	*
	*	*	8	9	9	*	*	6	7	*
L397D, F420M	2 0	*	*	*	6	*	*	0	3	*
	*	*	*	9	9	*	*	*	9	8
	3	*	*	3	2	*	*	1	2	3
L397E, F420V	2 1	*	*	0	0	*	*	1	2	2
	0	*	*	8	8	*	*	7	8	8
	2	*	*	6	6	*	*	6	7	7
L397Q, F420V	2 0	*	*	3	5	*	*	5	4	6
	0	*	*	1	*	*	*	0	1	3
	0	*	*	6	7	*	*	7	7	7
	0	*	*	8	9	*	*	9	8	9
	2	*	*	2	2	*	*	5	4	7
	*	*	*	3	3	*	*	4	4	4
	1	*	*	9	9	*	*	8	8	9
	0	*	*	0	2	*	*	0	1	1
	0	*	*	0	3	*	*	2	4	5
	0	*	*	0	0	*	*	4	5	5
	0	*	*	7	7	*	*	6	7	7
L397E, F420M	2 0	*	*	1	2	*	*	2	2	2
	0	*	*	0	0	*	*	1	1	3
	0	*	*	2	1	*	*	0	1	1
L397Q, F420M	2 0	*	*	0	0	*	*	3	2	3
	1	*	*	6	7	*	*	7	7	7
	1	*	*	6	7	*	*	7	7	8
	1	*	*	1	3	*	*	2	3	4
	0	*	*	0	3	*	*	0	4	5
	0	*	*	0	0	*	*	2	4	4
	*	*	*	5	6	*	*	6	6	6

2	*	*	4	6	*	*	6	6	*
3	*	*	0	0	*	*	3	5	5
1	*	*	0	1	*	*	2	1	2
0	*	*	1	2	*	*	2	3	4

Table 7 c) Soybean tolerance to PPO Inhibitor compounds: Wild type and transgenic segregating T1 individuals from 2 independent events

Amino acid substitution	SEQ ID NO		saflufenacil	1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione	Fomesafen	Flumioxazin	Sulfentrazone	Sulfentrazone	Oxyfluorfen	Oxyfluorfen
		Unsprayed	150 g ai/ha	100 g ai/ha	600 g ai/ha	150 g ai/ha	350 g ai/ha	700 g ai/ ha	600 g ai/ha	1200 g ai/ha
none	none	0	9	9	5	9	9	9	8	9
		0	9	9	5	9	9	9	7	9
		0	9	9	4	9	7	9	8	9
		0	9	9	4	9	7	9	9	9
		0	9	9	4	9	8	9	7	8
		1	9	8	5	9	9	9	8	9
		*	9	9	4	9	9	9	9	9
		*	9	9	5	9	9	9	7	8
L397D_F420V	2	0	3	9	1	9	9	2	4	5
Event A		0	5	4	2	6	*	3	4	5
		0	3	4	0	6	6	7	5	4
		0	4	4	0	5	3	7	6	4
		0	6	4	1	5	5	4	6	3
		0	4	9	0	4	3	4	9	4
		*	3	4	2	4	5	6	4	4

		*	5	4	4	6	6	4	4	5
L397D_F420V	2	1	3	4	2	5	4	4	6	5
Event B		0	2	3	4	6	4	5	4	5
		0	3	3	1	6	4	5	8	4
		0	3	3	3	5	5	4	4	5
		0	4	4	2	6	5	5	4	4
		0	4	3	1	4	4	6	5	6
		*	3	4	3	5	6	6	4	5
		*	4	4	3	4	4	5	6	5

WO 2013/189984 PCT/EP2013/062744

The following gives a definition of the injury scores measured above:

Score	Description of injury
0	No Injury
1	Minimal injury, only a few patches of leaf injury or chlorosis.
2	Minimal injury with slightly stronger chlorosis. Overall growth points remain undamaged.
3	Slightly stronger injury on secondary leaf tissue, but primary leaf and growth points are still undamaged.
4	Overall plant morphology is slightly different, some chlorosis and necrosis in secondary growth points and leaf tissue. Stems are intact. Regrowth is highly probable within 1 week.
5	Overall plant morphology is clearly different, some chlorosis and necrosis on a few leaves and growth points, but primary growth point is intact. Stem tissue is still green. Regrowth is highly probably within 1 week.
6	Strong injury can be seen on the new leaflet growth. Plant has a high probability to survive only through regrowth at different growth points. Most of the leaves are chlorotic/ necrotic but stem tissue is still green. May have regrowth but with noticeable injured appearance.
7	Most of the active growth points are necrotic. There may be a single growth point that could survive and may be partially chlorotic or green and partially necrotic. Two leaves may still be chlorotic with some green; the rest of the plant including stem is necrotic.
8	Plant will likely die, and all growth points are necrotic. One leaf may still be chlorotic with some green. The remainder of the plant is necrotic.
9	Plant is dead.
*	Not tested

