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(54) Title: DNA MOLECULES ENCODING PLANT PROTOPORPHYRINOGEN OXIDASE AND INHIBITOR-RESISTANT MUTANTS THEREOF

(57) Abstract

The present invention provides novel DNA sequences coding for plant protoporphyrinogen oxidase (protox) enzymes from soybean, wheat, cotton, sugar beet, grape, rice and sorghum. In addition, the present invention teaches modified forms of protox enzymes that are herbicide tolerant. Plants expressing herbicide tolerant protox enzymes taught herein are also provided. These plants may be engineered for resistance to protox inhibitors via mutation of the native protox gene to a resistant form or they may be transformed with a gene encoding an inhibitor-resistant form of a plant protox enzyme.

DNA MOLECULES ENCODING PLANT PROTOPORPHYRINOGEN OXIDASE AND INHIBITOR-RESISTANT MUTANTS THEREOF

FIELD OF THE INVENTION

The invention relates generally to the plant enzyme protoporphyrinogen oxidase ("protox"). In particular, the invention relates to DNA molecules encoding this enzyme and to modified, inhibitor-resistant forms of this enzyme. The invention further relates to methods for tissue culture selection and herbicide application based on these modified forms.

BACKGROUND OF THE INVENTION

I. The Protox Enzyme and its Involvement in the Chlorophyll/Heme Biosynthetic Pathway

The biosynthetic pathways that lead to the production of chlorophyll and heme share a number of common steps. Chlorophyll is a light harvesting pigment present in all green photosynthetic organisms. Heme is a cofactor of hemoglobin, cytochromes, P450 mixed-function oxygenases, peroxidases, and catalyses (*see, e.g.* Lehninger, Biochemistry. Worth Publishers, New York (1975)), and is therefore a necessary component for all aerobic organisms.

The last common step in chlorophyll and heme biosynthesis is the oxidation of protoporphyrinogen IX to protoporphyrin IX. Protoporphyrinogen oxidase (referred to herein as "protox") is the enzyme that catalyzes this last oxidation step (Matringe *et al., Biochem. J. 260:* 231 (1989)).

The protox enzyme has been purified either partially or completely from a number of organisms including the yeast *Saccharomyces cerevisiae* (Labbe-Bois and Labbe, In Biosynthesis of Heme and Chlorophyll, E.H. Dailey, ed. McGraw Hill: New York, pp. 235-285 (1990)), barley etioplasts (Jacobs and Jacobs, *Biochem. J. 244:* 219 (1987)), and mouse liver (Dailey and Karr, *Biochem. 26*: 2697 (1987)). Genes encoding protox have been isolated from two prokaryotic organisms, *Escherichia coli* (Sasarman *et al., Can. J. Microbiol. 39:* 1155 (1993)) and *Bacillus subtilis* (Dailey *et al., J. Biol. Chem. 269:* 813 (1994)). These genes share no sequence similarity; neither do their predicted protein products share any amino acid sequence identity. The *E. coli* protein is approximately 21 kDa, and associates with the cell membrane. The *B. subtilis* protein is 51 kDa, and is a soluble, cytoplasmic activity.

Protox encoding genes have now also been isolated from humans (*see* Nishimura *et al., J. Biol. Chem. 270(14):* 8076-8080 (1995) and plants (International application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659).

II. The Protox Gene as a Herbicide Target

The use of herbicides to control undesirable vegetation such as weeds or plants in crops has become an almost universal practice. The relevant market exceeds a billion dollars annually. Despite this extensive use, weed control remains a significant and costly problem for farmers.

Effective use of herbicides requires sound management. For instance, time and method of application and stage of weed plant development are critical to getting good weed control with herbicides. Since various weed species are resistant to herbicides, the production of effective herbicides becomes increasingly important.

Unfortunately, herbicides that exhibit greater potency, broader weed spectrum and more rapid degradation in soil can also have greater crop phytotoxicity. One solution applied to this problem has been to develop crops that are resistant or tolerant to herbicides. Crop hybrids or varieties resistant to the herbicides allow for the use of the herbicides without attendant risk of damage to the crop. Development of resistance can allow application of a herbicide to a crop where its use was previously precluded or limited (*e.g.* to pre-emergence use) due to sensitivity of the crop to the herbicide. For example, U.S. Patent No. 4,761,373 to Anderson *et al.* is directed to plants resistant to various imidazolinone or sulfonamide herbicides. The resistance is conferred by an altered acetohydroxyacid synthase (AHAS) enzyme. U.S. Patent No. 4,975,374 to Goodman *et al.* relates to plant cells and plants containing a gene encoding a mutant glutamine synthetase (GS) resistant to inhibition by herbicides that were known to inhibit GS, *e.g.* phosphinothricin and methionine sulfoximine. U.S. Patent No. 5,013,659 to Bedbrook *et al.* is directed to plants that express a mutant acetolactate synthase that renders the plants resistant to inhibition by sulfonylurea herbicides. U.S. Patent No. 5,162,602 to Somers *et al.* discloses plants tolerant to inhibition by cyclohexanedione and aryloxyphenoxypropanoic acid herbicides. The tolerance is conferred by an altered acetyl coenzyme A carboxylase(ACCase).

The protox enzyme serves as the target for a variety of herbicidal compounds. The herbicides that inhibit protox include many different structural classes of molecules (Duke *et al., Weed Sci. 39*: 465 (1991); Nandihalli *et al., Pesticide Biochem. Physiol. 43:* 193 (1992); Matringe *et al., FEBS Lett. 245:* 35 (1989); Yanase and Andoh, *Pesticide Biochem. Physiol. 35*: 70 (1989)). These herbicidal compounds include the diphenylethers {e.g. acifluorfen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobezoic 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-(3*H*)-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.

Typically, the inhibitory effect on protox is determined by measuring fluorescence at about 622 to 635 nm, after excitation at about 395 to 410 nM (see, *e.g.* Jacobs and Jacobs, *Enzyme 28:* 206 (1982); Sherman *et al., Plant Physiol. 97:* 280 (1991)). This assay is based on the fact that protoporphyrin IX is a fluorescent pigment, and protoporphyrinogen IX is nonfluorescent.

The predicted mode of action of protox-inhibiting herbicides involves the accumulation of protoporphyrinogen IX in the chloroplast. This accumulation is thought to lead to leakage of protoporphyrinogen IX into the cytosol where it is oxidized by a peroxidase activity to protoporphyrin IX. When exposed to light, protoporphyrin IX can cause formation of singlet oxygen in the cytosol. This singlet oxygen can in turn lead to the formation of other reactive oxygen species, which can cause lipid peroxidation and membrane disruption leading to rapid cell death (Lee *et al., Plant Physiol. 102*: 881 (1993)).

Not all protox enzymes are sensitive to herbicides that inhibit plant protox enzymes. Both of the protox enzymes encoded by genes isolated from *Escherichia coli* (Sasarman *et* *al., Can. J. Microbiol. 39:* 1155 (1993)) and *Bacillus subtilis* (Dailey *et al., J. Biol. Chem. 269*: 813 (1994)) are resistant to these herbicidal inhibitors. In addition, mutants of the unicellular alga *Chlamydomonas reinhardtii* resistant to the phenylimide herbicide S-23142 have been reported (Kataoka *et al., J. Pesticide Sci. 15:* 449 (1990); Shibata *et al.*, In Research in Photosynthesis, Vol. III, N. Murata, ed. Kluwer:Netherlands. pp. 567-570 (1992)). At least one of these mutants appears to have an altered protox 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., Z. Naturforsch. 48c:* 339 (1993); Sato *et al.*, In ACS Symposium on Porphyric Pesticides, S. Duke, ed. ACS Press: Washington, D.C. (1994)). A mutant tobacco cell line has also been reported that is resistant to the inhibitor S-21432 (Che *et al.,. Z. Naturforsch. 48c:* 350 (1993).

SUMMARY OF THE INVENTION

The present invention provides isolated DNA molecules and chimeric genes encoding the protoporphyrinogen oxidase (protox) enzyme from wheat, soybean, cotton, sugar beet, rape, rice, and sorghum. The sequence of such isolated DNA molecules are set forth in SEQ ID NOs: 9 (wheat), 11 (soybean), 15 (cotton), 17 (sugar beet), 19 (rape), 21 (rice), and 23 (sorghum).

The present invention also provides modified forms of plant protoporphyrinogen oxidase (protox) enzymes that are resistant to compounds that inhibit unmodified naturally occurring plant protox enzymes, and DNA molecules coding for such inhibitor-resistant plant protox enzymes. The present invention includes chimeric genes and modified forms of naturally occurring protox genes that can express the inhibitor-resistant plant protox enzymes in plants.

Genes encoding inhibitor-resistant plant protox enzymes can be used to confer resistance to protox-inhibitory herbicides in whole plants and as a selectable marker in plant cell transformation methods. Accordingly, the present invention also includes plants, including the descendants thereof, plant tissues and plant seeds containing plant expressible genes encoding these modified protox enzymes. These plants, plant tissues and plant seeds are resistant to protox-inhibitors at levels that normally are inhibitory to the naturally occurring protox activity in the plant. Plants encompassed by the invention especially include those that would be potential targets for protox inhibiting herbicides, particularly agronomically important crops such as maize and other cereal crops such as barley, wheat, sorghum, rye, oats, turf and forage grasses, millet and rice. Also comprised are other crop plants such as sugar cane, soybean, cotton, sugar beet, oilseed rape and tobacco.

The present invention is directed further to methods for the production of plants, including plant material, such as for example plant tissues, protoplasts, cells, calli, organs, plant seeds, embryos, pollen, egg cells, zygotes, together with any other propagating material and plant parts, such as for example flowers, stems, fruits, leaves, roots originating in transgenic plants or their progeny previously transformed by means of the process of the invention, which produce an inhibitor-resistant form of the plant protox enzyme provided herein. Such plants may be stably transformed with a structural gene encoding the resistant protox, or prepared by direct selection techniques whereby herbicide resistant lines are isolated, characterized and developed. Furthermore, the present invention encompasses using plastid transformation technology to express protox genes within the plant chloroplast.

The present invention is further directed to probes and methods for detecting the presence of genes encoding inhibitor-resistant forms of the plant protox enzyme and quantitating levels of inhibitor-resistant protox transcripts in plant tissue. These methods may be used to identify or screen for plants or plant tissue containing and/or expressing a gene encoding an inhibitor-resistant form of the plant protox enzyme.

DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID NO:1:	DNA coding sequence for an *Arabidopsis thaliana* protox-1 protein.
SEQ ID NO:2:	*Arabidopsis* protox-1 amino acid sequence encoded by SEQ ID NO:1.
SEQ ID NO:3:	DNA coding sequence for an *Arabidopsis thaliana* protox-2 protein.
SEQ ID NO:4:	*Arabidopsis* protox-2 amino acid sequence encoded by SEQ ID NO:3.
SEQ ID NO:5:	DNA coding sequence for a maize protox-1 protein.
SEQ ID NO:6:	Maize protox-1 amino acid sequence encoded by SEQ ID NO:5.
SEQ ID NO:7:	DNA coding sequence for a maize protox-2 protein.
SEQ ID NO:8:	Maize protox-2 amino acid sequence encoded by SEQ ID NO:7.
SEQ ID NO:9:	DNA coding sequence for a wheat protox-1 protein.
SEQ ID NO:10:	Wheat protox-1 amino acid sequence encoded by SEQ ID NO:9.
SEQ ID NO:11:	DNA coding sequence for a soybean protox-1 protein.
SEQ ID NO:12:	Soybean protox-1 protein encoded by SEQ ID NO:11.
SEQ ID NO:13:	Promoter sequence from *Arabidopsis thaliana* protox-1 gene.
SEQ ID NO:14:	Promoter sequence from maize protox-1 gene.
SEQ ID NO:15:	DNA coding sequence for a cotton protox-1 protein.
SEQ ID NO:16:	Cotton protox-1 amino acid sequence encoded by SEQ ID NO:15.
SEQ ID NO:17:	DNA coding sequence for a sugar beet protox-1 protein.
SEQ ID NO:18:	Sugar beet protox-1 amino acid sequence encoded by SEQ ID NO:17.
SEQ ID NO:19:	DNA coding sequence for a rape protox-1 protein.
SEQ ID NO:20:	Rape protox-1 amino acid sequence encoded by SEQ ID NO:19.
SEQ ID NO:21:	DNA coding sequence for a rice protox-1 protein.
SEQ ID NO:22:	Rice protox-1 amino acid sequence encoded by SEQ ID NO:21.
SEQ ID NO:23:	DNA coding sequence for a sorghum protox-1 protein.
SEQ ID NO:24:	Sorghum protox-1 amino acid sequence encoded by SEQ ID NO:23.
SEQ ID NO:25:	Maize protox-1 intron sequence.
SEQ ID NO:26:	Promoter sequence from sugar beet protox-1 gene.
SEQ ID NO:27:	Pclp_P1a - plastid clpP gene promoter top strand PCR primer.
SEQ ID NO:28:	Pclp_P1b - plastid clpP gene promoter bottom strand PCR primer.
SEQ ID NO:29:	Pclp_P2b - plastid clpP gene promoter bottom strand PCR primer.
SEQ ID NO:30:	Trps16_P1a - plastid rps16 gene top strand PCR primer.
SEQ ID NO:31:	Trps16_p1b - plastid rps16 gene bottom strand PCR primer.
SEQ ID NO:32:	minpsb_U - plastid psbA gene top strand primer.
SEQ ID NO:33:	minpsb_L - plastid psbA gene bottom strand primer.

SEQ ID NO:34: APRTXP1a - top strand PCR primer.

SEQ ID NO:35: APRTXP1b - bottom strand PCR primer.

DEPOSITS

The following vector molecules have been deposited with Agricultural Research Service, Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A on the dates indicated below:

Wheat Protox-1a, in the pBluescript SK vector, was deposited March 19, 1996, as pWDC-13 (NRRL #B21545).

Soybean Protox-1, in the pBluescript SK vector, was deposited December 15, 1995 as pWDC-12 (NRRL #B-21516).

Cotton Protox-1, in the pBluescript SK vector, was deposited July 1, 1996 as pWDC-15 (NRRL #B-21594).

Sugar beet Protox-1, in the pBluescript SK vector, was deposited July 29, 1996, as pWDC-16 (NRRL #B-21595N).

Rape Protox-1, in the pBluescript SK vector, was deposited August 23, 1996, as pWDC-17 (NRRL #B-21615).

Rice Protox-1, in the pBluescript SK vector, was deposited December 6, 1996, as pWDC-18 (NRRL #B-21648).

Sorghum Protox-1, in the pBluescript SK vector, was deposited December 6, 1996, as pWDC-19 (NRRL #B-21649).

Resistant mutant pAraC-2Cys, in the pMut-1 plasmid, was deposited on November 14, 1994 under the designation pWDC-7 with the Agricultural Research Culture Collection and given the deposit designation NRRL #21339N.

AraPT1Pro containing the *Arabidopsis* Protox-1 promoter was deposited December 15, 1995, as pWDC-11 (NRRL #B-21515)

A plasmid containing the maize Protox-1 promoter fused to the remainder of the maize Protox-1 coding sequence was deposited March 19, 1996 as pWDC-14 (NRRL #B-21546).

A plasmid containing the Sugar Beet Protox-1 promoter was deposited December 6, 1996, as pWDC-20 (NRRL #B-21650).

DETAILED DESCRIPTION OF THE INVENTION

I. Plant Protox Coding Sequences

In one aspect, the present invention is directed to an isolated DNA molecule that encodes protoporphyrinogen oxidase (referred to herein as "protox"), the enzyme that catalyzes the oxidation of protoporphyrinogen IX to protoporphyrin IX, from wheat, soybean, cotton, sugar beet, rape, rice, and sorghum. The DNA coding sequence and corresponding amino acid sequence for a wheat protox enzyme are provided as SEQ ID NOs:9 and 10, respectively. The DNA coding sequence and corresponding amino acid sequence for a soybean protox enzyme are provided as SEQ ID NOs:11 and 12, respectively. The DNA coding sequence and corresponding amino acid sequence for a cotton protox enzyme are provided as SEQ ID NOs:15 and 16, respectively. The DNA coding sequence and corresponding amino acid sequence for a sugar beet protox enzyme are provided as SEQ ID NOs:17 and 18, respectively. The DNA coding sequence and corresponding amino acid sequence for a rape protox enzyme are provided as SEQ ID NOs:19 and 20, respectively. The DNA coding sequence and corresponding amino acid sequence for a rice protox enzyme are provided as SEQ ID NOs:21 and 22, respectively. The DNA coding sequence and corresponding amino acid sequence for a sorghum protox enzyme are provided as SEQ ID NOs:23 and 24, respectively.

The DNA coding sequences and corresponding amino acid sequences for protox enzymes from *Arabidopsis thaliana* and maize that have been previously isolated are reproduced herein as SEQ ID NOs:1-4 (*Arabidopsis*) and SEQ ID NOs:5-8 (maize).

The invention therefore primarily is directed to a DNA molecule encoding a protoporphyrinogen oxidase (protox) comprising a eukaryotic protox selected from the group consisting of a wheat protox enzyme, a soybean protox enzyme, a cotton protox enzyme, a sugar beet protox enzyme, a rape protox enzyme, a rice protox enzyme and a sorghum protox enzyme.

Preferred within the scope of the invention are isolated DNA molecules encoding the protoporphyrinogen oxidase (protox) enzyme from dicotyledonous plants, but especially from soybean plants, cotton plants, sugar beet plants and rape plants, such as those given in SEQ ID NOS: 11, 15, 17 and 19. More preferred are isolated DNA molecules encoding the protoporphyrinogen oxidase (protox) enzyme from soybean, such as given in SEQ ID NO:11, and sugar beet, such as given in SEQ ID NO:17.

Also preferred are isolated DNA molecules encoding the protoporphyrinogen oxidase (protox) enzyme from monocotyledonous plants, but especially from wheat plants, rice plants and sorghum plants, such as those given in SEQ ID NOS: 9, 21 and 23. More preferred are isolated DNA molecules encoding the protoporphyrinogen oxidase (protox) enzyme from wheat such as given in SEQ ID NO:9.

In another aspect, the present invention is directed to isolated DNA molecules encoding the protoporphyrinogen oxidase (protox) enzyme protein from a dicotyledonous plant, wherein said protein comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 16, 18 and 20. Further comprised are isolated DNA molecules encoding the protoporphyrinogen oxidase (protox) enzyme protein from a monocotyledonous plant, wherein said protein comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 10, 22 and 24. More preferred is an isolated DNA molecule encoding the protoporphyrinogen oxidase (protox) enzyme wherein said protein comprises the amino acid sequence from wheat such as given in SEQ ID NO:10. More preferred is an isolated DNA molecule encoding the protoporphyrinogen oxidase (protox) enzyme wherein said protein comprises the amino acid sequence from soybean, such as given in SEQ ID NO:12 and sugar beet, such as given in SEQ ID NO:18.

Using the information provided by the present invention, the DNA coding sequence for the protoporphyrinogen oxidase (protox) enzyme from any eukaryotic organism may be obtained using standard methods.

In another aspect, the present invention is directed to an isolated DNA molecule that encodes a wheat protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:9 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

In yet another aspect, the present invention is directed to an isolated DNA molecule that encodes a soybean protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:11 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

In still another aspect, the present invention is directed to an isolated DNA molecule that encodes a cotton protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:15 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

In another aspect, the present invention is directed to an isolated DNA molecule that encodes a sugar beet protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:17 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

In another aspect, the present invention is directed to an isolated DNA molecule that encodes a rape protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:19 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

In another aspect, the present invention is directed to an isolated DNA molecule that encodes a rice protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:21 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

In another aspect, the present invention is directed to an isolated DNA molecule that encodes a sorghum protox enzyme and that has a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:23 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

The isolated eukaryotic protox sequences taught by the present invention may be manipulated according to standard genetic engineering techniques to suit any desired purpose. For example, the entire protox sequence or portions thereof may be used as probes capable of specifically hybridizing to protox coding sequences and messenger RNA's. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among protox coding sequences and are preferably at least 10 nucleotides in length, and most preferably at least 20 nucleotides in length. Such probes may be used to amplify and analyze protox coding sequences from a chosen organism via the well known process of polymerase chain reaction (PCR). This technique may be useful to isolate additional protox coding sequences from a desired organism or as a diagnostic assay to determine the presence of protox coding sequences in an organism.

Factors that affect the stability of hybrids determine the stringency of the hybridization. One such factor is the melting temperature T_m, which can be easily calculated according to the formula provided in DNA PROBES, George H. Keller and Mark M. Manak , Macmillan Publishers Ltd, 1993, Section one: Molecular Hybridization Technology; page 8 ff. The preferred hybridization temperature is in the range of about 25°C below the calculated melting temperature T_m and preferably in the range of about 12-15°C below the calculated melting temperature T_m and in the case of oligonucleotides in the range of about 5-10°C below the melting temperature T_m.

Comprised by the present invention are DNA molecules that hybridize to a DNA molecule according to the invention as defined hereinbefore, but preferably to an oligonucleotide probe obtainable from said DNA molecule comprising a contiguous portion of the sequence of the said protoporphyrinogen oxidase (protox) enzyme at least 10 nucleotides in length, under moderately stringent conditions.

The invention further embodies the use of a nucleotide probe capable of specifically hybridizing to a plant protox gene or mRNA of at least 10 nucleotides length in a polymerase chain reaction (PCR).

In a further embodiment, the present invention provides probes capable of specifically hybridizing to a eukaryotic DNA sequence encoding a protoporphyrinogen oxidase activity or to the respective mRNA and methods for detecting the said DNA sequences in eukaryotic organisms using the probes according to the invention.

Protox specific hybridization probes may also be used to map the location of the native eukaryotic protox gene(s) in the genome of a chosen organism using standard techniques based on the selective hybridization of the probe to genomic protox sequences. These techniques include, but are not limited to, identification of DNA polymorphisms identified or contained within the protox probe sequence, and use of such polymorphisms to follow segregation of the protox gene relative to other markers of known map position in a mapping population derived from self fertilization of a hybrid of two polymorphic parental lines (see e.g. Helentjaris *et al.*, *Plant Mol. Biol. 5:* 109 (1985). Sommer *et al. Biotechniques 12:*82 (1992); D'Ovidio *et al.*, *Plant Mol. Biol. 15:* 169 (1990)). While any eukaryotic protox sequence is contemplated to be useful as a probe for mapping protox genes from any eukaryotic organism, preferred probes are those protox sequences from organisms more closely related to the chosen organism, and most preferred probes are those protox sequences from the chosen organism. Mapping of protox genes in this manner is contemplated to be particularly useful in plants for breeding purposes. For instance, by knowing the genetic map position of a mutant protox gene that confers herbicide resistance, flanking DNA markers can be identified from a reference genetic map (see, *e.g.*, Helentjaris, *Trends Genet. 3:* 217 (1987)). During introgression of the herbicide resistance trait into a new breeding line, these markers can then be used to monitor the extent of protox-linked flanking chromosomal DNA still present in the recurrent parent after each round of back-crossing.

Protox specific hybridization probes may also be used to quantitate levels of protox mRNA in an organism using standard techniques such as Northern blot analysis. This technique may be useful as a diagnostic assay to detect altered levels of protox expression that may be associated with particular adverse conditions such as autosomal dominant disorder in humans characterized by both neuropsychiatric symptoms and skin lesions, which are associated with decreased levels of protox activity (Brenner and Bloomer, *New Engl. J. Med. 302:* 765 (1980)).

A further embodiment of the invention is a method of producing a DNA molecule comprising a DNA portion encoding a protein having protoporphyrinogen oxidase (protox) enzyme activity comprising:

(a) preparing a nucleotide probe capable of specifically hybridizing to a plant protox gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for a protox protein from a plant of at least 10 nucleotides length;

(b) probing for other protox coding sequences in populations of cloned genomic DNA fragments or cDNA fragments from a chosen organism using the nucleotide probe prepared according to step (a); and

(c) isolating and multiplying a DNA molecule comprising a DNA portion encoding a protein having protoporphyrinogen oxidase (protox) enzyme activity.

A further embodiment of the invention is a method of isolating a DNA molecule from any plant comprising a DNA portion encoding a protein having protoporphyrinogen oxidase (protox) enzyme activity.

(a) preparing a nucleotide probe capable of specifically hybridizing to a plant protox gene or mRNA, wherein said probe comprises a contiguous portion of the coding sequence for a protox protein from a plant of at least 10 nucleotides length;

(b) probing for other protox coding sequences in populations of cloned genomic DNA fragments or cDNA fragments from a chosen organism using the nucleotide probe prepared according to step (a); and

(c) isolating a DNA molecule comprising a DNA portion encoding a protein having protoporphyrinogen oxidase (protox) enzyme activity.

The invention further comprises a method of producing an essentially pure DNA sequence coding for a protein exhibiting protoporphyrinogen oxidase (protox) enzyme activity, which method comprises:

(a) preparing a genomic or a cDNA library from a suitable source organism using an appropriate cloning vector;

(b) hybridizing the library with a probe molecule; and

(c) identifying positive hybridizations of the probe to the DNA clones from the library that is clones potentially containing the nucleotide sequence corresponding to the amino acid sequence for protoporphyrinogen oxidase (protox).

The invention further comprises a method of producing an essentially pure DNA sequence coding for a protein exhibiting protoporphyrinogen oxidase (protox) enzyme activity, which method comprises:

(a) preparing total DNA from a genomic or a cDNA library;

(b) using the DNA of step (a) as a template for PCR reaction with primers representing low degeneracy portions of the amino acid sequence of protoporphyrinogen oxidase (protox).

A further object of the invention is an assay to identify inhibitors of protoporphyrinogen oxidase (protox) enzyme activity that comprises:

(a) incubating a first sample of protoporphyrinogen oxidase (protox) and its substrate;

(b) measuring an uninhibited reactivity of the protoporphyrinogen oxidase (protox) from step (a);

(c) incubating a first sample of protoporphyrinogen oxidase (protox) and its substrate in the presence of a second sample comprising an inhibitor compound;

(d) measuring an inhibited reactivity of the protoporphyrinogen oxidase (protox) enzyme from step (c); and

(e) comparing the inhibited reactivity to the uninhibited reactivity of protoporphyrinogen oxidase (protox) enzyme.

A further object of the invention is an assay to identify inhibitor-resistant protoporphyrinogen oxidase (protox) mutants that comprises:

(a) incubating a first sample of protoporphyrinogen oxidase (protox) enzyme and its substrate in the presence of a second sample comprising a protoporphyrinogen oxidase (protox) enzyme inhibitor;

(b) measuring an unmutated reactivity of the protoporphyrinogen oxidase (protox) enzyme from step (a);

(c) incubating a first sample of a mutated protoporphyrinogen oxidase (protox) enzyme and its substrate in the presence of a second sample comprising protoporphyrinogen oxidase (protox) enzyme inhibitor;

(d) measuring a mutated reactivity of the mutated protoporphyrinogen oxidase (protox) enzyme from step (c); and

(e) comparing the mutated reactivity to the unmutated reactivity of the protoporphyrinogen oxidase (protox) enzyme.

A further object of the invention is a protox enzyme inhibitor obtained by a method according to the invention.

For recombinant production of the enzyme in a host organism, the protox coding sequence may be inserted into an expression cassette designed for the chosen host and introduced into the host where it is recombinantly produced. The choice of specific regulatory sequences such as promoter, signal sequence, 5' and 3' untranslated sequences, and enhancer, is within the level of skill of the routineer in the art. The resultant molecule, containing the individual elements linked in proper reading frame, may be inserted into a vector capable of being transformed into the host cell. Suitable expression vectors and methods for recombinant production of proteins are well known for host organisms such as *E. coli* (see, *e.g.* Studier and Moffatt, *J. Mol. Biol. 189:* 113 (1986); Brosius, *DNA 8:* 759 (1989)), yeast (see, *e.g.*, Schneider and Guarente, *Meth. Enzymol. 194*: 373 (1991)) and insect cells (see, *e.g.*, Luckow and Summers, *Bio/Technol. 6:* 47 (1988)). Specific examples include plasmids such as pBluescript (Stratagene, La Jolla, CA), pFLAG (International Biotechnologies, Inc., New Haven, CT), pTrcHis (Invitrogen, La Jolla, CA), and baculovirus expression vectors, e.g., those derived from the genome of *Autographica californica* nuclear polyhedrosis virus (AcMNPV). A preferred baculovirus/insect system is pVl11392/Sf21 cells (Invitrogen, La Jolla, CA).

Recombinantly produced eukaryotic protox enzyme is useful for a variety of purposes. For example, it may be used to supply protox enzymatic activity *in vitro*. It may also be used in an *in vitro* assay to screen known herbicidal chemicals whose target has not been identified to determine if they inhibit protox. Such an *in vitro* assay may also be used as a more general screen to identify chemicals that inhibit protox activity and that are therefore herbicide candidates. Recombinantly produced eukaryotic protox enzyme may also be used in an assay to identify inhibitor-resistant protox mutants (*see* International application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659, incorporated by reference herein in its entirety). Alternatively, recombinantly produced protox enzyme may be used to further characterize its association with known inhibitors in order to rationally design new inhibitory herbicides as well as herbicide tolerant forms of the enzyme.

II. Inhibitor Resistant Plant Protox Enzymes

In another aspect, the present invention teaches modifications that can be made to the amino acid sequence of any plant protoporphyrinogen oxidase (referred to herein as "protox") enzyme to yield an inhibitor-resistant form of this enzyme. The present invention is directed to inhibitor-resistant plant protox enzymes having the modifications taught herein, and to DNA molecules encoding these modified enzymes, and to genes capable of expressing these modified enzymes in plants.

The present invention is thus directed to an isolated DNA molecule encoding a modified protoporphyrinogen oxidase (protox) having at least one amino acid modification, wherein said amino acid modification having the property of conferring resistance to a protox inhibitor, that is wherein said modified protox is tolerant to a herbicide in amounts that inhibit said eukaryotic protox. As used herein 'inhibit' refers to a reduction in enzymatic activity observed in the presence of a subject herbicide compared to the level of activity observed in the absence of the subject herbicide, wherein the percent level of reduction is preferably at least 10%, more preferably at least 50%, and most preferably at least 90%.

Preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a eukaryotic protox selected from the group consisting of a wheat protox enzyme, a soybean protox enzyme, a cotton protox enzyme, a sugar beet protox enzyme, a rape protox enzyme, a rice protox enzyme and a sorghum protox enzyme having at least one amino acid modification, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the cysteine occurring at the position corresponding to amino acid 159 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is said DNA molecule wherein said cysteine is replaced with a phenylalanine or lysine, most preferred, wherein said cysteine is replaced with a phenylalanine.

Also preferred is a DNA encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the isoleucine occurring at the position corresponding to amino acid 419 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule, wherein said isoleucine is replaced with a threonine, histidine, glycine or asparagine most preferred, wherein said isoleucine is replaced with a threonine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the alanine occurring at the position corresponding to amino acid 164 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said alanine is replaced with a threonine, leucine or valine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the glycine occurring at the position corresponding to amino acid 165 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said glycine is replaced with a serine or leucine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 370 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said tyrosine is replaced with a isoleucine or methionine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the valine occurring at the position corresponding to amino acid 356 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said valine is replaced with a leucine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the serine occurring at the position corresponding to amino acid 421 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said serine is replaced with a proline.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the valine occurring at the position corresponding to amino acid 502 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said valine is replaced with a alanine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the alanine occurring at the position corresponding to amino acid 211 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said alanine is replaced with a valine or threonine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the glycine occurring at the position corresponding to amino acid 212 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said glycine is replaced with a serine.

Also preferred is a DNA encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the isoleucine occurring at the position corresponding to amino acid 466 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said isoleucine is replaced with a threonine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the proline occurring at the position corresponding to amino acid 369 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said proline is replaced with a serine or histidine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the alanine occurring at the position corresponding to amino acid 226 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule, wherein said alanine is replaced with a threonine or leucine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the valine occurring at the position corresponding to amino acid 517 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said valine is replaced with a alanine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 432 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said tyrosine is replaced with a leucine or isoleucine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the proline occurring at the position corresponding to amino acid 365 of SEQ ID NO:16 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said proline is replaced with a serine.

Also preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 428 of SEQ ID NO:16 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said tyrosine is replaced with a cysteine or arginine.

Also preferred is a DNA encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 449 of SEQ ID NO:18 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity. Particularly preferred is a DNA molecule wherein said tyrosine is replaced with a cysteine, leucine, isoleucine, valine or methionine.

The present invention is further directed to a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox having a first amino acid substitution and a second amino acid substitution; said first amino acid substitution having the property of conferring resistance to a protox inhibitor; and said second amino acid substitution having the property of enhancing said resistance conferred by said first amino acid substitution. Preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox, wherein said plant is selected from the group consisting of maize, wheat, soybean, cotton, sugar beet, rape, rice, sorghum and *Arabidopsis*. More preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox, wherein said plant is selected from the group consisting of maize, wheat, soybean, sugar beet, and *Arabidopsis*.

Preferred is a DNA molecule wherein said second amino acid substitution occurs at a position selected from the group consisting of:

(i) the position corresponding to the serine at amino acid 305 of SEQ ID NO:2;
(ii) the position corresponding to the threonine at amino acid 249 of SEQ ID NO:2;
(iii) the position corresponding to the proline at amino acid 118 of SEQ ID NO:2;

(iv) the position corresponding to the asparagine at amino acid 425 of SEQ ID NO:2; and

(v) the position corresponding to the tyrosine at amino acid 498 of SEQ ID NO:2.

Also preferred is a DNA molecule wherein said first amino acid substitution occurs at a position selected from the group consisting of:

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6;
(b) the position corresponding to the glycine at amino acid 165 of SEQ ID NO:6;
(c) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6;
(d) the position corresponding to the cysteine at amino acid 159 of SEQ ID NO:6;
(e) the position corresponding to the isoleucine at amino acid 419 of SEQ ID NO:6.
(f) the position corresponding to the valine at amino acid 356 of SEQ ID NO:10;
(g) the position corresponding to the serine at amino acid 421 of SEQ ID NO:10;
(h) the position corresponding to the valine at amino acid 502 of SEQ ID NO:10;
(i) the position corresponding to the alanine at amino acid 211 of SEQ ID NO:10;
(k) the position corresponding to the glycine at amino acid 212 of SEQ ID NO:10;
(l) the position corresponding to the isoleucine at amino acid 466 of SEQ ID NO:10;
(m) the position corresponding to the proline at amino acid 369 of SEQ ID NO:12;
(n) the position corresponding to the alanine at amino acid 226 of SEQ ID NO:12;
(o) the position corresponding to the tyrosine at amino acid 432 of SEQ ID NO:12;
(p) the position corresponding to the valine at amino acid 517 of SEQ ID NO:12;
(q) the position corresponding to the tyrosine at amino acid 428 of SEQ ID NO:16;
(r) the position corresponding to the proline at amino acid 365 of SEQ ID NO:16; and

(s) the position corresponding to the tyrosine at amino acid 449 of SEQ ID NO:18.

Particularly preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein said plant protox comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 16, 18, 20 and 22. Most preferred is a DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox, wherein said plant protox comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, and 18.

More preferred is a DNA molecule, wherein said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6;

(b) the position corresponding to the glycine at amino acid 165 of SEQ ID NO:6;

(c) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6;

(d) the position corresponding to the cysteine at amino acid 159 of SEQ ID NO:6;

(e) the position corresponding to the isoleucine at amino acid 419 of SEQ ID NO:6.

More preferred is a DNA molecule wherein said second amino acid substitution occurs at the position corresponding to the serine at amino acid 305 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6;

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

Particularly preferred is a DNA molecule wherein said serine occurring at the position corresponding to amino acid 305 of SEQ ID NO:2 is replaced with leucine.

More preferred is a DNA molecule wherein said second amino acid substitution occurs at the position corresponding to the threonine at amino acid 249 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

Particularly preferred is a DNA wherein said threonine occurring at the position corresponding to amino acid 249 of SEQ ID NO:2 is replaced with an amino acid selected from the group consisting of isoleucine and alanine.

More preferred is a DNA molecule wherein said second amino acid substitution occurs at the position corresponding to the proline at amino acid 118 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

Particularly preferred is a DNA molecule wherein said proline occurring at the position corresponding to amino acid 118 of SEQ ID NO:2 is replaced with a leucine.

More preferred is a DNA molecule wherein said second amino acid substitution occurs at the position corresponding to the asparagine at amino acid 425 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and
(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

Particularly preferred is a DNA molecule wherein said asparagine occurring at the position corresponding to amino acid 425 of SEQ ID NO:2 is replaced with a serine.

More preferred is a DNA molecule wherein said second amino acid substitution occurs the position corresponding to the tyrosine at amino acid 498 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and
(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

Particularly preferred is a DNA molecule wherein said tyrosine occurring at the position corresponding to amino acid 498 of SEQ ID NO:2 is replaced with a cysteine.

More preferred is a DNA molecule wherein said tyrosine occurring at the position corresponding to amino acid 370 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of cysteine, isoleucine, leucine, threonine, valine and methionine.

Particularly preferred is a DNA molecule wherein said tyrosine occurring at the position corresponding to amino acid 370 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of cysteine, isoleucine, leucine, threonine and methionine.

More preferred is a DNA molecule wherein said alanine occurring at the position corresponding to residue 164 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of valine, threonine, leucine, cysteine and tyrosine.

More preferred is a DNA molecule wherein said glycine occurring at the position corresponding to residue 165 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of serine and leucine.

Particularly preferred is a DNA molecule wherein said glycine occurring at the position corresponding to residue 165 of SEQ ID NO:6 is replaced with a serine.

More preferred is a DNA molecule wherein said cysteine occurring at the position corresponding to residue 159 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of phenylalanine and lysine.

Particularly preferred is a DNA molecule wherein said cysteine occurring at the position corresponding to residue 159 of SEQ ID NO:6 is replaced with a phenylalanine.

More preferred is a DNA molecule wherein said isoleucine occurring at the position corresponding to residue 419 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of threonine, histidine, glycine and asparagine.

Particularly preferred is a DNA molecule wherein said isoleucine occurring at the position corresponding to residue 419 of SEQ ID NO:6 is replaced with a threonine.

More preferred is a DNA molecule wherein said valine occurring at the position corresponding to residue 356 of SEQ ID NO:10 is replaced with a leucine.

More preferred is a DNA molecule wherein said serine occurring at the position corresponding to residue 421 of SEQ ID NO:10 is replaced with a proline.

More preferred is a DNA molecule wherein said valine occurring at the position corresponding to residue 502 of SEQ ID NO:10 is replaced with a alanine.

More preferred is a DNA molecule wherein said isoleucine occurring at the position corresponding to residue 466 of SEQ ID NO:10 is replaced with a threonine.

More preferred is a DNA molecule wherein said glycine occurring at the position corresponding to residue 212 of SEQ ID NO:10 is replaced with a serine.

More preferred is a DNA molecule wherein said alanine occurring at the position corresponding to residue 211 of SEQ ID NO:10 is replaced with a valine or threonine.

More preferred is a DNA molecule wherein said proline occurring at the position corresponding to residue 369 of SEQ ID NO:12 is replaced with a serine or a histidine.

More preferred is a DNA molecule wherein said alanine occurring at the position corresponding to residue 226 of SEQ ID NO:12 is replaced with a leucine or threonine.

More preferred is a DNA molecule wherein said tyrosine occurring at the position corresponding to residue 432 of SEQ ID NO:12 is replaced with a leucine or isoleucine.

More preferred is a DNA molecule wherein said valine occurring at the position corresponding to residue 517 of SEQ ID NO:12 is replaced with a alanine.

More preferred is a DNA molecule wherein said tyrosine occurring at the position corresponding to residue 428 of SEQ ID NO:16 is replaced with cysteine or arginine.

More preferred is a DNA molecule wherein said proline occurring at the position corresponding to residue 365 of SEQ ID NO:16 is replaced with serine.

More preferred is a DNA molecule wherein said proline occurring at the position corresponding to residue 449 of SEQ ID NO:18 is replaced with an amino acid selected from the group consisting of leucine, isoleucine, valine and methionine.

The present invention is directed to expression cassettes and recombinant vectors comprising said expression cassettes comprising essentially a promoter, but especially a promoter that is active in a plant, operably linked to a DNA molecule encoding the protoporphyrinogen oxidase (protox) enzyme from a eukaryotic organism according to the invention. The expression cassette according to the invention may in addition further comprise a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the chloroplast or the mitochondria.

The invention relates to a chimeric gene, which comprises an expression cassette comprising essentially a promoter, but especially a promoter that is active in a plant, operably linked to a heterologous DNA molecule encoding a protoporphyrinogen oxidase (protox) enzyme from a eukaryotic organism according to the invention. Preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of *Arabidopsis*, sugar cane, soybean, barley, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf and forage grasses, millet, forage and rice. More preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of soybean, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf grass, and rice. Particularly preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of wheat, soybean, cotton, sugar beet, rape, rice and sorghum. Most preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of soybean, sugar beet, and wheat.

More preferred is a chimeric gene comprising a promoter active in a plant operably linked to a heterologous DNA molecule encoding a protoporphyrinogen oxidase (protox) selected from the group consisting of a wheat protox comprising the sequence set forth in SEQ ID NO:10, a soybean protox comprising the sequence set forth in SEQ ID NO:12, cotton protox comprising the sequence set forth in SEQ ID NO:16, a sugar beet protox comprising the sequence set forth in SEQ ID NO:18, a rape protox comprising the sequence set forth in SEQ ID NO:20, a rice protox comprising the sequence set forth in SEQ ID NO:22 and a sorghum protox comprising the sequence set forth in SEQ ID NO:24. More preferred is a chimeric gene, wherein the protoporphyrinogen oxidase (protox) is selected from the group consisting of a wheat protox comprising the sequence set forth in SEQ ID NO:10, a soybean protox comprising the sequence set forth in SEQ ID NO:12, and a sugar beet protox comprising the sequence set forth in SEQ ID NO:18.

As used herein 'protox-1' refers to a chloroplast protox whereas 'protox-2' refers to a mitochondrial protox.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from an *Arabidopsis* species having protox-1 activity or protox-2 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from maize having protox-1 activity or protox-2 activity, preferably wherein said protein comprises the amino acid sequence set forth in set forth in SEQ ID NO:6 or SEQ ID NO:8.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from wheat having protox-1 activity preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:10.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from soybean having protox-1 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:12.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from cotton having protox-1 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:16.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from sugar beet having protox-1 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:18.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from rape having protox-1 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:20.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from rice having protox-1 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:22.

