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

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<th>(51) International Patent Classification</th>
<th>C12N 15/82 // A01H 1/00, 5/10</th>
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<td>(11) International Publication Number</td>
<td>WO 98/08963</td>
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<td>(43) International Publication Date</td>
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<td>29 August 1997 (29.08.97)</td>
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<td>(30) Priority Data:</td>
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<td>(60) Parent Application or Grant</td>
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<td>(63) Related by Continuation</td>
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Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

| (54) Title: GENE COMBINATIONS FOR HERBICIDE TOLERANCE IN CORN |
| (57) Abstract |

The present invention provides methods for preparing herbicide tolerant corn plants. Also provided are herbicide tolerant corn plants, as well as seeds and progeny derived from these plants.
FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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GENE COMBINATIONS FOR HERBICIDE TOLERANCE IN CORN

Background of the Invention

The use of selective herbicides for controlling specific weeds or plants in crops has become almost a universal practice. The market for these herbicides approaches a billion dollars annually. Even with this extensive use, weed control remains a significant and costly problem for the farmer.

Present day herbicides used singly or in so-called tank mixes require careful management to be effective. Time and method of application and stage of weed plant development are critical to getting good weed control with herbicides. Application of large amounts of preemergence herbicides can result in a commitment to grow the same crop in subsequent years because of chemical persistence in the soil which prevents rotation with a crop sensitive to that herbicide. Furthermore, some weed species are simply resistant to the available herbicides. Therefore, the development of effective herbicides increases in importance every year, especially as other weeds are controlled and thus reduce interplant competition.

Weed control in maize is currently accomplished by soil application of herbicides that are applied before the crop emerges and prior to the observation of a weed problem. The preemergence herbicides currently used adequately control most dicot and monocot (grass) weeds in maize. However, annual grass weeds such as wild proso millet and wooly cupgrass and perennial grass weeds commonly escape preemergence weed control. Preemergence herbicides require rainfall for activation, and under low rainfall conditions they fail to control grass weeds in maize. Furthermore, some preemergence herbicides persist in the soil and several have been detected as groundwater contaminants. The options for controlling these escape grass weeds are very limited.

A postemergence herbicide for grass weed control in maize would be very beneficial. An attractive alternative to developing new herbicides to combat this weed control problem in maize and/or to decrease the amount of herbicide carryover and groundwater contamination in maize fields from the
existing herbicides is to develop maize hybrids or varieties that are tolerant to other existing herbicides that normally kill all monocot (grass) species. The herbicide POAST™ (BASF Corp., Parsippany, New Jersey), the active ingredient of which is sethoxydim, kills most grasses, and is applied at lower rates than many preemergence herbicides. POAST™ is nonpersistent in the environment and therefore does not represent a groundwater contamination threat. POAST™ tolerant maize would provide the producer with increased weed management flexibility because POAST™ could be applied when a grass weed problem was detected without risk of damage to the crop and only to the areas with a weed problem. Therefore, postemergence control of local weed problems would further decrease the amount of herbicide applied compared to existing preemergence weed control strategies.

The sensitivity of maize to some herbicides is the result of the presence of herbicide sensitive forms of acetyl CoA carboxylase (ACCase) in those plants. ACCase is an enzyme involved in many important metabolic pathways in plant, animal and bacterial cells. Structurally, ACCases are biotinylated and are quite large enzymes consisting of one or more subunits. For example, most ACCases of animals, higher plants, and yeast are dimers of 420 to 700 kD native MW and contain subunits of 200 to 280 kD.

Two forms of ACCase, termed ACCase I and ACCase II, can be isolated from maize. These forms differ in their size, charge, cellular location, immunoreactivity with ACCase I antiserum, and sensitivity to herbicide inhibition. The predominate form, ACCase I, is plastid localized and is sensitive to herbicide inhibition.