EXAMPLE 8. Herbicide Selection Using Tissue Culture.

Media was selected for use and kill curves developed as specified above. For selection, different techniques were utilized. Either a step wise selection was applied, or an immediate lethal level of herbicide was applied. In either case, all of the calli were transferred for each new round of selection. Selection was 4-5 cycles of culture with 3-5 weeks for each cycle. Cali were placed onto nylon membranes to facilitate transfer (200 micron pore sheets, Biodesign, Saco, Maine). Membranes were cut to fit 100x20 mm Petri dishes and were autoclaved prior to use 25-35 calli (average weight/calli being 22mg) were utilized in every plate. In addition, one set of calli were subjected to selection in liquid culture media with weekly subcultures followed by further selection on semi-solid media.

Mutant lines were selected using saflufenacil, 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-

(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione flumioxazin, butafenacil, acifluorfen, lactofen, bifenox, sulfentrazone. Efficiencies of obtaining mutants was high either based on a percentage of calli that gave rise to a regenerable, mutant line or the number of lines as determined by the gram of tissue utilized.

EXAMPLE 9 Screening of mutagenized algae cells to identify herbicide tolerant clones and identification of causative mutations in PPO genes

To generate mutations in PPO genes conferring "PPO-inhibiting herbicides" resistance, screening with chemical or UV mutagenized cell populations can be used. Especially unicellular organisms like Chlamydomonas reinhardtii are useful for identifying dominant mutations conferring herbicide resistance (Kataoka M, et al.; 1990; J. of Pest. Sci. 15: 449-451; Oshio H, et al.; 1993; Zeitschrift für Naturforschung 48: 339-344).

Algae cells of Chlamydomonas reinhardtii strains CC-503 and CC-1691 (Duke University, Durham, USA) were propagated in TAP medium (Gorman and Levine; 1965; PNAS 54: 1665-1669) by constant shaking at 100 rpm, 22°C and 30 µmol Phot * m^{-2} * s^{-2} light illumination. Compound screening was performed at 450 µmol Phot * m^{-2} * s^{-2} illumination.

Sensitive strains of Chlamydomonas reinhardtii were mutagenized with 0.14 M ethylmethanesulfonate (EMS) for 1 h as described by Loppes (1969, Mol Gen Genet 104: 172-177). Tolerant strains are identified by screening of mutagenized cells on solid TAP medium plates containing "PPO-inhibiting herbicide" like saflufenacil or 1,5-dimethyl-6-thioxo-3-(2,2,7-trifluoro-3-oxo-4-(prop-2-ynyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)-1,3,5-triazinane-2,4-dione at wildype-lethal concentrations depending on compound activity in CC-503 or CC-1691 strain.

Standard techniques were used for isolation of RNA and cDNA synthesis as described by Sambrock et al. (Molecular cloning (2001) Cold Spring Harbor Laboratory Press). Amplification of PPO genes from wild-type and resistant Chlamydomonas reinhardii from genomic DNA or copy DNA as template were performed by standard PCR techniques with DNA oligonucleotides as listed in Table 5. The resulting DNA molecules were cloned in standard sequencing vector (pJET1) and sequenced by standard sequencing techniques. Mutations were identified by comparing wildtype and mutant PPO sequences by sequence alignment tool Align X (Vector NTI Advance Software Version 10.3, Invitrogen, Carlsbad, CA, USA).