Particularly preferred is a chimeric gene, wherein the DNA molecule encodes a protein from sorghum having protox-1 activity, preferably wherein said protein comprises the amino acid sequence set forth in SEQ ID NO:24.

The invention also embodies a chimeric gene, which comprises an expression cassette comprising essentially a promoter, but especially a promoter that is active in a plant, operably linked to the DNA molecule encoding an protoporphyrinogen oxidase (protox) enzyme from a eukaryotic organism according to the invention, which is resistant to herbicides at levels that inhibit the corresponding unmodified version of the enzyme. Preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of *Arabidopsis*, sugar cane, soybean, barley, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf and forage grasses, millet, forage and rice. More preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of soybean, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf grass, and rice. Particularly preferred is a chimeric gene, wherein the DNA molecule encodes an protoporphyrinogen oxidase (protox) enzyme from a plant selected from the group consisting of *Arabidopsis*, soybean, cotton, sugar beet, oilseed rape, maize, wheat, sorghum, and rice.

Encompassed by the present invention is a chimeric gene comprising a promoter that is active in a plant operably linked to the DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a eukaryotic protox having at least one amino acid modification, wherein said amino acid modification having the property of conferring resistance to a protox inhibitor.

Also encompassed by the present invention is a chimeric gene comprising a promoter that is active in a plant operably linked to the DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox having a first amino acid substitution and a second amino acid substitution; said first amino acid substitution having the property of conferring resistance to a protox inhibitor; and said second amino acid substitution having the property of enhancing said resistance conferred by said first amino acid substitution. Preferred is said chimeric gene additionally comprising a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the chloroplast or in the mitochondria.

The chimeric gene according to the invention may in addition further comprise a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the chloroplast. The chimeric gene according to the invention may in addition further comprise a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the mitochondria.

Also encompassed by the present invention is any of the DNA sequences mentioned herein before, which is stably integrated into a host genome.

The invention further relates to a recombinant DNA molecule comprising a plant protoporphyrinogen oxidase (protox) or a functionally equivalent derivative thereof.

The invention further relates to a recombinant DNA vector comprising said recombinant DNA molecule.

A further object of the invention is a recombinant vector comprising the chimeric gene according to the invention, wherein said vector is capable of being stably transformed into a host cell.

A further object of the invention is a recombinant vector comprising the chimeric gene according to the invention, wherein said vector is capable of being stably transformed into a plant, plant seeds, plant tissue or plant cell. Preferred is a recombinant vector comprising the chimeric gene according to the invention, wherein said vector is capable of being stably transformed into a plant. The plant, plant seeds, plant tissue or plant cell stably transformed with the vector is capable of expressing the DNA molecule encoding a protoporphyrinogen oxidase (protox). Preferred is a recombinant vector, wherein the plant, plant seeds, plant tissue or plant cell stably transformed with the said vector is capable of expressing the DNA molecule encoding a protoporphyrinogen oxidase (protox) from a plant that is resistant to herbicides at levels that inhibit the corresponding unmodified version of the enzyme.

Preferred is a recombinant vector comprising the chimeric gene comprising a promoter active in a plant operably linked to a heterologous DNA molecule encoding a protoporphyrinogen oxidase (protox) selected from the group consisting of a wheat protox comprising the sequence set forth in SEQ ID NO:10, a soybean protox comprising the sequence set forth in SEQ ID NO:12, cotton protox comprising the sequence set forth in SEQ ID NO:16, a sugar beet protox comprising the sequence set forth in SEQ ID NO:18, a rape protox comprising the sequence set forth in SEQ ID NO:20, a rice protox comprising the sequence set forth in SEQ ID NO:22 and a sorghum protox comprising the sequence set forth in SEQ ID NO:24, wherein said vector is capable of being stably transformed into a host cell.

Also preferred is recombinant vector comprising the chimeric gene comprising a promoter that is active in a plant operably linked to the DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox having a first amino acid substitution and a second amino acid substitution; said first amino acid substitution having the property of conferring resistance to a protox inhibitor; and said second amino acid substitution having the property of enhancing said resistance conferred by said first amino acid substitution, wherein said vector is capable of being stably transformed into a plant cell.

Also encompassed by the present invention is a host cell stably transformed with the vector according to the invention, wherein said host cell is capable of expressing said DNA molecule. Preferred is a host cell selected from the group consisting of a plant cell, a bacterial cell, a yeast cell, and an insect cell.

The present invention is further directed to plants and the progeny thereof, plant tissue and plant seeds tolerant to herbicides that inhibit the naturally occurring protox activity in these plants, wherein the tolerance is conferred by a gene expressing a modified inhibitor-resistant protox enzyme as taught herein. Representative plants include any plants to which these herbicides may be applied for their normally intended purpose. Preferred are agronomically important crops, i.e., angiosperms and gymnosperms such as *Arabidopsis*, sugar cane, soybean, barley, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf and forage grasses , millet, forage and rice and the like. More preferred are agronomically important crops, i.e., angiosperms and gymnosperms such as *Arabidopsis*, cotton, soybean, rape, sugar beet, maize, rice, wheat, barley, oats, rye, sorghum, millet, turf, forage, turf grasses. Particularly preferred are agronomically important crops, i.e., angiosperms and gymnosperms such as *Arabidopsis*, soybean, cotton, sugar beet, oilseed rape, maize, wheat, sorghum, and rice.

Preferred is a plant comprising the DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox having a first amino acid substitution and a second amino acid substitution; said first amino acid substitution having the property of conferring resistance to a protox inhibitor; and said second amino acid substitution having the property of enhancing said resistance conferred by said first amino acid substitution, wherein said DNA molecule is expressed in said plant and confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity. Preferred is a plant, wherein said DNA molecule replaces a corresponding naturally occurring protox coding sequence. Comprised by the present invention is a plant and the progeny thereof comprising the chimeric gene according to the invention, wherein said chimeric gene confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

Encompassed by the present invention are transgenic plant tissue, including plants and the progeny thereof, seeds, and cultured tissue, stably transformed with at least one chimeric gene according to the invention. Preferred is transgenic plant tissue, including plants, seeds, and cultured tissue, stably transformed with at least one chimeric gene that comprises an expression cassette comprising essentially a promoter, but especially a promoter that is active in a plant, operably linked to the DNA molecule encoding an protoporphyrinogen oxidase (protox) enzyme that is resistant to herbicides at levels that inhibit the corresponding unmodified version of the enzyme in the plant tissue.

The recombinant DNA molecules of the invention can be introduced into the plant cell in a number of art-recognized ways. Those skilled in the art will appreciate that the choice of method might depend on the type of plant, i.e. monocot or dicot, targeted for transformation. Suitable methods of transforming plant cells include microinjection (Crossway *et al.*, *BioTechniques 4*:320-334 (1986)), electroporation (Riggs *et al*, *Proc. Natl. Acad. Sci. USA 83*:5602-5606 (1986), *Agrobacterium* mediated transformation (Hinchee *et al.*, *Biotechnology 6*:915-921 (1988)), direct gene transfer (Paszkowski *et al.*, *EMBO J. 3*:2717-2722 (1984)), ballistic particle acceleration using devices available from Agracetus, Inc., Madison, Wisconsin and Dupont, Inc., Wilmington, Delaware (*see, for example,* Sanford *et al.*, U.S. Patent 4,945,050; and McCabe *et al., Biotechnology 6*:923-926 (1988)), and protoplast transformation/regeneration methods (*see* U.S. Patent No. 5,350,689 issued Sept. 27, 1994 to Ciba-Geigy Corp.). Also see, Weissinger *et al., Annual Rev. Genet. 22*:421-477 (1988); Sanford *et al., Particulate Science and Technology 5*:27-37 (1987)(onion); Christou *et al., Plant Physiol. 87*:671-674 (1988)(soybean); McCabe *et al., Bio/Technology 6*:923-926 (1988)(soybean); Datta *et al., Bio/Technology 8*:736-740 (1990)(rice); Klein *et al., Proc. Natl. Acad. Sci. USA, 85*:4305-4309 (1988)(maize); Klein *et al., Bio/Technology 6*:559-563 (1988)(maize); Klein *et al., Plant Physiol. 91*:440-444 (1988)(maize); Fromm *et al.,* *Bio/Technology 8*:833-839 (1990); and Gordon-Kamm *et al., Plant Cell 2*:603-618 (1990) (maize).

Comprised within the scope of the present invention are transgenic plants, in particular transgenic fertile plants transformed by means of the aforedescribed processes and their asexual and/or sexual progeny, which still are resistant or at least tolerant to inhibition by a herbicide at levels that normally are inhibitory to the naturally occurring protox activity in the plant. Progeny plants also include plants with a different genetic background than the parent plant, which plants result from a backcrossing program and still comprise in their genome the herbicide resistance trait according to the invention. Very especially preferred are hybrid plants that are resistant or at least tolerant to inhibition by a herbicide at levels that normally are inhibitory to the naturally occurring protox activity in the plant.

The transgenic plant according to the invention may be a dicotyledonous or a monocotyledonous plant. Preferred are monocotyledonous plants of the *Graminaceae* family involving *Lolium, Zea, Triticum, Triticale, Sorghum, Saccharum, Bromus, Oryzae, Avena, Hordeum, Secale* and *Setaria* plants. More preferred are transgenic maize, wheat, barley, sorghum, rye, oats, turf and forage grasses, millet and rice. Especially preferred are maize, wheat, sorghum, rye, oats, turf grasses and rice.

Among the dicotyledonous plants Arabidopsis, soybean, cotton, sugar beet, sugar cane, oilseed rape, tobacco and sunflower are more preferred herein. Especially preferred are soybean, cotton, tobacco, sugar beet and oilseed rape.

The expression 'progeny' is understood to embrace both, "asexually" and "sexually" generated progeny of transgenic plants. This definition is also meant to include all mutants and variants obtainable by means of known processes, such as for example cell fusion or mutant selection and that still exhibit the characteristic properties of the initial transformed plant, together with all crossing and fusion products of the transformed plant material. This also includes progeny plants that result from a backcrossing program, as long as the said progeny plants still contain the herbicide resistant trait according to the invention.

Another object of the invention concerns the proliferation material of transgenic plants. The proliferation material of transgenic plants is defined relative to the invention as any plant material that may be propagated sexually or asexually *in vivo* or *in vitro*.

Particularly preferred within the scope of the present invention are protoplasts, cells, calli, tissues, organs, seeds, embryos, pollen, egg cells, zygotes, together with any other propagating material obtained from transgenic plants.

Parts of plants, such as for example flowers, stems, fruits, leaves, roots originating in transgenic plants or their progeny previously transformed by means of the process of the invention and therefore consisting at least in part of transgenic cells, are also an object of the present invention.

A further object of the invention is a method of producing plants, protoplasts, cells, calli, tissues, organs, seeds, embryos, pollen, egg cells, zygotes, together with any other propagating material, parts of plants, such as for example flowers, stems, fruits, leaves, roots originating in transgenic plants or their progeny previously transformed by means of the process of the invention, which therefore produce an inhibitor resistant form of a plant protox enzyme by transforming the plant, plant parts with the DNA according to the invention. Preferred is a method of producing a host cell comprising an isolated DNA molecule encoding a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity comprising transforming the said host cell with a recombinant vector molecule according to the invention. Further preferred is a method of producing a plant cell comprising an isolated DNA molecule encoding a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity comprising transforming the said plant cell with a recombinant vector molecule according to the invention. Preferred is a method of producing transgenic progeny of a transgenic parent plant comprising an isolated DNA molecule encoding a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity comprising transforming the said parent plant with a recombinant vector molecule according to the invention and transferring the herbicide tolerant trait to the progeny of the said transgenic parent plant involving known plant breeding techniques.

Preferred is a method for the production of plants, plant tissues, plant seeds and plant parts, which produce an inhibitor-resistant form of the plant protox enzyme, wherein the plants, plant tissues, plant seeds and plant parts have been stably transformed with a structural gene encoding the resistant protox enzyme. Particularly preferred is a method for the production of plants, plant tissues, plant seeds and plant parts, wherein the plants, plant tissues, plant seeds and plant parts have been stably transformed with the DNA according to the invention. Especially preferred is a method for the production of said plants, plant tissues, plant seeds and plant parts, which produce an inhibitor-resistant form of the plant protox enzyme, wherein the plants, plant tissues, plant seeds and plant parts have been prepared by direct selection techniques whereby herbicide resistant lines are isolated, characterized and developed.

The genetic properties engineered into the transgenic seeds and plants described above are passed on by sexual reproduction or vegetative growth and can thus be maintained and propagated in progeny plants. Generally said maintenance and propagation make use of known agricultural methods developed to fit specific purposes such as tilling, sowing or harvesting. Specialized processes such as hydroponics or greenhouse technologies can also be applied. As the growing crop is vulnerable to attack and damages caused by insects or infections as well as to competition by weed plants, measures are undertaken to control weeds, plant diseases, insects, nematodes, and other adverse conditions to improve yield. These include mechanical measures such a tillage of the soil or removal of weeds and infected plants, as well as the application of agrochemicals such as herbicides, fungicides, gametocides, nematicides, growth regulants, ripening agents and insecticides.

Use of the advantageous genetic properties of the transgenic plants and seeds according to the invention can further be made in plant breeding that aims at the development of plants with improved properties such as tolerance of pests, herbicide tolerance, or stress tolerance, improved nutritional value, increased yield, or improved structure causing less loss from lodging or shattering. The various breeding steps are characterized by well-defined human intervention such as selecting the lines to be crossed, directing pollination of the parental lines, or selecting appropriate progeny plants. Depending on the desired properties different breeding measures are taken. The relevant techniques are well known in the art and include but are not limited to hybridization, inbreeding, backcross breeding, multiline breeding, variety blend, interspecific hybridization, aneuploid techniques, etc. Hybridization techniques also include the sterilization of plants to yield male or female sterile plants by mechanical, chemical or biochemical means. Cross pollination of a male sterile plant with pollen of a different line assures that the genome of the male sterile but female fertile plant will uniformly obtain properties of both parental lines. Thus, the transgenic seeds and plants according to the invention can be used for the breeding of improved plant lines that for example increase the effectiveness of conventional methods such as herbicide or pesticide treatment or allow to dispense with said methods due to their modified genetic properties. Alternatively new crops with improved stress tolerance can be obtained that, due to their optimized genetic "equipment", yield harvested product of better quality than products that were not able to tolerate comparable adverse developmental conditions.

In seeds production germination quality and uniformity of seeds are essential product characteristics, whereas germination quality and uniformity of seeds harvested and sold by the farmer is not important. As it is difficult to keep a crop free from other crop and weed seeds, to control seedborne diseases, and to produce seed with good germination, fairly extensive and well-defined seed production practices have been developed by seed producers, who are experienced in the art of growing, conditioning and marketing of pure seed. Thus, it is common practice for the farmer to buy certified seed meeting specific quality standards instead of using seed harvested from his own crop. Propagation material to be used as seeds is customarily treated with a protectant coating comprising herbicides, insecticides, fungicides, bactericides, nematicides, molluscicides or mixtures thereof. Customarily used protectant coatings comprise compounds such as captan, carboxin, thiram (TMTD®), methalaxyl (Apron®), and pirimiphos-methyl (Actellic®). If desired these compounds are formulated together with further carriers, surfactants or application-promoting adjuvants customarily employed in the art of formulation to provide protection against damage caused by bacterial, fungal or animal pests. The protectant coatings may be applied by impregnating propagation material with a liquid formulation or by coating with a combined wet or dry formulation. Other methods of application are also possible such as treatment directed at the buds or the fruit.

It is thus a further object of the present invention to provide plant propagation material for cultivated plants, but especially plant seed that is treated with an seed protectant coating customarily used in seed treatment.

It is a further aspect of the present invention to provide new agricultural methods such as the methods exemplified above, which are characterized by the use of transgenic plants, transgenic plant material, or transgenic seed according to the present invention. Comprised by the present invention is an agricultural method, wherein a transgenic plant or the progeny thereof is used comprising a chimeric gene according to the invention in an amount sufficient to express herbicide resistant forms of herbicide target proteins in a plant to confer tolerance to the herbicide.

To breed progeny from plants transformed according to the method of the present invention, a method such as that which follows may be used: maize plants produced as described in the examples set forth below are grown in pots in a greenhouse or in soil, as is known in the art, and permitted to flower. Pollen is obtained from the mature tassel and used to pollinate the ears of the same plant, sibling plants, or any desirable maize plant. Similarly, the ear developing on the transformed plant may be pollinated by pollen obtained from the same plant, sibling plants, or any desirable maize plant. Transformed progeny obtained by this method may be distinguished from non-transformed progeny by the presence of the introduced gene(s) and/or accompanying DNA (genotype), or the phenotype conferred. The transformed progeny may similarly be selfed or crossed to other plants, as is normally done with any plant carrying a desirable trait. Similarly, tobacco or other transformed plants produced by this method may be selfed or crossed as is known in the art in order to produce progeny with desired characteristics. Similarly, other transgenic organisms produced by a combination of the methods known in the art and this invention may be bred as is known in the art in order to produce progeny with desired characteristics.

The modified inhibitor-resistant protox enzymes of the invention have at least one amino acid substitution, addition or deletion relative to their naturally occurring counterpart (i.e. inhibitor-sensitive forms that occur naturally in a plant without being manipulated, either directly *via* recombinant DNA methodology or indirectly *via* selective breeding, etc., by man). Amino acid positions that may be modified to yield an inhibitor-resistant form of the protox enzyme, or enhance inhibitor resistance, are indicated in bold type in Table 1 in the context of plant protox-1 sequences from *Arabidopsis,* maize, soybean, cotton, sugar beet, rape, rice, sorghum and wheat. The skilled artisan will appreciate that equivalent changes may be made to any plant protox gene having a structure sufficiently similar to the protox enzyme sequences shown herein to allow alignment and identification of those amino acids that are modified according to the invention to generate inhibitor-resistant forms of the enzyme. Such additional plant protox genes may be obtained using standard techniques as described in International application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659 whose relevant parts are herein incorporated by reference.

DNA molecules encoding the herbicide resistant protox coding sequences taught herein may be genetically engineered for optimal expression in a crop plant. This may include altering the coding sequence of the resistance allele for optimal expression in the crop species of interest. Methods for modifying coding sequences to achieve optimal expression in a particular crop species are well known (see, *e.g.* Perlak *et al.*, *Proc. Natl. Acad. Sci. USA 88:* 3324 (1991); Koziel *et al.*, *Bio/technol. 11:* 194 (1993)).

Genetically engineering a protox coding sequence for optimal expression may also include operably linking the appropriate regulatory sequences (i.e. promoter, signal sequence, transcriptional terminators). Examples of promoters capable of functioning in plants or plant cells (i.e., those capable of driving expression of the associated structural genes such as protox in plant cells) include the cauliflower mosaic virus (CaMV) 19S or 35S promoters and CaMV double promoters; nopaline synthase promoters; pathogenesis-related (PR) protein promoters; small subunit of ribulose bisphosphate carboxylase (ssuRUBISCO) promoters, heat shock protein promoter from Brassica with reference to EPA 0 559 603 (hsp80 promoter), Arabidopsis actin promoter and the SuperMas promoter with reference to WO 95/14098 and the like. Preferred promoters will be those that confer high level constitutive expression or, more preferably, those that confer specific high level expression in the tissues susceptible to damage by the herbicide. Preferred promoters are the rice actin promoter (McElroy *et al., Mol. Gen. Genet. 231*: 150 (1991)), maize ubiquitin promoter (EP 0 342 926; Taylor *et al., Plant Cell Rep. 12:* 491 (1993)), and the PR-1 promoter from tobacco, *Arabidopsis*, or maize (see U.S. Patent Application Serial Nos. EP-332 104 and 08/181,271 to Ryals *et al.*, incorporated by reference herein in their entirety). The promoters themselves may be modified to manipulate promoter strength to increase protox expression, in accordance with art-recognized procedures.

The inventors have also discovered that another preferred promoter for use with the inhibitor-resistant protox coding sequences is the promoter associated with the native protox gene (i.e. the protox promoter; *see* copending, co-owned International Application No __________ (docket number PH/5-20756/P1/CGC1846) entitled "Promoters from Protoporphyrinogen Oxidase Genes", filed on the same day as the present application and incorporated by reference herein in its entirety.) The promoter sequence from an *Arabidopsis* protox-1 gene is set forth in SEQ ID NO:13, the promoter sequence from a maize protox-1 gene is set forth in SEQ ID NO:14, and the promoter sequence from a sugar beet protox-1 gene is set forth in SEQ ID NO:26.

Since the protox promoter itself is suitable for expression of inhibitor-resistant protox coding sequences, the modifications taught herein may be made directly on the native protox gene present in the plant cell genome without the need to construct a chimeric gene with heterologous regulatory sequences. Such modifications can be made via directed mutagenesis techniques such as homologous recombination and selected for based on the resulting herbicide-resistance phenotype (*see, e.g.* Example 10, Pazkowski *et al., EMBO J. 7:* 4021-4026 (1988), and U.S. Patent No. 5,487,992, particularly columns 18-19 and Example 8). An added advantage of this approach is that besides containing the native protox promoter, the resulting modified gene will also include any other regulatory elements, such as signal or transit peptide coding sequences, which are part of the native gene.

Signal or transit peptides may be fused to the protox coding sequence in chimeric DNA constructs of the invention to direct transport of the expressed protox enzyme to the desired site of action. Examples of signal peptides include those natively linked to the plant pathogenesis-related proteins, e.g. PR-1, PR-2, and the like. *See, e.g.*, Payne *et al., Plant Mol. Biol. 11*:89-94 (1988). Examples of transit peptides include the chloroplast transit peptides such as those described in Von Heijne *et al., Plant Mol. Biol. Rep. 9*:104-126 (1991); Mazur *et al., Plant Physiol. 85*: 1110 (1987); Vorst *et al., Gene 65*: 59 (1988), and mitochondrial transit peptides such as those described in Boutry *et al., Nature 328*:340-342 (1987). Chloroplast and mitochondrial transit peptides are contemplated to be particularly useful with the present invention as protox enzymatic activity typically occurs within the mitochondria and chloroplast. Most preferred for use are chloroplast transit peptides as inhibition of the protox enzymatic activity in the chloroplasts is contemplated to be the primary basis for the action of protox-inhibiting herbicides (Witkowski and Halling, *Plant Physiol. 87:* 632 (1988); Lehnen *et al., Pestic. Biochem. Physiol. 37*: 239 (1990); Duke *et al., Weed Sci. 39*: 465 (1991)). Also included are sequences that result in localization of the encoded protein to various cellular compartments such as the vacuole. *See, for example*, Neuhaus *et al., Proc. Natl. Acad. Sci. USA 88:* 10362-10366 (1991) and Chrispeels, *Ann. Rev. Plant Physiol. Plant Mol. Biol. 42*: 21-53 (1991). The relevant disclosures of these publications are incorporated herein by reference in their entirety.

Chimeric DNA construct(s) of the invention may contain multiple copies of a promoter or multiple copies of the protox structural genes. In addition, the construct(s) may include coding sequences for markers and coding sequences for other peptides such as signal or transit peptides, each in proper reading frame with the other functional elements in the DNA molecule. The preparation of such constructs are within the ordinary level of skill in the art.

Useful markers include peptides providing herbicide, antibiotic or drug resistance, such as, for example, resistance to hygromycin, kanamycin, G418, gentamycin, lincomycin, methotrexate, glyphosate, phosphinothricin, or the like. These markers can be used to select cells transformed with the chimeric DNA constructs of the invention from untransformed cells. Other useful markers are peptidic enzymes that can be easily detected by a visible reaction, for example a color reaction, for example luciferase, ß-glucuronidase, or ß-galactosidase.

The method of positive selection of genetically transformed cells into which a desired nucleotide sequence can be incorporated by providing the transformed cells with a selective advantage is herein incorporated by reference as WO 94/20627.

Plastid expression, in which genes are inserted by homologous recombination into all of the several thousand copies of the circular plastid genome present in each plant cell, takes advantage of the enormous copy number advantage over nuclear-expressed genes to permit expression levels that may exceed 10% of the total soluble plant protein. In addition, plastid expression is desirable because plastid-encoded traits are not pollen transmissable; hence, potential risks of inadvertent transgene escape to wild relatives of transgenic plants is obviated. Plastid transformation technology is extensively described in U.S. Patent Nos. 5,451,513, 5,545,817, and 5,545,818, all of which are hereby expressly incorporated by reference in their entireties; in PCT application no. WO 95/16783, which is hereby incorporated by reference in its entirety; and in McBride et al., Proc. Natl. Acad. Sci. USA 91: 7301-7305 (1994), which is also hereby incorporated by reference in its entirety. The basic technique for tobacco chloroplast transformation was developed and refined in the laboratory of Dr. Pal Maliga at Rutgers University (Piscattaway, New Jersey) and involves the particle bombardment of leaf tissue with regions of cloned plastid DNA flanking a selectable antibiotic resistance marker. The 1 to 1.5 kb flanking regions, termed targeting sequences, facilitate homologous recombination with the plastid genome and thus allow the replacement or modification of specific regions of the 156 kb tobacco plastome. Initially, point mutations in the chloroplast 16S rRNA and rps12 genes conferring resistance to spectinomycin and/or streptomycin were utilized as selectable markers for transformation (Svab, Z., Hajdukiewicz, P., and Maliga, P. (1990) Proc. Natl. Acad. Sci. USA 87, 8526-8530, hereby incorporated by reference; Staub, J. M., and Maliga, P. (1992) Plant Cell 4, 39-45, hereby incorporated by reference). This resulted in stable homoplasmic transformants at a frequency of approximately one per 100 bombardments of target leaves. The presence of cloning sites between these markers allowed creation of a plastid targeting vector for introduction of foreign genes (Staub, J.M., and Maliga, P., EMBO J. 12: 601-606 (1993), hereby incorporated by reference). Substantial increases in transformation frequency were obtained by replacement of the recessive rRNA or r-protein antibiotic resistance genes with a dominant selectable marker, the bacterial aadA gene encoding the spectinomycin-detoxifying enzyme aminoglycoside-3'-adenyltransferase (Svab, Z., and Maliga, P. (1993) Proc. Natl. Acad. Sci. USA 90, 913-917, hereby incorporated by reference). Previously, this marker had been used successfully for high-frequency transformation of the plastid genome of the green alga Chlamydomonas reinhardtii (Goldschmidt-Clermont, M. (1991) Nucl. Acids Res. 19, 4083-4089, hereby incorporated by reference).

Therefore, the present invention further encompasses a chimeric gene comprising a plant plastid promoter operably linked to an isolated DNA molecule that either encodes a native plant protox enzyme or a modified plant protox enzyme, such as a DNA molecule that encodes a native or modified wheat, soybean, cotton, sugar beet, rape, rice, or sorghum protox enzyme. An especially preferred plant plastid promoter is a *clp*P gene promoter. The chimeric gene preferably further comprises a 5' untranslated sequence (5'UTR) from the plastid promoter and a plastid gene 3' untranslated sequence (3' UTR) operably linked to the isolated DNA molecule. Preferably, the 3' UTR is a plastid *rps*16 gene 3' untranslated sequence.

The present invention also encompasses a plastid transformation vector comprising the chimeric gene described immediately above, as well as a plant plastid transformed with such a plastid transformation vector, wherein said modified plant protox enzyme is expressed in said plant plastid. The invention also encompasses a plant or plant cell, including the progeny thereof, comprising this plant plastid, wherein a modified plant protox enzyme is expressed in the plant and confers upon the plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

Where a herbicide resistant protox allele is obtained via directed mutation of the native gene in a crop plant or plant cell culture from which a crop plant can be regenerated, it may be moved into commercial varieties using traditional breeding techniques to develop a herbicide tolerant crop without the need for genetically engineering the modified coding sequence and transforming it into the plant. Alternatively, the herbicide resistant gene may be isolated, genetically engineered for optimal expression and then transformed into the desired variety.

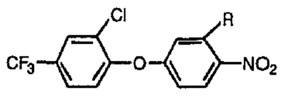
Genes encoding altered protox resistant to a protox inhibitor can also be used as selectable markers in plant cell transformation methods. For example, plants, plant tissue or plant cells transformed with a transgene can also be transformed with a gene encoding an altered protox capable of being expressed by the plant. The thus-transformed cells are transferred to medium containing the protox inhibitor wherein only the transformed cells will survive. Protox inhibitors contemplated to be particularly useful as selective agents are the diphenylethers {e.g. acifluorfen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobezoic 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-(3*H*)-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 and bicyclic Triazolones as disclosed in the International patent application WO 92/04827; EP 532146).

The method is applicable to any plant cell capable of being transformed with an altered protox-encoding gene, and can be used with any transgene of interest. Expression of the transgene and the protox gene can be driven by the same promoter functional on plant cells, or by separate promoters.

Modified inhibitor-resistant protox enzymes of the present invention are resistant to herbicides that inhibit the naturally occurring protox activity. The herbicides that inhibit protox include many different structural classes of molecules (Duke *et al.*, *Weed Sci. 39*: 465 (1991); Nandihalli *et al.*, *Pesticide Biochem. Physiol. 43:* 193 (1992); Matringe *et al.*, *FEBS Lett. 245:* 35 (1989); Yanase and Andoh, *Pesticide Biochem. Physiol. 35:* 70 (1989)), including the diphenylethers {e.g. acifluorifen, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobezoic 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-(3*H*)-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.

The diphenylethers of particular significance are those having the general formula



(Formula I)

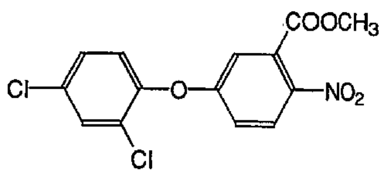
wherein R equals -COONa (Formula II), $-CONHSO_2CH_3$ (Formula III) or $-COOCH_2COOC_2H_5$ (Formula IV; *see* Maigrot *et al., Brighton Crop Protection Conference-Weeds:* 47-51 (1989)).

Additional diphenylethers of interest are those where R equals:

$$\begin{array}{l} NOCH_2COOCH_3 \\ \| \\ CCH_2OCH_3 \end{array}$$

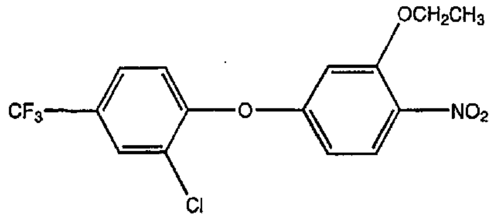
(Formula IVa; *see* Hayashi *et al., Brighton Crop Protection Conference-Weeds:* 53-58 (1989)).

An additional diphenylether of interest is one having the formula:



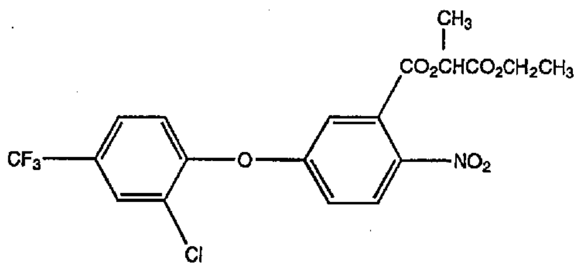
(Formula IVb; bifenox, *see* Dest *et al., Proc. Northeast Weed Sci. Conf. 27:* 31 (1973)).

A further diphenylether of interest is one having the formula:



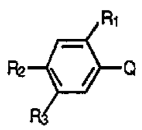
(Formula IVc; oxyfluorfen; *see* Yih and Swithenbank, *J. Agric. Food Chem.*, 23: 592 (1975))

Yet another diphenylether of interest is one having the formula:



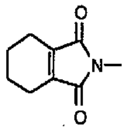
(Formula IVd; lactofen, *see* page 623 of "The Pesticide Manual", 10th ed., ed. by C. Tomlin, British Crop Protection Council, Surrey (1994))

Also of significance are the class of herbicides known as imides, having the general formula

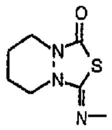


(Formula V)

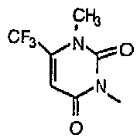
wherein Q equals

 OR

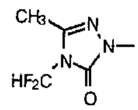
(Formula VI)

 OR

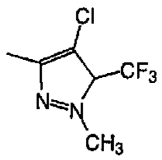
(Formula VII)

 OR

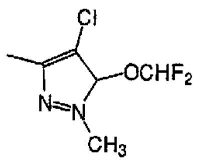
(Formula VIII)



(Formula IX)

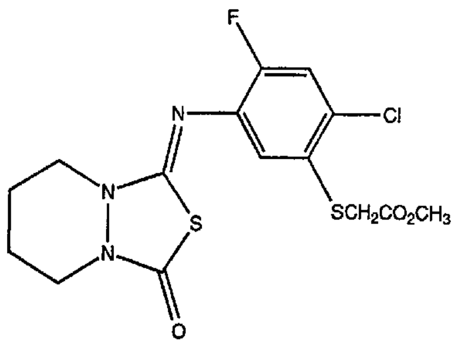
OR 

(Formula IXa)

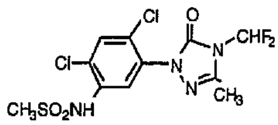
OR 

(Formula IXb)

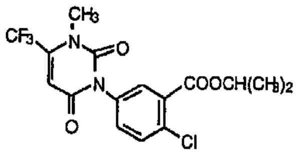
(*see* Hemper *et al.* (1995) *in* "Proceedings of the Eighth International Congress of Pesticide Chemistry", Ragdale *et al.*, eds., Amer. Chem. Soc, Washington, D.C., pp.42-48 (1994)); and R_1 equals H, Cl or F, R_2 equals Cl and R_3 is an optimally substituted ether, thioether, ester, amino or alkyl group. Alternatively, R_2 and R_3 together may form a 5 or 6 membered heterocyclic ring. Examples of imide herbicides of particular interest are



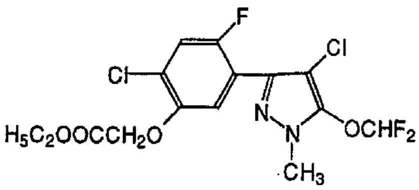
(Formula VIIa; fluthiacet-methyl, *see* Miyazawa *et al.*, *Brighton Crop Protection Conference-Weeds*, pp. 23-28 (1993))



(Formula X sulfentrazone, *see* Van Saun *et al.*, *Brighton Crop Protection Conference-Weeds*, pp. 77-82 (1991)).

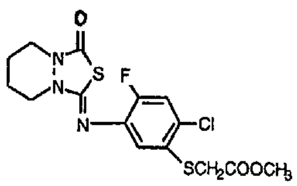


(Formula XI)

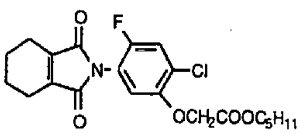


(Formula XII)

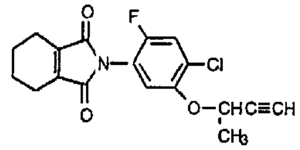
(*see* Miura *et al.*, *Brighton Crop Protection Conference-Weeds:* 35-40 (1993))



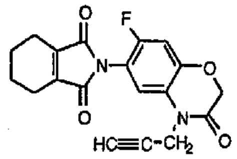
(Formula XIII)



(Formula XIV)



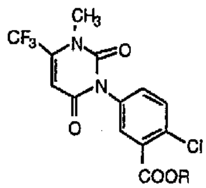
(Formula XV)



(Formula XVI)

The herbicidal activity of the above compounds is described in the *Proceedings of the 1991 Brighton Crop Protection Conference, Weeds* (British Crop Protection Council) (Formulae X and XVI), *Proceedings of the 1993 Brighton Crop Protection Conference, Weeds* (British Crop Protection Council) (Formulae XII and XIII), U.S. Patent No. 4,746,352 (Formula XI) and *Abstracts of the Weed Science Society of America* vol. 33, pg. 9 (1993)(Formula XIV).

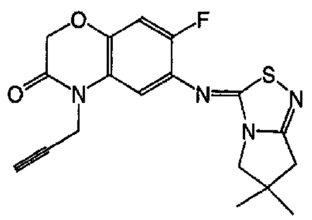
The most preferred imide herbicides are those classified as aryluracils and having the general formula



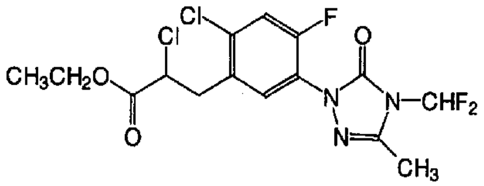
(Formula XVII)

wherein R signifies the group (C_{2-6}-alkenyloxy)carbonyl-C_{1-4}-alkyl, as disclosed in U.S. Patent No. 5,183,492, herein incorporated by reference.

Also of significance are herbicides having the general formula:

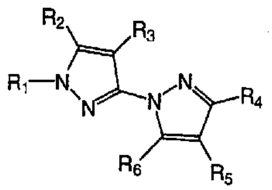


(Formula XVIII; thiadiazimin)
(*see* Weiler *et al., Brighton Crop Protection Conference-Weeds*, pp. 29-34 (1993));



(Formula XIX; carfentrazone)
(*see* Van Saun *et al., Brighton Crop Protection Conference-Weeds:* pp. 19-22 (1993));

N-substituted pyrazoles of the general formula:



(Formula XX)

wherein R_1 is C_1-C_4-alkyl, optionally substituted by one or more halogen atoms;

R_2 is hydrogen, or a C_1-C_4-alkoxy, each of which is optionally substituted by one or more halogen atoms, or

R_1 and R_2 together from the group $-(CH_2)_n-X-$, where X is bound at R_2;

R_3 is hydrogen or halogen,

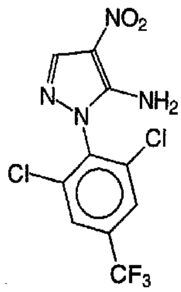
R_4 is hydrogen or C_1-C_4-alkyl,

R_5 is hydrogen, nitro, cyano or the group $-COOR_6$ or $-CONR_7R_8$, and

R_6 is hydrogen, C_1-C_6-alkyl, C_2-C_6-alkenyl or C_2-C_6-alkynyl;

(*see* international patent publications WO 94/08999, WO 93/10100, and U. S. Patent No. 5,405,829 assigned to Schering);

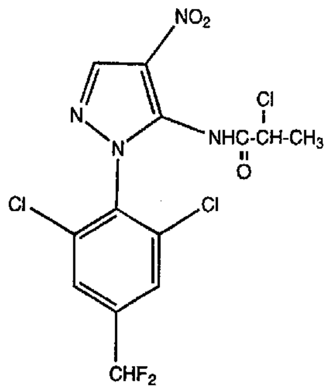
N-phenylpyrazoles, such as:



(Formula XXI; nipyraclofen)

(*see* page 621 of "The Pesticide Manual", 9th ed., ed. by C.R. Worthing, British Crop Protection Council, Surrey (1991));

and 3-substituted-2-aryl-4,5,6,7-tetrahydroindazoles (Lyga *et al.* *Pesticide Sci.* *42*:29-36 (1994)).



(Formula XXIa; BAY 11340)

Also of significance are phenylpyrazoles of the type described in WO 96/01254 and WO 97/00246, both of which are hereby incorporated by reference. (Formula XXII).

Levels of herbicide that normally are inhibitory to the activity of protox include application rates known in the art, and that depend partly on external factors such as environment, time and method of application. For example, in the case of the imide herbicides represented by Formulae V through IX, and more particularly those represented by Formulae X through XVII, the application rates range from 0.0001 to 10 kg/ha, preferably from 0.005 to 2 kg/ha. This dosage rate or concentration of herbicide may be different, depending on the desired action and particular compound used, and can be determined by methods known in the art.

A further object of the invention is a method for controlling the growth of undesired vegetation that comprises applying to a population of the plant selected from a group consisting of *Arabidopsis*, sugar cane, soybean, barley, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf and forage grasses , millet, forage and rice and the like an effective amount of a protox-inhibiting herbicide. Preferred is a method for controlling the growth of undesired vegetation, which comprises applying to a population of the selected from the group consisting of selected from the group consisting of soybean, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf grasses and rice an effective amount of a protox-inhibiting herbicide. Particularly preferred is a method for controlling the growth of undesired vegetation, which comprises applying to a population of the selected from the group consisting of *Arabidopsis*, soybean, cotton, sugar beet, oilseed rape, maize, wheat, sorghum, and rice.

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

EXAMPLES

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by T. Maniatis, E. F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory manual, Cold Spring Harbor laboratory, Cold Spring Harbor, NY (1989) and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984).

Section A. Isolation And Characterization Of Plant Protoporphyrinogen Oxidase (Protox) Genes

Example 1: Isolation of a Wheat Protox-1 cDNA Based on Sequence Homology to a Maize Protox-1 Coding Sequence

Total RNA prepared from *Triticum aestivum* (cv Kanzler) was submitted to Clontech for custom cDNA library construction in the Lambda Uni-Zap vector. Approximately 50,000 pfu of the cDNA library were plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto nitrocellulose membranes (Schleicher and Schuell). The plaque lifts were probed with the maize Protox-1 cDNA (SEQ ID NO:5; *see* Example 2 of International application no. PCT/IB95/00452, filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert, *Proc. Natl. Acad. Sci. USA 81:* 1991-1995 (1984), hereby incorporated by reference in its entirety.) Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. The sequences of the cDNA inserts were determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest wheat Protox-1 cDNA obtained from initial screening efforts, designated "wheat Protox-1", was 1489 bp in length. Wheat Protox-1 lacks coding sequence for the transit peptide plus approximately 126 amino acids of the mature coding sequence based on comparison with the other known plant protox peptide sequences.

A second screen was performed to obtain a longer wheat protox cDNA. For this screen, a *Triticum aestivum* (cv Kanzler) cDNA library was prepared internally using the lambda Uni-Zap vector. Approximately 200,000 pfu of the cDNA library was screened as indicated above, except that the wheat Protox-1 cDNA was used as a probe and hybridization and wash conditions were at 65° C instead of 50° C. The longest wheat cDNA obtained from this screening effort, designated "wheat Protox-1a", was 1811 bp in length. The nucleotide sequence of this cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs:9 and 10, respectively. Based on comparison with the other known plant protox peptide sequences and with corresponding genomic sequence, this cDNA is either full-length or missing only a few transit peptide codons (Table 1). This wheat protein sequence is 91% identical (95% similar) to the maize Protox-1 protein sequence set forth in SEQ ID NO:6.