Table 8: PCR primer for amplification of CrPPO

Primer name	Primer sequence (5' – 3')
Cr_PPO1_Fw	ATGATGTTGACCCAGACTCCTGGGAC
Cr_PPO1_Rv	TTAGGCCTTGACTGCGGCCTTGGAC

***

In some aspects, embodiments of the present invention as described herein include the following items:

Item 1. A method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:

a) planting or sowing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a mutated protoporphyrinogen oxidase (mut-PPO) which is resistant or tolerant to a "PPO inhibiting herbicide" and has oxidase activity
b) applying to said site an effective amount of said herbicide,

wherein the nucleotide sequence of a) comprises the sequence of SEQ ID NO: 1, or a variant thereof, having at least 90%, at least 91%, a least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99,5% sequence identity over the full length of the nucleotide sequence SEQ ID NO: 1, and

wherein the mut-PPO is a variant of SEQ ID NO: 2, which comprises the following:

a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Val,

or

b) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Met; and

c) the encoded polypeptide is resistant or tolerant to the "PPO inhibiting herbicide" and has oxidase activity.

Item 2. The method according to item 1, wherein the plant comprises at least one additional heterologous nucleic acid comprising a nucleotide sequence encoding an herbicide tolerance enzyme.

Item 3. The method according to item 1 or 2 wherein the PPO inhibiting herbicide is applied in conjunction with one or more additional herbicides.

Item 4. An expression cassette comprising an isolated nucleic acid encoding a mut-PPO polypeptide, wherein the nucleic acid has at least 90%, at least 91%, a least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99,5% sequence identity over the full length of the nucleotide sequence of SEQ ID NO: 1,
wherein the encoded mut-PPO is a variant of SEQ ID NO: 2, in which

a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Val,

or

b) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Met, and

wherein said mut-PPO polypeptide has oxidase activity and confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

Item 5. A mut-PPO polypeptide comprising a sequence which is at least 90%, at least 91%, a least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99,5% identical over the full length of SEQ ID NO: 2, in which

a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Val,

or

b) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Met, and

wherein said mut-PPO polypeptide has oxidase activity and confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

Item 6. A transgenic plant cell transformed by and comprising the expression cassette as defined in item 4.

Item 7. A seed cell produced by a transgenic plant comprising the plant cell as defined in item 6, and which comprises the expression cassette as defined in item 4.

Item 8. A method of producing a transgenic plant comprising, (a) transforming a plant cell with the expression cassette as defined in item 4, and (b) generating a plant with an increased resistance to PPO inhibiting herbicide from the plant cell compared to a wild-type plant.

Item 9. The method of item 8, wherein the expression cassette further comprises a transcription initiation regulatory region and a translation initiation regulatory region that are functional in the plant.

Claims:

1. A method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:
   a) planting or sowing, at said site, a plant that comprises at least one nucleic acid comprising a nucleotide sequence encoding a mutated protoporphyrinogen oxidase (mut-PPO) which is resistant or tolerant to a "PPO inhibiting herbicide" and has oxidase activity
   b) applying to said site an effective amount of said herbicide,
   wherein the nucleotide sequence of a) comprises the sequence of SEQ ID NO: 1, or a variant thereof, having at least 90%, at least 91%, a least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99,5% sequence identity over the full length of the nucleotide sequence SEQ ID NO: 1, and
   wherein the mut-PPO is a variant of SEQ ID NO: 2, which comprises the following:
      a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Val,
      or
      b) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Met; and
      c) the encoded polypeptide is resistant or tolerant to the "PPO inhibiting herbicide" and has oxidase activity.

2. The method according to claim 1, wherein the plant comprises at least one additional heterologous nucleic acid comprising a nucleotide sequence encoding an herbicide tolerance enzyme.

3. The method according to claim 1 or 2 wherein the PPO inhibiting herbicide is applied in conjunction with one or more additional herbicides.

4. An expression cassette comprising an isolated nucleic acid encoding a mut-PPO polypeptide, wherein the nucleic acid has at least 90%, at least 91%, a least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99,5% sequence identity over the full length of the nucleotide sequence of SEQ ID NO: 1,
   wherein the encoded mut-PPO is a variant of SEQ ID NO: 2, in which
      a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Val,
      or
      b) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Met, and
   wherein said mut-PPO polypeptide has oxidase activity and confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

5. A mut-PPO polypeptide comprising a sequence which is at least 90%, at least 91%, a least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99,5% identical over the full length of SEQ ID NO: 2, in which

a) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Val,

or

b) the amino acid corresponding to position 397 is Gly, Ala, Ser, Thr, Cys, Val, Ile, Met, Pro, Phe, Tyr, Trp, His, Lys, Arg, Asn, Asp, Glu, or Gln; and the amino acid corresponding to position 420 is Met, and

wherein said mut-PPO polypeptide has oxidase activity and confers increased resistance or tolerance to a PPO inhibiting herbicide in a plant as compared to a wild type plant.