Wheat Protox-1a, in the pBluescript SK vector, was deposited March 19, 1996, as pWDC-13 (NRRL #B21545).

Example 2: Isolation of a Soybean Protox-1 cDNA Based on Sequence Homology to an *Arabidopsis* Protox-1 Coding Sequence

A Lambda Uni-Zap cDNA library prepared from soybean (v Williams 82, epicotyls) was purchased from Stratagene. Approximately 50,000 pfu of the library was plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto Colony/Plaque Screen membranes (NEN Dupont). The plaque lifts were probed with the *Arabidopsis* Protox-1 cDNA (SEQ ID NO:1; see Example 1 of International application no. PCT/IB95/00452, filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659)) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert (1984)). Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. The sequence of the cDNA inserts was determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest soybean cDNA obtained, designated "soybean Protox-1", is full-length based on comparison with the other known plant protox peptide sequences (Table 1). Soybean Protox-1 is 1847 bp in length and encodes a protein of 58.8 kDa. The nucleotide sequence of this cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs:11 and 12, respectively. The soybean protein is 78% identical (87% similar) to the *Arabidopsis* Protox-1 protein.

Soybean Protox-1, in the pBluescript SK vector, was deposited December 15, 1995 as pWDC-12 (NRRL #B-21516).

An alignment of the predicted amino acid sequences of the respective proteins encoded by the sequences shown in SEQ ID NOS: 2, 6, 10, 12, 15, 17, 19, 21, 23 and are set forth in Table 1. An alignment of the predicted amino acid sequences of the respective proteins encoded by the sequences shown in SEQ ID NOS: 4 and 8 are set forth in Table 2.

TABLE 1

Comparison of Protox-1 Amino Acid Sequences from Arabidopsis ("Arabpt-1"; SEQ ID NO:2), Maize ("Mzpt-1"; SEQ ID NO:6), Wheat ("Wtpt-1"; SEQ ID NO:10), Soybean ("Soybeanpt-1"; SEQ ID NO:12), Cotton ("Cottonpt-1"; SEQ ID NO:16), Sugar beet ("Sugpt-1"; SEQ ID NO:18), Rape ("Rapept-1"; SEQ ID NO:20), Rice ("Ricept-1"; SEQ ID NO:22), and Sorghum ("Sorghumpt-1"; SEQ ID NO:24)

Alignment is performed using the PileUp program (GCG package, University of Wisconsin, Madison, WI). Positions that may be modified according to the teachings herein to confer or enhance inhibitor resistance are shown in bold type.

```
             1                                                50
   Rapept-1  .......... .......... MDLSLLRP.. QPFLSPFSNP FPRSRPYKPL
   Arabpt-1  .......... .......... MELSLLRPTT QSLLPSFSKP NLRLNVYKPL
Sorghumpt-1  .......... .......... .......... .......... ..........
     Mzpt-1  .......... .......... .......... .......... ..........
     Wtpt-1  .......... .......... .........M ATATVAAASP LRGRVTGRPH
   Ricept-1  .......... .......... .......... .......... ..........
 Cottonpt-1  .......... ......MTAL IDLSLLRSSP SVSPFSIPHH QHPPRFRKPF
 Soybeanpt1  ........MV SVFNEILFPP NQTLLRPSLH SPTSFFTSPT RKFPRSRPNP
    Sugpt-1  MKSMALSNCI PQTQCMPLRS SGHYRGNCIM LSIPCSLIGR RGYYSHKKRR

             51                                              100
   Rapept-1  NLRCSVSGGS VVGSSTIEGG GGGKTVTADC VIVGGGISGL CIAQALVTKH
   Arabpt-1  RLRCSVAGGP TVGSSKIEGG GGT.TITTDC VIVGGGISGL CIAQALATKH
Sorghumpt-1  .......... .......... .......... .......... ..........
     Mzpt-1  .......... .......... .......ADC VVVGGGISGL CTAQALATRH
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    Wtpt-1  RVRPRCATAS SATETPAAPG VRL...SAEC VIVGAGISGL CTAQALATRY
  Ricept-1  .......... .......... .......... .......... ..........
Cottonpt-1  KLRCSLAEGP TISSSKIDGG ESS...IADC VIVGGGISGL CIAQALATKH
Soybeanpt1  ILRCSIAEES TASPPKTR.. DSA...PVDC VVVGGGVSGL CIAQALATKH
   Sugpt-1  MSMSCSTSSG SKSAVKEAGS GSGAGGLLDC VIVGGGISGL CIAQALCTKH

            101                                            150
  Rapept-1  PDA..AKNVM VTEAKDRVGG NIIT..REEQ GFLWEEGPNS FQPSDPMLTM
   Arabpt-1 PDA..APNLI VTEAKDRVGG NIIT..REEN GFLWEEGPNS FQPSDPMLTM
Sorghumpt-1 .......... .......... ..STVERPEE GYLWEEGPNS FQPSDPVLSM
     Mzpt-1 ..G..VGDVL VTEARARPGG NITTVERPEE GYLWEEGPNS FQPSDPVLTM
     Wtpt-1 ..G..VSDLL VTEARDRPGG NITTVERPDE GYLWEEGPNS FQPSDPVLTM
   Ricept-1 .......... .......... .......... .......... ..........
 Cottonpt-1 RDV..ASNVI VTEARDRVGG NITTVER..D GYLWEEGPNS FQPSDPILTM
 Soybeanpt1 ..A..NANVV VTEARDRVGG NITTMER..D GYLWEEGPNS FQPSDPMLTM
    Sugpt-1 SSSSLSPNFI VTEAKDRVGG NIVTVE..AD GYIWEEGPNS FQPSDAVLTM

            151                                            200
   Rapept-1 VVDSGLKDDL VLGDPTAPRF VLWNGKLRPV PSKLTDLPFF DLMSIGGKIR
   Arabpt-1 VVDSGLKDDL VLGDPTAPRF VLWNGKLRPV PSKLTDLPFF DLMSIGGKIR
Sorghumpt-1 AVDSGLKDDL VFGDPNAPRF VLWEGKLRPV PSKPADLPFF DLMSIPGKLR
     Mzpt-1 AVDSGLKDDL VFGDPNAPRF VLWEGKLRPV PSKPADLPFF DLMSIPGKLR
     Wtpt-1 AVDSGLKDDL VFGDPNAPRF VLWEGKLRPV PSKPGDLPFF SLMSIPGKLR
   Ricept-1 .......... .......... .......... .......... ..........
 Cottonpt-1 AVDSGLKDDL VLGDPNAPRF VLWEGKLRPV PSKPTDLPFF DLMSIAGKLR
 Soybeanpt1 VVDSGLKDEL VLGDPDAPRF VLWNRKLRPV PGKLTDLPFF DLMSIGGKIR
    Sugpt-1 AVDSGLKDEL VLGDPNAPRF VLWNDKLRPV PSSLTDLPFF DLMTIPGKIR

            201                                            250
   Rapept-1 AGFGAIGIRP SPPGREESVE EFVRRNLGDE VFERLIEPFC SGVYAGDPAK
   Arabpt-1 AGFGALGIRP SPPGREESVE EFVRRNLGDE VFERLIEPFC SGVYAGDPSK
Sorghumpt-1 AGLGALGIRP PAPGREESVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK
     Mzpt-1 AGLGALGIRP PPPGREESVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK
     Wtpt-1 AGLGALGIRP PPPGREESVE EFVRRNLGAE VFERLIEPFC SGVYAGDPSK
   Ricept-1 .......... .......... .......... .......... ..........
 Cottonpt-1 AGFGAIGIRP PPPGYEESVE EFVRRNLGAE VFERFIEPFC SGVYAGDPSK
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```
Soybeanpt1   AGFGALGIRP PPPGHEESVE EFVRRNLGDE VFERLIEPFC SGVYAGDPSK
   Sugpt-1   AALGALGFRP SPPPHEESVE HFVRRNLGDE VFERLIEPFC SGVYAGDPAK

             251                                              300
 Rapept-1    LSMKAAFGKV WKLEENGGSI IGGAFKAIQA KNKAPKTTRD PRLPKPKGQT
  Arabpt-1   LSMKAAFGKV WKLEQNGGSI IGGTFKAIQE RKNAPKAERD PRLPKPQGQT
Sorghumpt-1  LSMKAAFGKV WRLEEAGGSI IGGTIKTIQE RGKNPKPPRD PRLPKPKGQT
    Mzpt-1   LSMKAAFGKV WRLEETGGSI IGGTIKTIQE RSKNPKPPRD ARLPKPKGQT
    Wtpt-1   LSMKAAFGKV WRLEEIGGSI IGGTIKAIQD KGKNPKPPRD PRLPAPKGQT
  Ricept-1   RALKAAFGKV WRLEDTGGSI IGGTIKTIQE RGKNPKPPRD PRLPTPKGQT
 Cottonpt-1  LSMKAAFGRV WKLEEIGGSI IGGTFKTIQE RNKTPKPPRD PRLPKPKGQT
Soybeanpt1   LSMKAAFGKV WKLEKNGGSI IGGTFKAIQE RNGASKPPRD PRLPKPKGQT
   Sugpt-1   LSMKAAFGKV WKLEQKGGSI IGGTLKAIQE RGSNPKPPRD QRLPKPKGQT

             301                                              350
  Rapept-1   VGSFRKGLTM LPEAISARLG DKVKVSWKLS SITKLASGEY SLTYETPEGI
  Arabpt-1   VGSFRKGLRM LPEAISARLG SKVKLSWKLS GITKLESGGY NLTYETPDGL
Sorghumpt-1  VASFRKGLAM LPNAITSSLG SKVKLSWKLT SMTKSDGKGY VLEYETPEGV
    Mzpt-1   VASFRKGLAM LPNAITSSLG SKVKLSWKLT SITKSDDKGY VLEYETPEGV
    Wtpt-1   VASFRKGLAM LPNAIASRLG SKVKLSWKLT SITKADNQGY VLGYETPEGL
  Ricept-1   VASFRKGLTM LPDAITSRLG SKVKLSWKLT SITKSDNKGY ALVYETPEGV
 Cottonpt-1  VGSFRKGLTM LPEAIANSLG SNVKLSWKLS SITKLGNGGY NLTFETPEGM
Soybeanpt1   VGSFRKGLTM LPDAISARLG NKVKLSWKLS SISKLDSGEY SLTYETPEGV
   Sugpt-1   VGSFRKGLVM LPTAISARLG SRVKLSWTLS SIVKSLNGEY SLTYDTPDGL

             351                                              400
  Rapept-1   VTVQSKSVVM TVPSHVASSL LRPLSDSAAE ALSKLYYPPV AAVSISYAKE
  Arabpt-1   VSVQSKSVVM TVPSHVASGL LRPLSESAAN ALSKLYYPPV AAVSISYPKE
Sorghumpt-1  VLVQAKSVIM TIPSYVASDI LRPLSGDAAD VLSRFYYPPV AAVTVSYPKE
    Mzpt-1   VSVQAKSVIM TIPSYVASNI LRPLSSDAAD ALSRFYYPPV AAVTVSYPKE
    Wtpt-1   VSVQAKSVIM TIPSYVASDI LRPLSIDAAD ALSKFYYPPV AAVTVSYPKE
  Ricept-1   VSVQAKTVVM TIPSYVASDI LRPLSSDAAD ALSIFYYPPV AAVTVSYPKE
 Cottonpt-1  VSLQSRSVVM TIPSHVASNL LHPLSAAAAD ALSQFYYPPV ASVTVSYPKE
Soybeanpt1   VSLQCKTVVL TIPSYVASTL LRPLSAAAAD ALSKFYYPPV AAVSISYPKE
   Sugpt-1   VSVRTKSVVM TVPSYVASRL LRPLSDSAAD SLSKFYYPPV AAVSLSYPKE
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             401                                                    450
Rapept-1     AIRSECLIDG ELKGFGQLHP RTQKVETLGT IYSSSLFPNR APPGRVLLLN
Arabpt-1     AIRTECLIDG ELKGFGQLHP RTQGVETLGT IYSSSLFPNR APPGRILLLN
Sorghumpt-1  AIRKECLIDG ELQGFGQLHP RSQGVETLGT IYSSSLFPNR APAGRVLLLN
Mzpt-1       AIRKECLIDG ELQGFGQLHP RSQGVETLGT IYSSSLFPNR APDGRVLLLN
Wtpt-1       AIRKECLIDG ELQGFGQLHP RSQGVETLGT IYSSSLFPNR APAGRVLLLN
Ricept-1     AIRKECLIDG ELQGFGQLHP RSQGVETLGT IYSSSLFPNR APAGRVLLLN
Cottonpt-1   AIRKECLIDG ELKGFGQLHP RSQGIETLGT IYSSSLFPNR APSGRVLLLN
Soybeanpt1   AIRSECLIDG ELKGFGQLHP RSQGVETLGT IYSSSLFPNR APPGRVLLLN
Sugpt-1      AIRSECLING ELQGFGQLHP RSQGVETLGT IYSSSLFPGR APPGRILILS

             451                                                    500
Rapept-1     YIGGATNTGI LSKSEGELVE AVDRDLRKML IKPSSTDPLV LGVKLWPQAI
Arabpt-1     YIGGSTNTGI LSKSEGELVE AVDRDLRKML IKPNSTDPLK LGVRVWPQAI
Sorghumpt-1  YIGGATNTGI VSKTESELVE AVDRDLRKML INPTAVDPLV LGVRVWPQAI
Mzpt-1       YIGGATNTGI VSKTESELVE AVDRDLRKML INSTAVDPLV LGVRVWPQAI
Wtpt-1       YIGGSTNTGI VSKTESDLVG AVDRDLRKML INPRAADPLA LGVRVWPQAI
Ricept-1     YIGGSTNTGI VSKTESELVE AVDRDLRKML INPRAVDPLV LGVRVWPQAI
Cottonpt-1   YIGGATNTGI LSKTEGELVE AVDRDLRKML INPNAKDPLV LGVRVWPKAI
Soybeanpt1   YIGGATNTGI LSKTDSELVE TVDRDLRKIL INPNAQDPFV VGVRLWPQAI
Sugpt-1      YIGGAKNPGI LNKSKDELAK TVDKDLRRML INPDAKLPRV LGVRVWPQAI

             501                                                    550
Rapept-1     PQFLIGHIDL VDAAKASLSS SGHEGLFLGG NYVAGVALGR CVEGAYETAT
Arabpt-1     PQFLVGHFDI LDTAKSSLTS SGYEGLFLGG NYVAGVALGR CVEGAYETAI
Sorghumpt-1  PQFLVGHLDL LEAAKSALDQ GGYNGLFLGG NYVAGVALGR CIEGAYESAA
Mzpt-1       PQFLVGHLDL LEAAKAALDR GGYDGLFLGG NYVAGVALGR CVEGAYESAS
Wtpt-1       PQFLIGHLDR LAAAKSALGQ GGYDGLFLGG KYVAGVALGR CIEGAYESAS
Ricept-1     PQFLIGHLDH LEAAKSALGK GGYDGLFLGG NYVAGVALGR CVEGAYESAS
Cottonpt-1   PQFLVGHLDL LDSAKMALRD SGFHGLFLGG NYVSGVALGR CVEGAYEVAA
Soybeanpt1   PQFLVGHLDL LDVAKASIRN TGFEGLFLGG NYVSGVALGR CVEGAYEVAA
Sugpt-1      PQFSIGHFDL LDAAKAALTD TGVKGLFLGG NYVSGVALGR CIEGAYESAA

             551        563
Rapept-1     QVNDFMSRYA YK*
Arabpt-1     EVNNFMSRYA YK*
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Sorghumpt-1  QIYDFLTKYA YK*
     Mzpt-1  QISDFLTKYA YK*
     Wtpt-1  QVSDFLTKYA YK*
  Ricept-1  QISDYLTKYA YK*
 Cottonpt-1  EVKEFLSQYA YK*
Soybeanpt1  EVNDFLTNRV YK*
    Sugpt-1  EVVDFLSQYS DK*
```

TABLE 2

Comparison of the Arabidopsis (SEQ ID NO:4) and
Maize (SEQ ID NO:8) Protox-2 Amino Acid Sequences

Identical residues are denoted by the vertical bar between the two sequences. Alignment is performed using the GAP program described in Deveraux *et al., Nucleic Acids Res. 12*:387-395 (1984).

Percent Similarity: 75.889 Percent Identity: 57.905
Protox-2.Pep x Mzprotox-2.Pep

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  1 ............................MASGAVAD.HQIEAVSGKRVAV 21
                                .|  |:|: .:  |..::.|||
  1 MLALTASASSASSHPYRHASAHTRRPRLRAVLAMAGSDDPRAAPARSVAV 50

 22 VGAGVSGLAAAYKLKSRGLNVTVFEADGRVGGKLRSVMQNGLIWDEGANT 71
    ||||||||||||:|: .|:|||||||.:|.|||:|.  :.|::|||||||
 51 VGAGVSGLAAAYRLRQSGVNVTVFEAADRAGGKIRTNSEGGFVWDEGANT 100

 72 MTEAEPEVGSLLDDLGLREKQQFPISQKKRYIVRNGVPVMLPTNPIELVT 121
    |||:| |.:.|:|||||.:|||:| ||.|||||::|.|.::|.:||.|:.
101 MTEGEWEASRLIDDLGLQDKQQYPNSQHKRYIVKDGAPALIPSDPISLMK 150

122 SSVLSTQSKFQILLEPFLWKK....KSSKVSDASAEESVSEFFQRHFGQE 167
    ||||||.||:.:::||||:||    .|:|||:.  .|||:.| :||||.|
151 SSVLSTKSKIALFFEPFLYKKANTRNSGKVSEEHLSESVGSFCERHFGRE 200
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168 VVDYLIDPFVGGTSAADPDSLSMKHSFPDLWNVEKSFGSIIVGAIRTKFA 217
    ||||::||||:||||:||:|||::|.||.|||:|:.:||:|||||  .|:|
201 VVDYFVDPFVAGTSAGDPESLSIRHAFPALWNLERKYGSVIVGAILSKLA 250

218 AKGGKSRDTKSSPGTKKGSRGSFSFKGGMQILPDTLCKSLSHDEINLDSK 267
    |||:.  :.  ..|.|.::..|.||||.|||| | :.| ..::.|::.|:..
251 AKGDPVKTRHDSSGKRRNRRVSFSFHGGMQSLINALHNEVGDDNVKLGTE 300

268 VLSLS..YNSGSRQENWSLSCVSHNETQRQ...NPHYDAVIMTAPLCNVK 312
    ||||.  ::::..  :.||:|. |.:..:::    |. :|||||||||:||:
301 VLSLACTFDGVPALGRWSISVDSKDSGDKDLASNQTFDAVIMTAPLSNVR 350

313 EMKVMKGGQPFQLNFLPEINYMPLSVLITTFTKEKVKRPLEGFGVLIPSK 362
     ||. |||.|. |:|||.::|:|||:::|.|.|:.||:|||||||||| |
351 RMKFTKGGAPVVLDFLPKMDYLPLSLMVTAFKKDDVKKPLEGFGVLIPYK 400

363 E.QKHGFKTLGTLFSSMMFPDRSPSDVHLYTTFIGGSRNQELAKASTDEL 411
    | ||||:||||||||||||||||.|.| .|||||:|||:|.:|| |.|. |
401 EQQKHGLKTLGTLFSSMMFPDRAPDDQYLYTTFVGGSHNRDLAGAPTSIL 450

412 KQVVTSDLQRLLGVEGEPVSVNHYYWRKAFPLYDSSYDSVMEAIDKMEND 461
    ||:|||||.:|||||||:|. |.| || .|||||: .|.||:|||:||||.:
451 KQLVTSDLKKLLGVEGQPTFVKHVYWGNAFPLYGHDYSSVLEAIEKMEKN 500

462 LPGFFYAGNHRGGLSVGKSIASGCKAADLVISYLESCSNDKKPNDSL* 509
    ||||||||| ::||.||. ||||:|||||.|||||| ......:
501 LPGFFYAGNSKDGLAVGSVIASGSKAADLAISYLESHTKHNNSH*... 545
```

Example 3: Isolation of a Cotton Protox-1 cDNA Based on Sequence Homology to a Maize Protox-1 Coding Sequence

A Lambda Uni-Zap cDNA library prepared from *Gossypium hirsutum L.* (72 hr. dark grown cotyledons) was obtained from Dr. Dick Trelease, Dept. of Botany, Arizona State University (Ni W. and Trelease R.N., *Arch. Biochem. Biophys.* 289: 237-243 (1991)). Approximately 50,000 pfu of the library was plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto Colony/Plaque Screen membranes (NEN Dupont). The plaque lifts were probed with the maize Protox-1 cDNA (SEQ ID NO:5) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert (1984)). Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. The sequence of the cDNA inserts was determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest cotton cDNA obtained, designated "cotton Protox-1", appears to be full-length based on comparison with the other known plant protox peptide sequences (Table 1). Cotton Protox-1 is 1826 bp in length and encodes a protein of 58.2 kDa. The nucleotide sequence of this cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs:13 and 14, respectively. The cotton protein is 77% identical (86% similar) to the Maize Protox-1 protein.

Cotton Protox-1, in the pBluescript SK vector, was deposited July 1, 1996 as pWDC-15 (NRRL #B-21594).

Example 4: Isolation of a Sugar Beet Protox-1 cDNA Based on Sequence Homology to an *Arabidopsis* Protox-1 Coding Sequence

A Lambda-Zap cDNA library prepared from *Beta vulgaris* was obtained from Dr. Philip Rea, Dept. of Botany, Plant Science Institute, Philadelphia, PA (Yongcheol Kim, Eugene J. Kim, and Philip A. Rea, *Plant Physiol.* 106: 375-382 (1994)). Approximately 50,000 pfu of the cDNA library were plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto nitrocellulose membranes (Schleicher and Schuell). The plaque lifts were probed with the *Arabidopsis* Protox-1 cDNA (SEQ ID NO:1) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert (1984)). Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. The sequences of the cDNA inserts were determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest sugar beet Protox-1 cDNA obtained, designated "sugar beet Protox-1", is full-length based on comparison with the other known plant protox peptide sequences (Table 1). Sugar beet

Protox-1 is 1910 bp in length and encodes a protein of 60 kDa. The nucleotide sequence of this cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs:15 and 16, respectively. The sugar beet protein is 73% identical (82% similar) to the *Arabidopsis* Protox-1 protein.

Sugar beet Protox-1, in the pBluescript SK vector, was deposited July 29, 1996, as pWDC-16 (NRRL #B-21595N).

Example 5: Isolation of a Rape Protox-1 cDNA Based on Sequence Homology to an *Arabidopsis* Protox-1 Coding Sequence

A Lambda Uni-Zap II cDNA library prepared from *Brassica napus* (3-4 wk. mature green leaves) was obtained from Dr. Guenther Ochs, Institut Fuer Allgemeine Botanik, Johannes Gutenberg-Universitaet Mainz, Germany (Günther Ochs, Gerald Schock, and Aloysius Wild, *Plant Physiol.* 103: 303-304 (1993)). Approximately 50,000 pfu of the cDNA library were plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto nitrocellulose membranes (Schleicher and Schuell). The plaque lifts were probed with the *Arabidopsis* Protox-1 cDNA (SEQ ID NO:1) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert (1984)). Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. The sequences of the cDNA inserts were determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest rape Protox-1 cDNA obtained, designated "rape Protox-1", is full-length based on comparison with the other known plant protox peptide sequences (Table 1). Rape Protox-1 is 1784 bp in length and encodes a protein of 57.3kD. The nucleotide sequence of this cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs: 17 and 18, respectively. The rape protein is 87% identical (92% similar) to the *Arabidopsis* Protox-1 protein.

Rape Protox-1, in the pBluescript SK vector, was deposited August 23, 1996, as pWDC-17 (NRRL #B-21615).

Example 6: Isolation of a Rice Protox-1 cDNA Based on Sequence Homology to a Maize Protox-1 Coding Sequence

A Lambda gt11 cDNA library prepared from *Oryza sativa* (5 day etiolated shoots) was purchased from Clontech. Approximately 50,000 pfu of the cDNA library were plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto nitrocellulose membranes (Schleicher and Schuell). The plaque lifts were probed with the maize Protox-1 cDNA (SEQ ID NO:5) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert (1984)). Positively hybridizing plaques were purified, and lambda DNA was prepared using the Wizard Lambda-Prep kit (Promega). The cDNA inserts were subcloned as EcoRI fragments into the pBluescript SK vector using standard techniques. The sequences of the cDNA inserts were determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest rice Protox-1 cDNA obtained, designated "rice Protox-1", was 1224 bp in length. Rice Protox-1 lacks coding sequence for the transit peptide plus approximately 172 amino acids of the mature coding sequence based on comparison with the other known plant protox peptide sequences (Table 1). The nucleotide sequence of this partial cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs:19 and 20, respectively.

Rice Protox-1, in the pBluescript SK vector, was deposited December 6, 1996, as pWDC-18 (NRRL #B-21648).

Example 7: Isolation of a Sorghum Protox-1 cDNA Based on Sequence Homology to a Maize Protox-1 Coding Sequence

A Lambda-Zap II cDNA library prepared from Sorghum bicolor (3-6 day green seedlings) was obtained from Dr. Klaus Pfizenmaier, Institute of Cell Biology and Immunology, University of Stuttgart, Germany (Harald Wajant, Karl-Wolfgang Mundry, and Klaus Pfizenmaier, *Plant Mol. Biol.* 26: 735-746 (1994)). Approximately 50,000 pfu of the cDNA library were plated at a density of approximately 5,000 pfu per 10 cm Petri dish and duplicate filter lifts were made onto nitrocellulose membranes (Schleicher and Schuell). The plaque lifts were probed with the maize Protox-1 cDNA (SEQ ID NO:5) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization conditions were 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C. Wash conditions were 2X SSC, 1% SDS at 50° C. (Church and Gilbert (1984)). Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. The sequences of the cDNA inserts were determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). The longest sorghum Protox-1 cDNA obtained, designated "sorghum Protox-1", was 1590 bp in length. Sorghum Protox-1 lacks coding sequence for the transit peptide plus approximately 44 amino acids of the mature coding sequence based on comparison with the other known plant protox peptide sequences (Table 1). The nucleotide sequence of this partial cDNA and the amino acid sequence it encodes are set forth in SEQ ID NOs:21 and 22, respectively.

Sorghum Protox-1, in the pBluescript SK vector, was deposited December 6, 1996, as pWDC-19 (NRRL #B-21649).

Example 8: Demonstration of Plant Protox Clone Sensitivity to Protox Inhibitory Herbicides in a Bacterial System

Liquid cultures of Protox-1/SASX38, Protox-2/SASX38 and pBluescript/XL1-Blue were grown in L amp^{100}. One hundred microliter aliquots of each culture were plated on L amp^{100} media containing various concentrations (1.0nM-10mM) of a protox inhibitory aryluracil herbicide of formula XVII. Duplicate sets of plates were incubated for 18 hours at 37° C.

The $protox^{+}$ *E. coli* strain XL1-Blue showed no sensitivity to the herbicide at any concentration, consistent with reported resistance of the native bacterial enzyme to similar herbicides. The Protox-1/SASX38 was clearly sensitive, with the lawn of bacteria almost entirely eliminated by inhibitor concentrations as low as 10nM. The Protox-2/SASX38 was also sensitive, but only at a higher concentration (10µM) of the herbicide. The herbicide was effective even on plates maintained almost entirely in the dark. The toxicity of the herbicide was entirely eliminated by the addition of 20 µg/ml hematin to the plates.

The different herbicide tolerance between the two plant Protox strains is likely the result of differential expression from these two plasmids, rather than any inherent difference in enzyme sensitivity. Protox-1/SASX38 grows much more slowly than Protox-2/SASX38 in any heme-deficient media. In addition, the MzProtox-2/SASX38 strain, with a growth rate comparable to Arab Protox-1/SASX38, is also very sensitive to herbicide at the lower (10-100nM) concentrations.

Section B: Identification and Characterization of Plant Protox Genes Resistant to Protox-Inhibitory Herbicides

Example 9: Selecting for Plant Protox Genes Resistant to Protox-Inhibitory Herbicides in the E. coli Expression System

An *Arabidopsis thaliana* (Landsberg) cDNA library in the plasmid vector pFL61 (Minet *et al., Plant J.* 2:417-422 (1992) was obtained and amplified. The *E. coli hemG* mutant SASX38 (Sasarman *et al., J. Gen. Microbiol.* 113:297(1979)) was obtained and maintained on L media containing 20ug/ml hematin (United States Biochemicals). The plasmid library was transformed into SASX38 by electroporation using the Bio-Rad Gene Pulser and the manufacturer's conditions. The electroporated cells were plated on L agar containing 100ug/ml ampicillin at a density of approximately 500,000 transformants/10cm plate. The cells were then incubated at 37°C for 40 hours in low light and selected for the ability to grow without the addition of exogenous heme. Heme prototrophs were recovered at a frequency of $400/10^7$ from the pFL61 library. Sequence analysis of twenty-two complementing clones showed that nine are of the type designated "Protox-1," the protox gene expected to express a chloroplastic protox enzyme.

The pFL61 library is a yeast expression library, with the *Arabidopsis* cDNAs inserted bidirectionally. These cDNAs can also be expressed in bacteria. The protox cDNAs apparently initiate at an in-frame ATG in the yeast PGK 3' sequence approximately 10 amino acids 5' to the NotI cloning site in the vector and are expressed either from the lacZ promoter 300bp further upstream or from an undefined cryptic bacterial promoter. Because Protox-1 cDNAs that included significant portions of a chloroplast transit sequence inhibited the growth of the *E. coli* SASX38 strain, the clone with the least amount of chloroplast transit sequence attached was chosen for mutagenesis/herbicide selection experiments. This clone, pSLV19, contains only 17 amino acids of the putative chloroplast transit peptide, with the DNA sequence beginning at bp 151 of the *Arabidopsis* Protox-1 cDNA (SEQ ID NO:1).

The plasmid pSLV19 was transformed into the random mutagenesis strain XL1-Red (Stratagene, La Jolla, CA). The transformation was plated on L media containing 50ug/ml ampicillin and incubated for 48 hours at 37° C. Lawns of transformed cells were scraped from the plates and plasmid DNA prepared using the Wizard Megaprep kit (Promega, Madison, WI). Plasmid DNA isolated from this mutator strain is predicted to contain approximately one random base change per 2000 nucleotides (see Greener *et al.*, *Strategies* 7(2):32-34 (1994).

The mutated plasmid DNA was transformed into the *hemG* mutant SASX38 (Sasarman *et al.*, *J. Gen. Microbiol.* 113:297 (1979) and plated on L media containing various concentrations of protox-inhibiting herbicide. The plates were incubated for 2 days at 37° C. Plasmid DNA was isolated from all colonies that grew in the presence of herbicide concentrations that effectively killed the wild type strain. The isolated DNA was then transformed into SASX38 and plated again on herbicide to ensure that the resistance observed was plasmid-borne. The protox coding sequence from plasmids passing this screen was excised by NotI digestion, recloned into an unmutagenized vector, and tested again for the ability to confer herbicide tolerance. The DNA sequence of protox cDNAs that conferred herbicide resistance was then determined and mutations identified by comparison with the wild type *Arabidopsis* Protox-1 sequence (SEQ ID NO:1).

A single coding sequence mutant was recovered from the first mutagenesis experiment. This mutant leads to enhanced herbicide "resistance" only by increasing growth rate. It contains a C to A mutation at nucleotide 197 in SEQ ID NO:1 in the truncated chloroplast transit sequence of pSLV19, converting an ACG codon for threonine to an AAG codon for lysine at amino acid 56 of SEQ ID NO:2, and resulting in better complementation of the bacterial mutant. This plasmid also contains a silent coding sequence mutation at nucleotide 1059, with AGT (Ser) changing to AGC (Ser). This plasmid was designated pMut-1.

The pMut-1 plasmid was then transformed into the mutator XL1-Red strain as described above and the mutated DNA was isolated and plated on an herbicide concentration that is lethal to the unmutagenized pMut-1 protox gene. Herbicide tolerant colonies were isolated after two days at 37° C and analyzed as described above. Multiple plasmids were shown to contain herbicide resistant protox coding sequences. Sequence analysis indicated that the resistant genes fell into two classes. One resistance mutation identified was a C to T change at nucleotide 689 in the *Arabidopsis* Protox-1 sequence set forth in SEQ ID NO:1. This change converts a GCT codon for alanine at amino acid 220 of SEQ ID NO:2 to a GTT codon for valine, and was designated pAraC-1Val.

A second class of herbicide resistant mutant contains an A to G change at nucleotide 1307 in the *Arabidopsis* Protox-1 sequence. This change converts a TAC codon for tyrosine at amino acid 426 to a TGC codon for cysteine, and was designated pAraC-2Cys.

A third resistant mutant has a G to A change at nucleotide 691 in the *Arabidopsis* Protox-1 sequence. This mutation converts a GGT codon for glycine at amino acid 221 to an AGT codon for serine at the codon position adjacent to the mutation in pAraC-1. This plasmid was designated pAraC-3Ser.

Resistant mutant pAraC-2Cys, in the pMut-1 plasmid, was deposited on November 14, 1994 under the designation pWDC-7 with the Agricultural Research Culture Collection and given the deposit designation NRRL #21339N.

Example 10: Additional Herbicide-Resistant Codon Substitutions at Positions Identified in the Random Screen

The amino acids identified as herbicide resistance sites in the random screen are replaced by other amino acids and tested for function and for herbicide tolerance in the bacterial system. Oligonucleotide-directed mutagenesis of the *Arabidopsis* Protox-1 sequence is performed using the Transformer Site-Directed Mutagenesis Kit (Clontech, Palo Alto, CA). After amino acid changes are confirmed by sequence analysis, the mutated plasmids are transformed into SASX38 and plated on L-amp^{100} media to test for function and on various concentrations of protox-inhibiting herbicide to test for tolerance.

This procedure is applied to the alanine codon at nucleotides 688-690 and to the tyrosine codon at nucleotides 1306-1308 of the *Arabidopsis* Protox-1 sequence (SEQ ID NO:1). The results demonstrate that the alanine codon at nucleotides 688-690 can be changed to a codon for valine, threonine, leucine, cysteine, or isoleucine to yield an herbicide-resistant protox enzyme that retains function. The results further demonstrate that the tyrosine codon at nucleotides 1306-1308 can be changed to a codon for cysteine, isoleucine, leucine, threonine, methionine, valine, or alanine to yield an herbicide-resistant protox enzyme that retains function.

Example 11: Isolation of Additional Mutations that Increase Enzyme Function and/or Herbicide Tolerance of Previously Identified Resistant Mutants

Plasmids containing herbicide resistant protox genes are transformed into the mutator strain XL1-Red and mutated DNA is isolated as described above. The mutated plasmids are transformed into SASX38 and the transformants are screened on herbicide concentrations sufficient to inhibit growth of the original "resistant" mutant. Tolerant colonies are isolated and the higher tolerance phenotype is verified as being coding sequence dependent as described above. The sequence of these mutants is determined and mutations identified by comparison to the progenitor sequence.

This procedure was applied to the pAraC-1Val mutant described above. The results demonstrate that the serine codon at amino acid 305 (SEQ ID NO:2) can be changed to a codon for leucine to yield an enzyme with higher tolerance to protox-inhibiting herbicides than the pAraC-1Val mutant alone. This second site mutation is designated AraC305Leu. The same results are demonstrated for the threonine codon at amino acid 249, where a change to either isoleucine or to alanine leads to a more tolerant enzyme . These changes are designated AraC249Ile and AraC249Ala, respectively.

The procedure was also applied to the pAraC-2Cys mutant described above. The results demonstrate that the proline codon at amino acid 118 (SEQ ID NO:2) can be changed to a codon for leucine to yield an enzyme with higher tolerance to protox-inhibiting herbicides than the pAraC-1Cys mutant alone. This mutation is designated AraC118Leu. The same results are demonstrated for the serine codon at amino acid 305, where a change to leucine leads to a more tolerant pAraC-2Cys enzyme. This change was also isolated with the pAraC-1Val mutant as described above and is designated AraC305Leu. Additional mutations that enhance the herbicide resistance of the pAraC-2Cys mutant include an asparagine to serine change at amino acid 425, designated AraC425Ser, and a tyrosine to cysteine at amino acid 498, designated AraC498Cys.

These changes are referred to as "second site" mutations, because they are not sufficient to confer herbicide tolerance alone, but rather enhance the function and/or the herbicide tolerance of an already mutant enzyme. This does not preclude the possibility that other amino acid substitutions at these sites could suffice to produce an herbicide tolerant enzyme since exhaustive testing of all possible replacements has not been performed.

Example 12: Combining Identified Resistance Mutations with Identified Second Site Mutations to Create Highly Functional/Highly Tolerant Protox Enzymes

The AraC305Leu mutation described above was found to enhance the function/herbicide resistance of both the AraC-1Val and the AraC-2Cys mutant plasmids. In an effort to test the general usefulness of this second site mutation, it was combined with the AraC-2Leu, AraC-2Val, and AraC-2Ile mutations and tested for herbicide tolerance. In each case, the AraC305Leu change significantly increased the growth rate of the resistant protox mutant on protox-inhibiting herbicide. Combinations of the AraC-2Ile resistant mutant with either the second site mutant AraC249Ile or AraC118Leu also produced more highly tolerant mutant protox enzymes. The AraC249Ile mutation demonstrates that a second site mutation identified as enhancing an AraC-1 mutant may also increase the resistance of an AraC-2 mutant. A three mutation plasmid containing AraC-2Ile, AraC305Leu, and AraC249Ile has also been shown to produce a highly functional, highly herbicide tolerant protox-1 enzyme.

Example 13: Identification of Sites in the Maize Protox-1 Gene that can be Mutated to Give Herbicide Tolerance

The pMut-1 *Arabidopsis* Protox -1 plasmid described above is very effective when used in mutagenesis/screening experiments in that it gives a high frequency of genuine coding sequence mutants, as opposed to the frequent up-promoter mutants that are isolated when other plasmids are used. In an effort to create an efficient plasmid screening system for maize Protox-1, the maize cDNA was engineered into the pMut-1 vector in approximately the same sequence context as the *Arabidopsis* cDNA. Using standard methods of overlapping PCR fusion, the 5' end of the pMut-1 *Arabidopsis* clone (including 17 amino acids of chloroplast transit peptide with one mis-sense mutation as described above) was fused to the maize Protox-1 cDNA sequence starting at amino acid number 14 (SEQ ID NO:6) of the maize sequence. The 3' end of the maize cDNA was unchanged. NotI restriction sites were placed on both ends of this fusion, and the chimeric gene was cloned into the pFL61 plasmid backbone from pMut-1. Sequence analysis revealed a single nucleotide PCR-derived silent mutation that converts the ACG codon at nucleotides 745-747 (SEQ ID NO:5) to an ACT codon, both of which encode threonine. This chimeric Arab-maize Protox-1 plasmid is designated pMut-3.

The pMut-3 plasmid was transformed into the mutator XL1-Red strain as described above and the mutated DNA was isolated and plated on an herbicide concentration that was lethal to the unmutagenized pMut-3 maize protox gene. Herbicide tolerant colonies were isolated after two days at 37° C and analyzed as described above. This analysis revealed multiple plasmids containing herbicide resistant protox coding sequences. Sequence analysis showed 5 single base changes that individually result in an herbicide tolerant maize Protox-1 enzyme. Three of these mutations correspond to amino acid changes previously shown to confer tolerance at the homologous position in the *Arabidopsis* Protox-1 gene. Two of the three are pMzC-1Val and pMzC-1Thr, converting the alanine (GCT) at amino acid 164 (SEQ ID NO:6) to either valine (GAT) or to threonine (ACT). This position corresponds to the pAraC-1 mutations described above. The third analogous change converts the glycine (GGT) at amino acid 165 to Serine (AGT), corresponding to the AraC-3Ser mutation described above. These results serve to validate the expectation that herbicide-tolerant mutations identified in one plant protox gene may also confer herbicide tolerance in an equivalent plant protox gene from another species.

Two of the mutations isolated from the maize Protox-1 screen result in amino acid changes at residues not previously identified as herbicide resistance sites. One change converts cysteine (TGC) to phenylalanine (TTC) at amino acid 159 of the maize Protox-1 sequence (SEQ ID NO:6). The second converts isoleucine (ATA) to threonine (ACA) at amino acid 419.

Additional amino acid substitutions were made and tested at three of the maize mutant sites. Tolerance was demonstrated when glycine 165 was changed to leucine or when cysteine 159 was changed to either leucine or to lysine. Tolerant enzymes were also created by changing isoleucine 419 to histidine, glycine, or asparagine.

Individual amino acid changes that produced highly herbicide tolerant *Arabidopsis* Protox-1 enzymes were engineered into the maize Protox-1 gene by site-directed mutagenesis as described above. Bacterial testing demonstrated that changing the alanine (GCT) at amino acid 164 (SEQ ID NO:6) to leucine (CTT) produced a highly tolerant maize enzyme. No mutation analogous to the AraC-2 site in *Arabidopsis* was isolated in the maize random screen. However, changing this site, tyrosine 370 in the maize enzyme (SEQ ID NO:6), to either isoleucine or methionine did produce an herbicide tolerant enzyme.

Example 14: Identification of Sites in the Wheat Protox-1 Gene that can be Mutated to Give Herbicide Tolerance

To create an efficient plasmid screening system for wheat Protox-1, the wheat cDNA was engineered into the pMut-1 vector as described above for the maize cDNA. This chimeric Arab-wheat Protox-1 plasmid is designated pMut-4. The pMut-4 DNA was mutated and screened for herbicide tolerance as described above. This analysis revealed multiple plasmids containing herbicide resistant protox coding sequences. Sequence analysis showed 7 single base changes that individually result in an herbicide tolerant wheat Protox-1 enzyme. Four of these mutations correspond to amino acid changes previously shown to confer tolerance at the homologous position in the *Arabidopsis* and/or in the maize Protox-1 gene. Two convert the alanine (GCT) at amino acid 211 (SEQ ID NO:10) to either valine (GAT) or to threonine (ACT). This position corresponds to the pAraC-1 mutations described above. The third analogous change converts the glycine (GGT) at amino acid 212 to Serine (AGT), corresponding to the AraC-3Ser mutation described above. The fourth converts isoleucine (ATA) to threonine (ACA) at amino acid 466, corresponding to the Mz419Thr mutant from maize.

Three of the mutations isolated from the wheat Protox-1 screen result in amino acid changes at residues not previously identified as herbicide resistance sites. One change converts valine (GTT) to leucine (CTT) at amino acid 356 of the wheat Protox-1 sequence (SEQ ID NO:10). A second converts serine (TCT) to proline (CCT) at amino acid 421. The third converts valine (GTT) to alanine (GCT) at amino acid 502.

Example 15: Identification of Sites in the Soybean Protox-1 Gene that can be Mutated to Give Herbicide Tolerance

To create an efficient plasmid screening system for soybean Protox-1, the soybean cDNA was engineered into the pMut-1 vector as described above for the maize cDNA. This chimeric Arab-soybean Protox-1 plasmid is designated pMut-5. The pMut-5 DNA was mutated and screened for herbicide tolerance as described above. This analysis revealed multiple plasmids containing herbicide resistant protox coding sequences. Sequence analysis showed 4 single base changes that individually result in an herbicide tolerant soybean Protox-1 enzyme. Two of these mutations correspond to amino acid changes previously shown to confer tolerance at the homologous position in the *Arabidopsis* and/or in the wheat Protox-1 gene. One converts the alanine (GCA) at amino acid 226 (SEQ ID NO:12) to threonine (ACA). This position corresponds to the pAraC-1Thr mutation described above. The second analogous change converts the valine (GTT) at amino acid 517 to alanine (GCT), corresponding to the Wht502Val mutation from wheat.

Two of the mutations isolated from the soybean Protox-1 screen result in amino acid changes at a residue not previously identified as an herbicide resistance site. One change converts proline (CCT) to serine (TCT) at amino acid 369 of the soybean Protox-1 sequence (SEQ ID NO:12). A second converts this same proline369 to histidine (CAT).

Individual amino acid changes that produced highly herbicide tolerant *Arabidopsis* Protox-1 enzymes were engineered into the soybean Protox-1 gene by site directed mutagenesis as described above. Bacterial testing demonstrated that changing the alanine (GCA) at amino acid 226 (SEQ ID NO:12) to leucine produced a tolerant soybean enzyme. Changing the tyrosine (TAC) at amino acid 432 (SEQ ID NO:12) to either leucine or isoleucine also produced an herbicide tolerant enzyme.

Example 16: Identification of Sites in the Sugar Beet Protox-1 Gene that can be Mutated to Give Herbicide Tolerance

To create an efficient plasmid screening system for sugar beet Protox-1, the sugar beet cDNA was engineered into the pMut-1 vector as described above for the maize cDNA. This chimeric Arab-sugar beet Protox-1 plasmid is designated pMut-6. The pMut-6 DNA was mutated and screened for herbicide tolerance as described above. This analysis revealed multiple plasmids containing herbicide resistant protox coding sequences. Sequence analysis showed a single base change that results in an herbicide tolerant sugar beet Protox-1 enzyme. This change converts tyrosine (TAC) at amino acid 449 to cysteine (TGC) and is analogous to the AraC-2 mutation in *Arabidopsis*.

Individual amino acid changes that produced highly herbicide tolerant *Arabidopsis* Protox-1 enzymes were engineered into the sugar beet Protox-1 gene by site directed mutagenesis as described above. Bacterial testing demonstrated that changing the tyrosine (TAC) at amino acid 449 to either leucine, isoleucine, valine, or methionine produced an herbicide tolerant sugar beet enzyme.

Example 17: Identification of Sites in the Cotton Protox-1 Gene that can be Mutated to Give Herbicide Tolerance

In an effort to create an efficient plasmid screening system for cotton Protox-1, the cotton cDNA was engineered into the pMut-1 vector as described above for the maize cDNA. This chimeric Arab-cotton Protox-1 plasmid is designated pMut-7. The pMut-7 DNA was mutated and screened for herbicide tolerance as described above. This analysis revealed multiple plasmids containing herbicide resistant protox coding sequences. Sequence analysis showed 3 single base changes that individually result in an herbicide tolerant cotton Protox-1 enzyme. Two mutants change tyrosine (TAC) at amino acid 428 (SEQ ID NO:16) to cysteine (TGC) and to arginine (CGC), respectively. Arginine is a novel substitution giving tolerance at this previously identified AraC-2 site. The third mutation converts proline (CCC) to serine (TCC) at amino acid 365. This change corresponds to the soybean mutant Soy369Ser.

Example 18: Demonstration of Resistant Mutations' Cross-Tolerance to Various Protox-Inhibiting Compounds

Resistant mutant plasmids, originally identified based on resistance against a single protox inhibitory herbicide, were tested against a spectrum of other protox inhibiting compounds. For this test, the SASX38 strain containing the wild-type plasmid is plated on a range of concentrations of each compound to determine the lethal concentration for each one. Resistant mutant plasmids in SASX38 are plated and scored for the ability to survive on a concentration of each compound at least 10 fold higher than the concentration that is lethal to the SASX38 strain containing the wild-type plasmid.

Results from bacterial cross-tolerance testing, illustrated in Tables 3A and 3B below, show that each of the mutations identified confers tolerance to a variety of protox inhibiting compounds.

Table 3A

Cross Tolerance of Plant Protox Mutants to Various Protox Inhibitors

Formula	AraC-1Val	AraC-2Cys	AraC-1Thr	AraC-3Thr	MzC-1Val
XVII	+	+	+	+	+
VIIa	+	+	+	-	+
IV	++	-	++	++	-
XV	+	+	+	+	+
XI	-	+	+	++	+
XVI	-	-	-	-	+
XII	+	-	++	++	++
XIV	+	-	+	+	+
*X					

+ = 10X or more tolerant than WT

++ = 100X or more tolerant than WT

- = no cross tolerance

* = this compound was tested but provided no information

Table 3B

Cross Tolerance of Plant Protox Mutants to Various Protox Inhibitors

	AraC-1Leu	AraC-2Ile	AraC-1Leu + AraC-2Met	AraC-1Leu + AraC-2Leu	AraC-2Ile + AraC305Leu	AraC-2Cys + AraC425 Ser	AraC-2Leu + AraC425 Ser	AraC-2Met + AraC425 Ser
XVII	+	+	+	+	+	+	+	+
VIIa	++	++	++	++	++	++	++	++
IV	++	-	+	++	+	-	+	+
XV	++	+++	+++	+++	+++	++	+++	++
XI	++	++	++	++	++	++	++	++
XVI	+++	+++	+++	+++	+++	+	++	++
XII								
XIV	++	++	++	++	++	-	++	++

Section C: Expression of Herbicide-Resistant Protox Genes in Transgenic Plants

Example 19: Engineering of plants tolerant to protox-inhibiting herbicides by homologous recombination or gene conversion

Because the described mutant coding sequences effectively confer herbicide tolerance when expressed under the control of the native protox promoter, targeted changes to the protox coding sequence in its native chromosomal location represent an alternative means for generating herbicide tolerant plants and plant cells. A fragment of protox DNA containing the desired mutations, but lacking its own expression signals (either promoter or 3' untranslated region) can be introduced by any of several art-recognized methods (for instance, Agrobacterium transformation, direct gene transfer to protoplasts, microprojectile bombardment), and herbicide-tolerant transformants selected. The introduced DNA fragment also contains a diagnostic restriction enzyme site or other sequence polymorphism that is introduced by site-directed mutagenesis in vitro without changing the encoded amino acid sequence (i.e. a silent mutation). As has been previously reported for various selectable marker and herbicide tolerance genes (see, e.g., Paszkowski et al., EMBO J. 7: 4021-4026 (1988); Lee et al., Plant Cell 2: 415-425 (1990); Risseeuw et al., Plant J. 7: 109-119 (1995)). some transformants are found to result from homologous integration of the mutant DNA into the protox chromosomal locus, or from conversion of the native protox chromosomal sequence to the introduced mutant sequence. These transformants are recognized by the combination of their herbicide-tolerant phenotype, and the presence of the diagnostic restriction enzyme site in their protox chromosomal locus.

Example 20: Construction of Plant Transformation Vectors

Numerous transformation vectors are available for plant transformation, and the genes of this invention can be used in conjunction with any such vectors. The selection of vector for use will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the *nptII* gene, which confers resistance to kanamycin and related antibiotics (Messing & Vierra, *Gene 19:* 259-268 (1982); Bevan *et al., Nature 304:*184-187 (1983)), the *bar* gene, which confers resistance to the herbicide phosphinothricin (White *et al., Nucl Acids Res 18:* 1062 (1990), Spencer *et al.* Theor Appl Genet 79: 625-631(1990)), the *hph* gene, which confers resistance to the antibiotic hygromycin (Blochinger & Diggelmann, *Mol Cell Biol 4:* 2929-2931), and the *dhfr* gene, which confers resistance to methotrexate (Bourouis *et al.*, *EMBO J. 2(7):* 1099-1104 (1983)).

I. Construction of Vectors Suitable for *Agrobacterium* Transformation

Many vectors are available for transformation using *Agrobacterium tumefaciens.* These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, *Nucl. Acids Res.* (1984)) and pXYZ. Below the construction of two typical vectors is described.

Construction of pCIB200 and pCIB2001: The binary vectors pCIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and was constructed in the following manner. pTJS75kan was created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, *J Bacteriol. 164*: 446-455 (1985)) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII (Messing & Vierra, *Gene 19*: 259-268 (1982); Bevan *et al.*, *Nature 304*: 184-187 (1983); McBride *et al.*, *Plant Molecular Biology 14*: 266-276 (1990)). *XhoI* linkers were ligated to the *EcoRV* fragment of pCIB7, which contains the left and right T-DNA borders, a plant selectable *nos/nptII* chimeric gene and the pUC polylinker (Rothstein *et al.*, *Gene 53:* 153-161 (1987)), and the *XhoI*-digested fragment was cloned into *SalI*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19). pCIB200 contains the following unique polylinker restriction sites: *EcoRI, SstI, KpnI, BglII, XbaI,* and *SalI. pCIB2001* is a derivative of pCIB200, which is created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of pCIB2001 are *EcoRI, SstI, KpnI, BglII, XbaI, SalI, MluI, BclI, AvrII, ApaI, HpaI,* and *StuI.* pCIB2001, in addition to containing these unique restriction sites also has plant and bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated transformation, the RK2-derived *trfA* function for mobilization between *E. coli* and other hosts, and the *OriT* and *OriV* functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

Construction of pCIB10 and Hygromycin Selection Derivatives Thereof: The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants, T-DNA right and left border sequences and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium*. Its construction is described by Rothstein *et al., Gene 53*: 153-161 (1987). Various derivatives of pCIB10 have been constructed that incorporate the gene for hygromycin B phosphotransferase described by Gritz *et al., Gene 25:* 179-188 (1983)). These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).

II. Construction of Vectors Suitable for non-*Agrobacterium* Transformation.

Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above that contain T-DNA sequences. Transformation techniques that do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (*e.g.* PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Below, the construction of some typical vectors is described.

Construction of pCIB3064: pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide basta (or phosphinothricin). The plasmid pCIB246 comprises the CaMV 35S promoter in operational fusion to the *E. coli* GUS gene and the CaMV 35S transcriptional terminator and is described in the PCT published application WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites were mutated using standard PCR techniques in such a way as to remove the ATG's and generate the restriction sites *SspI* and *PvuII*. The new restriction sites were 96 and 37 bp away from the unique *SalI* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 was designated pCIB3025. The GUS gene was then excised from pCIB3025 by digestion with *SalI* and *SacI*, the termini rendered blunt and religated to generate plasmid pCIB3060. The plasmid pJIT82 was obtained from the John Innes Centre, Norwich and the a 400 bp *SmaI* fragment containing the *bar* gene from *Streptomyces viridochromogenes* was excised and inserted into the *HpaI* site of pCIB3060 (Thompson *et al.* EMBO J 6: 2519-2523 (1987)). This generated pCIB3064, which comprises the *bar* gene under the control of the CaMV 35S promoter and terminator for herbicide selection, a gene for ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *SphI, PstI, HindIII,* and *BamHI*. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

Construction of pSOG19 and pSOG35: pSOG35 is a transformation vector that utilizes the *E. coli* gene dihydrofolate reductase (DHFR) as a selectable marker conferring resistance to methotrexate. PCR was used to amplify the 35S promoter (~800 bp), intron 6 from the maize Adh1 gene (~550 bp) and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250 bp fragment encoding the *E. coli* dihydrofolate reductase type II gene was also amplified by PCR and these two PCR fragments were assembled with a *SacI-PstI* fragment from pBI221 (Clontech), which comprised the pUC19 vector backbone and the nopaline synthase terminator. Assembly of these fragments generated pSOG19, which contains the 35S promoter in fusion with the intron 6 sequence, the GUS leader, the DHFR gene and the nopaline synthase terminator. Replacement of the GUS leader in pSOG19 with the leader sequence from Maize Chlorotic Mottle Virus (MCMV) generated the vector pSOG35. pSOG19 and pSOG35 carry the pUC gene for ampicillin resistance and have *HindIII, SphI, PstI* and *EcoRI* sites available for the cloning of foreign sequences.

Example 21: Construction of Plant Expression Cassettes

Gene sequences intended for expression in transgenic plants are firstly assembled in expression cassettes behind a suitable promoter and upstream of a suitable transcription terminator. These expression cassettes can then be easily transferred to the plant transformation vectors described above in Example 20.

I. Promoter Selection

The selection of a promoter used in expression cassettes will determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters will express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and this selection will reflect the desired location of expression of the transgene. Alternatively, the selected promoter may drive expression of the gene under a light-induced or other temporally regulated promoter. A further alternative is that the selected promoter be chemically regulated. This would provide the possibility of inducing expression of the transgene only when desired and caused by treatment with a chemical inducer.

II. Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators are those that are known to function in plants and include the CaMV 35S terminator, the *tml* terminator, the nopaline synthase terminator, the pea *rbcS* E9 terminator, as well as terminators naturally associated with the plant protox gene (i.e. "protox terminators"). These can be used in both monocotyledons and dicotyledons.

III. Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adh1* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*, Genes Develop. 1: 1183-1200 (1987)). In the same experimental system, the intron from the maize *bronze1* gene had a similar effect in enhancing expression (Callis *et al., supra*). Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells. Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (*e.g.* Gallie *et al. Nucl. Acids Res. 15*: 8693-8711 (1987); Skuzeski *et al.* Plant Molec. Biol. 15: 65-79 (1990))

IV. Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence that is found at the amino terminal end of various proteins and that is cleaved during chloroplast import yielding the mature protein (*e.g.* Comai *et al.* J. *Biol. Chem. 263*: 15104-15109 (1988)). These signal sequences can be fused to heterologous gene products to effect the import of heterologous products into the chloroplast (van den Broeck *et al. Nature 313*: 358-363 (1985)). DNA encoding for appropriate signal sequences can be isolated from the 5' end of the cDNAs encoding the RUBISCO protein, the CAB protein, the EPSP synthase enzyme, the GS2 protein and many other proteins that are known to be chloroplast localized.

Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (*e.g.* Unger *et al. Plant Molec. Biol. 13*: 411-418 (1989)). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles. Examples of such sequences are the nuclear-encoded ATPases and specific aspartate amino transferase isoforms for mitochondria. Targeting to cellular protein bodies has been described by Rogers *et al., Proc. Natl. Acad. Sci. USA 82*: 6512-6516 (1985)).

In addition, sequences have been characterized that cause the targeting of gene products to other cell compartments. Amino terminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, *Plant Cell 2:* 769-783 (1990)). Additionally, amino terminal sequences in conjunction with carboxy terminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al., Plant Molec. Biol. 14*: 357-368 (1990)).

By the fusion of the appropriate targeting sequences described above to transgene sequences of interest it is possible to direct the transgene product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in frame to the amino terminal ATG of the transgene. The signal sequence selected should include the known cleavage site and the fusion constructed should take into account any amino acids after the cleavage site that are required for cleavage. In some cases this requirement may be fulfilled by the addition of a small number of amino acids between the cleavage site and the transgene ATG or alternatively replacement of some amino acids within the transgene sequence. Fusions constructed for chloroplast import can be tested for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions followed by *in vitro* chloroplast uptake using techniques described by (Bartlett *et al.* In: Edelmann *et al.* (Eds.) Methods in Chloroplast Molecular Biology, Elsevier. pp. 1081-1091 (1982); Wasmann *et al. Mol. Gen. Genet. 205*: 446-453 (1986)). These construction techniques are well known in the art and are equally applicable to mitochondria and peroxisomes. The choice of targeting that may be required for expression of the transgenes will depend on the cellular localization of the precursor required as the starting point for a given pathway. This will usually be cytosolic or chloroplastic, although it may is some cases be mitochondrial or peroxisomal. The products of transgene expression will not normally require targeting to the ER, the apoplast or the vacuole.

The above described mechanisms for cellular targeting can be utilized not only in conjunction with their cognate promoters, but also in conjunction with heterologous promoters so as to effect a specific cell targeting goal under the transcriptional regulation of a promoter that has an expression pattern different to that of the promoter from which the targeting signal derives.

Example 22: Transformation of Dicotyledons

Transformation techniques for dicotyledons are well known in the art and include *Agrobacterium*-based techniques and techniques that do not require *Agrobacterium*. Non-*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This can be accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are described by Paszkowski *et al., EMBO J 3:* 2717-2722 (1984), Potrykus *et al., Mol. Gen. Genet. 199:* 169-177 (1985), Reich *et al., Biotechnology 4:* 1001-1004 (1986), and Klein *et al., Nature 327*: 70-73 (1987). In each case the transformed cells are regenerated to whole plants using standard techniques known in the art.

Agrobacterium-mediated transformation is a preferred technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. The many crop species that are routinely transformable by *Agrobacterium* include tobacco, tomato, sunflower, cotton, oilseed rape, potato, soybean, alfalfa and poplar (EP 0 317 511 (cotton), EP 0 249 432 (tomato, to Calgene), WO 87/07299 (*Brassica*, to Calgene), US 4,795,855 (poplar)).

Transformation of the target plant species by recombinant *Agrobacterium* usually involves co-cultivation of the *Agrobacterium* with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.

Example 23: Transformation of Monocotyledons

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, and particle bombardment into callus tissue. Transformations can be undertaken with a single DNA species or multiple DNA species (*i.e.* co-transformation) and both these techniques are suitable for use with this invention. Co-transformation may have the advantage of avoiding complex vector construction and of generating transgenic plants with unlinked loci for the gene of interest and the selectable marker, enabling the removal of the selectable marker in subsequent generations, should this be regarded desirable. However, a disadvantage of the use of co-transformation is the less than 100% frequency with which separate DNA species are integrated into the genome (Schocher *et al. Biotechnology 4:* 1093-1096 (1986)).

Patent Applications EP 0 292 435 (to Ciba-Geigy), EP 0 392 225 (to Ciba-Geigy) and WO 93/07278 (to Ciba-Geigy) describe techniques for the preparation of callus and protoplasts from an élite inbred line of maize, transformation of protoplasts using PEG or electroporation, and the regeneration of maize plants from transformed protoplasts. Gordon-Kamm *et al., Plant Cell 2:* 603-618 (1990)) and Fromm *et al., Biotechnology 8:* 833-839 (1990)) have published techniques for transformation of A188-derived maize line using particle bombardment. Furthermore, application WO 93/07278 (to Ciba-Geigy) and Koziel *et al., Biotechnology 11:* 194-200 (1993)) describe techniques for the transformation of élite inbred lines of maize by particle bombardment. This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days after pollination and a PDS-1000He Biolistics device for bombardment.

Transformation of rice can also be undertaken by direct gene transfer techniques utilizing protoplasts or particle bombardment. Protoplast-mediated transformation has been described for *Japonica*-types and *Indica*-types (Zhang *et al.*, *Plant Cell Rep 7*: 379-384 (1988); Shimamoto *et al. Nature 338*: 274-277 (1989); Datta *et al. Biotechnology 8:* 736-740 (1990)). Both types are also routinely transformable using particle bombardment (Christou *et al. Biotechnology 9:* 957-962 (1991)).