6. A transgenic plant cell transformed by and comprising the expression cassette as defined in claim 4.

7. A seed cell produced by a transgenic plant comprising the plant cell as defined in claim 6, and which comprises the expression cassette as defined in claim 4.

8. A method of producing a transgenic plant comprising, (a) transforming a plant cell with the expression cassette as defined in claim 4, and (b) generating a plant with an increased resistance to PPO inhibiting herbicide from the plant cell compared to a wild-type plant.

9. The method of claim 8, wherein the expression cassette further comprises a transcription initiation regulatory region and a translation initiation regulatory region that are functional in the plant.

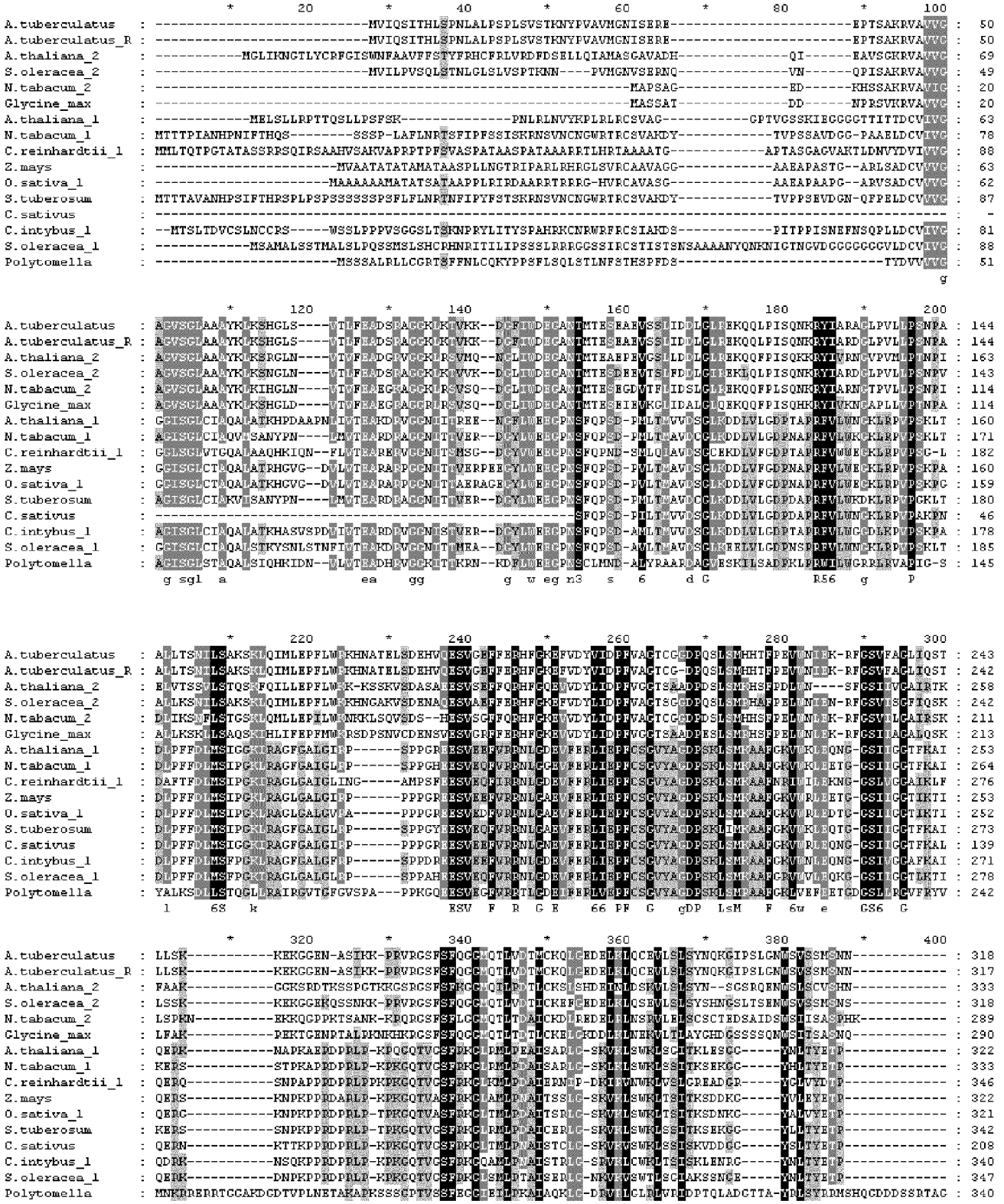


Figure 1

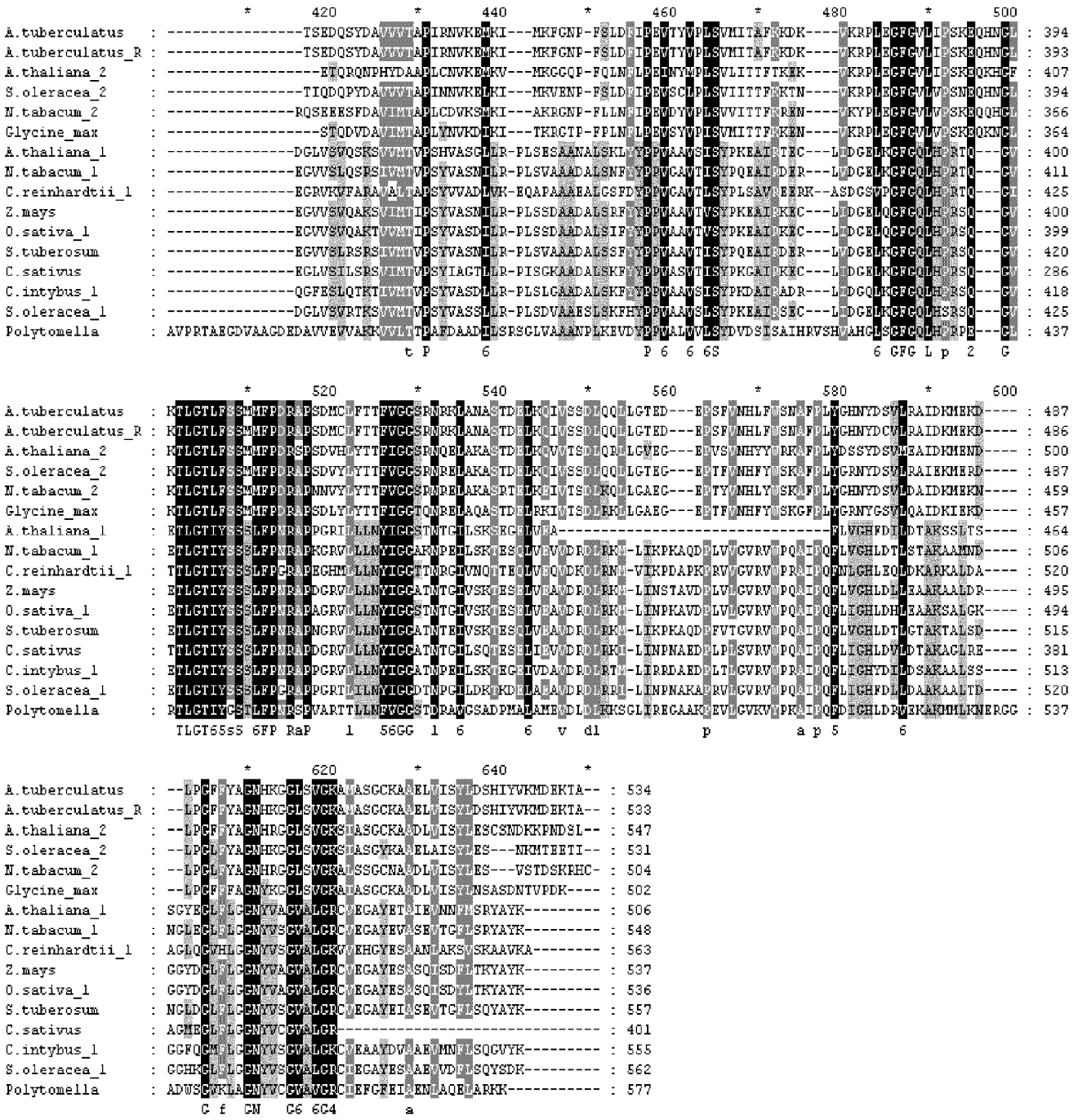
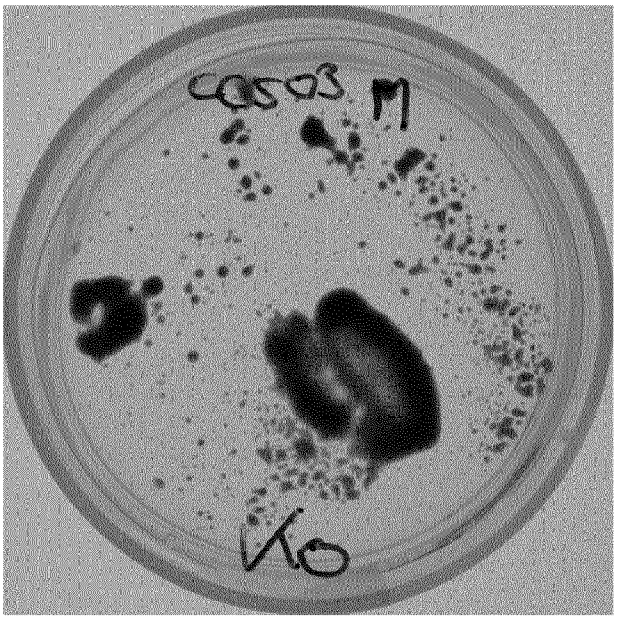