Patent Application EP 0 332 581 (to Ciba-Geigy) describes techniques for the generation, transformation and regeneration of Pooideae protoplasts. These techniques allow the transformation of *Dactylis* and wheat. Furthermore, wheat transformation was been described by Vasil *et al., Biotechnology 10:* 667-674 (1992)) using particle bombardment into cells of type C long-term regenerable callus, and also by Vasil *et al., Biotechnology 11:* 1553-1558 (1993)) and Weeks *et al., Plant Physiol. 102*: 1077-1084 (1993) using particle bombardment of immature embryos and immature embryo-derived callus. A preferred technique for wheat transformation, however, involves the transformation of wheat by particle bombardment of immature embryos and includes either a high sucrose or a high maltose step prior to gene delivery. Prior to bombardment, any number of embryos (0.75-1 mm in length) are plated onto MS medium with 3% sucrose (Murashige & Skoog, *Physiologia Plantarum 15*: 473-497 (1962)) and 3 mg/l 2,4-D for induction of somatic embryos, which is allowed to proceed in the dark. On the chosen day of bombardment, embryos are removed from the induction medium and placed onto the osmoticum (*i.e.* induction medium with sucrose or maltose added at the desired concentration, typically 15%). The embryos are allowed to plasmolyze for 2-3 h and are then bombarded. Twenty embryos per target plate is typical, although not critical. An appropriate gene-carrying plasmid (such as pCIB3064 or pSG35) is precipitated onto micrometer size gold particles using standard procedures. Each plate of embryos is shot with the DuPont Biolistics› helium device using a burst pressure of ~1000 psi using a standard 80 mesh screen. After bombardment, the embryos are placed back into the dark to recover for about 24 h (still on osmoticum). After 24 hrs, the embryos are removed from the osmoticum and placed back onto induction medium where they stay for about a month before regeneration. Approximately one month later the embryo explants with developing embryogenic callus are transferred to regeneration medium (MS + 1 mg/liter NAA, 5 mg/liter GA), further containing the appropriate selection agent (10 mg/l basta in the case of pCIB3064 and 2 mg/l methotrexate in the case of pSOG35). After approximately one month, developed shoots are transferred to larger sterile containers known as "GA7s" that contained half-strength MS, 2% sucrose, and the same concentration of selection agent. Patent application WO 94/13822 describes methods for wheat transformation and is hereby incorporated by reference.

Example 24: Isolation of the *Arabidopsis thaliana* Protox-1 Promoter Sequence

A Lambda Zap II genomic DNA library prepared from *Arabidopsis thaliana* (Columbia, whole plant) was purchased from Stratagene. Approximately 125,000 phage were plated at a density of 25,000 pfu per 15 cm Petri dish and duplicate lifts were made onto Colony/Plaque Screen membranes (NEN Dupont). The plaque lifts were probed with the *Arabidopsis* Protox-1 cDNA (SEQ ID NO:1 labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization and wash conditions were at 65°C as described in Church and Gilbert, *Proc. Natl. Acad. Sci.* USA 81: 1991-1995 (1984). Positively hybridizing plaques were purified and in vivo excised into pBluescript plasmids. Sequence from the genomic DNA inserts was determined by the chain termination method using dideoxy terminators labeled with fluorescent dyes (Applied Biosystems, Inc.). One clone, AraPT1Pro, was determined to contain 580 bp of *Arabidopsis* sequence upstream from the initiating methionine (ATG) of the Protox-1 protein coding sequence. This clone also contains coding sequence and introns that extend to bp 1241 of the Protox-1 cDNA sequence. The 580 bp 5' noncoding fragment is the putative *Arabidopsis* Protox-1 promoter, and the sequence is set forth in SEQ ID NO:13.

AraPT1Pro was deposited December 15, 1995, as pWDC-11 (NRRL #B-21515)

Example 25: Construction of Plant Transformation Vectors Expressing Altered Protox-1 Genes Behind the Native *Arabidopsis* Protox-1 Promoter

A full-length cDNA of the appropriate altered *Arabidopsis* Protox-1 cDNA was isolated as an EcoRI-XhoI partial digest fragment and cloned into the plant expression vector pCGN1761ENX (see Example 9 of International application no. PCT/IB95/00452 filed June 8, 1995, published Dec. 21, 1995 as WO 95/34659). This plasmid was digested with NcoI and BamHI to produce a fragment comprised of the complete Protox-1 cDNA plus a transcription terminator from the 3' untranslated sequence of the tml gene of *Agrobacterium tumefaciens*. The AraPT1Pro plasmid described above was digested with NcoI and BamHI to produce a fragment comprised of pBluescript and the 580 bp putative *Arabidopsis* Protox-1 promoter. Ligation of these two fragments produced a fusion of the altered protox cDNA to the native protox promoter. The expression cassette containing the Protox-1 promoter/Protox-1 cDNA/tml terminator fusion was excised by digestion with KpnI and cloned into the binary vector pCIB200. The binary plasmid was transformed by electroporation into Agrobacterium and then into *Arabidopsis* using the vacuum infiltration method (Bechtold *et al., C.R. Acad. Sci. Paris* 316: 1194-1199 (1993). Transformants expressing altered protox genes were selected on kanamycin or on various concentrations of protox inhibiting herbicide.

Example 26: Production of Herbicide Tolerant Plants by Expression of a Native Protox-1 Promoter/Altered Protox-1 Fusion

Using the procedure described above, an *Arabidopsis* Protox-1 cDNA containing a TAC to ATG (Tyrosine to Methionine) change at nucleotides 1306-1308 in the Protox-1 sequence (SEQ ID NO:1) was fused to the native Protox-1 promoter fragment and transformed into *Arabidopsis thaliana*. This altered Protox-1 enzyme (AraC-2Met) has been shown to be >10-fold more tolerant to various protox-inhibiting herbicides than the naturally occurring enzyme when tested in the previously described bacterial expression system. Seed from the vacuum infiltrated plants was collected and plated on a range (10.0nM-1.0uM) of a protox inhibitory aryluracil herbicide of formula XVII. Multiple experiments with wild type *Arabidopsis* have shown that a 10.0nM concentration of this compound is sufficient to prevent normal seedling germination. Transgenic seeds expressing the AraC-2Met altered enzyme fused to the native Protox-1 promoter produced normal *Arabidopsis* seedlings at herbicide concentrations up to 500nM, indicating at least 50-fold higher herbicide tolerance when compared to wild-type *Arabidopsis*. This promoter/altered protox enzyme fusion therefore functions as an effective selectable marker for plant transformation. Several of the plants that germinated on 100.0nM of protox-inhibiting herbicide were transplanted to soil, grown 2-3 weeks, and tested in a spray assay with various concentrations of the protox-inhibiting herbicide. When compared to empty vector control transformants, the AraPT1Pro/AraC-2Met transgenics were >10-fold more tolerant to the herbicide spray.

EXAMPLE 27: Demonstration of resistant mutations' cross-tolerance to various protox-inhibiting compounds in an Arabidopsis germination assay.

Using the procedure described above, an Arabidopsis Protox-1 cDNA containing both a TAC to ATC (tyrosine to isoleucine) change at nucleotides 1306-1308 and a TCA to TTA (serine to leucine) change at nucleotides 945-947 in the Protox-1 sequence (SEQ ID NO:1) was fused to the native Protox-1 promoter fragment and transformed into *Arabidopsis thaliana.* This altered Protox-1 enzyme (AraC-2Ile + AraC305Leu) has been shown to be >10-fold more tolerant to a protox inhibitory aryluracil herbicide of formula XVII than the naturally occurring enzyme when tested in a bacterial system (see Examples 8-12). Homozygous Arabidopsis lines containing this fusion were generated from transformants that showed high tolerance to a protox inhibiting herbicide in a seedling germination assay as described above. The seed from one line was tested for cross-tolerance to various protox-inhibitory compounds by repeating the germination assay on concentrations of the compounds that had been shown to inhibit germination of wild-type Arabidopsis. The results from these experiments are shown in Table 4.

Table 4

Cross Tolerance to Various Protox Inhibitors in a Seed Germination Assay

Formula	Common name	Tolerance
II	acifluorofen	+
III	fomasafen	+
IV	fluoroglycofen	±
IVb	bifenox	+
IVc	oxyfluorofen	+
IVd	lactofen	±
VIIa	fluthiacet-methyl	++
X	sulfentrazone	+
XI	flupropazil	++
XIV	flumiclorac	+

XVI	flumioxazin	+++
XVII		++
XXIa	BAY 11340	+
XXII		++

± ≤ 10X more tolerant than wt

+ ≥ 10X more tolerant than wt

++ ≥ 100X more tolerant than wt

+++ ≥ 1000X more tolerant than wt

Example 28: Isolation of a Maize Protox-1 Promoter Sequence

A Zea Mays (Missouri 17 inbred, etiolated seedlings) genomic DNA library in the Lambda FIX II vector was purchased from Stratagene. Approximately 250,000 pfu of the library was plated at a density of 50,000 phage per 15 cm plate and duplicate lifts were made onto Colony/Plaque screen membranes (NEN Dupont). The plaque lifts were probed with the maize Protox-1 cDNA (SEQ ID NO:5) labeled with 32P-dCTP by the random priming method (Life Technologies). Hybridization and wash conditions were at 65°C as described in Church and Gilbert, *Proc. Natl. Acad. Sci. USA 81:* 1991-1995 (1984). Lambda phage DNA was isolated from three positively hybridizing phage using the Wizard Lambda Preps DNA Purification System (Promega). Analysis by restriction digest, hybridization patterns, and DNA sequence analysis identified a lambda clone containing approximately 3.5 kb of maize genomic DNA located 5' to the maize Protox-1 coding sequence previously isolated as a cDNA clone. This fragment includes the maize Protox-1 promoter. The sequence of this fragment is set forth in SEQ ID NO:14. From nucleotide 1 to 3532, this sequence is comprised of 5' noncoding sequence. From nucleotide 3533 to 3848, this sequence encodes the 5' end of the maize Protox-1 protein.

A plasmid containing the sequence of SEQ ID NO:14 fused to the remainder of the maize Protox-1 coding sequence was deposited March 19, 1996 as pWDC-14 (NRRL #B-21546).

Example 29: Construction of Plant Transformation Vectors Expressing Altered Protox-1 Genes Behind the Native Maize Protox-1 Promoter

The 3848 bp maize genomic fragment (SEQ ID NO:14) was excised from the isolated lambda phage clone as a SalI-KpnI partial digest product and ligated to a KpnI-NotI fragment derived from an altered maize Protox-1 cDNA that contained an alanine to leucine change at amino acid 164 (SEQ ID NO:6). This created a fusion of the native maize Protox-1 promoter to a full length cDNA that had been shown to confer herbicide tolerance in a bacterial system (Examples 8-13). This fusion was cloned into a pUC18 derived vector containing the CaMV 35S terminator sequence to create a protox promoter/altered protox cDNA/terminator cassette. The plasmid containing this cassette was designated pWCo-1.

A second construct for maize transformation was created by engineering the first intron found in the coding sequence from the maize genomic clone back into the maize cDNA. The insertion was made using standard overlapping PCR fusion techniques. The intron (SEQ ID NO:25) was 93 bp long and was inserted between nucleotides 203 and 204 of SEQ ID NO:6, exactly as it appeared in natural context in the lambda clone described in Example 28. This intron-containing version of the expression cassette was designated pWCo-2.

Example 30: Demonstration of Maize Protox-1 Promoter Activity in Transgenic Maize Plants

Maize plants transformed with maize protox promoter/altered protox fusions were identified using PCR analysis with primers specific for the transgene. Total RNA was prepared from the PCR positive plants and reverse-transcribed using Superscript M-MLV (Life Technologies) under recommended conditions. Two microliters of the reverse transcription reaction was used in a PCR reaction designed to be specific for the altered protox sequence. While untransformed controls give no product in this reaction, approximately 85% of plants transformed with pWCo-1 gave a positive result, indicating the presence of mRNA derived from the transgene. This demonstrates some level of activity for the maize protox promoter. The RNA's from the transgenic maize plants were also subjected to standard northern blot analysis using the radiolabeled maize protox cDNA fragment from SEQ ID NO:6 as a probe. Protox-1 mRNA levels significantly above those of untransformed controls were detected in some of the transgenic maize plants. This elevated mRNA level is presumed to be due to expression of altered protox-1 mRNA from the cloned maize protox promoter.

Example 31: Isolation of a Sugar Beet Protox-1 Promoter Sequence

A genomic sugar beet library was prepared by Stratagene in the Lambda Fix II vector. Approximately 300,000 pfu of the library was plated and probed with the sugar beet protox-1 cDNA sequence (SEQ ID NO:17) as described for maize in Example 28. Analysis by restriction digest, hybridization patterns and DNA sequence analysis identified a lambda clone containing approximately 7 kb of sugar beet genomic DNA located 5' to the sugar beet coding sequence previously isolated as a cDNA clone. A PstI-SalI fragment of 2606 bb was subcloned from the lambda clone into a pBluescript vector. This fragment contains 2068 bp of 5' noncoding sequence and includes the sugar beet protox-1 promoter sequence. It also includes the first 453 bp of the protox-1 coding sequence and the 85 bp first intron contained in the coding sequence. The sequence of this fragment is set forth in SEQ ID NO:26.

A plasmid containing the sequence of SEQ ID NO:26 was deposited December 6, 1996 as pWDC-20 (NRRL #B-21650).

Example 32: Construction of Plant Transformation Vectors Expressing Altered Sugar Beet Protox-1 Genes Behind the Native Sugar Beet Protox-1 Promoter

The sugar beet genomic fragment (SEQ ID NO:26) was excised from the genomic subclone described in Example 31 as a SacI-BsrGI fragment that includes 2068 bp of 5' noncoding sequence and the first 300 bp of the sugar beet Protox-1 coding sequence. This fragment was ligated to a BsrGI-NotI fragment derived from an altered sugar beet Protox-1 cDNA that contained a tyrosine to methionine change at amino acid 449 (SEQ ID NO:18). This created a fusion of the native sugar beet Protox-1 promoter to a full length cDNA that had been shown to confer herbicide tolerance in a bacterial system (Examples 8-13). This fusion was cloned into a pUC18 derived vector containing the CaMV 35S terminator sequence to create a protox promoter/altered protox cDNA/terminator cassette. The plasmid containing this cassette was designated pWCo-3.

Example 33: Production of Herbicide Tolerant Plants by Expression of a Native Sugar Beet Protox-1 Promoter/Altered Sugar Beet Protox-1 Fusion

The expression cassette from pWCo-3 is transformed into sugar beet using any of the transformation methods applicable to dicot plants, including Agrobacterium, protoplast, and biolistic transformation techniques. Transgenic sugar beets expressing the altered protox-1 enzyme are identified by RNA-PCR and tested for tolerance to protox-inhibiting herbicides at concentrations that are lethal to untransformed sugar beets.

Section D: Expression of Protox Genes in Plant Plastids

Example 34: Preparation of a Chimeric Gene Containing the Tobacco Plastid *clpP* Gene Promoter and Native *clpP* 5' Untranslated Sequence Fused to a GUS Reporter Gene and Plastid *rps16* Gene 3' Untranslated Sequence in a Plastid Transformation Vector

I. Amplification of the Tobacco Plastid clpP Gene Promoter and Complete 5' Untranslated RNA (5' UTR).

Total DNA from *N. tabacum* c.v. "Xanthi NC" was used as the template for PCR with a left-to-right "top strand" primer comprising an introduced EcoRI restriction site at position -197 relative to the ATG start codon of the constitutively expressed plastid *clpP* gene (primer Pclp_P1a: 5'-gcggaattcatacttatttatcattagaaag-3' (SEQ ID NO:27); EcoRI restriction site underlined) and a right-to-left "bottom strand" primer homologous to the region from -21 to -1 relative to the ATG start codon of the *clpP* promoter that incorporates an introduced NcoI restriction site at the start of translation (primer Pclp_P2b: 5'-gcgccatggtaaatgaaagaaagaactaaa-3' (SEQ ID NO:28); NcoI restriction site underlined). This PCR reaction was undertaken with *Pfu* thermostable DNA polymerase (Stratagene, La Jolla CA) in a Perkin Elmer Thermal Cycler 480 according to the manufacturer's recommendations (Perkin Elmer/Roche, Branchburg, NJ) as follows: 7 min 95°C, followed by 4 cycles of 1 min 95°C / 2 min 43°C / 1 min 72°C, then 25 cycles of 1 min 95°C / 2 min 55°C / 1 min 72°C. The 213 bp amplification product comprising the promoter and 5' untranslated region of the *clpP* gene containing an EcoRI site at its left end and an NcoI site at its right end and corresponding to nucleotides 74700 to 74505 of the *N. tabacum* plastid DNA sequence (Shinozaki et al., *EMBO J.* 5: 2043–2049 (1986)) was gel purified using standard procedures and digested with EcoRI and NcoI (all restriction enzymes were purchased from New England Biolabs, Beverly, MA).

II. Amplification of the Tobacco Plastid rps16 Gene 3' Untranslated RNA Sequence (3'UTR).

Total DNA from *N. tabacum* c.v. "Xanthi NC" was used as the template for PCR as described above with a left-to-right "top strand" primer comprising an introduced XbaI restriction site immediately following the TAA stop codon of the plastid *rps16* gene encoding ribosomal protein S16 (primer rps16P_1a (5'-GCGTCTAGATCAACCGAAATTCAATTAAGG-3' (SEQ ID NO:30); XbaI restriction site underlined) and a right-to-left "bottom strand" primer homologous to the region from +134 to +151 relative to the TAA stop codon of *rps16* that incorporates an introduced HindIII restriction site at the 3' end of the *rps16* 3' UTR (primer rps16P_1b (5'-CGCAAGCTTCAATGGAAGCAATGATAA-3' (SEQ ID NO:31); *HindIII* restriction site underlined). The 169 bp amplification product comprising the 3' untranslated region of the *rps16* gene containing an XbaI site at its left end and a HindIII site at its right end and containing the region corresponding to nucleotides 4943 to 5093 of the *N. tabacum* plastid DNA sequence (Shinozaki et al., 1986) was gel purified and digested with XbaI and HindIII.

III. Ligation of a GUS Reporter Gene Fragment to the clpP Gene Promoter and 5' and 3' UTR's.

An 1864 bp b-galacturonidase (GUS) reporter gene fragment derived from plasmid pRAJ275 (Clontech) containing an NcoI restriction site at the ATG start codon and an XbaI site following the native 3' UTR was produced by digestion with NcoI and XbaI. This fragment was ligated in a four-way reaction to the 201 bp EcoRI/NcoI *clpP* promoter fragment, the 157 bp XbaI/HindIII *rps16* 3'UTR fragment, and a 3148 bp EcoRI/HindIII fragment from cloning vector pGEM3Zf(-) (Promega, Madison WI) to construct plasmid pPH138. Plastid transformation vector pPH140 was constructed by digesting plasmid pPRV111a (Zoubenko et al. 1994) with EcoRI and HindIII and ligating the resulting 7287 bp fragment to a 2222 bp EcoRI/HindIII fragment of pPH138.

Example 35: Preparation of a Chimeric Gene Containing the Tobacco Plastid *clpP* Gene Promoter Plus Tobacco Plastid *psbA* Gene Minimal 5' Untranslated Sequence

Fused to a GUS Reporter Gene and Plastid *rps16* Gene 3' Untranslated Sequence in a Plastid Transformation Vector

Amplification of the tobacco plastid clpP gene promoter and truncated 5' untranslated RNA (5' UTR): Total DNA from *N. tabacum* c.v. "Xanthi NC" was used as the template for PCR as described above with the left-to-right "top strand" primer Pclp_P1a (SEQ ID NO:27) and a right-to-left "bottom strand" primer homologous to the region from -34 to -11 relative to the ATG start codon of the *clpP* promoter that incorporates an introduced XbaI restriction site in the *clpP* 5' UTR at position -11 (primer Pclp_P1b: 5'-gcgtctagaaagaactaaatactatatttcac-3' (SEQ ID NO:29); XbaI restriction site underlined). The 202 bp amplification product comprising the promoter and truncated 5' UTR of the *clpP* gene containing an EcoRI site at its left end and an XbaI site at its right end was gel purified and digested with XbaI. The XbaI site was subsequently filled in with Klenow DNA polymerase (New England Biolabs) and the fragment digested with EcoRI. This was ligated in a five-way reaction to a double stranded DNA fragment corresponding to the final 38 nucleotides and ATG start codon of the tobacco plastid *psbA* gene 5' UTR (with an NcoI restriction site overhang introduced into the ATG start codon) that was created by annealing the synthetic oligonucleotides minpsb_U (top strand: 5'-gggagtccctgatgattaaataaaccaagattttac-3' (SEQ ID NO:32)) and minpsb_L (bottom strand: 5'-catggtaaaatcttggtttatttaatcatcagggactccc-3' (SEQ ID NO:33); NcoI restriction site 5' overhang underlined), the NcoI/XbaI GUS reporter gene fragment described above, the XbaI/HindIII *rps16* 3'UTR fragment described above, and the EcoRI/HindIII pGEM3Zf(-) fragment described above to construct plasmid pPH139. Plastid transformation vector pPH144 was constructed by digesting plasmid pPRV111a (Zoubenko, *et al.*, *Nucleic Acids Res* 22: 3819-3824 (1994)) with EcoRI and HindIII and ligating the resulting 7287 bp fragment to a 2251 bp EcoRI/HindIII fragment of pPH139.

Example 36: Preparation of a Chimeric Gene Containing the Tobacco Plastid *clpP* Gene Promoter and Complete 5' Untranslated Sequence Fused to the *Arabidopsis thaliana* Protox-1 Coding Sequence and Plastid *rps16* Gene 3' Untranslated Sequence in a Vector for Tobacco Plastid Transformation

Miniprep DNA from plasmid AraC-2Met carrying an *Arabidopsis thaliana* NotI insert that includes cDNA sequences from the Protoporphyrinogen IX Oxidase ("PROTOX") gene encoding a portion of the amino terminal plastid transit peptide, the full-length cDNA and a portion of the 3' untranslated region was used as the template for PCR as described above using a left-to-right "top strand" primer (with homology to nucleotides +172 to +194 relative to the ATG start codon of the full length precursor protein) comprising an introduced NcoI restriction site and new ATG start codon at the deduced start of the mature PROTOX protein coding sequence (primer APRTXP1a: 5'-GGGACCATGGATTGTGTGATTGTCGGCGGAGG-3' (SEQ ID NO:34); *NcoI* restriction site underlined) and a right-to-left "bottom strand" primer homologous to nucleotides +917 to +940 relative to the native ATG start codon of the PROTOX precursor protein (primer APRTXP1b: 5'- CTCCGCTCTCCAGCTTAGTGATAC-3' (SEQ ID NO:35)). The 778 bp product was digested with NcoI and SfuI and the resulting 682 bp fragment ligated to an 844 bp SfuI/NotI DNA fragment of AraC-2Met comprising the 3' portion of the PROTOX coding sequence and a 2978 bp NcoI/NotI fragment of the cloning vector pGEM5Zf(+) (Promega, Madison WI) to construct plasmid pPH141. Plastid transformation vector pPH143 containing the *clpP* promoter driving the 276'854-resistance SV1-Met PROTOX gene with the *rps16* 3' UTR was constructed by digesting pPH141 with NcoI and SspI and isolating the 1491 bp fragment containing the complete PROTOX coding sequence, digesting the rps16P_1a and rps16P_1b PCR product described above with HindIII, and ligating these to a 7436 bp NcoI/HindIII fragment of pPH140.

Example 37: Preparation of a Chimeric Gene Containing the Tobacco Plastid *clpP* Gene Promoter Plus Tobacco Plastid *psbA* Gene Minimal 5' Untranslated Sequence Fused to the *Arabidopsis thaliana* Protox-1 Coding Sequence and Plastid *rps16* Gene 3' Untranslated Sequence in a Vector for Tobacco Plastid Transformation

Plastid transformation vector pPH145 containing the *clpP* promoter/*psbA* 5' UTR fusion driving the 276'854-resistance SV1-Met PROTOX gene with the *rps16* 3' UTR was constructed by digesting pPH141 with NcoI and SspI and isolating the 1491 bp fragment containing the complete PROTOX coding sequence, digesting the rps16P_1a and rps16P_1b PCR product described above with HindIII, and ligating these to a 7465 bp NcoI/HindIII fragment of pPH144.

Example 38: Biolistic Transformation of the Tobacco Plastid Genome

Seeds of *Nicotiana tabacum* c.v. 'Xanthi nc' were germinated seven per plate in a 1" circular array on T agar medium and bombarded 12-14 days after sowing with 1 μm tungsten particles (M10, Biorad, Hercules, CA) coated with DNA from plasmids pPH143 and pPH145 essentially as described (Svab, Z. and Maliga, P. (1993) *PNAS* 90, 913–917). Bombarded seedlings were incubated on T medium for two days after which leaves were excised and placed abaxial side up in bright light (350-500 μmol photons/m^2/s) on plates of RMOP medium (Svab, Z., Hajdukiewicz, P. and Maliga, P. (1990) *PNAS* 87, 8526–8530) containing 500 μg/ml spectinomycin dihydrochloride (Sigma, St. Louis, MO). Resistant shoots appearing underneath the bleached leaves three to eight weeks after bombardment were subcloned onto the same selective medium, allowed to form callus, and secondary shoots isolated and subcloned. Complete segregation of transformed plastid genome copies (homoplasmicity) in independent subclones was assessed by standard techniques of Southern blotting (Sambrook et al., (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor). BamHI/EcoRI-digested total cellular DNA (Mettler, I. J. (1987) *Plant Mol Biol Reporter* 5, 346–349) was separated on 1% Tris-borate (TBE) agarose gels, transferred to nylon membranes (Amersham) and probed with ^{32}P-labeled random primed DNA sequences corresponding to a 0.7 kb BamHI/HindIII DNA fragment from pC8 containing a portion of the *rps7/12* plastid targeting sequence. Homoplasmic shoots are rooted aseptically on spectinomycin-containing MS/IBA medium (McBride, K. E. et al. (1994) *PNAS* 91, 7301-7305) and transferred to the greenhouse.

Various modifications of the invention described herein will become apparent to those skilled in the art. Such modifications are intended to fall within the scope of the appended claims.

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

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgement or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: DNA Molecules Encoding Plant Protoporphyrinogen Oxidase and Inhibitor-Resistant Mutants Thereof

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(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1719 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-2 (NRRL B-21238)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 31..1644
(D) OTHER INFORMATION: /product= "Arabidopsis protox-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TGACAAAATT CCGAATTCTC TGCGATTTCC ATG GAG TTA TCT CTT CTC CGT CCG      54
                                 Met Glu Leu Ser Leu Leu Arg Pro
                                   1               5
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ACG ACT CAA TCG CTT CTT CCG TCG TTT TCG AAG CCC AAT CTC CGA TTA      102
Thr Thr Gln Ser Leu Leu Pro Ser Phe Ser Lys Pro Asn Leu Arg Leu
     10                  15                  20

AAT GTT TAT AAG CCT CTT AGA CTC CGT TGT TCA GTG GCC GGT GGA CCA      150
Asn Val Tyr Lys Pro Leu Arg Leu Arg Cys Ser Val Ala Gly Gly Pro
 25                  30                  35                  40

ACC GTC GGA TCT TCA AAA ATC GAA GGC GGA GGA GGC ACC ACC ATC ACG      198
Thr Val Gly Ser Ser Lys Ile Glu Gly Gly Gly Gly Thr Thr Ile Thr
                 45                  50                  55

ACG GAT TGT GTG ATT GTC GGC GGA GGT ATT AGT GGT CTT TGC ATC GCT      246
Thr Asp Cys Val Ile Val Gly Gly Gly Ile Ser Gly Leu Cys Ile Ala
             60                  65                  70

CAG GCG CTT GCT ACT AAG CAT CCT GAT GCT GCT CCG AAT TTA ATT GTG      294
Gln Ala Leu Ala Thr Lys His Pro Asp Ala Ala Pro Asn Leu Ile Val
         75                  80                  85

ACC GAG GCT AAG GAT CGT GTT GGA GGC AAC ATT ATC ACT CGT GAA GAG      342
Thr Glu Ala Lys Asp Arg Val Gly Gly Asn Ile Ile Thr Arg Glu Glu
     90                  95                 100

AAT GGT TTT CTC TGG GAA GAA GGT CCC AAT AGT TTT CAA CCG TCT GAT      390
Asn Gly Phe Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp
105                 110                 115                 120

CCT ATG CTC ACT ATG GTG GTA GAT AGT GGT TTG AAG GAT GAT TTG GTG      438
Pro Met Leu Thr Met Val Val Asp Ser Gly Leu Lys Asp Asp Leu Val
                125                 130                 135

TTG GGA GAT CCT ACT GCG CCA AGG TTT GTG TTG TGG AAT GGG AAA TTG      486
Leu Gly Asp Pro Thr Ala Pro Arg Phe Val Leu Trp Asn Gly Lys Leu
            140                 145                 150

AGG CCG GTT CCA TCG AAG CTA ACA GAC TTA CCG TTC TTT GAT TTG ATG      534
Arg Pro Val Pro Ser Lys Leu Thr Asp Leu Pro Phe Phe Asp Leu Met
        155                 160                 165

AGT ATT GGT GGG AAG ATT AGA GCT GGT TTT GGT GCA CTT GGC ATT CGA      582
Ser Ile Gly Gly Lys Ile Arg Ala Gly Phe Gly Ala Leu Gly Ile Arg
    170                 175                 180

CCG TCA CCT CCA GGT CGT GAA GAA TCT GTG GAG GAG TTT GTA CGG CGT      630
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Pro Ser Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg
185 190 195 200

AAC CTC GGT GAT GAG GTT TTT GAG CGC CTG ATT GAA CCG TTT TGT TCA 678
Asn Leu Gly Asp Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser
205 210 215

GGT GTT TAT GCT GGT GAT CCT TCA AAA CTG AGC ATG AAA GCA GCG TTT 726
Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe
220 225 230

GGG AAG GTT TGG AAA CTA GAG CAA AAT GGT GGA AGC ATA ATA GGT GGT 774
Gly Lys Val Trp Lys Leu Glu Gln Asn Gly Gly Ser Ile Ile Gly Gly
235 240 245

ACT TTT AAG GCA ATT CAG GAG AGG AAA AAC GCT CCC AAG GCA GAA CGA 822
Thr Phe Lys Ala Ile Gln Glu Arg Lys Asn Ala Pro Lys Ala Glu Arg
250 255 260

GAC CCG CGC CTG CCA AAA CCA CAG GGC CAA ACA GTT GGT TCT TTC AGG 870
Asp Pro Arg Leu Pro Lys Pro Gln Gly Gln Thr Val Gly Ser Phe Arg
265 270 275 280

AAG GGA CTT CGA ATG TTG CCA GAA GCA ATA TCT GCA AGA TTA GGT AGC 918
Lys Gly Leu Arg Met Leu Pro Glu Ala Ile Ser Ala Arg Leu Gly Ser
285 290 295

AAA GTT AAG TTG TCT TGG AAG CTC TCA GGT ATC ACT AAG CTG GAG AGC 966
Lys Val Lys Leu Ser Trp Lys Leu Ser Gly Ile Thr Lys Leu Glu Ser
300 305 310

GGA GGA TAC AAC TTA ACA TAT GAG ACT CCA GAT GGT TTA GTT TCC GTG 1014
Gly Gly Tyr Asn Leu Thr Tyr Glu Thr Pro Asp Gly Leu Val Ser Val
315 320 325

CAG AGC AAA AGT GTT GTA ATG ACG GTG CCA TCT CAT GTT GCA AGT GGT 1062
Gln Ser Lys Ser Val Val Met Thr Val Pro Ser His Val Ala Ser Gly
330 335 340

CTC TTG CGC CCT CTT TCT GAA TCT GCT GCA AAT GCA CTC TCA AAA CTA 1110
Leu Leu Arg Pro Leu Ser Glu Ser Ala Ala Asn Ala Leu Ser Lys Leu
345 350 355 360

TAT TAC CCA CCA GTT GCA GCA GTA TCT ATC TCG TAC CCG AAA GAA GCA 1158
Tyr Tyr Pro Pro Val Ala Ala Val Ser Ile Ser Tyr Pro Lys Glu Ala

365 370 375

ATC CGA ACA GAA TGT TTG ATA GAT GGT GAA CTA AAG GGT TTT GGG CAA 1206
Ile Arg Thr Glu Cys Leu Ile Asp Gly Glu Leu Lys Gly Phe Gly Gln
380 385 390

TTG CAT CCA CGC ACG CAA GGA GTT GAA ACA TTA GGA ACT ATC TAC AGC 1254
Leu His Pro Arg Thr Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser
395 400 405

TCC TCA CTC TTT CCA AAT CGC GCA CCG CCC GGA AGA ATT TTG CTG TTG 1302
Ser Ser Leu Phe Pro Asn Arg Ala Pro Pro Gly Arg Ile Leu Leu Leu
410 415 420

AAC TAC ATT GGC GGG TCT ACA AAC ACC GGA ATT CTG TCC AAG TCT GAA 1350
Asn Tyr Ile Gly Gly Ser Thr Asn Thr Gly Ile Leu Ser Lys Ser Glu
425 430 435 440

GGT GAG TTA GTG GAA GCA GTT GAC AGA GAT TTG AGG AAA ATG CTA ATT 1398
Gly Glu Leu Val Glu Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile
445 450 455

AAG CCT AAT TCG ACC GAT CCA CTT AAA TTA GGA GTT AGG GTA TGG CCT 1446
Lys Pro Asn Ser Thr Asp Pro Leu Lys Leu Gly Val Arg Val Trp Pro
460 465 470

CAA GCC ATT CCT CAG TTT CTA GTT GGT CAC TTT GAT ATC CTT GAC ACG 1494
Gln Ala Ile Pro Gln Phe Leu Val Gly His Phe Asp Ile Leu Asp Thr
475 480 485

GCT AAA TCA TCT CTA ACG TCT TCG GGC TAC GAA GGG CTA TTT TTG GGT 1542
Ala Lys Ser Ser Leu Thr Ser Ser Gly Tyr Glu Gly Leu Phe Leu Gly
490 495 500

GGC AAT TAC GTC GCT GGT GTA GCC TTA GGC CGG TGT GTA GAA GGC GCA 1590
Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala
505 510 515 520

TAT GAA ACC GCG ATT GAG GTC AAC AAC TTC ATG TCA CGG TAC GCT TAC 1638
Tyr Glu Thr Ala Ile Glu Val Asn Asn Phe Met Ser Arg Tyr Ala Tyr
525 530 535

AAG TAAATGTAAA ACATTAAATC TCCCAGCTTG CGTGAGTTTT ATTAAATATT 1691
Lys

TTGAGATATC CAAAAAAAAA AAAAAAAA 1719

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 537 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Leu Ser Leu Leu Arg Pro Thr Thr Gln Ser Leu Leu Pro Ser
1 5 10 15

Phe Ser Lys Pro Asn Leu Arg Leu Asn Val Tyr Lys Pro Leu Arg Leu
20 25 30

Arg Cys Ser Val Ala Gly Gly Pro Thr Val Gly Ser Ser Lys Ile Glu
35 40 45

Gly Gly Gly Gly Thr Thr Ile Thr Thr Asp Cys Val Ile Val Gly Gly
50 55 60

Gly Ile Ser Gly Leu Cys Ile Ala Gln Ala Leu Ala Thr Lys His Pro
65 70 75 80

Asp Ala Ala Pro Asn Leu Ile Val Thr Glu Ala Lys Asp Arg Val Gly
85 90 95

Gly Asn Ile Ile Thr Arg Glu Glu Asn Gly Phe Leu Trp Glu Glu Gly
100 105 110

Pro Asn Ser Phe Gln Pro Ser Asp Pro Met Leu Thr Met Val Val Asp
115 120 125

Ser Gly Leu Lys Asp Asp Leu Val Leu Gly Asp Pro Thr Ala Pro Arg
130 135 140

Phe Val Leu Trp Asn Gly Lys Leu Arg Pro Val Pro Ser Lys Leu Thr
145 150 155 160

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Asp Leu Pro Phe Phe Asp Leu Met Ser Ile Gly Gly Lys Ile Arg Ala
                165                 170                 175

Gly Phe Gly Ala Leu Gly Ile Arg Pro Ser Pro Pro Gly Arg Glu Glu
            180                 185                 190

Ser Val Glu Glu Phe Val Arg Arg Asn Leu Gly Asp Glu Val Phe Glu
        195                 200                 205

Arg Leu Ile Glu Pro Phe Cys Ser Gly Val Tyr Ala Gly Asp Pro Ser
    210                 215                 220

Lys Leu Ser Met Lys Ala Ala Phe Gly Lys Val Trp Lys Leu Glu Gln
225                 230                 235                 240

Asn Gly Gly Ser Ile Ile Gly Gly Thr Phe Lys Ala Ile Gln Glu Arg
                245                 250                 255

Lys Asn Ala Pro Lys Ala Glu Arg Asp Pro Arg Leu Pro Lys Pro Gln
            260                 265                 270

Gly Gln Thr Val Gly Ser Phe Arg Lys Gly Leu Arg Met Leu Pro Glu
        275                 280                 285

Ala Ile Ser Ala Arg Leu Gly Ser Lys Val Lys Leu Ser Trp Lys Leu
    290                 295                 300

Ser Gly Ile Thr Lys Leu Glu Ser Gly Gly Tyr Asn Leu Thr Tyr Glu
305                 310                 315                 320

Thr Pro Asp Gly Leu Val Ser Val Gln Ser Lys Ser Val Val Met Thr
                325                 330                 335

Val Pro Ser His Val Ala Ser Gly Leu Leu Arg Pro Leu Ser Glu Ser
            340                 345                 350

Ala Ala Asn Ala Leu Ser Lys Leu Tyr Tyr Pro Pro Val Ala Ala Val
        355                 360                 365

Ser Ile Ser Tyr Pro Lys Glu Ala Ile Arg Thr Glu Cys Leu Ile Asp
    370                 375                 380

Gly Glu Leu Lys Gly Phe Gly Gln Leu His Pro Arg Thr Gln Gly Val
385                 390                 395                 400
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Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu Phe Pro Asn Arg Ala
                405                 410                 415

Pro Pro Gly Arg Ile Leu Leu Leu Asn Tyr Ile Gly Gly Ser Thr Asn
            420                 425                 430

Thr Gly Ile Leu Ser Lys Ser Glu Gly Glu Leu Val Glu Ala Val Asp
        435                 440                 445

Arg Asp Leu Arg Lys Met Leu Ile Lys Pro Asn Ser Thr Asp Pro Leu
    450                 455                 460

Lys Leu Gly Val Arg Val Trp Pro Gln Ala Ile Pro Gln Phe Leu Val
465                 470                 475                 480

Gly His Phe Asp Ile Leu Asp Thr Ala Lys Ser Ser Leu Thr Ser Ser
                485                 490                 495

Gly Tyr Glu Gly Leu Phe Leu Gly Gly Asn Tyr Val Ala Gly Val Ala
            500                 505                 510

Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu Thr Ala Ile Glu Val Asn
        515                 520                 525

Asn Phe Met Ser Arg Tyr Ala Tyr Lys
    530                 535
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1738 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Arabidopsis thaliana

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(vii) IMMEDIATE SOURCE:
      (B) CLONE: pWDC-1 (NRRL B-21237)

 (ix) FEATURE:
      (A) NAME/KEY: CDS
      (B) LOCATION: 70..1596
      (D) OTHER INFORMATION: /product= "Arabidopsis protox-2"


 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTTTTACTT ATTTCCGTCA CTGCTTTCGA CTGGTCAGAG ATTTTGACTC TGAATTGTTG      60

CAGATAGCA ATG GCG TCT GGA GCA GTA GCA GAT CAT CAA ATT GAA GCG         108
          Met Ala Ser Gly Ala Val Ala Asp His Gln Ile Glu Ala
            1               5                  10

GTT TCA GGA AAA AGA GTC GCA GTC GTA GGT GCA GGT GTA AGT GGA CTT      156
Val Ser Gly Lys Arg Val Ala Val Val Gly Ala Gly Val Ser Gly Leu
    15                  20                  25

GCG GCG GCT TAC AAG TTG AAA TCG AGG GGT TTG AAT GTG ACT GTG TTT      204
Ala Ala Ala Tyr Lys Leu Lys Ser Arg Gly Leu Asn Val Thr Val Phe
 30                  35                  40                  45

GAA GCT GAT GGA AGA GTA GGT GGG AAG TTG AGA AGT GTT ATG CAA AAT      252
Glu Ala Asp Gly Arg Val Gly Gly Lys Leu Arg Ser Val Met Gln Asn
                 50                  55                  60

GGT TTG ATT TGG GAT GAA GGA GCA AAC ACC ATG ACT GAG GCT GAG CCA      300
Gly Leu Ile Trp Asp Glu Gly Ala Asn Thr Met Thr Glu Ala Glu Pro
             65                  70                  75

GAA GTT GGG AGT TTA CTT GAT GAT CTT GGG CTT CGT GAG AAA CAA CAA      348
Glu Val Gly Ser Leu Leu Asp Asp Leu Gly Leu Arg Glu Lys Gln Gln
         80                  85                  90

TTT CCA ATT TCA CAG AAA AAG CGG TAT ATT GTG CGG AAT GGT GTA CCT      396
Phe Pro Ile Ser Gln Lys Lys Arg Tyr Ile Val Arg Asn Gly Val Pro
     95                 100                 105

GTG ATG CTA CCT ACC AAT CCC ATA GAG CTG GTC ACA AGT AGT GTG CTC      444
Val Met Leu Pro Thr Asn Pro Ile Glu Leu Val Thr Ser Ser Val Leu
110                 115                 120                 125
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TCT ACC CAA TCT AAG TTT CAA ATC TTG TTG GAA CCA TTT TTA TGG AAG 492
Ser Thr Gln Ser Lys Phe Gln Ile Leu Leu Glu Pro Phe Leu Trp Lys
130 135 140

AAA AAG TCC TCA AAA GTC TCA GAT GCA TCT GCT GAA GAA AGT GTA AGC 540
Lys Lys Ser Ser Lys Val Ser Asp Ala Ser Ala Glu Glu Ser Val Ser
145 150 155

GAG TTC TTT CAA CGC CAT TTT GGA CAA GAG GTT GTT GAC TAT CTC ATC 588
Glu Phe Phe Gln Arg His Phe Gly Gln Glu Val Val Asp Tyr Leu Ile
160 165 170

GAC CCT TTT GTT GGT GGA ACA AGT GCT GCG GAC CCT GAT TCC CTT TCA 636
Asp Pro Phe Val Gly Gly Thr Ser Ala Ala Asp Pro Asp Ser Leu Ser
175 180 185

ATG AAG CAT TCT TTC CCA GAT CTC TGG AAT GTA GAG AAA AGT TTT GGC 684
Met Lys His Ser Phe Pro Asp Leu Trp Asn Val Glu Lys Ser Phe Gly
190 195 200 205

TCT ATT ATA GTC GGT GCA ATC AGA ACA AAG TTT GCT GCT AAA GGT GGT 732
Ser Ile Ile Val Gly Ala Ile Arg Thr Lys Phe Ala Ala Lys Gly Gly
210 215 220

AAA AGT AGA GAC ACA AAG AGT TCT CCT GGC ACA AAA AAG GGT TCG CGT 780
Lys Ser Arg Asp Thr Lys Ser Ser Pro Gly Thr Lys Lys Gly Ser Arg
225 230 235

GGG TCA TTC TCT TTT AAG GGG GGA ATG CAG ATT CTT CCT GAT ACG TTG 828
Gly Ser Phe Ser Phe Lys Gly Gly Met Gln Ile Leu Pro Asp Thr Leu
240 245 250

TGC AAA AGT CTC TCA CAT GAT GAG ATC AAT TTA GAC TCC AAG GTA CTC 876
Cys Lys Ser Leu Ser His Asp Glu Ile Asn Leu Asp Ser Lys Val Leu
255 260 265

TCT TTG TCT TAC AAT TCT GGA TCA AGA CAG GAG AAC TGG TCA TTA TCT 924
Ser Leu Ser Tyr Asn Ser Gly Ser Arg Gln Glu Asn Trp Ser Leu Ser
270 275 280 285

TGT GTT TCG CAT AAT GAA ACG CAG AGA CAA AAC CCC CAT TAT GAT GCT 972
Cys Val Ser His Asn Glu Thr Gln Arg Gln Asn Pro His Tyr Asp Ala
290 295 300

GTA ATT ATG ACG GCT CCT CTG TGC AAT GTG AAG GAG ATG AAG GTT ATG 1020

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Val Ile Met Thr Ala Pro Leu Cys Asn Val Lys Glu Met Lys Val Met
            305                 310                 315

AAA GGA GGA CAA CCC TTT CAG CTA AAC TTT CTC CCC GAG ATT AAT TAC      1068
Lys Gly Gly Gln Pro Phe Gln Leu Asn Phe Leu Pro Glu Ile Asn Tyr
        320                 325                 330

ATG CCC CTC TCG GTT TTA ATC ACC ACA TTC ACA AAG GAG AAA GTA AAG      1116
Met Pro Leu Ser Val Leu Ile Thr Thr Phe Thr Lys Glu Lys Val Lys
    335                 340                 345

AGA CCT CTT GAA GGC TTT GGG GTA CTC ATT CCA TCT AAG GAG CAA AAG      1164
Arg Pro Leu Glu Gly Phe Gly Val Leu Ile Pro Ser Lys Glu Gln Lys
350                 355                 360                 365

CAT GGT TTC AAA ACT CTA GGT ACA CTT TTT TCA TCA ATG ATG TTT CCA      1212
His Gly Phe Lys Thr Leu Gly Thr Leu Phe Ser Ser Met Met Phe Pro
                370                 375                 380

GAT CGT TCC CCT AGT GAC GTT CAT CTA TAT ACA ACT TTT ATT GGT GGG      1260
Asp Arg Ser Pro Ser Asp Val His Leu Tyr Thr Thr Phe Ile Gly Gly
            385                 390                 395

AGT AGG AAC CAG GAA CTA GCC AAA GCT TCC ACT GAC GAA TTA AAA CAA      1308
Ser Arg Asn Gln Glu Leu Ala Lys Ala Ser Thr Asp Glu Leu Lys Gln
        400                 405                 410

GTT GTG ACT TCT GAC CTT CAG CGA CTG TTG GGG GTT GAA GGT GAA CCC      1356
Val Val Thr Ser Asp Leu Gln Arg Leu Leu Gly Val Glu Gly Glu Pro
    415                 420                 425

GTG TCT GTC AAC CAT TAC TAT TGG AGG AAA GCA TTC CCG TTG TAT GAC      1404
Val Ser Val Asn His Tyr Tyr Trp Arg Lys Ala Phe Pro Leu Tyr Asp
430                 435                 440                 445

AGC AGC TAT GAC TCA GTC ATG GAA GCA ATT GAC AAG ATG GAG AAT GAT      1452
Ser Ser Tyr Asp Ser Val Met Glu Ala Ile Asp Lys Met Glu Asn Asp
                450                 455                 460

CTA CCT GGG TTC TTC TAT GCA GGT AAT CAT CGA GGG GGC CTC TCT GTT      1500
Leu Pro Gly Phe Phe Tyr Ala Gly Asn His Arg Gly Gly Leu Ser Val
            465                 470                 475

GGG AAA TCA ATA GCA TCA GGT TGC AAA GCA GCT GAC CTT GTG ATC TCA      1548
Gly Lys Ser Ile Ala Ser Gly Cys Lys Ala Ala Asp Leu Val Ile Ser
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480 485 490

TAC CTG GAG TCT TGC TCA AAT GAC AAG AAA CCA AAT GAC AGC TTA TAACATTGTC 1603
Tyr Leu Glu Ser Cys Ser Asn Asp Lys Lys Pro Asn Asp Ser Leu
495 500 505

AAGGTTCGTC CCTTTTTATC ACTTACTTTG TAAACTTGTA AAATGCAACA AGCCGCCGTG 1663

CGATTAGCCA ACAACTCAGC AAAACCCAGA TTCTCATAAG GCTCACTAAT TCCAGAATAA 1723

ACTATTTATG TAAAA 1738

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 508 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Ala Ser Gly Ala Val Ala Asp His Gln Ile Glu Ala Val Ser Gly
1 5 10 15

Lys Arg Val Ala Val Val Gly Ala Gly Val Ser Gly Leu Ala Ala Ala
20 25 30

Tyr Lys Leu Lys Ser Arg Gly Leu Asn Val Thr Val Phe Glu Ala Asp
35 40 45

Gly Arg Val Gly Gly Lys Leu Arg Ser Val Met Gln Asn Gly Leu Ile
50 55 60

Trp Asp Glu Gly Ala Asn Thr Met Thr Glu Ala Glu Pro Glu Val Gly
65 70 75 80

Ser Leu Leu Asp Asp Leu Gly Leu Arg Glu Lys Gln Gln Phe Pro Ile
85 90 95

Ser Gln Lys Lys Arg Tyr Ile Val Arg Asn Gly Val Pro Val Met Leu
100 105 110

Pro Thr Asn Pro Ile Glu Leu Val Thr Ser Ser Val Leu Ser Thr Gln
115 120 125

Ser Lys Phe Gln Ile Leu Leu Glu Pro Phe Leu Trp Lys Lys Lys Ser
130 135 140

Ser Lys Val Ser Asp Ala Ser Ala Glu Glu Ser Val Ser Glu Phe Phe
145 150 155 160

Gln Arg His Phe Gly Gln Glu Val Val Asp Tyr Leu Ile Asp Pro Phe
165 170 175

Val Gly Gly Thr Ser Ala Ala Asp Pro Asp Ser Leu Ser Met Lys His
180 185 190

Ser Phe Pro Asp Leu Trp Asn Val Glu Lys Ser Phe Gly Ser Ile Ile
195 200 205

Val Gly Ala Ile Arg Thr Lys Phe Ala Ala Lys Gly Gly Lys Ser Arg
210 215 220

Asp Thr Lys Ser Ser Pro Gly Thr Lys Lys Gly Ser Arg Gly Ser Phe
225 230 235 240

Ser Phe Lys Gly Gly Met Gln Ile Leu Pro Asp Thr Leu Cys Lys Ser
245 250 255

Leu Ser His Asp Glu Ile Asn Leu Asp Ser Lys Val Leu Ser Leu Ser
260 265 270

Tyr Asn Ser Gly Ser Arg Gln Glu Asn Trp Ser Leu Ser Cys Val Ser
275 280 285

His Asn Glu Thr Gln Arg Gln Asn Pro His Tyr Asp Ala Val Ile Met
290 295 300

Thr Ala Pro Leu Cys Asn Val Lys Glu Met Lys Val Met Lys Gly Gly
305 310 315 320

Gln Pro Phe Gln Leu Asn Phe Leu Pro Glu Ile Asn Tyr Met Pro Leu
325 330 335

Ser Val Leu Ile Thr Thr Phe Thr Lys Glu Lys Val Lys Arg Pro Leu
340 345 350

Glu Gly Phe Gly Val Leu Ile Pro Ser Lys Glu Gln Lys His Gly Phe
355 360 365

Lys Thr Leu Gly Thr Leu Phe Ser Ser Met Met Phe Pro Asp Arg Ser
370 375 380

Pro Ser Asp Val His Leu Tyr Thr Thr Phe Ile Gly Gly Ser Arg Asn
385 390 395 400

Gln Glu Leu Ala Lys Ala Ser Thr Asp Glu Leu Lys Gln Val Val Thr
405 410 415

Ser Asp Leu Gln Arg Leu Leu Gly Val Glu Gly Glu Pro Val Ser Val
420 425 430

Asn His Tyr Tyr Trp Arg Lys Ala Phe Pro Leu Tyr Asp Ser Ser Tyr
435 440 445

Asp Ser Val Met Glu Ala Ile Asp Lys Met Glu Asn Asp Leu Pro Gly
450 455 460

Phe Phe Tyr Ala Gly Asn His Arg Gly Gly Leu Ser Val Gly Lys Ser
465 470 475 480

Ile Ala Ser Gly Cys Lys Ala Ala Asp Leu Val Ile Ser Tyr Leu Glu
485 490 495

Ser Cys Ser Asn Asp Lys Lys Pro Asn Asp Ser Leu
500 505

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1691 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Zea mays (maize)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-4 (NRRL B-21260)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1443
(D) OTHER INFORMATION: /product= "Maize protox-1 cDNA "

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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GCG GAC TGC GTC GTG GTG GGC GGA GGC ATC AGT GGC CTC TGC ACC GCG      48
Ala Asp Cys Val Val Val Gly Gly Gly Ile Ser Gly Leu Cys Thr Ala
  1               5                  10                  15

CAG GCG CTG GCC ACG CGG CAC GGC GTC GGG GAC GTG CTT GTC ACG GAG      96
Gln Ala Leu Ala Thr Arg His Gly Val Gly Asp Val Leu Val Thr Glu
             20                  25                  30

GCC CGC GCC CGC CCC GGC GGC AAC ATT ACC ACC GTC GAG CGC CCC GAG     144
Ala Arg Ala Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Glu
         35                  40                  45

GAA GGG TAC CTC TGG GAG GAG GGT CCC AAC AGC TTC CAG CCC TCC GAC     192
Glu Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp
     50                  55                  60

CCC GTT CTC ACC ATG GCC GTG GAC AGC GGA CTG AAG GAT GAC TTG GTT     240
Pro Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val
 65                  70                  75                  80

TTT GGG GAC CCA AAC GCG CCG CGT TTC GTG CTG TGG GAG GGG AAG CTG     288
Phe Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu
                 85                  90                  95

AGG CCC GTG CCA TCC AAG CCC GCC GAC CTC CCG TTC TTC GAT CTC ATG     336
Arg Pro Val Pro Ser Lys Pro Ala Asp Leu Pro Phe Phe Asp Leu Met
            100                 105                 110

AGC ATC CCA GGG AAG CTC AGG GCC GGT CTA GGC GCG CTT GGC ATC CGC     384
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Ser Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg
        115                 120                 125

CCG CCT CCT CCA GGC CGC GAA GAG TCA GTG GAG GAG TTC GTG CGC CGC      432
Pro Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg
    130                 135                 140

AAC CTC GGT GCT GAG GTC TTT GAG CGC CTC ATT GAG CCT TTC TGC TCA      480
Asn Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser
145                 150                 155                 160

GGT GTC TAT GCT GGT GAT CCT TCT AAG CTC AGC ATG AAG GCT GCA TTT      528
Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe
                165                 170                 175

GGG AAG GTT TGG CGG TTG GAA GAA ACT GGA GGT AGT ATT ATT GGT GGA      576
Gly Lys Val Trp Arg Leu Glu Glu Thr Gly Gly Ser Ile Ile Gly Gly
            180                 185                 190

ACC ATC AAG ACA ATT CAG GAG AGG AGC AAG AAT CCA AAA CCA CCG AGG      624
Thr Ile Lys Thr Ile Gln Glu Arg Ser Lys Asn Pro Lys Pro Pro Arg
        195                 200                 205

GAT GCC CGC CTT CCG AAG CCA AAA GGG CAG ACA GTT GCA TCT TTC AGG      672
Asp Ala Arg Leu Pro Lys Pro Lys Gly Gln Thr Val Ala Ser Phe Arg
    210                 215                 220

AAG GGT CTT GCC ATG CTT CCA AAT GCC ATT ACA TCC AGC TTG GGT AGT      720
Lys Gly Leu Ala Met Leu Pro Asn Ala Ile Thr Ser Ser Leu Gly Ser
225                 230                 235                 240

AAA GTC AAA CTA TCA TGG AAA CTC ACG AGC ATT ACA AAA TCA GAT GAC      768
Lys Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ser Asp Asp
                245                 250                 255

AAG GGA TAT GTT TTG GAG TAT GAA ACG CCA GAA GGG GTT GTT TCG GTG      816
Lys Gly Tyr Val Leu Glu Tyr Glu Thr Pro Glu Gly Val Val Ser Val
            260                 265                 270

CAG GCT AAA AGT GTT ATC ATG ACT ATT CCA TCA TAT GTT GCT AGC AAC      864
Gln Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asn
        275                 280                 285

ATT TTG CGT CCA CTT TCA AGC GAT GCT GCA GAT GCT CTA TCA AGA TTC      912
Ile Leu Arg Pro Leu Ser Ser Asp Ala Ala Asp Ala Leu Ser Arg Phe
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290 295 300

TAT TAT CCA CCG GTT GCT GCT GTA ACT GTT TCG TAT CCA AAG GAA GCA 960
Tyr Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala
305 310 315 320

ATT AGA AAA GAA TGC TTA ATT GAT GGG GAA CTC CAG GGC TTT GGC CAG 1008
Ile Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln
325 330 335

TTG CAT CCA CGT AGT CAA GGA GTT GAG ACA TTA GGA ACA ATA TAC AGT 1056
Leu His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser
340 345 350

TCC TCA CTC TTT CCA AAT CGT GCT CCT GAC GGT AGG GTG TTA CTT CTA 1104
Ser Ser Leu Phe Pro Asn Arg Ala Pro Asp Gly Arg Val Leu Leu Leu
355 360 365

AAC TAC ATA GGA GGT GCT ACA AAC ACA GGA ATT GTT TCC AAG ACT GAA 1152
Asn Tyr Ile Gly Gly Ala Thr Asn Thr Gly Ile Val Ser Lys Thr Glu
370 375 380

AGT GAG CTG GTC GAA GCA GTT GAC CGT GAC CTC CGA AAA ATG CTT ATA 1200
Ser Glu Leu Val Glu Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile
385 390 395 400

AAT TCT ACA GCA GTG GAC CCT TTA GTC CTT GGT GTT CGA GTT TGG CCA 1248
Asn Ser Thr Ala Val Asp Pro Leu Val Leu Gly Val Arg Val Trp Pro
405 410 415

CAA GCC ATA CCT CAG TTC CTG GTA GGA CAT CTT GAT CTT CTG GAA GCC 1296
Gln Ala Ile Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Glu Ala
420 425 430

GCA AAA GCT GCC CTG GAC CGA GGT GGC TAC GAT GGG CTG TTC CTA GGA 1344
Ala Lys Ala Ala Leu Asp Arg Gly Gly Tyr Asp Gly Leu Phe Leu Gly
435 440 445

GGG AAC TAT GTT GCA GGA GTT GCC CTG GGC AGA TGC GTT GAG GGC GCG 1392
Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala
450 455 460

TAT GAA AGT GCC TCG CAA ATA TCT GAC TTC TTG ACC AAG TAT GCC TAC 1440
Tyr Glu Ser Ala Ser Gln Ile Ser Asp Phe Leu Thr Lys Tyr Ala Tyr
465 470 475 480

AAG TGATGAAAGA AGTGGAGCGC TACTTGTTAA TCGTTTATGT TGCATAGATG 1493
Lys

AGGTGCCTCC GGGGAAAAAA AAGCTTGAAT AGTATTTTTT ATTCTTATTT TGTAAATTGC 1553

ATTTCTGTTC TTTTTTCTAT CAGTAATTAG TTATATTTTA GTTCTGTAGG AGATTGTTCT 1613

GTTCACTGCC CTTCAAAAGA AATTTTATTT TTCATTCTTT TATGAGAGCT GTGCTACTTA 1673

AAAAAAAAAA AAAAAAAA 1691

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 481 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Asp Cys Val Val Val Gly Gly Gly Ile Ser Gly Leu Cys Thr Ala
1 5 10 15

Gln Ala Leu Ala Thr Arg His Gly Val Gly Asp Val Leu Val Thr Glu
20 25 30

Ala Arg Ala Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Glu
35 40 45

Glu Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp
50 55 60

Pro Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val
65 70 75 80

Phe Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu
85 90 95

Arg Pro Val Pro Ser Lys Pro Ala Asp Leu Pro Phe Phe Asp Leu Met
100 105 110

Ser Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg
115 120 125

Pro Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg
130 135 140

Asn Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser
145 150 155 160

Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe
165 170 175

Gly Lys Val Trp Arg Leu Glu Glu Thr Gly Gly Ser Ile Ile Gly Gly
180 185 190

Thr Ile Lys Thr Ile Gln Glu Arg Ser Lys Asn Pro Lys Pro Pro Arg
195 200 205

Asp Ala Arg Leu Pro Lys Pro Lys Gly Gln Thr Val Ala Ser Phe Arg
210 215 220

Lys Gly Leu Ala Met Leu Pro Asn Ala Ile Thr Ser Ser Leu Gly Ser
225 230 235 240

Lys Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ser Asp Asp
245 250 255

Lys Gly Tyr Val Leu Glu Tyr Glu Thr Pro Glu Gly Val Val Ser Val
260 265 270

Gln Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asn
275 280 285

Ile Leu Arg Pro Leu Ser Ser Asp Ala Ala Asp Ala Leu Ser Arg Phe
290 295 300

Tyr Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala
305 310 315 320

Ile Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln
325 330 335

Leu His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser
340 345 350

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Ser Ser Leu Phe Pro Asn Arg Ala Pro Asp Gly Arg Val Leu Leu Leu
        355                 360                 365

Asn Tyr Ile Gly Gly Ala Thr Asn Thr Gly Ile Val Ser Lys Thr Glu
    370                 375                 380

Ser Glu Leu Val Glu Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile
385                 390                 395                 400

Asn Ser Thr Ala Val Asp Pro Leu Val Leu Gly Val Arg Val Trp Pro
                405                 410                 415

Gln Ala Ile Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Glu Ala
            420                 425                 430

Ala Lys Ala Ala Leu Asp Arg Gly Gly Tyr Asp Gly Leu Phe Leu Gly
        435                 440                 445

Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala
    450                 455                 460

Tyr Glu Ser Ala Ser Gln Ile Ser Asp Phe Leu Thr Lys Tyr Ala Tyr
465                 470                 475                 480

Lys
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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 2061 base pairs
  (B) TYPE: nucleic acid
  (C) STRANDEDNESS: single
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
  (A) ORGANISM: Zea mays (maize)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-3 (NRRL B-21259)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 64..1698
(D) OTHER INFORMATION: /product= "Maize protox-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

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CTCTCCTACC TCCACCTCCA CGACAACAAG CAAATCCCCA TCCAGTTCCA AACCCTAACT      60

CAA ATG CTC GCT TTG ACT GCC TCA GCC TCA TCC GCT TCG TCC CAT CCT       108
    Met Leu Ala Leu Thr Ala Ser Ala Ser Ser Ala Ser Ser His Pro
      1               5                  10                  15

TAT CGC CAC GCC TCC GCG CAC ACT CGT CGC CCC CGC CTA CGT GCG GTC       156
Tyr Arg His Ala Ser Ala His Thr Arg Arg Pro Arg Leu Arg Ala Val
                 20                  25                  30

CTC GCG ATG GCG GGC TCC GAC GAC CCC CGT GCA GCG CCC GCC AGA TCG       204
Leu Ala Met Ala Gly Ser Asp Asp Pro Arg Ala Ala Pro Ala Arg Ser
             35                  40                  45

GTC GCC GTC GTC GGC GCC GGG GTC AGC GGG CTC GCG GCG GCG TAC AGG       252
Val Ala Val Val Gly Ala Gly Val Ser Gly Leu Ala Ala Ala Tyr Arg
         50                  55                  60

CTC AGA CAG AGC GGC GTG AAC GTA ACG GTG TTC GAA GCG GCC GAC AGG       300
Leu Arg Gln Ser Gly Val Asn Val Thr Val Phe Glu Ala Ala Asp Arg
     65                  70                  75

GCG GGA GGA AAG ATA CGG ACC AAT TCC GAG GGC GGG TTT GTC TGG GAT       348
Ala Gly Gly Lys Ile Arg Thr Asn Ser Glu Gly Gly Phe Val Trp Asp
 80                  85                  90                  95

GAA GGA GCT AAC ACC ATG ACA GAA GGT GAA TGG GAG GCC AGT AGA CTG       396
Glu Gly Ala Asn Thr Met Thr Glu Gly Glu Trp Glu Ala Ser Arg Leu
                100                 105                 110

ATT GAT GAT CTT GGT CTA CAA GAC AAA CAG CAG TAT CCT AAC TCC CAA       444
Ile Asp Asp Leu Gly Leu Gln Asp Lys Gln Gln Tyr Pro Asn Ser Gln
            115                 120                 125
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CAC AAG CGT TAC ATT GTC AAA GAT GGA GCA CCA GCA CTG ATT CCT TCG      492
His Lys Arg Tyr Ile Val Lys Asp Gly Ala Pro Ala Leu Ile Pro Ser
        130                 135                 140

GAT CCC ATT TCG CTA ATG AAA AGC AGT GTT CTT TCG ACA AAA TCA AAG      540
Asp Pro Ile Ser Leu Met Lys Ser Ser Val Leu Ser Thr Lys Ser Lys
    145                 150                 155

ATT GCG TTA TTT TTT GAA CCA TTT CTC TAC AAG AAA GCT AAC ACA AGA      588
Ile Ala Leu Phe Phe Glu Pro Phe Leu Tyr Lys Lys Ala Asn Thr Arg
160                 165                 170                 175

AAC TCT GGA AAA GTG TCT GAG GAG CAC TTG AGT GAG AGT GTT GGG AGC      636
Asn Ser Gly Lys Val Ser Glu Glu His Leu Ser Glu Ser Val Gly Ser
                180                 185                 190

TTC TGT GAA CGC CAC TTT GGA AGA GAA GTT GTT GAC TAT TTT GTT GAT      684
Phe Cys Glu Arg His Phe Gly Arg Glu Val Val Asp Tyr Phe Val Asp
            195                 200                 205

CCA TTT GTA GCT GGA ACA AGT GCA GGA GAT CCA GAG TCA CTA TCT ATT      732
Pro Phe Val Ala Gly Thr Ser Ala Gly Asp Pro Glu Ser Leu Ser Ile
        210                 215                 220

CGT CAT GCA TTC CCA GCA TTG TGG AAT TTG GAA AGA AAG TAT GGT TCA      780
Arg His Ala Phe Pro Ala Leu Trp Asn Leu Glu Arg Lys Tyr Gly Ser
    225                 230                 235

GTT ATT GTT GGT GCC ATC TTG TCT AAG CTA GCA GCT AAA GGT GAT CCA      828
Val Ile Val Gly Ala Ile Leu Ser Lys Leu Ala Ala Lys Gly Asp Pro
240                 245                 250                 255

GTA AAG ACA AGA CAT GAT TCA TCA GGG AAA AGA AGG AAT AGA CGA GTG      876
Val Lys Thr Arg His Asp Ser Ser Gly Lys Arg Arg Asn Arg Arg Val
                260                 265                 270

TCG TTT TCA TTT CAT GGT GGA ATG CAG TCA CTA ATA AAT GCA CTT CAC      924
Ser Phe Ser Phe His Gly Gly Met Gln Ser Leu Ile Asn Ala Leu His
            275                 280                 285

AAT GAA GTT GGA GAT GAT AAT GTG AAG CTT GGT ACA GAA GTG TTG TCA      972
Asn Glu Val Gly Asp Asp Asn Val Lys Leu Gly Thr Glu Val Leu Ser
        290                 295                 300

TTG GCA TGT ACA TTT GAT GGA GTT CCT GCA CTA GGC AGG TGG TCA ATT     1020
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Leu Ala Cys Thr Phe Asp Gly Val Pro Ala Leu Gly Arg Trp Ser Ile
305 310 315

TCT GTT GAT TCG AAG GAT AGC GGT GAC AAG GAC CTT GCT AGT AAC CAA 1068
Ser Val Asp Ser Lys Asp Ser Gly Asp Lys Asp Leu Ala Ser Asn Gln
320 325 330 335

ACC TTT GAT GCT GTT ATA ATG ACA GCT CCA TTG TCA AAT GTC CGG AGG 1116
Thr Phe Asp Ala Val Ile Met Thr Ala Pro Leu Ser Asn Val Arg Arg
340 345 350

ATG AAG TTC ACC AAA GGT GGA GCT CCG GTT GTT CTT GAC TTT CTT CCT 1164
Met Lys Phe Thr Lys Gly Gly Ala Pro Val Val Leu Asp Phe Leu Pro
355 360 365

AAG ATG GAT TAT CTA CCA CTA TCT CTC ATG GTG ACT GCT TTT AAG AAG 1212
Lys Met Asp Tyr Leu Pro Leu Ser Leu Met Val Thr Ala Phe Lys Lys
370 375 380

GAT GAT GTC AAG AAA CCT CTG GAA GGA TTT GGG GTC TTA ATA CCT TAC 1260
Asp Asp Val Lys Lys Pro Leu Glu Gly Phe Gly Val Leu Ile Pro Tyr
385 390 395

AAG GAA CAG CAA AAA CAT GGT CTG AAA ACC CTT GGG ACT CTC TTT TCC 1308
Lys Glu Gln Gln Lys His Gly Leu Lys Thr Leu Gly Thr Leu Phe Ser
400 405 410 415

TCA ATG ATG TTC CCA GAT CGA GCT CCT GAT GAC CAA TAT TTA TAT ACA 1356
Ser Met Met Phe Pro Asp Arg Ala Pro Asp Asp Gln Tyr Leu Tyr Thr
420 425 430

ACA TTT GTT GGG GGT AGC CAC AAT AGA GAT CTT GCT GGA GCT CCA ACG 1404
Thr Phe Val Gly Gly Ser His Asn Arg Asp Leu Ala Gly Ala Pro Thr
435 440 445

TCT ATT CTG AAA CAA CTT GTG ACC TCT GAC CTT AAA AAA CTC TTG GGC 1452
Ser Ile Leu Lys Gln Leu Val Thr Ser Asp Leu Lys Lys Leu Leu Gly
450 455 460

GTA GAG GGG CAA CCA ACT TTT GTC AAG CAT GTA TAC TGG GGA AAT GCT 1500
Val Glu Gly Gln Pro Thr Phe Val Lys His Val Tyr Trp Gly Asn Ala
465 470 475

TTT CCT TTG TAT GGC CAT GAT TAT AGT TCT GTA TTG GAA GCT ATA GAA 1548
Phe Pro Leu Tyr Gly His Asp Tyr Ser Ser Val Leu Glu Ala Ile Glu

480 485 490 495

AAG ATG GAG AAA AAC CTT CCA GGG TTC TTC TAC GCA GGA AAT AGC AAG 1596
Lys Met Glu Lys Asn Leu Pro Gly Phe Phe Tyr Ala Gly Asn Ser Lys
500 505 510

GAT GGG CTT GCT GTT GGA AGT GTT ATA GCT TCA GGA AGC AAG GCT GCT 1644
Asp Gly Leu Ala Val Gly Ser Val Ile Ala Ser Gly Ser Lys Ala Ala
515 520 525

GAC CTT GCA ATC TCA TAT CTT GAA TCT CAC ACC AAG CAT AAT AAT TCA 1692
Asp Leu Ala Ile Ser Tyr Leu Glu Ser His Thr Lys His Asn Asn Ser
530 535 540

CAT TGAAAGTGTC TGACCTATCC TCTAGCAGTT GTCGACAAAT TTCTCCAGTT 1745
His
545

CATGTACAGT AGAAACCGAT GCGTTGCAGT TTCAGAACAT CTTCACTTCT TCAGATATTA 1805

ACCCTTCGTT GAACATCCAC CAGAAAGGTA GTCACATGTG TAAGTGGGAA AATGAGGTTA 1865

AAAACTATTA TGGCGGCCGA AATGTTCCTT TTTGTTTTCC TCACAAGTGG CCTACGACAC 1925

TTGATGTTGG AAATACATTT AAATTTGTTG AATTGTTTGA GAACACATGC GTGACGTGTA 1985

ATATTTGCCT ATTGTGATTT TAGCAGTAGT CTTGGCCAGA TTATGCTTTA CGCCTTTAAA 2045

AAAAAAAAAA AAAAAA 2061

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 544 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Met Leu Ala Leu Thr Ala Ser Ala Ser Ser Ala Ser Ser His Pro Tyr
1 5 10 15

Arg His Ala Ser Ala His Thr Arg Arg Pro Arg Leu Arg Ala Val Leu
20 25 30

Ala Met Ala Gly Ser Asp Asp Pro Arg Ala Ala Pro Ala Arg Ser Val
35 40 45

Ala Val Val Gly Ala Gly Val Ser Gly Leu Ala Ala Ala Tyr Arg Leu
50 55 60

Arg Gln Ser Gly Val Asn Val Thr Val Phe Glu Ala Ala Asp Arg Ala
65 70 75 80

Gly Gly Lys Ile Arg Thr Asn Ser Glu Gly Gly Phe Val Trp Asp Glu
85 90 95

Gly Ala Asn Thr Met Thr Glu Gly Glu Trp Glu Ala Ser Arg Leu Ile
100 105 110

Asp Asp Leu Gly Leu Gln Asp Lys Gln Gln Tyr Pro Asn Ser Gln His
115 120 125

Lys Arg Tyr Ile Val Lys Asp Gly Ala Pro Ala Leu Ile Pro Ser Asp
130 135 140

Pro Ile Ser Leu Met Lys Ser Ser Val Leu Ser Thr Lys Ser Lys Ile
145 150 155 160

Ala Leu Phe Phe Glu Pro Phe Leu Tyr Lys Lys Ala Asn Thr Arg Asn
165 170 175

Ser Gly Lys Val Ser Glu Glu His Leu Ser Glu Ser Val Gly Ser Phe
180 185 190

Cys Glu Arg His Phe Gly Arg Glu Val Val Asp Tyr Phe Val Asp Pro
195 200 205

Phe Val Ala Gly Thr Ser Ala Gly Asp Pro Glu Ser Leu Ser Ile Arg
210 215 220

His Ala Phe Pro Ala Leu Trp Asn Leu Glu Arg Lys Tyr Gly Ser Val
225 230 235 240

Ile Val Gly Ala Ile Leu Ser Lys Leu Ala Ala Lys Gly Asp Pro Val
245 250 255

Lys Thr Arg His Asp Ser Ser Gly Lys Arg Arg Asn Arg Arg Val Ser
260 265 270

Phe Ser Phe His Gly Gly Met Gln Ser Leu Ile Asn Ala Leu His Asn
275 280 285

Glu Val Gly Asp Asp Asn Val Lys Leu Gly Thr Glu Val Leu Ser Leu
290 295 300

Ala Cys Thr Phe Asp Gly Val Pro Ala Leu Gly Arg Trp Ser Ile Ser
305 310 315 320

Val Asp Ser Lys Asp Ser Gly Asp Lys Asp Leu Ala Ser Asn Gln Thr
325 330 335

Phe Asp Ala Val Ile Met Thr Ala Pro Leu Ser Asn Val Arg Arg Met
340 345 350

Lys Phe Thr Lys Gly Gly Ala Pro Val Val Leu Asp Phe Leu Pro Lys
355 360 365

Met Asp Tyr Leu Pro Leu Ser Leu Met Val Thr Ala Phe Lys Lys Asp
370 375 380

Asp Val Lys Lys Pro Leu Glu Gly Phe Gly Val Leu Ile Pro Tyr Lys
385 390 395 400

Glu Gln Gln Lys His Gly Leu Lys Thr Leu Gly Thr Leu Phe Ser Ser
405 410 415

Met Met Phe Pro Asp Arg Ala Pro Asp Asp Gln Tyr Leu Tyr Thr Thr
420 425 430

Phe Val Gly Gly Ser His Asn Arg Asp Leu Ala Gly Ala Pro Thr Ser
435 440 445

Ile Leu Lys Gln Leu Val Thr Ser Asp Leu Lys Lys Leu Leu Gly Val
450 455 460

Glu Gly Gln Pro Thr Phe Val Lys His Val Tyr Trp Gly Asn Ala Phe
465 470 475 480

Pro Leu Tyr Gly His Asp Tyr Ser Ser Val Leu Glu Ala Ile Glu Lys
485 490 495

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Met Glu Lys Asn Leu Pro Gly Phe Phe Tyr Ala Gly Asn Ser Lys Asp
            500                 505                 510

Gly Leu Ala Val Gly Ser Val Ile Ala Ser Gly Ser Lys Ala Ala Asp
        515                 520                 525

Leu Ala Ile Ser Tyr Leu Glu Ser His Thr Lys His Asn Asn Ser His
    530                 535                 540
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1811 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Triticum aestivum (wheat)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-13 (NRRL B-21545)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..1589
(D) OTHER INFORMATION: /product= "wheat protox-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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GC GCA ACA ATG GCC ACC GCC ACC GTC GCG GCC GCG TCG CCG CTC CGC        47
   Ala Thr Met Ala Thr Ala Thr Val Ala Ala Ala Ser Pro Leu Arg
     1               5                  10                  15

GGC AGG GTC ACC GGG CGC CCA CAC CGC GTC CGC CCG CGT TGC GCT ACC       95
Gly Arg Val Thr Gly Arg Pro His Arg Val Arg Pro Arg Cys Ala Thr
                20                  25                  30
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GCG AGC AGC GCG ACC GAG ACT CCG GCG GCG CCC GGC GTG CGG CTG TCC 143
Ala Ser Ser Ala Thr Glu Thr Pro Ala Ala Pro Gly Val Arg Leu Ser
35 40 45

GCG GAA TGC GTC ATT GTG GGC GCC GGC ATC AGC GGC CTC TGC ACC GCG 191
Ala Glu Cys Val Ile Val Gly Ala Gly Ile Ser Gly Leu Cys Thr Ala
50 55 60

CAG GCG CTG GCC ACC CGA TAC GGC GTC AGC GAC CTG CTC GTC ACG GAG 239
Gln Ala Leu Ala Thr Arg Tyr Gly Val Ser Asp Leu Leu Val Thr Glu
65 70 75

GCC CGC GAC CGC CCG GGC GGC AAC ATC ACC ACC GTC GAG CGT CCC GAC 287
Ala Arg Asp Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Asp
80 85 90 95

GAG GGG TAC CTG TGG GAG GAG GGA CCC AAC AGC TTC CAG CCC TCC GAC 335
Glu Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp
100 105 110

CCG GTC CTC ACC ATG GCC GTG GAC AGC GGG CTC AAG GAT GAC TTG GTG 383
Pro Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val
115 120 125

TTC GGG GAC CCC AAC GCG CCC CGG TTC GTG CTG TGG GAG GGG AAG CTG 431
Phe Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu
130 135 140

AGG CCG GTG CCG TCG AAG CCA GGC GAC CTG CCT TTC TTC AGC CTC ATG 479
Arg Pro Val Pro Ser Lys Pro Gly Asp Leu Pro Phe Phe Ser Leu Met
145 150 155

AGT ATC CCT GGG AAG CTC AGG GCC GGC CTT GGC GCG CTC GGC ATT CGC 527
Ser Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg
160 165 170 175

CCA CCT CCT CCA GGG CGC GAG GAG TCG GTG GAG GAG TTT GTG CGC CGC 575
Pro Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg
180 185 190

AAC CTC GGT GCC GAG GTC TTT GAG CGC CTC ATC GAG CCT TTC TGC TCA 623
Asn Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser
195 200 205

GGT GTA TAT GCT GGT GAT CCT TCG AAG CTT AGT ATG AAG GCT GCA TTT 671

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Gly Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe
        210                 215                 220

GGG AAG GTC TGG AGG TTG GAG GAG ATT GGA GGT AGT ATT ATT GGT GGA       719
Gly Lys Val Trp Arg Leu Glu Glu Ile Gly Gly Ser Ile Ile Gly Gly
    225                 230                 235

ACC ATC AAG GCG ATT CAG GAT AAA GGG AAG AAC CCC AAA CCG CCA AGG       767
Thr Ile Lys Ala Ile Gln Asp Lys Gly Lys Asn Pro Lys Pro Pro Arg
240                 245                 250                 255

GAT CCC CGA CTT CCG GCA CCA AAG GGA CAG ACG GTG GCA TCT TTC AGG       815
Asp Pro Arg Leu Pro Ala Pro Lys Gly Gln Thr Val Ala Ser Phe Arg
                260                 265                 270

AAG GGT CTA GCC ATG CTC CCG AAT GCC ATC GCA TCT AGG CTG GGT AGT       863
Lys Gly Leu Ala Met Leu Pro Asn Ala Ile Ala Ser Arg Leu Gly Ser
            275                 280                 285

AAA GTC AAG CTG TCA TGG AAG CTT ACG AGC ATT ACA AAG GCG GAC AAC       911
Lys Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ala Asp Asn
        290                 295                 300

CAA GGA TAT GTA TTA GGT TAT GAA ACA CCA GAA GGA CTT GTT TCA GTG       959
Gln Gly Tyr Val Leu Gly Tyr Glu Thr Pro Glu Gly Leu Val Ser Val
    305                 310                 315

CAG GCT AAA AGT GTT ATC ATG ACC ATC CCG TCA TAT GTT GCT AGT GAT      1007
Gln Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asp
320                 325                 330                 335

ATC TTG CGC CCA CTT TCA ATT GAT GCA GCA GAT GCA CTC TCA AAA TTC      1055
Ile Leu Arg Pro Leu Ser Ile Asp Ala Ala Asp Ala Leu Ser Lys Phe
                340                 345                 350

TAT TAT CCG CCA GTT GCT GCT GTA ACT GTT TCA TAT CCA AAA GAA GCT      1103
Tyr Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala
            355                 360                 365

ATT AGA AAA GAA TGC TTA ATT GAT GGG GAG CTC CAG GGT TTC GGC CAG      1151
Ile Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln
        370                 375                 380

TTG CAT CCA CGT AGC CAA GGA GTC GAG ACT TTA GGG ACA ATA TAT AGC      1199
Leu His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser
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    385                 390                 395

TCT TCT CTC TTT CCT AAT CGT GCT CCT GCT GGA AGA GTG TTA CTT CTG      1247
Ser Ser Leu Phe Pro Asn Arg Ala Pro Ala Gly Arg Val Leu Leu Leu
400                 405                 410                 415

AAC TAT ATC GGG GGT TCT ACA AAT ACA GGG ATC GTC TCC AAG ACT GAG      1295
Asn Tyr Ile Gly Gly Ser Thr Asn Thr Gly Ile Val Ser Lys Thr Glu
                420                 425                 430

AGT GAC TTA GTA GGA GCC GTT GAC CGT GAC CTC AGA AAA ATG TTG ATA      1343
Ser Asp Leu Val Gly Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile
            435                 440                 445

AAC CCT AGA GCA GCA GAC CCT TTA GCA TTA GGG GTT CGA GTG TGG CCA      1391
Asn Pro Arg Ala Ala Asp Pro Leu Ala Leu Gly Val Arg Val Trp Pro
        450                 455                 460

CAA GCA ATA CCA CAG TTT TTG ATT GGG CAC CTT GAT CGC CTT GCT GCT      1439
Gln Ala Ile Pro Gln Phe Leu Ile Gly His Leu Asp Arg Leu Ala Ala
    465                 470                 475

GCA AAA TCT GCA CTG GGC CAA GGC GGC TAC GAC GGG TTG TTC CTA GGA      1487
Ala Lys Ser Ala Leu Gly Gln Gly Gly Tyr Asp Gly Leu Phe Leu Gly
480                 485                 490                 495

GGA AAC TAC GTC GCA GGA GTT GCC TTG GGC CGA TGC ATC GAG GGT GCG      1535
Gly Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Ile Glu Gly Ala
                500                 505                 510

TAC GAG AGT GCC TCA CAA GTA TCT GAC TTC TTG ACC AAG TAT GCC TAC      1583
Tyr Glu Ser Ala Ser Gln Val Ser Asp Phe Leu Thr Lys Tyr Ala Tyr
            515                 520                 525

AAG TGA TGGAAGTAGT GCATCTCTTC ATTTTGTTGC ATATACGAGG TGAGGCTAGG       1639
Lys

ATCGGTAAAA CATCATGAGA TTCTGTAGTG TTTCTTTAAT TGAAAAAACA AATTTTAGTG    1699

ATGCAATATG TGCTCTTTCC TGTAGTTCGA GCATGTACAT CGGTATGGGA TAAAGTAGAA    1759

TAAGCTATTC TGCAAAAGCA GTGATTTTTT TTGAAAAAAA AAAAAAAAAA AA            1811
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 528 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ala Thr Met Ala Thr Ala Thr Val Ala Ala Ala Ser Pro Leu Arg Gly
1 5 10 15

Arg Val Thr Gly Arg Pro His Arg Val Arg Pro Arg Cys Ala Thr Ala
20 25 30

Ser Ser Ala Thr Glu Thr Pro Ala Ala Pro Gly Val Arg Leu Ser Ala
35 40 45

Glu Cys Val Ile Val Gly Ala Gly Ile Ser Gly Leu Cys Thr Ala Gln
50 55 60

Ala Leu Ala Thr Arg Tyr Gly Val Ser Asp Leu Leu Val Thr Glu Ala
65 70 75 80

Arg Asp Arg Pro Gly Gly Asn Ile Thr Thr Val Glu Arg Pro Asp Glu
85 90 95

Gly Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Pro
100 105 110

Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Asp Leu Val Phe
115 120 125

Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Glu Gly Lys Leu Arg
130 135 140

Pro Val Pro Ser Lys Pro Gly Asp Leu Pro Phe Phe Ser Leu Met Ser
145 150 155 160

Ile Pro Gly Lys Leu Arg Ala Gly Leu Gly Ala Leu Gly Ile Arg Pro
165 170 175

Pro Pro Pro Gly Arg Glu Glu Ser Val Glu Glu Phe Val Arg Arg Asn

180 185 190

Leu Gly Ala Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser Gly
195 200 205

Val Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe Gly
210 215 220

Lys Val Trp Arg Leu Glu Glu Ile Gly Gly Ser Ile Ile Gly Gly Thr
225 230 235 240

Ile Lys Ala Ile Gln Asp Lys Gly Lys Asn Pro Lys Pro Pro Arg Asp
245 250 255

Pro Arg Leu Pro Ala Pro Lys Gly Gln Thr Val Ala Ser Phe Arg Lys
260 265 270

Gly Leu Ala Met Leu Pro Asn Ala Ile Ala Ser Arg Leu Gly Ser Lys
275 280 285

Val Lys Leu Ser Trp Lys Leu Thr Ser Ile Thr Lys Ala Asp Asn Gln
290 295 300

Gly Tyr Val Leu Gly Tyr Glu Thr Pro Glu Gly Leu Val Ser Val Gln
305 310 315 320

Ala Lys Ser Val Ile Met Thr Ile Pro Ser Tyr Val Ala Ser Asp Ile
325 330 335

Leu Arg Pro Leu Ser Ile Asp Ala Ala Asp Ala Leu Ser Lys Phe Tyr
340 345 350

Tyr Pro Pro Val Ala Ala Val Thr Val Ser Tyr Pro Lys Glu Ala Ile
355 360 365

Arg Lys Glu Cys Leu Ile Asp Gly Glu Leu Gln Gly Phe Gly Gln Leu
370 375 380

His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser
385 390 395 400

Ser Leu Phe Pro Asn Arg Ala Pro Ala Gly Arg Val Leu Leu Leu Asn
405 410 415

Tyr Ile Gly Gly Ser Thr Asn Thr Gly Ile Val Ser Lys Thr Glu Ser

420 425 430

Asp Leu Val Gly Ala Val Asp Arg Asp Leu Arg Lys Met Leu Ile Asn
435 440 445

Pro Arg Ala Ala Asp Pro Leu Ala Leu Gly Val Arg Val Trp Pro Gln
450 455 460

Ala Ile Pro Gln Phe Leu Ile Gly His Leu Asp Arg Leu Ala Ala Ala
465 470 475 480

Lys Ser Ala Leu Gly Gln Gly Gly Tyr Asp Gly Leu Phe Leu Gly Gly
485 490 495

Asn Tyr Val Ala Gly Val Ala Leu Gly Arg Cys Ile Glu Gly Ala Tyr
500 505 510

Glu Ser Ala Ser Gln Val Ser Asp Phe Leu Thr Lys Tyr Ala Tyr Lys
515 520 525

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1847 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: soybean

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-12 (NRRL B-21516)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 55..1683
(D) OTHER INFORMATION: /product= "soybean protox-1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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CTTTAGCACA GTGTTGAAGA TAACGAACGA ATAGTGCCAT TACTGTAACC AACC ATG      57
                                                            Met
                                                              1

GTT TCC GTC TTC AAC GAG ATC CTA TTC CCG CCG AAC CAA ACC CTT CTT    105
Val Ser Val Phe Asn Glu Ile Leu Phe Pro Pro Asn Gln Thr Leu Leu
              5                  10                  15

CGC CCC TCC CTC CAT TCC CCA ACC TCT TTC TTC ACC TCT CCC ACT CGA    153
Arg Pro Ser Leu His Ser Pro Thr Ser Phe Phe Thr Ser Pro Thr Arg
         20                  25                  30

AAA TTC CCT CGC TCT CGC CCT AAC CCT ATT CTA CGC TGC TCC ATT GCG    201
Lys Phe Pro Arg Ser Arg Pro Asn Pro Ile Leu Arg Cys Ser Ile Ala
     35                  40                  45

GAG GAA TCC ACC GCG TCT CCG CCC AAA ACC AGA GAC TCC GCC CCC GTG    249
Glu Glu Ser Thr Ala Ser Pro Pro Lys Thr Arg Asp Ser Ala Pro Val
 50                  55                  60                  65

GAC TGC GTC GTC GTC GGC GGA GGC GTC AGC GGC CTC TGC ATC GCC CAG    297
Asp Cys Val Val Val Gly Gly Gly Val Ser Gly Leu Cys Ile Ala Gln
                 70                  75                  80

GCC CTC GCC ACC AAA CAC GCC AAT GCC AAC GTC GTC GTC ACG GAG GCC    345
Ala Leu Ala Thr Lys His Ala Asn Ala Asn Val Val Val Thr Glu Ala
             85                  90                  95

CGA GAC CGC GTC GGC GGC AAC ATC ACC ACG ATG GAG AGG GAC GGA TAC    393
Arg Asp Arg Val Gly Gly Asn Ile Thr Thr Met Glu Arg Asp Gly Tyr
        100                 105                 110

CTC TGG GAA GAA GGC CCC AAC AGC TTC CAG CCT TCT GAT CCA ATG CTC    441
Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Pro Met Leu
    115                 120                 125

ACC ATG GTG GTG GAC AGT GGT TTA AAG GAT GAG CTT GTT TTG GGG GAT    489
Thr Met Val Val Asp Ser Gly Leu Lys Asp Glu Leu Val Leu Gly Asp
130                 135                 140                 145

CCT GAT GCA CCT CGG TTT GTG TTG TGG AAC AGG AAG TTG AGG CCG GTG    537
Pro Asp Ala Pro Arg Phe Val Leu Trp Asn Arg Lys Leu Arg Pro Val
                150                 155                 160
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CCC GGG AAG CTG ACT GAT TTG CCT TTC TTT GAC TTG ATG AGC ATT GGT      585
Pro Gly Lys Leu Thr Asp Leu Pro Phe Phe Asp Leu Met Ser Ile Gly
            165                 170                 175

GGC AAA ATC AGG GCT GGC TTT GGT GCG CTT GGA ATT CGG CCT CCT CCT      633
Gly Lys Ile Arg Ala Gly Phe Gly Ala Leu Gly Ile Arg Pro Pro Pro
        180                 185                 190

CCA GGT CAT GAG GAA TCG GTT GAA GAG TTT GTT CGT CGG AAC CTT GGT      681
Pro Gly His Glu Glu Ser Val Glu Glu Phe Val Arg Arg Asn Leu Gly
    195                 200                 205

GAT GAG GTT TTT GAA CGG TTG ATA GAG CCT TTT TGT TCA GGG GTC TAT      729
Asp Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser Gly Val Tyr
210                 215                 220                 225

GCA GGC GAT CCT TCA AAA TTA AGT ATG AAA GCA GCA TTC GGG AAA GTT      777
Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe Gly Lys Val
                230                 235                 240

TGG AAG CTG GAA AAA AAT GGT GGT AGC ATT ATT GGT GGA ACT TTC AAA      825
Trp Lys Leu Glu Lys Asn Gly Gly Ser Ile Ile Gly Gly Thr Phe Lys
            245                 250                 255

GCA ATA CAA GAG AGA AAT GGA GCT TCA AAA CCA CCT CGA GAT CCG CGT      873
Ala Ile Gln Glu Arg Asn Gly Ala Ser Lys Pro Pro Arg Asp Pro Arg
        260                 265                 270

CTG CCA AAA CCA AAA GGT CAG ACT GTT GGA TCT TTC CGG AAG GGA CTT      921
Leu Pro Lys Pro Lys Gly Gln Thr Val Gly Ser Phe Arg Lys Gly Leu
    275                 280                 285

ACC ATG TTG CCT GAT GCA ATT TCT GCC AGA CTA GGC AAC AAA GTA AAG      969
Thr Met Leu Pro Asp Ala Ile Ser Ala Arg Leu Gly Asn Lys Val Lys
290                 295                 300                 305

TTA TCT TGG AAG CTT TCA AGT ATT AGT AAA CTG GAT AGT GGA GAG TAC     1017
Leu Ser Trp Lys Leu Ser Ser Ile Ser Lys Leu Asp Ser Gly Glu Tyr
                310                 315                 320

AGT TTG ACA TAT GAA ACA CCA GAA GGA GTG GTT TCT TTG CAG TGC AAA     1065
Ser Leu Thr Tyr Glu Thr Pro Glu Gly Val Val Ser Leu Gln Cys Lys
            325                 330                 335
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ACT GTT GTC CTG ACC ATT CCT TCC TAT GTT GCT AGT ACA TTG CTG CGT 1113
Thr Val Val Leu Thr Ile Pro Ser Tyr Val Ala Ser Thr Leu Leu Arg
340 345 350

CCT CTG TCT GCT GCT GCT GCA GAT GCA CTT TCA AAG TTT TAT TAC CCT 1161
Pro Leu Ser Ala Ala Ala Ala Asp Ala Leu Ser Lys Phe Tyr Tyr Pro
355 360 365

CCA GTT GCT GCA GTT TCC ATA TCC TAT CCA AAA GAA GCT ATT AGA TCA 1209
Pro Val Ala Ala Val Ser Ile Ser Tyr Pro Lys Glu Ala Ile Arg Ser
370 375 380 385

GAA TGC TTG ATA GAT GGT GAG TTG AAG GGG TTT GGT CAA TTG CAT CCA 1257
Glu Cys Leu Ile Asp Gly Glu Leu Lys Gly Phe Gly Gln Leu His Pro
390 395 400

CGT AGC CAA GGA GTG GAA ACA TTA GGA ACT ATA TAC AGC TCA TCA CTA 1305
Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu
405 410 415

TTC CCC AAC CGA GCA CCA CCT GGA AGG GTT CTA CTC TTG AAT TAC ATT 1353
Phe Pro Asn Arg Ala Pro Pro Gly Arg Val Leu Leu Leu Asn Tyr Ile
420 425 430

GGA GGA GCA ACT AAT ACT GGA ATT TTA TCG AAG ACG GAC AGT GAA CTT 1401
Gly Gly Ala Thr Asn Thr Gly Ile Leu Ser Lys Thr Asp Ser Glu Leu
435 440 445

GTG GAA ACA GTT GAT CGA GAT TTG AGG AAA ATC CTT ATA AAC CCA AAT 1449
Val Glu Thr Val Asp Arg Asp Leu Arg Lys Ile Leu Ile Asn Pro Asn
450 455 460 465

GCC CAG GAT CCA TTT GTA GTG GGG GTG AGA CTG TGG CCT CAA GCT ATT 1497
Ala Gln Asp Pro Phe Val Val Gly Val Arg Leu Trp Pro Gln Ala Ile
470 475 480

CCA CAG TTC TTA GTT GGC CAT CTT GAT CTT CTA GAT GTT GCT AAA GCT 1545
Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Asp Val Ala Lys Ala
485 490 495

TCT ATC AGA AAT ACT GGG TTT GAA GGG CTC TTC CTT GGG GGT AAT TAT 1593
Ser Ile Arg Asn Thr Gly Phe Glu Gly Leu Phe Leu Gly Gly Asn Tyr
500 505 510

GTG TCT GGT GTT GCC TTG GGA CGA TGC GTT GAG GGA GCC TAT GAG GTA 1641

Val Ser Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu Val
    515   520   525

GCA GCT GAA GTA AAC GAT TTT CTC ACA AAT AGA GTG TAC AAA 1683
Ala Ala Glu Val Asn Asp Phe Leu Thr Asn Arg Val Tyr Lys
530   535   540

TAGTAGCAGT TTTTGTTTTT GTGGTGGAAT GGGTGATGGG ACTCTCGTGT TCCATTGAAT 1743

TATAATAATG TGAAAGTTTC TCAAATTCGT TCGATAGGTT TTTGGCGGCT TCTATTGCTG 1803

ATAATGTAAA ATCCTCTTTA AGTTTGAAAA AAAAAAAAAA AAAA 1847

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 543 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID 12:

Met Val Ser Val Phe Asn Glu Ile Leu Phe Pro Pro Asn Gln Thr Leu
  1   5   10   15

Leu Arg Pro Ser Leu His Ser Pro Thr Ser Phe Phe Thr Ser Pro Thr
            20   25   30

Arg Lys Phe Pro Arg Ser Arg Pro Asn Pro Ile Leu Arg Cys Ser Ile
        35   40   45

Ala Glu Glu Ser Thr Ala Ser Pro Pro Lys Thr Arg Asp Ser Ala Pro
    50   55   60

Val Asp Cys Val Val Val Gly Gly Gly Val Ser Gly Leu Cys Ile Ala
 65   70   75   80

Gln Ala Leu Ala Thr Lys His Ala Asn Ala Asn Val Val Val Thr Glu
                85   90   95

Ala Arg Asp Arg Val Gly Gly Asn Ile Thr Thr Met Glu Arg Asp Gly
            100   105   110

Tyr Leu Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Pro Met
115 120 125

Leu Thr Met Val Val Asp Ser Gly Leu Lys Asp Glu Leu Val Leu Gly
130 135 140

Asp Pro Asp Ala Pro Arg Phe Val Leu Trp Asn Arg Lys Leu Arg Pro
145 150 155 160

Val Pro Gly Lys Leu Thr Asp Leu Pro Phe Phe Asp Leu Met Ser Ile
165 170 175

Gly Gly Lys Ile Arg Ala Gly Phe Gly Ala Leu Gly Ile Arg Pro Pro
180 185 190

Pro Pro Gly His Glu Glu Ser Val Glu Glu Phe Val Arg Arg Asn Leu
195 200 205

Gly Asp Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser Gly Val
210 215 220

Tyr Ala Gly Asp Pro Ser Lys Leu Ser Met Lys Ala Ala Phe Gly Lys
225 230 235 240

Val Trp Lys Leu Glu Lys Asn Gly Gly Ser Ile Ile Gly Gly Thr Phe
245 250 255

Lys Ala Ile Gln Glu Arg Asn Gly Ala Ser Lys Pro Pro Arg Asp Pro
260 265 270

Arg Leu Pro Lys Pro Lys Gly Gln Thr Val Gly Ser Phe Arg Lys Gly
275 280 285

Leu Thr Met Leu Pro Asp Ala Ile Ser Ala Arg Leu Gly Asn Lys Val
290 295 300

Lys Leu Ser Trp Lys Leu Ser Ser Ile Ser Lys Leu Asp Ser Gly Glu
305 310 315 320

Tyr Ser Leu Thr Tyr Glu Thr Pro Glu Gly Val Val Ser Leu Gln Cys
325 330 335

Lys Thr Val Val Leu Thr Ile Pro Ser Tyr Val Ala Ser Thr Leu Leu
340 345 350

Arg Pro Leu Ser Ala Ala Ala Ala Asp Ala Leu Ser Lys Phe Tyr Tyr
355 360 365

Pro Pro Val Ala Ala Val Ser Ile Ser Tyr Pro Lys Glu Ala Ile Arg
370 375 380

Ser Glu Cys Leu Ile Asp Gly Glu Leu Lys Gly Phe Gly Gln Leu His
385 390 395 400

Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser
405 410 415

Leu Phe Pro Asn Arg Ala Pro Pro Gly Arg Val Leu Leu Leu Asn Tyr
420 425 430

Ile Gly Gly Ala Thr Asn Thr Gly Ile Leu Ser Lys Thr Asp Ser Glu
435 440 445

Leu Val Glu Thr Val Asp Arg Asp Leu Arg Lys Ile Leu Ile Asn Pro
450 455 460

Asn Ala Gln Asp Pro Phe Val Val Gly Val Arg Leu Trp Pro Gln Ala
465 470 475 480

Ile Pro Gln Phe Leu Val Gly His Leu Asp Leu Leu Asp Val Ala Lys
485 490 495

Ala Ser Ile Arg Asn Thr Gly Phe Glu Gly Leu Phe Leu Gly Gly Asn
500 505 510

Tyr Val Ser Gly Val Ala Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu
515 520 525

Val Ala Ala Glu Val Asn Asp Phe Leu Thr Asn Arg Val Tyr Lys
530 535 540

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 583 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 1..583
(D) OTHER INFORMATION: /function= "arabidopsis protox-1 promoter"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAATTCCGAT CGAATTATAT AATTATCATA AATTTGAATA AGCATGTTGC CTTTTATTAA 60

AGAGGTTTAA TAAAGTTTGG TAATAATGGA CTTTGACTTC AAACTCGATT CTCATGTAAT 120

TAATTAATAT TTACATCAAA ATTTGGTCAC TAATATTACC AAATTAATAT ACTAAAATGT 180

TAATTCGCAA ATAAAACACT AATTCCAAAT AAAGGGTCAT TATGATAAAC ACGTATTGAA 240

CTTGATAAAG CAAAGCAAAA ATAATGGGTT TCAAGGTTTG GGTTATATAT GACAAAAAAA 300

AAAAAAGGTT TGGTTATATA TCTATTGGGC CTATAACCAT GTTATACAAA TTTGGGCCTA 360

ACTAAAATAA TAAAATAAAC GTAATGGTCC TTTTTATATT TGGGTCAAAC CCAACTCTAA 420

ACCCAAACCA AAGAAAAAGT ATACGGTACG GTACACAGAC TTATGGTGTG TGTGATTGCA 480

GGTGAATATT TCTCGTCGTC TTCTCCTTTC TTCTGAAGAA GATTACCCAA TCTGAAAAAA 540

ACCAAGAAGC TGACAAAATT CCGAATTCTC TGCGATTTCC ATG 583

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3848 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: promoter
(B) LOCATION: 1..3848
(D) OTHER INFORMATION: /function= "maize protox-1 promoter"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCGATCTTTC TAGGCTGATC CCCAAATCTT CCTCCGAAGC CCCTGGCGCC TCTGCCCCTT 60
GGAGCTGGTG GCCTGAAAGA GCTTTGCTGT TGCCCCGAAG ATTGTGAGGT ATATTGTGAC 120
CTCTGAGACT GACTTCCTTT GTCGTCACTT TGAGTGGAGT TATGGATTGA CCTGACGTGC 180
CTCAGATGGA TTCTTCCTCC GAAGCCCCTG GTCATTTCGG AGAATCTGTA ATCTTATTCC 240
CTTCTTTGGC GAAAATCTGT CAGCTTGGAT GTACTCATCC ATCTTCTGAA GCAGCTTCTC 300
CAGAGTTTGT GGAGGCTTCC TGGCGAAATA TTGGGCTGTA GGTCCTGGAC GAAGACCCTT 360
GATCATGGCC TCAATGACAA TCTCATTGGG CACCGTAGGC GCTTGTGCCC TCAATCGCAA 420
GAACCTTCGT ACATATGCCT GAAGGTATTC TTCGTGATCT TGTGTGCATT GGAACAGAGC 480
CTGAGCTGTG ACCGACTTCG TTTGAAAGCC TTGGAAGCTA GTAACCAACA TGTGCTTAAG 540
CTTCTGCCAC GACGTGATAG TCCCTGGCCG AAGAGAAGAA TACCATGTTT GGGCTACATT 600
CCGGACTGCC ATGACGAAGG ACTTCGCCAT GACTACAGTG TTGACCCCAT ACGAAGATAT 660
AGTTGCTTCG TAGCTCATCA GAAACTGCTT TGGATCTGAG TGCCCATCAT ACATGGGGAG 720
CTGAGGTGGC TTGTATGATG GGGGCCATGG GGTAGCCTGC AGTTCTGCTG CCAAGGGAGA 780
AGCATCATCA AAAGTAAAGG CATCATGATT AAAATCATCA TACCATCCAT CCTCGTTGAA 840
TAAGCCTTCT TGACGAAGCT CCCTGTGTTG GGGCCTTCGA TCTTGTTCAT CTTGAACAAG 900
ATGACGCACT TCTTCAGTGG CTTCGTCGAT CTTTCTTTGG AGATCAGCCA GTCGCACCAT 960
CTTCTCCTTC TTTCTTTGTA CTTGTTGATG GATGATCTCC ATGTCCCTGA TCTCTTGGTC 1020
CAACTCCTCC TCTTGGAGTG TCAGACTGGT GGCTTTCCTC TTCTGGCTTC GAGCCTCTCG 1080

AAGAGAAAGA GTTTCTTGAT TTGGGTCCAG CGGCTGCAGT GCAGTGGTCC CTGGTGCTGA 1140

AGCTTTCTTC GGTGGCATGA CAAAGGTCAG TGCTTGCCGA AGGTGGTCGA AAAGGGTTCA 1200

CTAGAGGTGG GAGCCAATGT TGGGGACTTC TCAAGTGCTA TGAGTTAAGA ACAAGGCAAC 1260

ACAAAATGTT AAATATTAAT AGCTTTCATC TTTCGAAGCA TTATTTCCCT TTGGGTATAA 1320

TGATCTTCAG ACGAAAGAGT CCTTCATCAT TGCGATATAT GTTAATAGAA GGAGGAGCAT 1380

ATGAAATGTA AGAGACAACA TGAACAATCG TGTAGCATTG TTAATTCATC ATCATTTTAT 1440

TATTATGGAA AAATAGAAAC AATATTGAAT TACAAATGTA CCTTTGGCTT GACAGAAGAT 1500

AAAAGTACAA GCTTGACGCA CGAGCAAGTA CAAGTCAGTG TGAACAGTAC GGGGGTACTG 1560

TTCATCTATT TATAGGCACA GGACACAGCC TGTGAGAAAT TACAGTCATG CCCTTTACAT 1620

TTACTATTGA CTTATAGAAA AATCTATGAG GACTGGATAG CCTTTTCCCC TTTAAGTCGG 1680

TGCCTTTTTC CGCGATTAAG CCGAATCTCC CTTGCGCATA GCTTCGGAGC ATCGGCAACC 1740

TTCGTCACGA TCATGCCCTT CTCATTGTGT ATGCTTTTAA TCCTGAATTC GAAGGTACCT 1800

GTCCATAAAC CATACTTGGA AGACATTGTT AAATTATGTT TTTGAGGACC TTCGGAGGAC 1860

GAAGGCCCCC AACAGTCGTG TTTTTGAGGA CCTTCGGAAG ATGAAGGCCC CCAACAAGAC 1920

CTATCCATAA AACCAACCTA TCCACAAAAC CGACCCCATT CACCCTTCAT TTGCCTCACC 1980

AACAACCCTA ATTAGGTTGT TGGTTTAAAT TTTTTAGGGT CAATTTGGTC ATCACCATCC 2040

ACTGTCACTC CACAAACTCA ATATCAATAA ACAGACTCAA TCACCCAAAC TGACCATACC 2100

CATAAAACCG CCCCACCCTT CTAGCGCCTC GCCAGAAACC AGAAACCCTG ATTCAGAGTT 2160

CAAACTTAAA ACGACCATAA CTTTCACCTT GGAACTCGAA TCAGGTCCAT TTTTTTCCAA 2220

ATCACACAAA ATTAAATTTC GCATCCGATA ATCAAGCCAT CTCTTCACTA TGGTTTTAAG 2280

TGTTGCTCAC ACTAGTGTAT TTATGGACTA ATCACCTGTG TATCTCATAC AATAACATAT 2340

CAGTACATCT AAGTTGTTAC TCAATTACCA AAACCGAATT ATAGCCTTCG AAAAAGGTTA 2400

TCGACTAGTC ACTCAATTAC CAAAACTAAA CTTTAGACTT TCATGTATGA CATCCAACAT 2460

GACACTGTAC TGGACTAAAC CACCTTTCAA GCTACACAAG GAGCAAAAAT AACTAATTTT 2520

CGTAGTTGTA GGAGCTAAAG TATATGTCCA CAACAATAGT TAAGGGAAGC CCCCAAGGAC 2580

TTAAAAGTCC TTTTACCTCT TGAAACTTTT GTCGTGGTCT ACTTTTTCAC TTTAAACTTC 2640

AAAATTTGAC ATTTTATCAC CCCTTAACTC TTAAAACCAT TTAAATTACA TTCTTACTAG 2700

ATTATAGATG ATTTTGTTGT GAAAAGTTTT TAAGACATGT TTACACATTG ATTAAAATCA 2760

TTTGTTCAAT TTCCTAGAGT TAAATCTAAT CTTATTAAAA CTATTAGAGA TACTTTCACG 2820

AGCTCTAAAT ATTTTTATTT TTTCATTATG GAATTTTGTT AGAATTCTTA TAGACCTTTT 2880

TTTGTGGTTT AAAAGCCTTG CCATGTTTTT AACAAGTTTT TTTTCTATTT TTTGAAATTT 2940

TCTTGGAAAC CACTTCTAAC CCGGTAGAAG ATTTATTTTG CTACACTTAT ATCTACAACA 3000

AAATCAACTT ATGAAATTGT CTTGGAAACT ACCTCTAACC CGGTAGAATG AATTTGAATG 3060

AAAATTAAAC CAACTTACGG AATCGCCCAA CATATGTCGA TTAAAGTGGA TATGGATACA 3120

TATGAAGAAG CCCTAGAGAT AATCTAAATG GTTTCAGAAT TGAGGGTTAT TTTTTGAAGT 3180

TTGATGGGAA GATAAGACCA TAACGGTAGT TCACAGAGAT AAAAGGGTTA TTTTTTTCAG 3240

AAATATTTGT GCTGCAATTG ATCCTGTGCC TCAAATTCAG CCTGCAACCA AGGCCAGGTT 3300

CTAGAGCGAA CAAGGCCCAC GTCACCCGTG GCCCGTCAGG CGAAGCAGGT CTTGTGCAGA 3360

CTTTGAGAGG GATTGGATAT CAACGGAACC AATCACGCAC GGCAATGCGA TTCCCAGCCC 3420

ACCTGTAACG TTCCAGTGGG CCATCCTTAA CTCCAAGCCC AACGGCCCTA CCCCATCTCG 3480

TCGTGTCATC CACTCCGCCG CACAGGCGCT CAGCTCCGCA ACGCCGCCGG AAATGGTCGC 3540

CGCCACAGCC ACCGCCATGG CCACCGCTGC ATCGCCGCTA CTCAACGGGA CCCGAATACC 3600

TGCGCGGCTC CGCCATCGAG GACTCAGCGT GCGCTGCGCT GCTGTGGCGG GCGGCGCGGC 3660

CGAGGCACCG GCATCCACCG GCGCGCGGCT GTCCGCGGAC TGCGTTGTGG TGGGCGGAGG 3720

CATCAGTGGC CTCTGCACCG CGCAGGCGCT GGCCACGCGG CACGGCGTCG GGGACGTGCT 3780

TGTCACGGAG GCCCGCGCCC GCCCCGGCGG CAACATTACC ACCGTCGAGC GCCCCGAGGA 3840

AGGGTACC 3848

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1826 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Gossypium hirsutum (cotton)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-15 (NRRL B-21594)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 31..1647
(D) OTHER INFORMATION: /product= "Cotton protox-1 coding region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCTCTCGCTC GCCTGGCCCC ACCACCAATC ATGACGGCTC TAATCGACCT TTCTCTTCTC 60

CGTTCCTCGC CCTCCGTTTC CCCTTTCTCC ATACCCCACC ACCAGCATCC GCCCCGCTTT 120

CGTAAACCTT TCAAGCTCCG ATGCTCCCTC GCCGAGGGTC CCACGATTTC CTCATCTAAA 180

ATCGACGGGG GAGAATCATC CATCGCGGAT TGCGTCATCG TTGGAGGTGG TATCAGTGGA 240

CTTTGCATTG CTCAAGCTCT CGCCACCAAG CACCGTGACG TCGCTTCCAA TGTGATTGTG 300

ACGGAGGCCA GAGACCGTGT TGGTGGCAAC ATCACTACCG TTGAGAGAGA TGGATATCTG 360

TGGGAAGAAG GCCCCAACAG TTTTCAGCCC TCCGATCCTA TTCTAACCAT GGCCGTGGAT 420

AGTGGATTGA AGGACGATTT GGTTTTAGGT GACCCTAATG CACCGCGATT TGTACTATGG 480

GAGGGAAAAC TAAGGCCTGT GCCCTCCAAG CCAACCGACT TGCCGTTTTT TGATTTGATG 540

AGCATTGCTG GAAAACTTAG GGCTGGGTTC GGGGCTATTG GCATTCGGCC TCCCCCTCCG 600

GGTTATGAAG AATCGGTGGA GGAGTTTGTG CGCCGTAATC TTGGTGCTGA GGTTTTTGAA 660

CGCTTTATTG AACCATTTTG TTCAGGTGTT TATGCAGGGG ATCCTTCAAA ATTAAGCATG 720

AAAGCAGCAT TTGGAAGAGT ATGGAAGCTA GAAGAGATTG GTGGCAGCAT CATTGGTGGC 780

ACTTTCAAGA CAATCCAGGA GAGAAATAAG ACACCTAAGC CACCCAGAGA CCCGCGTCTG 840

CCAAAACCGA AGGGCCAAAC AGTTGGATCT TTTAGGAAGG GACTTACCAT GCTGCCTGAG 900

GCAATTGCTA ACAGTTTGGG TAGCAATGTA AAATTATCTT GGAAGCTTTC CAGTATTACC 960

AAATTGGGCA ATGGAGGGTA TAACTTGACA TTTGAAACAC CTGAAGGAAT GGTATCTCTT 1020

CAGAGTAGAA GTGTTGTAAT GACCATTCCA TCCCATGTTG CCAGTAACTT GTTGCATCCT 1080

CTCTCGGCTG CTGCTGCAGA TGCATTATCC CAATTTTATT ATCCTCCAGT TGCATCAGTC 1140

ACAGTCTCCT ATCCAAAAGA AGCCATTCGA AAAGAATGTT TGATTGATGG TGAACTTAAG 1200

GGGTTTGGCC AGTTGCACCC ACGCAGCCAA GGAATTGAAA CTTTAGGGAC GATATACAGT 1260

TCATCACTTT TCCCCAATCG AGCTCCATCT GGCAGGGTGT TGCTCTTGAA CTACATAGGA 1320

GGAGCTACCA ACACTGGAAT TTTGTCCAAG ACTGAAGGGG AACTTGTAGA AGCAGTTGAT 1380

CGTGATTTGA GAAAAATGCT TATAAATCCT AATGCAAAGG ATCCTCTTGT TTTGGGTGTA 1440

AGAGTATGGC CAAAAGCCAT TCCACAGTTC TTGGTTGGTC ATTTGGATCT CCTTGATAGT 1500

GCAAAAATGG CTCTCAGGGA TTCTGGGTTT CATGGACTGT TTCTTGGGGG CAACTATGTA 1560

TCTGGTGTGG CATTAGGACG GTGTGTGGAA GGTGCTTACG AGGTTGCAGC TGAAGTGAAG 1620

GAATTCCTGT CACAATATGC ATACAAATAA TATTGAAATT CTTGTCAGGC TGCAAATGTA 1680

GAAGTCAGTT ATTGGATAGT ATCTCTTTAG CTAAAAAATT GGGTAGGGTT TTTTTTGTTA 1740

GTTCCTTGAC CACTTTTTGG GGTTTTCATT AGAACTTCAT ATTTGTATAT CATGTTGCAA 1800

TATCAAAAAA AAAAAAAAAA AAAAAA 1826

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 539 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Thr Ala Leu Ile Asp Leu Ser Leu Leu Arg Ser Ser Pro Ser Val
1 5 10 15

Ser Pro Phe Ser Ile Pro His His Gln His Pro Pro Arg Phe Arg Lys
20 25 30

Pro Phe Lys Leu Arg Cys Ser Leu Ala Glu Gly Pro Thr Ile Ser Ser
35 40 45

Ser Lys Ile Asp Gly Gly Glu Ser Ser Ile Ala Asp Cys Val Ile Val
50 55 60

Gly Gly Gly Ile Ser Gly Leu Cys Ile Ala Gln Ala Leu Ala Thr Lys
65 70 75 80

His Arg Asp Val Ala Ser Asn Val Ile Val Thr Glu Ala Arg Asp Arg
85 90 95

Val Gly Gly Asn Ile Thr Thr Val Glu Arg Asp Gly Tyr Leu Trp Glu
100 105 110

Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Pro Ile Leu Thr Met Ala
115 120 125

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Val Asp Ser Gly Leu Lys Asp Asp Leu Val Leu Gly Asp Pro Asn Ala
    130                 135                 140

Pro Arg Phe Val Leu Trp Glu Gly Lys Leu Arg Pro Val Pro Ser Lys
145                 150                 155                 160

Pro Thr Asp Leu Pro Phe Phe Asp Leu Met Ser Ile Ala Gly Lys Leu
                165                 170                 175

Arg Ala Gly Phe Gly Ala Ile Gly Ile Arg Pro Pro Pro Pro Gly Tyr
            180                 185                 190

Glu Glu Ser Val Glu Glu Phe Val Arg Arg Asn Leu Gly Ala Glu Val
        195                 200                 205

Phe Glu Arg Phe Ile Glu Pro Phe Cys Ser Gly Val Tyr Ala Gly Asp
    210                 215                 220

Pro Ser Lys Leu Ser Met Lys Ala Ala Phe Gly Arg Val Trp Lys Leu
225                 230                 235                 240

Glu Glu Ile Gly Gly Ser Ile Ile Gly Gly Thr Phe Lys Thr Ile Gln
                245                 250                 255

Glu Arg Asn Lys Thr Pro Lys Pro Pro Arg Asp Pro Arg Leu Pro Lys
            260                 265                 270

Pro Lys Gly Gln Thr Val Gly Ser Phe Arg Lys Gly Leu Thr Met Leu
        275                 280                 285

Pro Glu Ala Ile Ala Asn Ser Leu Gly Ser Asn Val Lys Leu Ser Trp
    290                 295                 300

Lys Leu Ser Ser Ile Thr Lys Leu Gly Asn Gly Gly Tyr Asn Leu Thr
305                 310                 315                 320

Phe Glu Thr Pro Glu Gly Met Val Ser Leu Gln Ser Arg Ser Val Val
                325                 330                 335

Met Thr Ile Pro Ser His Val Ala Ser Asn Leu Leu His Pro Leu Ser
            340                 345                 350

Ala Ala Ala Ala Asp Ala Leu Ser Gln Phe Tyr Tyr Pro Pro Val Ala
        355                 360                 365
```

```
Ser Val Thr Val Ser Tyr Pro Lys Glu Ala Ile Arg Lys Glu Cys Leu
    370                 375                 380

Ile Asp Gly Glu Leu Lys Gly Phe Gly Gln Leu His Pro Arg Ser Gln
385                 390                 395                 400

Gly Ile Glu Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu Phe Pro Asn
                405                 410                 415

Arg Ala Pro Ser Gly Arg Val Leu Leu Leu Asn Tyr Ile Gly Gly Ala
            420                 425                 430

Thr Asn Thr Gly Ile Leu Ser Lys Thr Glu Gly Glu Leu Val Glu Ala
        435                 440                 445

Val Asp Arg Asp Leu Arg Lys Met Leu Ile Asn Pro Asn Ala Lys Asp
    450                 455                 460

Pro Leu Val Leu Gly Val Arg Val Trp Pro Lys Ala Ile Pro Gln Phe
465                 470                 475                 480

Leu Val Gly His Leu Asp Leu Leu Asp Ser Ala Lys Met Ala Leu Arg
                485                 490                 495

Asp Ser Gly Phe His Gly Leu Phe Leu Gly Gly Asn Tyr Val Ser Gly
            500                 505                 510

Val Ala Leu Gly Arg Cys Val Glu Gly Ala Tyr Glu Val Ala Ala Glu
        515                 520                 525

Val Lys Glu Phe Leu Ser Gln Tyr Ala Tyr Lys
    530                 535
```