Figure 1 continued

A



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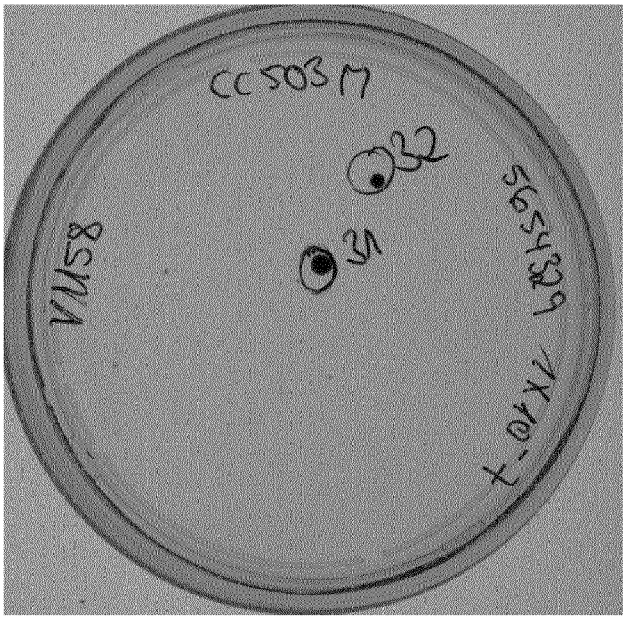
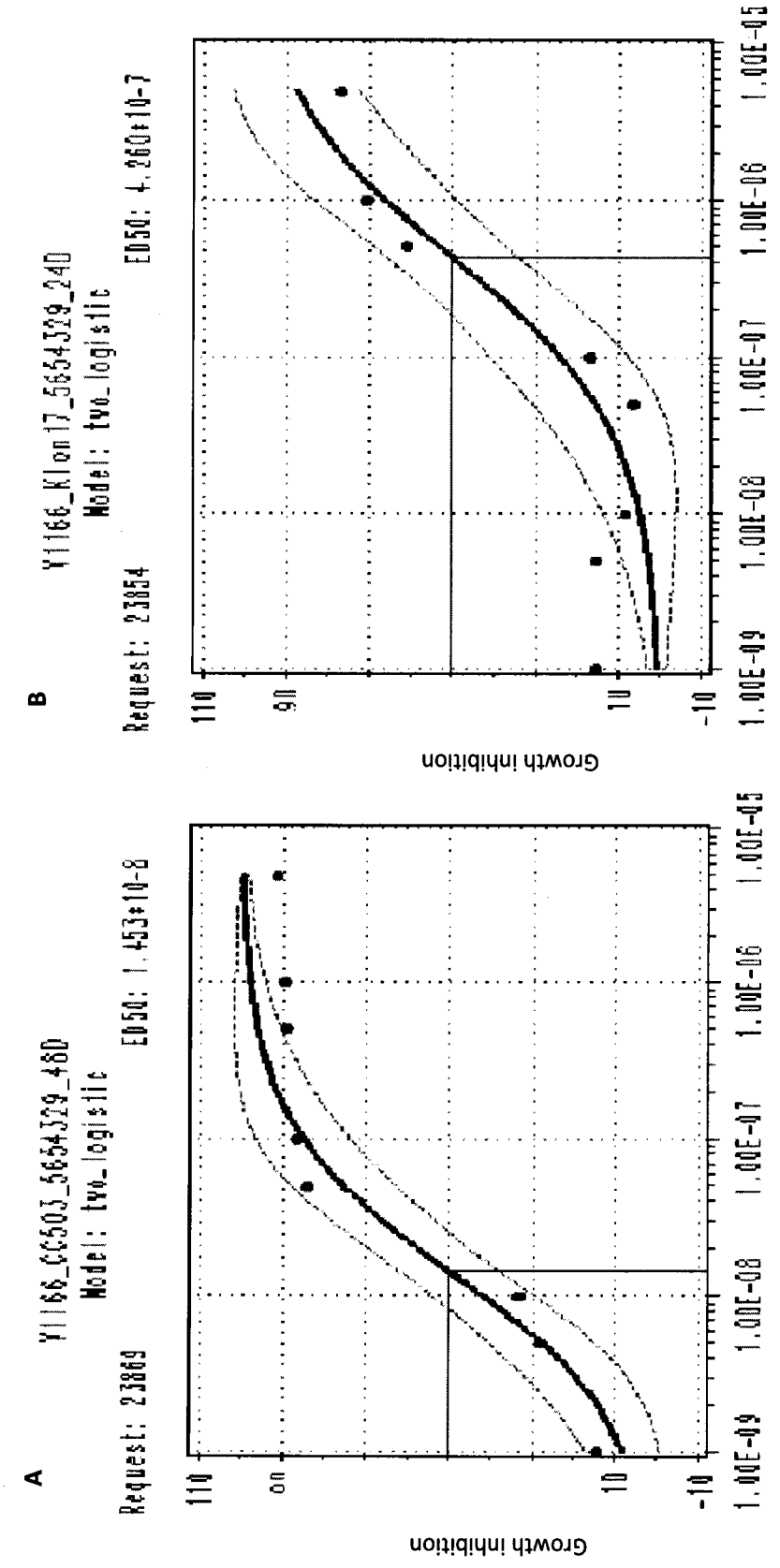