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1910 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Beta vulgaris (Sugar Beet)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-16 (NRRL B-21595N)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..1680
(D) OTHER INFORMATION: /product= "Sugar Beet Protox-1 coding region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGAAATCAA TGGCGTTATC AAACTGCATT CCACAGACAC AGTGCATGCC ATTGCGCAGC 60

AGCGGGCATT ACAGGGGTAA TTGTATCATG TTGTCAATTC CATGTAGTTT AATTGGAAGA 120

CGAGGTTATT ATTCACATAA GAAGAGGAGG ATGAGCATGA GTTGCAGCAC AAGCTCAGGC 180

TCAAAGTCAG CGGTTAAAGA AGCAGGATCA GGATCAGGTG CAGGAGGATT GCTAGACTGC 240

GTAATCGTTG GAGGTGGAAT TAGCGGGCTT TGCATCGCGC AGGCTCTTTG TACAAAACAC 300

TCCTCTTCCT CTTTATCCCC AAATTTTATA GTTACAGAGG CCAAAGACAG AGTTGGCGGC 360

AACATCGTCA CTGTGGAGGC CGATGGCTAT ATCTGGGAGG AGGGACCCAA TAGCTTCCAG 420

CCTTCCGACG CGGTGCTCAC CATGGCGGTC GACAGTGGCT TGAAAGATGA GTTGGTGCTC 480

GGAGATCCCA ATGCTCCTCG CTTTGTGCTA TGGAATGACA AATTAAGGCC CGTACCTTCC 540

AGTCTCACCG ACCTCCCTTT CTTCGACCTC ATGACCATTC CGGGCAAGAT TAGGGCTGCT 600

CTTGGTGCTC TCGGATTTCG CCCTTCTCCT CCACCTCATG AGGAATCTGT TGAACACTTT 660

GTGCGTCGTA ATCTCGGAGA TGAGGTCTTT GAACGCTTGA TTGAACCCTT TTGTTCAGGT 720

GTGTATGCCG GTGATCCTGC CAAGCTGAGT ATGAAAGCTG CTTTTGGGAA GGTCTGGAAG 780

TTGGAGCAAA AGGGTGGCAG CATAATTGGT GGCACTCTCA AAGCTATACA GGAAAGAGGG 840

AGTAATCCTA AGCCGCCCCG TGACCAGCGC CTCCCTAAAC CAAAGGGTCA GACTGTTGGA 900

TCCTTTAGAA AGGGACTCGT TATGTTGCCT ACCGCCATTT CTGCTCGACT TGGCAGTAGA 960

GTGAAACTAT CTTGGACCCT TTCTAGTATC GTAAAGTCAC TCAATGGAGA ATATAGTCTG 1020

ACTTATGATA CCCCAGATGG CTTGGTTTCT GTAAGAACCA AAAGTGTTGT GATGACTGTT 1080

CCATCATATG TTGCAAGTAG GCTTCTTCGT CCACTTTCAG ACTCTGCTGC AGATTCTCTT 1140

TCAAAATTTT ACTATCCACC AGTTGCAGCA GTGTCACTTT CCTATCCTAA AGAAGCGATC 1200

AGATCAGAAT GCTTGATTAA TGGTGAACTT CAAGGTTTCG GGCAACTACA TCCCCGCAGT 1260

CAGGGTGTGG AAACCTTGGG AACAATTTAT AGTTCGTCTC TTTTCCCTGG TCGAGCACCA 1320

CCTGGTAGGA TCTTGATCTT GAGCTACATC GGAGGTGCTA AAAATCCTGG CATATTAAAC 1380

AAGTCGAAAG ATGAACTTGC CAAGACAGTT GACAAGGACC TGAGAAGAAT GCTTATAAAT 1440

CCTGATGCAA AACTTCCTCG TGTACTGGGT GTGAGAGTAT GGCCTCAAGC AATACCCCAG 1500

TTTTCTATTG GGCACTTTGA TCTGCTCGAT GCTGCAAAAG CTGCTCTGAC AGATACAGGG 1560

GTCAAAGGAC TGTTTCTTGG TGGCAACTAT GTTTCAGGTG TTGCCTTGGG GCGGTGTATA 1620

GAGGGTGCTT ATGAGTCTGC AGCTGAGGTA GTAGATTTCC TCTCACAGTA CTCAGACAAA 1680

TAGAGCTTCA GCATCCTGTG TAATTCAACA CAGGCCTTTT TGTATCTGTT GTGCGCGCAT 1740

GTAGTCTGGT CGTGGTGCTA GGATTGATTA GTTGCTCTGC TGTGTGATCC ACAAGAATTT 1800

TGATGGAATT TTTCCAGATG TGGGCATTAT ATGTTGCTGT CTTATAAATC CTTAATTTGT 1860

ACGTTTAGTG AATTACACCG CATTTGATGA CTAAAAAAAA AAAAAAAAAA 1910

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 560 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Lys Ser Met Ala Leu Ser Asn Cys Ile Pro Gln Thr Gln Cys Met
1               5                   10                  15

Pro Leu Arg Ser Ser Gly His Tyr Arg Gly Asn Cys Ile Met Leu Ser
            20                  25                  30

Ile Pro Cys Ser Leu Ile Gly Arg Arg Gly Tyr Tyr Ser His Lys Lys
        35                  40                  45

Arg Arg Met Ser Met Ser Cys Ser Thr Ser Ser Gly Ser Lys Ser Ala
    50                  55                  60

Val Lys Glu Ala Gly Ser Gly Ser Gly Ala Gly Gly Leu Leu Asp Cys
65                  70                  75                  80

Val Ile Val Gly Gly Gly Ile Ser Gly Leu Cys Ile Ala Gln Ala Leu
                85                  90                  95

Cys Thr Lys His Ser Ser Ser Ser Leu Ser Pro Asn Phe Ile Val Thr
            100                 105                 110

Glu Ala Lys Asp Arg Val Gly Gly Asn Ile Val Thr Val Glu Ala Asp
        115                 120                 125

Gly Tyr Ile Trp Glu Glu Gly Pro Asn Ser Phe Gln Pro Ser Asp Ala
    130                 135                 140

Val Leu Thr Met Ala Val Asp Ser Gly Leu Lys Asp Glu Leu Val Leu
145                 150                 155                 160

Gly Asp Pro Asn Ala Pro Arg Phe Val Leu Trp Asn Asp Lys Leu Arg
                165                 170                 175

Pro Val Pro Ser Ser Leu Thr Asp Leu Pro Phe Phe Asp Leu Met Thr
            180                 185                 190

Ile Pro Gly Lys Ile Arg Ala Ala Leu Gly Ala Leu Gly Phe Arg Pro
        195                 200                 205
```

Ser Pro Pro Pro His Glu Glu Ser Val Glu His Phe Val Arg Arg Asn
    210   215   220

Leu Gly Asp Glu Val Phe Glu Arg Leu Ile Glu Pro Phe Cys Ser Gly
225   230   235   240

Val Tyr Ala Gly Asp Pro Ala Lys Leu Ser Met Lys Ala Ala Phe Gly
                245   250   255

Lys Val Trp Lys Leu Glu Gln Lys Gly Gly Ser Ile Ile Gly Gly Thr
            260   265   270

Leu Lys Ala Ile Gln Glu Arg Gly Ser Asn Pro Lys Pro Pro Arg Asp
        275   280   285

Gln Arg Leu Pro Lys Pro Lys Gly Gln Thr Val Gly Ser Phe Arg Lys
    290   295   300

Gly Leu Val Met Leu Pro Thr Ala Ile Ser Ala Arg Leu Gly Ser Arg
305   310   315   320

Val Lys Leu Ser Trp Thr Leu Ser Ser Ile Val Lys Ser Leu Asn Gly
                325   330   335

Glu Tyr Ser Leu Thr Tyr Asp Thr Pro Asp Gly Leu Val Ser Val Arg
            340   345   350

Thr Lys Ser Val Val Met Thr Val Pro Ser Tyr Val Ala Ser Arg Leu
        355   360   365

Leu Arg Pro Leu Ser Asp Ser Ala Ala Asp Ser Leu Ser Lys Phe Tyr
    370   375   380

Tyr Pro Pro Val Ala Ala Val Ser Leu Ser Tyr Pro Lys Glu Ala Ile
385   390   395   400

Arg Ser Glu Cys Leu Ile Asn Gly Glu Leu Gln Gly Phe Gly Gln Leu
                405   410   415

His Pro Arg Ser Gln Gly Val Glu Thr Leu Gly Thr Ile Tyr Ser Ser
            420   425   430

Ser Leu Phe Pro Gly Arg Ala Pro Pro Gly Arg Ile Leu Ile Leu Ser
        435   440   445

Tyr Ile Gly Gly Ala Lys Asn Pro Gly Ile Leu Asn Lys Ser Lys Asp
    450   455   460

Glu Leu Ala Lys Thr Val Asp Lys Asp Leu Arg Arg Met Leu Ile Asn
465   470   475   480

Pro Asp Ala Lys Leu Pro Arg Val Leu Gly Val Arg Val Trp Pro Gln
                485   490   495

Ala Ile Pro Gln Phe Ser Ile Gly His Phe Asp Leu Leu Asp Ala Ala
            500   505   510

Lys Ala Ala Leu Thr Asp Thr Gly Val Lys Gly Leu Phe Leu Gly Gly
        515   520   525

Asn Tyr Val Ser Gly Val Ala Leu Gly Arg Cys Ile Glu Gly Ala Tyr
    530   535   540

Glu Ser Ala Ala Glu Val Val Asp Phe Leu Ser Gln Tyr Ser Asp Lys
545   550   555   560

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1784 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Brassica napus (rape)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-17 (NRRL B-21615)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 47..1654

(D) OTHER INFORMATION: /product= "Rape Protox-1 coding region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGCCCCCCC CAAAATTGAG GATTCTCCTT CTCGCGGGCG ATCGCCATGG ATTTATCTCT 60

TCTCCGTCCG CAGCCATTCC TATCGCCATT CTCAAATCCA TTTCCTCGGT CGCGTCCCTA 120

CAAGCCTCTC AACCTCCGTT GCTCCGTATC CGGTGGATCC GTCGTCGGCT CTTCTACAAT 180

CGAAGGCGGA GGAGGAGGTA AAACCGTCAC GGCGGACTGC GTGATCGTCG GCGGAGGAAT 240

CAGCGGCCTG TGCATTGCGC AAGCGCTCGT GACGAAGCAC CCAGACGCTG CAAAGAATGT 300

GATGGTGACG GAGGCGAAGG ACCGTGTGGG AGGGAATATC ATCACGCGAG AGGAGCAAGG 360

GTTTCTATGG GAAGAAGGTC CCAATAGCTT TCAGCCGTCT GATCCTATGC TCACTATGGT 420

GGTAGATAGT GGTTTGAAAG ATGATCTAGT CTTGGGAGAT CCTACTGCTC CGAGGTTTGT 480

GTTGTGGAAT GGGAAGCTGA GGCCGGTTCC GTCGAAGCTA ACTGACTTGC CTTTCTTTGA 540

CTTGATGAGT ATTGGAGGGA AGATTAGAGC TGGGTTTGGT GCCATTGGTA TTCGACCTTC 600

ACCTCCGGGT CGTGAGGAAT CAGTGGAAGA GTTTGTAAGG CGTAATCTTG GTGATGAGGT 660

TTTTGAGCGC TTGATTGAAC CCTTTTGCTC AGGTGTTTAT GCGGGAGATC CTGCGAAACT 720

GAGTATGAAA GCAGCTTTTG GGAAGGTTTG GAAGCTAGAG GAGAATGGTG GGAGCATCAT 780

TGGTGGTGCT TTTAAGGCAA TTCAAGCGAA AAATAAAGCT CCCAAGACAA CCCGAGATCC 840

GCGTCTGCCA AAGCCAAAGG GCCAAACTGT TGGTTCTTTC AGGAAAGGAC TCACAATGCT 900

GCCAGAGGCA ATCTCCGCAA GGTTGGGTGA CAAGGTGAAA GTTTCTTGGA AGCTCTCAAG 960

TATCACTAAG CTGGCCAGCG GAGAATATAG CTTAACTTAC GAAACTCCGG AGGGTATAGT 1020

CACTGTACAG AGCAAAAGTG TAGTGATGAC TGTGCCATCT CATGTTGCTA GTAGTCTCTT 1080

GCGCCCTCTC TCTGATTCTG CAGCTGAAGC GCTCTCAAAA CTCTACTATC CGCCAGTTGC 1140

AGCCGTATCC ATCTCATACG CGAAAGAAGC AATCCGAAGC GAATGCTTAA TAGATGGTGA 1200

ACTAAAAGGG TTCGGCCAGT TGCATCCACG CACGCAAAAA GTGGAAACTC TTGGAACAAT 1260

ATACAGTTCA TCGCTCTTTC CCAACCGAGC ACCGCCTGGA AGAGTATTGC TATTGAACTA 1320

CATCGGTGGA GCTACCAACA CTGGGATCTT ATCAAAGTCG GAAGGTGAGT TAGTGGAAGC 1380

AGTAGATAGA GACTTGAGGA AGATGCTGAT AAAGCCAAGC TCGACCGATC CACTTGTACT 1440

TGGAGTAAAA TTATGGCCTC AAGCCATTCC TCAGTTTCTG ATAGGTCACA TTGATTTGGT 1500

AGACGCAGCG AAAGCATCGC TCTCGTCATC TGGTCATGAG GGCTTATTCT TGGGTGGAAA 1560

TTACGTTGCC GGTGTAGCAT TGGGTCGGTG TGTGGAAGGT GCTTATGAAA CTGCAACCCA 1620

AGTGAATGAT TTCATGTCAA GGTATGCTTA CAAGTAATGT AACGCAGCAA CGATTTGATA 1680

CTAAGTAGTA GATTTTGCAG TTTTGACTTT AAGAACACTC TGTTTGTGAA AAATTCAAGT 1740

CTGTGATTGA GTAAATTTAT GTATTATTAC TAAAAAAAAA AAAA 1784

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 536 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Asp Leu Ser Leu Leu Arg Pro Gln Pro Phe Leu Ser Pro Phe Ser
1 5 10 15

Asn Pro Phe Pro Arg Ser Arg Pro Tyr Lys Pro Leu Asn Leu Arg Cys
20 25 30

Ser Val Ser Gly Gly Ser Val Val Gly Ser Ser Thr Ile Glu Gly Gly
35 40 45

```
Gly Gly Gly Lys Thr Val Thr Ala Asp Cys Val Ile Val Gly Gly Gly
    50                  55                  60

Ile Ser Gly Leu Cys Ile Ala Gln Ala Leu Val Thr Lys His Pro Asp
65                  70                  75                  80

Ala Ala Lys Asn Val Met Val Thr Glu Ala Lys Asp Arg Val Gly Gly
                85                  90                  95

Asn Ile Ile Thr Arg Glu Glu Gln Gly Phe Leu Trp Glu Glu Gly Pro
            100                 105                 110

Asn Ser Phe Gln Pro Ser Asp Pro Met Leu Thr Met Val Val Asp Ser
        115                 120                 125

Gly Leu Lys Asp Asp Leu Val Leu Gly Asp Pro Thr Ala Pro Arg Phe
    130                 135                 140

Val Leu Trp Asn Gly Lys Leu Arg Pro Val Pro Ser Lys Leu Thr Asp
145                 150                 155                 160

Leu Pro Phe Phe Asp Leu Met Ser Ile Gly Gly Lys Ile Arg Ala Gly
                165                 170                 175

Phe Gly Ala Ile Gly Ile Arg Pro Ser Pro Pro Gly Arg Glu Glu Ser
            180                 185                 190

Val Glu Glu Phe Val Arg Arg Asn Leu Gly Asp Glu Val Phe Glu Arg
        195                 200                 205

Leu Ile Glu Pro Phe Cys Ser Gly Val Tyr Ala Gly Asp Pro Ala Lys
    210                 215                 220

Leu Ser Met Lys Ala Ala Phe Gly Lys Val Trp Lys Leu Glu Glu Asn
225                 230                 235                 240

Gly Gly Ser Ile Ile Gly Gly Ala Phe Lys Ala Ile Gln Ala Lys Asn
                245                 250                 255

Lys Ala Pro Lys Thr Thr Arg Asp Pro Arg Leu Pro Lys Pro Lys Gly
            260                 265                 270

Gln Thr Val Gly Ser Phe Arg Lys Gly Leu Thr Met Leu Pro Glu Ala
        275                 280                 285
```

Ile Ser Ala Arg Leu Gly Asp Lys Val Lys Val Ser Trp Lys Leu Ser
290 295 300

Ser Ile Thr Lys Leu Ala Ser Gly Glu Tyr Ser Leu Thr Tyr Glu Thr
305 310 315 320

Pro Glu Gly Ile Val Thr Val Gln Ser Lys Ser Val Val Met Thr Val
325 330 335

Pro Ser His Val Ala Ser Ser Leu Leu Arg Pro Leu Ser Asp Ser Ala
340 345 350

Ala Glu Ala Leu Ser Lys Leu Tyr Tyr Pro Pro Val Ala Ala Val Ser
355 360 365

Ile Ser Tyr Ala Lys Glu Ala Ile Arg Ser Glu Cys Leu Ile Asp Gly
370 375 380

Glu Leu Lys Gly Phe Gly Gln Leu His Pro Arg Thr Gln Lys Val Glu
385 390 395 400

Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu Phe Pro Asn Arg Ala Pro
405 410 415

Pro Gly Arg Val Leu Leu Leu Asn Tyr Ile Gly Gly Ala Thr Asn Thr
420 425 430

Gly Ile Leu Ser Lys Ser Glu Gly Glu Leu Val Glu Ala Val Asp Arg
435 440 445

Asp Leu Arg Lys Met Leu Ile Lys Pro Ser Ser Thr Asp Pro Leu Val
450 455 460

Leu Gly Val Lys Leu Trp Pro Gln Ala Ile Pro Gln Phe Leu Ile Gly
465 470 475 480

His Ile Asp Leu Val Asp Ala Ala Lys Ala Ser Leu Ser Ser Ser Gly
485 490 495

His Glu Gly Leu Phe Leu Gly Gly Asn Tyr Val Ala Gly Val Ala Leu
500 505 510

Gly Arg Cys Val Glu Gly Ala Tyr Glu Thr Ala Thr Gln Val Asn Asp
515 520 525

Phe Met Ser Arg Tyr Ala Tyr Lys
530 535

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1224 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Oryza sative (rice)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-18 (NRRL B-21648)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..936
(D) OTHER INFORMATION: /product= "Rice Protox-1 partial coding region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CGGGCTTTGA AGGCTGCATT TGGGAAGGTG TGGAGGCTGG AGGATACTGG AGGTAGCATT 60

ATTGGTGGAA CCATCAAGAC AATCCAGGAG AGGGGGAAAA ACCCCAAACC GCCGAGGGAT 120

CCCCGCCTTC CAACGCCAAA GGGGCAGACA GTTGCATCTT TCAGGAAGGG TCTGACTATG 180

CTCCCGGATG CTATTACATC TAGGTTGGGT AGCAAAGTCA AACTTTCATG GAAGTTGACA 240

AGCATTACAA AGTCAGACAA CAAAGGATAT GCATTAGTGT ATGAAACACC AGAAGGGGTG 300

GTCTCGGTGC AAGCTAAAAC TGTTGTCATG ACCATCCCAT CATATGTTGC TAGTGATATC 360

TTGCGGCCAC TTTCAAGTGA TGCAGCAGAT GCTCTGTCAA TATTCTATTA TCCACCAGTT 420

GCTGCTGTAA CTGTTTCATA TCCAAAAGAA GCAATTAGAA AAGAATGCTT AATTGACGGA 480

GAGCTCCAGG GTTTCGGCCA GCTGCATCCG CGTAGTCAGG GAGTTGAGAC TTTAGGAACA 540

ATATATAGCT CATCACTCTT TCCAAATCGT GCTCCAGCTG GAAGGGTGTT ACTTCTGAAC 600

TACATAGGAG GTTCTACAAA TACAGGGATT GTTTCCAAGA CTGAAAGTGA GCTGGTAGAA 660

GCAGTTGACC GTGACCTCAG GAAGATGCTG ATAAATCCTA GAGCAGTGGA CCCTTTGGTC 720

CTTGGCGTCC GGGTATGGCC ACAAGCCATA CCACAGTTCC TCATTGGCCA TCTTGATCAT 780

CTTGAGGCTG CAAAATCTGC CCTGGGCAAA GGTGGGTATG ATGGATTGTT CCTCGGAGGG 840

AACTATGTTG CAGGAGTTGC CCTGGGCCGA TGCGTTGAAG GTGCATATGA GAGTGCCTCA 900

CAAATATCTG ACTACTTGAC CAAGTACGCC TACAAGTGAT CAAAGTTGGC CTGCTCCTTT 960

TGGCACATAG ATGTGAGGCT TCTAGCAGCA AAAATTTCAT GGGCATCTTT TTATCCTGAT 1020

TCTAATTAGT TAGAATTTAG AATTGTAGAG GAATGTTCCA TTTGCAGTTC ATAATAGTTG 1080

TTCAGATTTC AGCCATTCAA TTTGTGCAGC CATTTACTAT ATGTAGTATG ATCTTGTAAG 1140

TACTACTAAG AACAAATCAA TTATATTTTC CTGCAAGTGA CATCTTAATC GTCAGCAAAT 1200

CCAGTTACTA GTAAAAAAAA AAAA 1224

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 312 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Arg Ala Leu Lys Ala Ala Phe Gly Lys Val Trp Arg Leu Glu Asp Thr

1 5 10 15

Gly Gly Ser Ile Ile Gly Gly Thr Ile Lys Thr Ile Gln Glu Arg Gly
20 25 30

Lys Asn Pro Lys Pro Pro Arg Asp Pro Arg Leu Pro Thr Pro Lys Gly
35 40 45

Gln Thr Val Ala Ser Phe Arg Lys Gly Leu Thr Met Leu Pro Asp Ala
50 55 60

Ile Thr Ser Arg Leu Gly Ser Lys Val Lys Leu Ser Trp Lys Leu Thr
65 70 75 80

Ser Ile Thr Lys Ser Asp Asn Lys Gly Tyr Ala Leu Val Tyr Glu Thr
85 90 95

Pro Glu Gly Val Val Ser Val Gln Ala Lys Thr Val Val Met Thr Ile
100 105 110

Pro Ser Tyr Val Ala Ser Asp Ile Leu Arg Pro Leu Ser Ser Asp Ala
115 120 125

Ala Asp Ala Leu Ser Ile Phe Tyr Tyr Pro Pro Val Ala Ala Val Thr
130 135 140

Val Ser Tyr Pro Lys Glu Ala Ile Arg Lys Glu Cys Leu Ile Asp Gly
145 150 155 160

Glu Leu Gln Gly Phe Gly Gln Leu His Pro Arg Ser Gln Gly Val Glu
165 170 175

Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu Phe Pro Asn Arg Ala Pro
180 185 190

Ala Gly Arg Val Leu Leu Leu Asn Tyr Ile Gly Gly Ser Thr Asn Thr
195 200 205

Gly Ile Val Ser Lys Thr Glu Ser Glu Leu Val Glu Ala Val Asp Arg
210 215 220

Asp Leu Arg Lys Met Leu Ile Asn Pro Arg Ala Val Asp Pro Leu Val
225 230 235 240

Leu Gly Val Arg Val Trp Pro Gln Ala Ile Pro Gln Phe Leu Ile Gly

```
                245                 250                 255

His Leu Asp His Leu Glu Ala Ala Lys Ser Ala Leu Gly Lys Gly Gly
            260                 265                 270

Tyr Asp Gly Leu Phe Leu Gly Gly Asn Tyr Val Ala Gly Val Ala Leu
        275                 280                 285

Gly Arg Cys Val Glu Gly Ala Tyr Glu Ser Ala Ser Gln Ile Ser Asp
    290                 295                 300

Tyr Leu Thr Lys Tyr Ala Tyr Lys
305                 310
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1590 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Sorghum bicolor (sorghum)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-19 (NRRL B-21649)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..1320
(D) OTHER INFORMATION: /product= "Sorghum Protox-1 partial coding region"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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TCCACCGTCG AGCGCCCCGA GGAAGGGTAC CTCTGGGAGG AGGGTCCCAA CAGCTTCCAG        60
```

CCATCCGACC CCGTTCTCTC CATGGCCGTG GACAGCGGGC TGAAGGATGA CCTGGTTTTT 120

GGGGACCCCA ACGCGCCACG GTTCGTGCTG TGGGAGGGGA AGCTGAGGCC CGTGCCATCC 180

AAGCCCGCCG ACCTCCCGTT CTTCGATCTC ATGAGCATCC CTGGCAAGCT CAGGGCCGGT 240

CTCGGCGCGC TTGGCATCCG CCCGCCTGCT CCAGGCCGCG AGGAGTCAGT GGAGGAGTTT 300

GTGCGCCGCA ACCTCGGTGC TGAGGTCTTT GAGCGCCTAA TTGAGCCTTT CTGCTCAGGT 360

GTCTATGCTG GCGATCCTTC CAAGCTCAGT ATGAAGGCTG CATTTGGGAA GGTGTGGCGG 420

TTAGAAGAAG CTGGAGGTAG TATTATTGGT GGAACCATCA AGACGATTCA GGAGAGGGGC 480

AAGAATCCAA AACCACCGAG GGATCCCCGC CTTCCGAAGC CAAAAGGGCA GACAGTTGCA 540

TCTTTCAGGA AGGGTCTTGC CATGCTTCCA AATGCCATCA CATCCAGCTT GGGTAGTAAA 600

GTCAAACTAT CATGGAAACT CACGAGCATG ACAAAATCAG ATGGCAAGGG GTATGTTTTG 660

GAGTATGAAA CACCAGAAGG GGTTGTTTTG GTGCAGGCTA AAAGTGTTAT CATGACCATT 720

CCATCATATG TTGCTAGCGA CATTTTGCGT CCACTTTCAG GTGATGCTGC AGATGTTCTA 780

TCAAGATTCT ATTATCCACC AGTTGCTGCT GTAACGGTTT CGTATCCAAA GGAAGCAATT 840

AGAAAAGAAT GCTTAATTGA TGGGGAACTC CAGGGTTTTG GCCAGTTGCA TCCACGTAGT 900

CAAGGAGTTG AGACATTAGG AACAATATAC AGCTCATCAC TCTTTCCAAA TCGTGCTCCT 960

GCTGGTAGGG TGTTACTTCT AAACTACATA GGAGGTGCTA CAAACACAGG AATTGTTTCC 1020

AAGACTGAAA GTGAGCTGGT AGAAGCAGTT GACCGTGACC TCCGAAAAAT GCTTATAAAT 1080

CCTACAGCAG TGGACCCTTT AGTCCTTGGT GTCCGAGTTT GGCCACAAGC CATACCTCAG 1140

TTCCTGGTAG GACATCTTGA TCTTCTGGAG GCCGCAAAAT CTGCCCTGGA CCAAGGTGGC 1200

TATAATGGGC TGTTCCTAGG AGGGAACTAT GTTGCAGGAG TTGCCCTGGG CAGATGCATT 1260

GAGGGCGCAT ATGAGAGTGC CGCGCAAATA TATGACTTCT TGACCAAGTA CGCCTACAAG 1320

TGATGGAAGA AGTGGAGCGC TGCTTGTTAA TTGTTATGTT GCATAGATGA GGTGAGACCA 1380

GGAGTAGTAA AAGGCGTCAC GAGTATTTTT CATTCTTATT TTGTAAATTG CACTTCTGTT 1440

TTTTTTTCCT GTCAGTAATT AGTTAGATTT TAGTTATGTA GGAGATTGTT GTGTTCACTG 1500

CCCTACAAAA GAATTTTTAT TTTGCATTCG TTTATGAGAG CTGTGCAGAC TTATGTAACG 1560

TTTTACTGTA AGTATCAACA AAATCAAATA 1590

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 440 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Ser Thr Val Glu Arg Pro Glu Glu Gly Tyr Leu Trp Glu Glu Gly Pro
1 5 10 15

Asn Ser Phe Gln Pro Ser Asp Pro Val Leu Ser Met Ala Val Asp Ser
20 25 30

Gly Leu Lys Asp Asp Leu Val Phe Gly Asp Pro Asn Ala Pro Arg Phe
35 40 45

Val Leu Trp Glu Gly Lys Leu Arg Pro Val Pro Ser Lys Pro Ala Asp
50 55 60

Leu Pro Phe Phe Asp Leu Met Ser Ile Pro Gly Lys Leu Arg Ala Gly
65 70 75 80

Leu Gly Ala Leu Gly Ile Arg Pro Pro Ala Pro Gly Arg Glu Glu Ser
85 90 95

Val Glu Glu Phe Val Arg Arg Asn Leu Gly Ala Glu Val Phe Glu Arg
100 105 110

Leu Ile Glu Pro Phe Cys Ser Gly Val Tyr Ala Gly Asp Pro Ser Lys
115 120 125

Leu Ser Met Lys Ala Ala Phe Gly Lys Val Trp Arg Leu Glu Glu Ala
130 135 140

Gly Gly Ser Ile Ile Gly Gly Thr Ile Lys Thr Ile Gln Glu Arg Gly
145 150 155 160

Lys Asn Pro Lys Pro Pro Arg Asp Pro Arg Leu Pro Lys Pro Lys Gly
165 170 175

Gln Thr Val Ala Ser Phe Arg Lys Gly Leu Ala Met Leu Pro Asn Ala
180 185 190

Ile Thr Ser Ser Leu Gly Ser Lys Val Lys Leu Ser Trp Lys Leu Thr
195 200 205

Ser Met Thr Lys Ser Asp Gly Lys Gly Tyr Val Leu Glu Tyr Glu Thr
210 215 220

Pro Glu Gly Val Val Leu Val Gln Ala Lys Ser Val Ile Met Thr Ile
225 230 235 240

Pro Ser Tyr Val Ala Ser Asp Ile Leu Arg Pro Leu Ser Gly Asp Ala
245 250 255

Ala Asp Val Leu Ser Arg Phe Tyr Tyr Pro Pro Val Ala Ala Val Thr
260 265 270

Val Ser Tyr Pro Lys Glu Ala Ile Arg Lys Glu Cys Leu Ile Asp Gly
275 280 285

Glu Leu Gln Gly Phe Gly Gln Leu His Pro Arg Ser Gln Gly Val Glu
290 295 300

Thr Leu Gly Thr Ile Tyr Ser Ser Ser Leu Phe Pro Asn Arg Ala Pro
305 310 315 320

Ala Gly Arg Val Leu Leu Leu Asn Tyr Ile Gly Gly Ala Thr Asn Thr
325 330 335

Gly Ile Val Ser Lys Thr Glu Ser Glu Leu Val Glu Ala Val Asp Arg
340 345 350

Asp Leu Arg Lys Met Leu Ile Asn Pro Thr Ala Val Asp Pro Leu Val
355 360 365

```
Leu Gly Val Arg Val Trp Pro Gln Ala Ile Pro Gln Phe Leu Val Gly
    370                 375                 380

His Leu Asp Leu Leu Glu Ala Ala Lys Ser Ala Leu Asp Gln Gly Gly
385                 390                 395                 400

Tyr Asn Gly Leu Phe Leu Gly Gly Asn Tyr Val Ala Gly Val Ala Leu
                405                 410                 415

Gly Arg Cys Ile Glu Gly Ala Tyr Glu Ser Ala Ala Gln Ile Tyr Asp
            420                 425                 430

Phe Leu Thr Lys Tyr Ala Tyr Lys
        435                 440
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "maize protox-1 intron sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
GTACGCTCCT CGCTGGCGCC GCAGCGTCTT CTTCTCAGAC TCATGCGCAG CCATGGAATT      60

GAGATGCTGA ATGGATTTTA TACGCGCGCG CAG                                   93
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2606 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Beta vulgaris (sugar beet)

(vii) IMMEDIATE SOURCE:
(B) CLONE: pWDC-20 (NRRL B-21650)

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "SalI site"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: complement (1..538)
(D) OTHER INFORMATION: /note= "partial cDNA of sugar beet protox-1 in 3' - 5' direction"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 539..2606
(D) OTHER INFORMATION: /note= "sugar beet protox-1 promoter region presented in 3' - 5' direction (partial sequence of the ~ 3 kb PstI-SalI fragment subcloned from pWDC-20)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GTCGACCTAC GCACATGCCA CATTCCACAT TCCACGTTAG GAATTGAATT GAATTGAATT 60

ATGATTATGA ATAATGAAGA GACAGAATTA CCGCCATGGT GAGCACCGCG TCGGAAGGCT 120

GGAAGCTATT GGGTCCCTCC TCCCAGATAT AGCCATCGGC CTCCACAGTG ACGATGTTGC 180

CGCCAACTCT GTCTTTGGCC TCTGTCACTA TAAAATTTGG GGATAAAGAG GACTGTTTTG 240

TACAAAGAGC CTGCGCGATG CAAAGCCCGC TAATTCCACC TCCAACGATT ACGCAGTCTA 300

GCAATCCTCC TGCTCCTGAT CCTGATCCTG ATCCTGCTTC TTTAACCGCT GACTTTGAGC 360

CTGAGCTTGT GCTGCAACTC ATGCTCATCC TCCTCTTCTT ATGTGAATAA TAACCTCGTC 420

TTCCAATTAA ACTACATGGA ATTGACAACA TGATACAATT GCCCCTGTAA TGCCCGCTGC 480

TGTGCAATGG CATGCACTGT GTCTGTGGAA TGCAGTTTGA TAACGCCATT GATTTCATCT 540

CTCTCTCGCT CTCTCGCCCT CCTTATCCTC TATATCCCCT TCTTGCTTGC TCGGGAATTC 600

TAATTAACCT TATATCAAAA TGAAACAACT GTTTCTAGTT AAAAAGTTTT TTATAAATAG 660

TACTCTAAAT AAACGATTAC ATGTATCTTC TAACCATACT TGTTTGGTGG AGGTGGTGCG 720

TAACCGGTAA CTTACCTTTG TAACTCACCT CAATACCTAC TTATGCTTAA GGATACGGAT 780

TCTTTTAAAC TCTCAGGCAT TGACCTATGT AGCTGGACTG ACTAACATCT GAATTTGTTT 840

CTCTGGTTAT ATATGCAATT TTAACTGAAT CGAAATTTCT CTGGATGCTA AAAATGTCTT 900

TAACGGGGTT TATGAGGACT AAATTATCTC CTTCAATGAG GAGGTTCTTG ATTTGCATGT 960

ATGAGCGTGA AAATGCATTC TTAACGGCTA TAGATTCAGT AATAAGTGGT GTTAAAAGTA 1020

AAAAGTACTT GGAAAAAATGA TTAAGCGACT TAATTTTTTT TATTTGTTTG AAAGTTGCCT 1080

TTTCTTGGCT ATCTTAACAT GTATTTATCA AACACCTTTT TTAATTACAT GGAAATCGAA 1140

AAGTTTGAAA AAAAAAAATC ATACTCACTA ACCGCCTTAA AATATAAGCT GAAGATGTCT 1200

CACTAACAGA GTGCATGTGA AGCACCCCCA AAGCAATTAT AACACAACAT CTCCGCCTCT 1260

TCAAAATTCC TACAAATACA TCTAATAAAC TTGTTGAAAC AATCAAAGTA ACATGGTGTG 1320

TCAATTGCGG ATGCTTCTCA TTCCAGACTT TATATAGTGA TTTTGTTTAA TCCATAGTCA 1380

ACAACTCACA TAATGGTACC CAAAGAATAC CCAAATTTTT TGCTCAAAAT CCCTAAACAT 1440

TGTAGCTGTG TAAGTTTGAC TAACATGTTT CAGCATGCTT GCCATGGGTA AATAAGACTT 1500

AGGGGCAAAT CTCGAATCCA CAAACTCATC ATTGGTTTTA GTTTGTCTCC AACGTAAAAC 1560

AATGATGTGA AATACACCAC AAAATTCATA CAATCTCGTT ATCTTGGAAG CTTGAAAGCC 1620

ATAATCTTGT TTGTACTTTC ACTACGTCGA GAAGACAAAA TTACAACTAA GAAGAGGTCA 1680

TTGCTCAGTG TCGTGTACTA CTTATCTTTC AACTCATAGA AACAAGCAAA CCAATTGTCA 1740

CCTATATACT GTACTTCTCC ATCATATACT TCCAACTTGC CTTAAACTCA ATACTATCAT 1800

AAAAACCACA AAGACATTTC ATAAAAGCAT AATAAAAATG TGTCATCACT CTTCAAAGTT 1860

CCAAAGTGAT TCTAACTACA TTCTAATGAA AATGACATTG GTGTAAACCT AATCCTTGTG 1920

TTATAAAACA CCTACATACC ACGATTATGT TAGAAATATA TTTATGAATG CAGTACCTAC 1980

ATAAAGCCAT TAAATAACCA GTTTTATGTT ATTTCGTGAC CAACATAGTT CCTAAAGATT 2040

ACGAAGTAAT TTATAGTCAT TTTGTGGCCA CTTAATTCAT TTAATACCCA GTATATTTAT 2100

AAGTTACCAG CTTAAGTAGT TTTGTGACCA TCTCTACATA CTTCCTCCGG TCCATAATAA 2160

GGGGGCGTTT GGTTGCAACG GGGTAAAGGG AATGGAATCA AGAAAGGGAG AGGAGAGGAA 2220

AGGAAAAGAA AACCCTTAGA TTTAGAGTGG TGTTTGGTTA AGATAATGTT AATTCTCTTT 2280

CTTCCTCTTT CTTACCCTTC TTCCACCCTA GCACCACCAC TCCTCCCTCT GTTACTATTC 2340

TCCACGCCGC CTCTCCCTAC CCCAGTAACA CCACCTTGTC GGCCCCCCGG TCTTCCCCTT 2400

CCCGCGACGG TTCCCCCCTC CCCTGCGCCG TCACGTCGTC CCCCTCACCT CCCTGCACCG 2460

TCGAGTTATC CCCCTCCCCT GCGCGTCGCG TTCTCCCCTC CCTCACCATC GCGTTCTCCC 2520

CTCCCTCACC GTCGCGTTCT CCCCTCCCTC ACCGTCGCGG TCTCCCCTCC CTCACCGTCG 2580

CGGTCTCTCT TTCCCTCCCC CTGCAG 2606

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Pclp_P1a - plastid clpP gene promoter top strand PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /note= "EcoRI restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GCGGAATTCA TACTTATTTA TCATTAGAAA G 31

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Pclp_P1b - plastid clpP gene promoter bottom strand PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /note= "XbaI restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GCGTCTAGAA AGAACTAAAT ACTATATTTC AC 32

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Pclp_P2b - plastid clpP gene promoter bottom strand PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /note= "NcoI restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCGCCATGGT AAATGAAAGA AAGAACTAAA 30

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Trps16_P1a - plastid rps16 gene 3' untranslated region XbaI/HindIII top strand PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /note= "XbaI restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCGTCTAGAT CAACCGAAAT TCAATTAAGG 30

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Trps16_p1b - plastid rps16 gene 3' untranslated region XbaI/HindIII bottom strand PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 4..9
(D) OTHER INFORMATION: /note= "HindIII restriction site"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CGCAAGCTTC AATGGAAGCA ATGATAA 27

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "minpsb_U - plastid psbA gene 5' untranslated region 38 nt (blunt/NcoI) including ATG

start codon, top strand primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GGGAGTCCCT GATGATTAAA TAAACCAAGA TTTTAC 36

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "minpsb_L - plastid psbA gene 5' untranslated region 38 nt (blunt/NcoI) including ATG start codon (bottom strand primer)"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CATGGTAAAA TCTTGGTTTA TTTAATCATC AGGGACTCCC 40

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "APRTXP1a - top strand PCR primer for amplifying the 5' portion of the mutant Arabidopsis protox gene"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 5..10
(D) OTHER INFORMATION: /note= "NcoI restriction site/ATG start codon"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGACCATGG ATTGTGTGAT TGTCGGCGGA GG 32

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "APRTXP1b - bottom strand PCR primer for amplifying the 5' portion of the mutant Arabidopsis protox gene"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CTCCGCTCTC CAGCTTAGTG ATAC 24

What is claimed is:

1. An isolated DNA molecule encoding a wheat protoporphyrinogen oxidase (protox) enzyme comprising the amino acid sequence set forth in SEQ ID NO:10.

2. The isolated DNA molecule of claim 1 comprising the nucleotide sequence set forth in SEQ ID NO:9.

3. An isolated DNA molecule encoding a soybean protox enzyme comprising the amino acid sequence set forth in SEQ ID NO:12.

4. The isolated DNA molecule of claim 3 comprising the nucleotide sequence set forth in SEQ ID NO:11.

5. An isolated DNA molecule encoding a cotton protox enzyme comprising the amino acid sequence set forth in SEQ ID NO:16.

6. The isolated DNA molecule of claim 5 comprising the nucleotide sequence set forth in SEQ ID NO:15.

7. An isolated DNA molecule encoding a sugar beet protox enzyme comprising the amino acid sequence set forth in SEQ ID NO:18.

8. The isolated DNA molecule of claim 7 comprising the nucleotide sequence set forth in SEQ ID NO:17.

9. An isolated DNA molecule encoding a rape protox enzyme comprising the amino acid sequence set forth in SEQ ID NO:20.

10. The isolated DNA molecule of claim 9 comprising the nucleotide sequence set forth in SEQ ID NO:19.

11. An isolated DNA molecule encoding a rice protox enzyme comprising the amino acid sequence set forth in SEQ ID NO:22.

12. The isolated DNA molecule of claim 11 comprising the nucleotide sequence set forth in SEQ ID NO:21.

13. An isolated DNA molecule encoding a sorghum protox enzyme comprising the amino acid sequence set forth in SEQ ID NO:24.

14. The isolated DNA molecule of claim 13 comprising the nucleotide sequence set forth in SEQ ID NO:23.

15. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the cysteine occurring at the position corresponding to amino acid 159 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit said plant protox.

16. The DNA molecule of claim 15 wherein said cysteine is replaced with a phenylalanine or lysine.

17. The DNA molecule of claim 16 wherein said cysteine is replaced with a phenylalanine.

18. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the isoleucine occurring at the position corresponding to amino acid 419 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

19. The DNA molecule of claim 18 wherein said isoleucine is replaced with a threonine, histidine, glycine or asparagine.

20. The DNA molecule of claim 18 wherein said isoleucine is replaced with a threonine.

21. DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the alanine occurring at the position corresponding to amino acid 164 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

22. The DNA molecule of claim 21 wherein said alanine is replaced with a threonine, leucine or valine.

23. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the glycine occurring at the position corresponding to amino acid 165 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

24. The DNA molecule of claim 23 wherein said glycine is replaced with a serine or leucine.

25. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 370 of SEQ ID NO:6 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

26. The DNA molecule of claim 25 wherein said tyrosine is replaced with a isoleucine or methionine.

27. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the valine occurring at the position corresponding to amino acid 356 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

28. The DNA molecule of claim 27 wherein said valine is replaced with a leucine.

29. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the serine occurring at the position corresponding to amino acid 421 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

30. The DNA molecule of claim 29 wherein said serine is replaced with a proline.

31. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the valine occurring at the position corresponding to amino acid 502 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

32. The DNA molecule of claim 31 wherein said valine is replaced with a alanine.

33. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the alanine occurring at the position corresponding to amino acid 211 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

34. The DNA molecule of claim 33 wherein said alanine is replaced with a valine or threonine.

35. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the glycine occurring at the position corresponding to amino acid 212 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

36. The DNA molecule of claim 35 wherein said glycine is replaced with a serine.

37. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the isoleucine occurring at the position corresponding to amino acid 466 of SEQ ID NO:10 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

38. The DNA molecule of claim 37 wherein said isoleucine is replaced with a threonine.

39. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the proline occurring at the position corresponding to amino acid 369 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

40. The DNA molecule of claim 39 wherein said proline is replaced with a serine or histidine.

41. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the alanine occurring at the position corresponding to amino acid 226 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

42. The DNA molecule of claim 41 wherein said alanine is replaced with a threonine or leucine.

43. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the valine occurring at the position corresponding to amino acid 517 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

44. The DNA molecule of claim 43 wherein said valine is replaced with a alanine.

45. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 432 of SEQ ID NO:12 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

46. The DNA molecule of claim 45 wherein said tyrosine is replaced with a leucine or isoleucine.

47. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the proline occurring at the position corresponding to amino acid 365 of SEQ ID NO:16 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

48. The DNA molecule of claim 47 wherein said proline is replaced with a serine.

49. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 428 of SEQ ID NO:16 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

50. The DNA molecule of claim 50 wherein said tyrosine is replaced with a cysteine or arginine.

51. A DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox wherein the tyrosine occurring at the position corresponding to amino acid 449 of SEQ ID NO:18 is replaced with another amino acid, wherein said modified protox is tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity.

52. The DNA molecule of claim 51 wherein said tyrosine is replaced with a cysteine, leucine, isoleucine, valine or methionine.

53. An isolated DNA molecule encoding a modified protoporphyrinogen oxidase (protox) comprising a plant protox having a first amino acid substitution and a second amino acid substitution,

said first amino acid substitution having the property of conferring resistance to a protox inhibitor; and

said second amino acid substitution having the property of enhancing said resistance conferred by said first amino acid substitution.

54. The DNA molecule of claim 53 wherein said second amino acid substitution occurs at a position selected from the group consisting of

(i) the position corresponding to the serine at amino acid 305 of SEQ ID NO:2;
(ii) the position corresponding to the threonine at amino acid 249 of SEQ ID NO:2;
(iii) the position corresponding to the proline at amino acid 118 of SEQ ID NO:2;
(iv) the position corresponding to the asparagine at amino acid 425 of SEQ ID NO:2; and
(v) the position corresponding to the tyrosine at amino acid 498 of SEQ ID NO:2.

55. The DNA molecule of claim 54, wherein said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6;
(b) the position corresponding to the glycine at amino acid 165 of SEQ ID NO:6;
(c) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6;
(d) the position corresponding to the cysteine at amino acid 159 of SEQ ID NO:6;
(e) the position corresponding to the isoleucine at amino acid 419 of SEQ ID NO:6.
(f) the position corresponding to the valine at amino acid 356 of SEQ ID NO:10;
(g) the position corresponding to the serine at amino acid 421 of SEQ ID NO:10;
(h) the position corresponding to the valine at amino acid 502 of SEQ ID NO:10;
(i) the position corresponding to the alanine at amino acid 211 of SEQ ID NO:10;
(k) the position corresponding to the glycine at amino acid 212 of SEQ ID NO:10;
(l) the position corresponding to the isoleucine at amino acid 466 of SEQ ID NO:10;
(m) the position corresponding to the proline at amino acid 369 of SEQ ID NO:12;
(n) the position corresponding to the alanine at amino acid 226 of SEQ ID NO:12;
(o) the position corresponding to the tyrosine at amino acid 432 of SEQ ID NO:12;
(p) the position corresponding to the valine at amino acid 517 of SEQ ID NO:12;
(q) the position corresponding to the tyrosine at amino acid 428 of SEQ ID NO:16;
(r) the position corresponding to the proline at amino acid 365 of SEQ ID NO:16;

(s) the position corresponding to the tyrosine at amino acid 449 of SEQ ID NO:18.

56. The DNA molecule of claim 54, wherein said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6;

(b) the position corresponding to the glycine at amino acid 165 of SEQ ID NO:6;

(c) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6;

(d) the position corresponding to the cysteine at amino acid 159 of SEQ ID NO:6;

(e) the position corresponding to the isoleucine at amino acid 419 of SEQ ID NO:6.

57. The DNA molecule of claim 54, wherein said second amino acid substitution occurs at the position corresponding to the serine at amino acid 305 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6;

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6

58. The DNA molecule of claim 57 wherein said serine occurring at the position corresponding to amino acid 305 of SEQ ID NO:2 is replaced with leucine.

59. The DNA molecule of claim 54 wherein said second amino acid substitution occurs at the position corresponding to the threonine at amino acid 249 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

60. The DNA molecule of claim 59 wherein said threonine occurring at the position corresponding to amino acid 249 of SEQ ID NO:2 is replaced with an amino acid selected from the group consisting of isoleucine and alanine.

61. The DNA molecule of claim 54 wherein said second amino acid substitution occurs at the position corresponding to the proline at amino acid 118 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

62. The DNA molecule of claim 61 wherein said proline occurring at the position corresponding to amino acid 118 of SEQ ID NO:2 is replaced with a leucine.

63. The DNA molecule of claim 54 wherein said second amino acid substitution occurs at the position corresponding to the asparagine at amino acid 425 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

64. The DNA molecule of claim 63 wherein said asparagine occurring at the position corresponding to amino acid 425 of SEQ ID NO:2 is replaced with a serine.

65. The DNA molecule of claim 54 wherein said second amino acid substitution occurs the position corresponding to the tyrosine at amino acid 498 of SEQ ID NO:2 and said first amino acid substitution occurs at a position selected from the group consisting of

(a) the position corresponding to the alanine at amino acid 164 of SEQ ID NO:6; and

(b) the position corresponding to the tyrosine at amino acid 370 of SEQ ID NO:6.

66. The DNA molecule of claim 65 wherein said tyrosine occurring at the position corresponding to amino acid 498 of SEQ ID NO:2 is replaced with a cysteine.

67. The DNA molecule of any of claims 50-66 wherein said tyrosine occurring at the position corresponding to amino acid 370 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of cysteine, isoleucine, leucine, threonine, valine and methionine.

68. The DNA molecule of any of claims 50-66 wherein said tyrosine occurring at the position corresponding to amino acid 370 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of cysteine, isoleucine, leucine, threonine and methionine.

69. The DNA molecule of claim 50-66 wherein said alanine occurring at the position corresponding to residue 164 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of valine, threonine, leucine, cysteine and tyrosine.

70. The DNA molecule of claim 56 wherein said glycine occurring at the position corresponding to residue 165 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of serine and leucine.

71. The DNA molecule of claim 56 wherein said glycine occurring at the position corresponding to residue 165 of SEQ ID NO:6 is replaced with a serine.

72. The DNA molecule of claim 56 wherein said cysteine occurring at the position corresponding to residue 159 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of phenylalanine and lysine.

73. The DNA molecule of claim 56 wherein said cysteine occurring at the position corresponding to residue 159 of SEQ ID NO:6 is replaced with a phenylalanine.

74. The DNA molecule of claim 56 wherein said isoleucine occurring at the position corresponding to residue 419 of SEQ ID NO:6 is replaced with an amino acid selected from the group consisting of threonine, histidine, glycine and asparagine.

75. The DNA molecule of claim 56 wherein said isoleucine occurring at the position corresponding to residue 419 of SEQ ID NO:6 is replaced with a threonine.

76. The DNA molecule of claim 55 wherein said valine occurring at the position corresponding to residue 356 of SEQ ID NO:10 is replaced with a leucine.

77. The DNA molecule of claim 55 wherein said serine occurring at the position corresponding to residue 421 of SEQ ID NO:10 is replaced with a proline.

78. The DNA molecule of claim 55 wherein said valine occurring at the position corresponding to residue 502 of SEQ ID NO:10 is replaced with a alanine.

79. The DNA molecule of claim 55 wherein said isoleucine occurring at the position corresponding to residue 466 of SEQ ID NO:10 is replaced with a threonine.

80. The DNA molecule of claim 55 wherein said glycine occurring at the position corresponding to residue 212 of SEQ ID NO:10 is replaced with a serine.

81. The DNA molecule of claim 55 wherein said alanine occurring at the position corresponding to residue 211 of SEQ ID NO:10 is replaced with a valine or threonine.

82. The DNA molecule of claim 55 wherein said proline occurring at the position corresponding to residue 369 of SEQ ID NO:12 is replaced with a serine or a histidine.

83. The DNA molecule of claim 55 wherein said alanine occurring at the position corresponding to residue 226 of SEQ ID NO:12 is replaced with a leucine or threonine.

84. The DNA molecule of claim 55 wherein said tyrosine occurring at the position corresponding to residue 432 of SEQ ID NO:12 is replaced with a leucine or isoleucine.

85. The DNA molecule of claim 55 wherein said valine occurring at the position corresponding to residue 517 of SEQ ID NO:12 is replaced with a alanine.

86. The DNA molecule of claim 55 wherein said tyrosine occurring at the position corresponding to residue 428 of SEQ ID NO:16 is replaced with cysteine or arginine.

87. The DNA molecule of claim 55 wherein said proline occurring at the position corresponding to residue 365 of SEQ ID NO:16 is replaced with serine.

88. The DNA molecule of claim 55 wherein said proline occurring at the position corresponding to residue 449 of SEQ ID NO:18 is replaced with an amino acid selected from the group consisting of leucine, isoleucine, valine and methionine.

89. The DNA molecule of claim 53 wherein said plant is selected from the group consisting of maize, wheat, soybean, cotton, sugar beet, rape, rice, sorghum and Arabidopsis.

90. The DNA molecule of claim 53 wherein said plant is selected from the group consisting of maize, wheat, soybean and Arabidopsis.

91. The DNA molecule of claim 53, wherein said plant protox comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 16, 18, 20, 22 and 24.

92. The DNA molecule of claim 53, wherein said plant protox comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, and 18.

93. A chimeric gene comprising a promoter active in a plant operably linked to a heterologous DNA molecule encoding a protoporphyrinogen oxidase (protox) selected from the group consisting of a

wheat protox comprising the sequence set forth in SEQ ID NO:10, a soybean protox comprising the sequence set forth in SEQ ID NO:12, cotton protox comprising the sequence set forth in SEQ ID NO:16, a sugar beet protox comprising the sequence set forth in SEQ ID NO:18, a rape protox comprising the sequence set forth in SEQ ID NO:20, a rice protox comprising the sequence set forth in SEQ ID NO:22 and a sorghum protox comprising the sequence set forth in SEQ ID NO:24.

94. A chimeric gene according to claim 93, wherein the a protoporphyrinogen oxidase (protox) is selected from the group consisting of a wheat protox comprising the sequence set forth in SEQ ID NO:10 and a soybean protox comprising the sequence set forth in SEQ ID NO:12.

95. The chimeric gene of claim 93 or 94 additionally comprising a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the chloroplast.

96. The chimeric gene of claim 93 or 94 additionally comprising a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the mitochondria.

97. A chimeric gene comprising a promoter that is active in a plant operably linked to the DNA molecule of any one of claims 15-52.

98. The chimeric gene of claim97 additionally comprising a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the chloroplast or into the mitochondria.

99. A chimeric gene comprising a promoter that is active in a plant operably linked to the DNA molecule of any one of claims 53 to 92.

100. The chimeric gene of claim 99 additionally comprising a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the chloroplast.

101. The chimeric gene of claim 99 additionally comprising a signal sequence operably linked to said DNA molecule, wherein said signal sequence is capable of targeting the protein encoded by said DNA molecule into the mitochondria.

102. A recombinant vector comprising the chimeric gene of any one of claims 93 to 101, wherein said vector is capable of being stably transformed into a plant cell.

103. A host cell stably transformed with a vector according to claim 102, wherein said host cell is capable of expressing said DNA molecule.

104. A host cell according to claim 103 wherein said host cell is selected from the group consisting of a plant cell, a bacterial cell, a yeast cell, and an insect cell.

105. A plant or plant cell including the progeny thereof comprising the DNA molecule of any one of claims 15 to 52 and 53 to 9253, wherein said DNA molecule is expressed in said plant and confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

106. A plant comprising the DNA molecule of any one of claims 15 to 52 and 53 to 92 , wherein said DNA molecule is expressed in said plant and confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

107. The plant or plant cell including the progeny thereof of claim 105 and 106, wherein said DNA molecule replaces a corresponding naturally occurring protox coding sequence.

108. A plant or plant cell including the progeny thereof comprising the chimeric gene of any one of claims 93 to 96, wherein said chimeric gene confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

109. A plant or plant cell including the progeny thereof comprising the chimeric gene of any one of claims 97 and 98 or 99 t0 101, wherein said chimeric gene confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

110. A plant comprising the chimeric gene of any one of claims 97 and 98 or 99 to 101, wherein said chimeric gene confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

111. The plant of any one of claims 105 to 110, wherein said plant is selected from the group consisting of Arabidopsis, sugar cane, soybean, barley, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf and forage grasses, millet, forage and rice.

112. The plant of any one of claims 105 to 110, wherein said plant is selected from the group consisting of maize, wheat, sorghum, rye, oats, turf grass, rice, soybean, cotton, tobacco, sugar beet and oilseed rape.

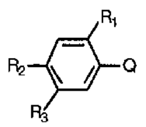
113. A method for controlling the growth of undesired vegetation, which comprises applying to a population of the plant of anyone of claims 105 to 112 an effective amount of a protox-inhibiting herbicide.

114. The method of claim 113 wherein said plant is selected from the group consisting of Arabidopsis, sugar cane, soybean, barley, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf and forage grasses, millet, forage and rice.

115. The method of claim 113 wherein said plant is selected from the group consisting of soybean, cotton, tobacco, sugar beet, oilseed rape, maize, wheat, sorghum, rye, oats, turf grass Arabidopsis and rice.

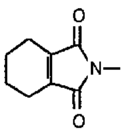
116. The method of claim 114 or 115 wherein said protox-inhibiting herbicide is selected from the group consisting of an aryluracil, a diphenylether, an oxidiazole, an imide, a phenyl pyrazole, a pyridine derivative, a 3-substituted-2-aryl-4,5,6,7-tetrahydroindazole, a phenopylate and O-phenylpyrrolidino- and piperidinocarbamate analogs of said phenopylate.

117. The method of claim 116 wherein said protox-inhibiting herbicide is an imide having the formula

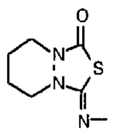


(Formula V)

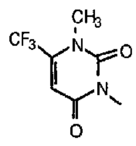
wherein Q equals



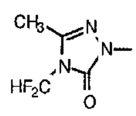
OR



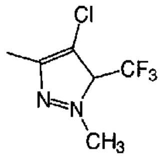
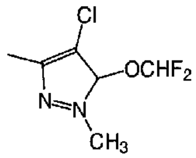
OR



OR

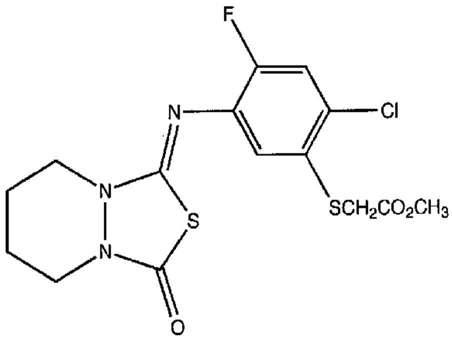


(Formula VI) (Formula VII) (Formula VIII) (Formula IX)

OR  OR 

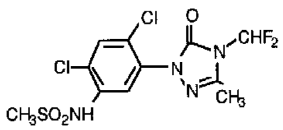
(Formula IXa) (Formula IXb)

and wherein R_1 equals H, Cl or F, R_2 equals Cl and R_3 is an optimally substituted ether, thioether, ester, amino or alkyl group, and wherein R_2 and R_3 together may form a 5 or 6 membered heterocyclic ring, or

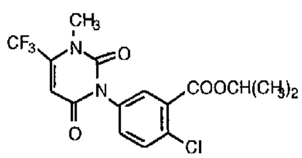


(Formula VIIa).

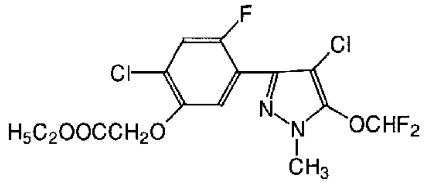
118. The method of claim 117 wherein said imide is selected from the group consisting of



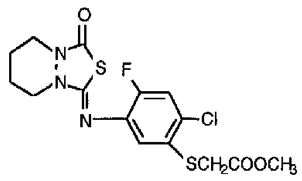
(Formula X);



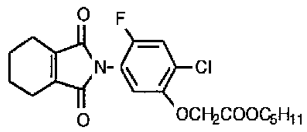
(Formula XI);



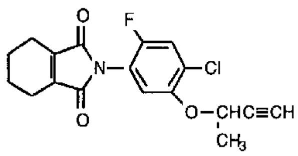
(Formula XII);



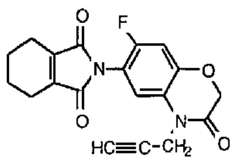
(Formula XIII);



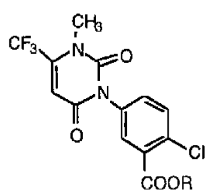
(Formula XIV);



(Formula XV);



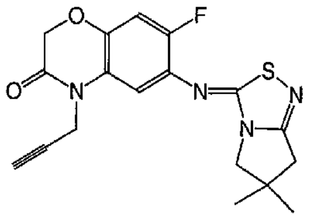
(Formula XVI); and



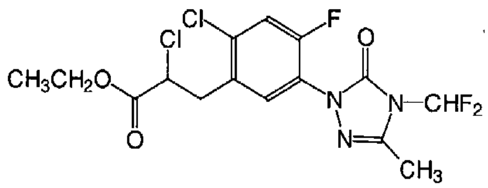
(Formula XVII)

wherein R signifies (C_{2-6}-alkenyloxy)carbonyl-C_{1-4}-alkyl.

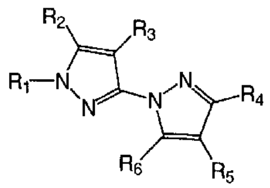
119. The method of claim 113 wherein said protox-inhibiting herbicide has the formula selected from the group consisting of



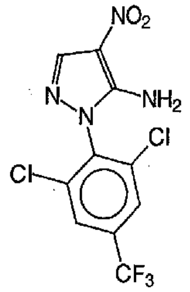
(Formula XVIII),



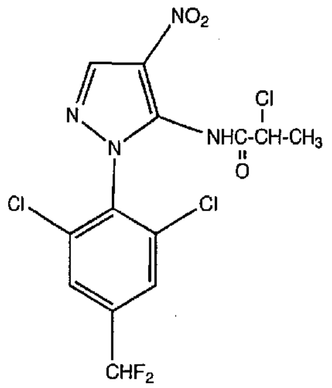
(Formula XIX),



(Formula XX),



(Formula XXI),



(Formula XXIa), and

(Formula XXII).

120. A method for the production of plants, plant tissues, and plant seeds that produce a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity wherein the plants, plant tissues, plant seeds and plant parts have been stably transformed with a structural gene encoding the protox enzyme comprising the amino acid sequence set forth in any one of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, and SEQ ID NO: 24.

121. A method of producing a plant cell comprising an isolated DNA molecule encoding a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity comprising transforming the said plant cell with a recombinant vector molecule according to claim 102.

122. . A method of producinga plant comprising an isolated DNA molecule encoding a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity comprising transforming the said parent plant with a recombinant vector molecule according to claim 102

123. A method of producing transgenic progeny of a transgenic parent plant comprising an isolated DNA molecule encoding a protein from a eukaryote having protoporphyrinogen oxidase (protox) activity comprising transforming the said parent plant with a recombinant vector molecule according to claim 102 and transferring the herbicide tolerant trait to the progeny of the said transgenic parent plant involving known plant breeding techniques.

124. An agricultural method, wherein a transgenic plant or the progeny thereof is used comprising a chimeric gene according to any one of claims 93 to 101 in an amount sufficient to express herbicide resistant forms of herbicide target proteins in a plant to confer tolerance to the herbicide.

125. A method for the production of plants, plant tissues, plant seeds and plant parts, that produce an inhibitor-resistant form of the plant protox enzyme, wherein the plants, plant tissues, plant seeds and plant parts have been stably transformed with a structural gene encoding the resistant protox enzyme according to any one of claims 15 to 52.

126. A method for the production of plants, plant tissues, plant seeds and plant parts, wherein the plants, plant tissues, plant seeds and plant parts have been stably transformed with the DNA of claims 53 to 92

127. An assay to identify inhibitors of protoporphyrinogen oxidase (protox) enzyme activity that comprises:

(a) incubating a first sample of protoporphyrinogen oxidase (protox)) selected from the group consisting of a wheat protox comprising the sequence set forth in SEQ ID NO:10, a soybean protox comprising the sequence set forth in SEQ ID NO:12, cotton protox comprising the sequence set forth in SEQ ID NO:16, a sugar beet protox comprising the sequence set forth in SEQ ID NO:18, a rape protox comprising the sequence set forth in SEQ ID NO:20, a rice protox comprising the sequence set forth in SEQ ID NO:22 and a sorghum protox comprising the sequence set forth in SEQ ID NO:24. and its substrate;

(b) measuring an uninhibited reactivity of the protoporphyrinogen oxidase (protox) from step (a);

(c) incubating a first sample of protoporphyrinogen oxidase (protox) and its substrate in the presence of a second sample comprising an inhibitor compound;

(d) measuring an inhibited reactivity of the protoporphyrinogen oxidase (protox) enzyme from step (c); and

(e) comparing the inhibited reactivity to the uninhibited reactivity of protoporphyrinogen oxidase (protox).

128. An assay to identify inhibitor-resistant protoporphyrinogen oxidase (protox) mutants comprises:

(a) incubating a first sample of protoporphyrinogen oxidase (protox) enzyme and its substrate in the presence of a second sample comprising a protoporphyrinogen oxidase (protox) enzyme inhibitor;

(b) measuring an unmutated reactivity of the protoporphyrinogen oxidase (protox) enzyme from step (a);

(c) incubating a first sample of a mutated protoporphyrinogen oxidase (protox) enzyme as disclosed in any one of claims 15 to 92 and its substrate in the presence of a second sample comprising protoporphyrinogen oxidase (protox) enzyme inhibitor;

(d) measuring a mutated reactivity of the mutated protoporphyrinogen oxidase (protox) enzyme from step (c); and

(e) comparing the mutated reactivity to the unmutated reactivity of the protoporphyrinogen oxidase (protox) enzyme.

129. A protox enzyme inhibitor obtained by a method according to claim 127 or 128.

130. A plant or plant cell including the progeny thereof comprising the DNA molecule according to claims 1 to 14.

131. An isolated DNA molecule that encodes a wheat protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:9 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

132. An isolated DNA molecule that encodes a soybean protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:11 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO4 pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

133. An isolated DNA molecule that encodes a cotton protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:15 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO4$ pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

134.An isolated DNA molecule that encodes a sugar beet protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:17 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO4$ pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

135. An isolated DNA molecule that encodes a rape protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:19 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO4$ pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

136. An isolated DNA molecule that encodes a rice protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:21 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO4$ pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

137. An isolated DNA molecule that encodes a sorghum protox enzyme, said DNA molecule having a nucleotide sequence that hybridizes to the nucleotide sequence of SEQ ID NO:23 under the following hybridization and wash conditions:

(a) hybridization in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO4$ pH 7.0, 1 mM EDTA at 50° C; and

(b) wash in 2X SSC, 1% SDS at 50° C.

138. A chimeric gene comprising a promoter active in a plant operably linked to the isolated DNA molecule of any one of claims131 to 137.

139. A recombinant vector comprising the chimeric gene of claim 138, wherein said vector is capable of being stably transformed into a host cell.

140. A host stably transformed with the recombinant vector of claim 139, wherein said host is capable of expressing said protox enzyme.

141. The host of claim 140, which is a plant or plant cell, including the progeny thereof.

142. A chimeric gene comprising a plant plastid promoter operably linked to the isolated DNA molecule of claim 1-52 .

143.The chimeric gene of claim 142, wherein said plant plastid promoter is a *clp*P gene promoter.

144. The chimeric gene of claim 142 further comprising a 5' untranslated sequence (5'UTR) from said plastid promoter and a plastid gene 3' untranslated sequence (3' UTR) operably linked to said isolated DNA molecule.

145. The chimeric gene of claim 144, wherein said plant plastid promoter is a *clp*P gene promoter, and wherein said 3' UTR is a plastid *rps*16 gene 3' untranslated sequence.

146.A plastid transformation vector comprising the chimeric gene of any one of claims 142 to 145.

147. A plant plastid transformed with the plastid transformation vector of claim 146, wherein said plant protox enzyme is expressed in said plant plastid.

148. A chimeric gene comprising a plant plastid promoter operably linked to the isolated DNA molecule of any one of claims 53 to 92.

149. The chimeric gene of claim 148, wherein said plant plastid promoter is a *clp*P gene promoter.

150. The chimeric gene of claim 148 further comprising a 5' untranslated sequence (5'UTR) from said plastid promoter and a plastid gene 3' untranslated sequence (3' UTR) operably linked to said isolated DNA molecule.

151. The chimeric gene of claim 150, wherein said plant plastid promoter is a *clp*P gene promoter, and wherein said 3' UTR is a plastid *rps*16 gene 3' untranslated sequence.

152. A plastid transformation vector comprising the chimeric gene of any one of claims 142 to 145 and 148 to 151..

153. A plant plastid transformed with the plastid transformation vector of claim 152, wherein said modified plant protox enzyme is expressed in said plant plastid.

154. A plant or plant cell, including the progeny thereof, comprising the plant plastid of claim 147 or claim 153, wherein said modified plant protox enzyme is expressed in said plant and confers upon said plant tolerance to a herbicide in amounts that inhibit naturally occurring protox activity.

155. A protoporphyrinogen oxidase (protox) selected from the group consisting of a wheat protox comprising the sequence set forth in SEQ ID NO:10, a soybean protox comprising the sequence set forth in SEQ ID NO:12, cotton protox comprising the sequence set forth in SEQ ID NO:16, a sugar beet protox comprising the sequence set forth in SEQ ID NO:18, a rape protox comprising the sequence set forth in SEQ ID NO:20, a rice protox comprising the sequence set forth in SEQ ID NO:22 and a sorghum protox comprising the sequence set forth in SEQ ID NO:24.

156. A modified protoporphyrinogen oxidase (protox) as disclosed in any one of claims 15 to 52, wherein said modified protox is tolerant to a herbicide in amounts that inhibit said plant protox.

157 A modified protoporphyrinogen oxidase (protox) as disclosed in any one of claims 53 to 92, wherein said modified protox is tolerant to a herbicide in amounts that inhibit said plant protox.

158 A protoporphyrinogen oxidase (protox) encoded by a DNA molecule according to any one of claims 131 to 137.

159. An isolated DNA molecule according to any one of claims 1-92, or a chimeric gene according to any one of claims 93-101, or a recombinant vector according to claim 102, or a host cell according to any one of claims 103 or 104, or a plant or plant cell according to any one of claims 105-112, or a method according to any one of claims 113-126, or an assay according to any one of claims 127 or 128, or a protox enzyme inhibitor according to claim 129, or a plant or plant cell

according to claim 130, or an isolated DNA molecule according to any one of claims 131-137, or a chimeric gene according to claim 138, or a recombinant vector according to claim 139, or a host according to any one of claims 140 or 141, or a chimeric gene according to any one of claims 142-145, or a plastid transformation vector according to claim 146, or a plant plastid according to claim 147, or a chimeric gene according to any one of claims 148-151, or a plastid transformation vector according to claim 152, or a plant plastid according to claim 153, or a plant or plant cell according to claim 154, or a protoporphyrinogen oxidase (protox) according to any one of claims 155 or 158, or a modified protoporphyrinogen oxidase (protox) according to any one of claims 156 or 157 substantially as herein before described with reference to the examples.

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