Figure 2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Growth Inhibition
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90
10
-10
1.00E-09
1.00E-08
1.00E-07
1.00E-06
1.00E-05
dose
B
Y1166_Klon17_5654329_24D
Model: two_logistic
Request: 23854
EC50: 4.260e10-7
Growth Inhibition
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90
10
-10
1.00E-09
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1.00E-07
1.00E-06
1.00E-05
dose

Figure 3

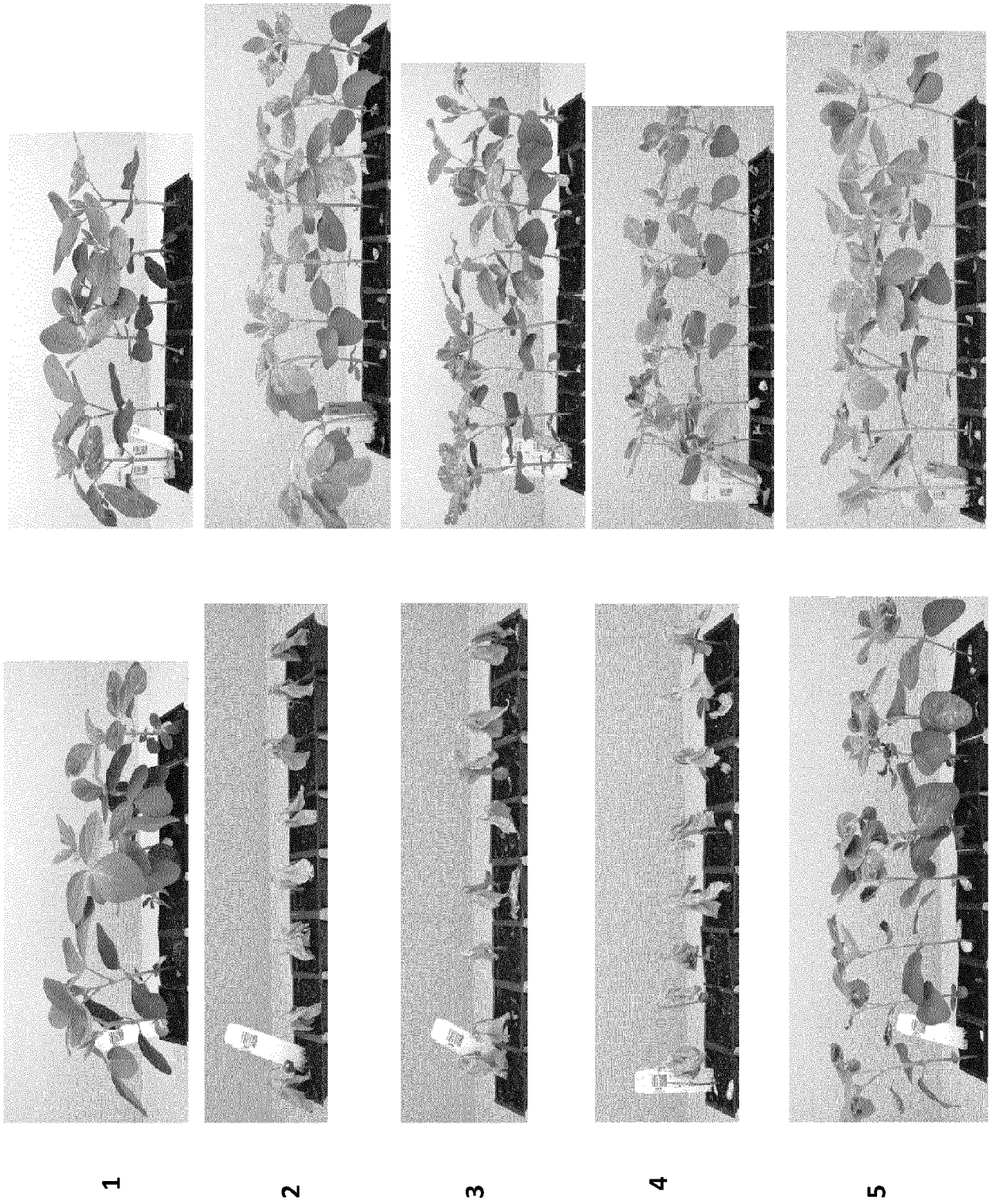
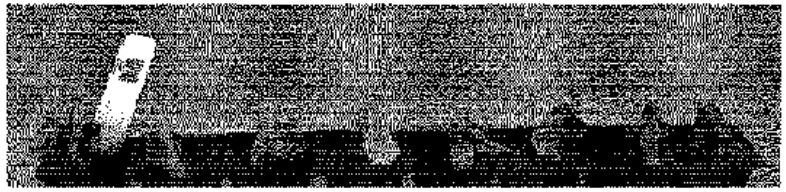
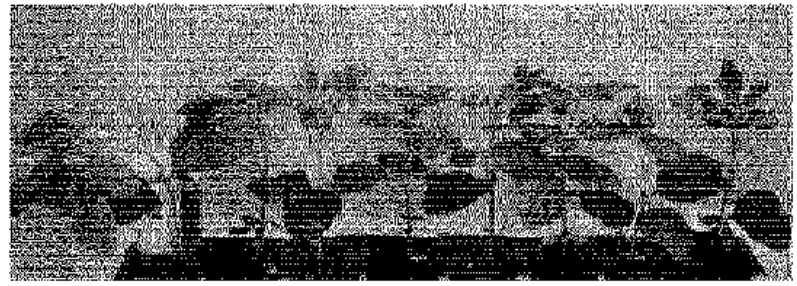
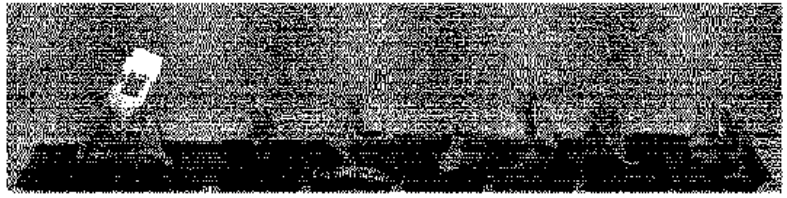


Figure 4

	A	B
1		
2		
3		
4		
5