Four ACCase genes have been identified in maize by Southern blot analyses (Lutz et al., Maize Genetics Conference (1995)). Restriction fragment length polymorphism (RFLP) analyses mapped one ACCase gene, termed Acc1, to chromosome 2 between umc131 and umc2b (Figure 1) in recombinant inbred lines from Tx303 x C059 (Egli et al., Maize Genetics Coop. Newsletter, 68, 92 (1994)). Mutations in the Acc1 gene can result an ACCase that is resistant or tolerant to herbicide inactivation ((Marshall et al., Theor. Appl. Genet., 83,435
(1992); Egli et al., *Plant Physiol.*, **101**, 499 (1993); Egli et al., *MNL*, **66**, 94 (1992)). However, the R0 plants which were regenerated from tissue cultures selected for resistance (or tolerance) to herbicides, by virtue of mutations in *Acc1*, exhibit only partial herbicide resistance, i.e., the symptoms induced by herbicide exposure are not prevented (see U.S. Patent No. 5,162,202).

Caffrey et al. (Maize Genetics Coop. Newsletter, **69**, 3 (1995)) disclose that RFLP analyses of recombinant inbreds derived from the crosses T3x303 x CO159 and T232 x CM37 showed that one ACCase gene maps to chromosome 2 between umc131 and uox while another ACCase gene maps to chromosome 10 between ncsu2 and umc155 (Figure 2). Caffrey et al. further disclose that the ACCase gene on chromosome 10 appears to correspond to an herbicide resistance locus described by Van Dee et al. (Agro. Abs., page 198 (1992)). The authors propose that the ACCase gene on chromosome 10 encodes an herbicide sensitive ACCase that is localized to the plastid while the ACCase gene on chromosome 2 encodes an herbicide resistant ACCase that is non-plastid localized.

Thus, there is a need for a method to prepare a maize plant with resistance or tolerance to herbicides.

**Summary of the Invention**

The invention provides a method to prepare maize (“corn” or *Zea mays* L.) plants with resistance or tolerance to cyclohexanedione or aryloxyphenoxypropionate herbicides, i.e., maize plants with high tolerance to field application rates of herbicide. The herbicide resistance or tolerance is the result of the plants having at least one copy of each of two herbicide resistant or tolerant acetyl CoA carboxylase (ACCase) genes, one of which is encoded on chromosome 2 (*Acc1*) and the other of which is encoded on chromosome 10 (*Acc2*), i.e., the plant is a double heterozygous mutant (e.g., *Acc1*-S2/++; *Acc2*-S5). In contrast, when a plant has only one copy of either of the herbicide resistant ACCase genes, e.g., *Acc1*-S2/++; *Acc2*-S5/+, the plant exhibits only partial tolerance to the herbicide, i.e., the expression of the
herbicide resistant ACCase gene does not prevent symptoms of herbicide
damage under standard field-application rates of herbicides.

In commercial corn breeding practices, the double heterozygous
genotype can be a hybrid variety planted by farmers. To prepare the double
heterozygote, a homozygous double mutant parent line (e.g., Acc1-S2/Acc1-
S2;Acc2-S5/Acc2-S5) is crossed to a normal (nonmutant) susceptible parent line.
Thus, corn breeders can maintain fewer homozygous double mutant parent lines
than would be needed with a system in which both parents must be homozygous
for a single mutant gene to obtain a fully herbicide resistant maize plant.

Thus, the invention provides a method of imparting cyclohexanedione or
arylxyphenoxypropanoic acid herbicide tolerance to a corn plant. The method
comprises crossing a first corn plant with a second corn plant so as to yield
progeny plants. The first plant is homozygous for an allele of Acc1 which
imparts cyclohexanedione or arylxyphenoxypropanoic acid herbicide tolerance.

The second plant is homozygous for an allele of Acc2 which imparts
cyclohexanedione or arylxyphenoxypropanoic acid herbicide tolerance. The
progeny plant is heterozygous for the Acc1 allele which imparts
cyclohexanedione or arylxyphenoxypropanoic acid herbicide tolerance and
heterozygous for the Acc2 allele which imparts cyclohexanedione or
arylxyphenoxypropanoic acid herbicide tolerance. A preferred embodiment of
the invention is a method of imparting herbicide tolerance to a hybrid plant.

The invention also provides a method of imparting tolerance to a corn
plant to an agent which inhibits acetyl CoA carboxylase, wherein the agent is
selected from the group consisting of 3-(2,4-dichlorophenyl)-perhydroindolizine-
2,4-dione (Babczinski et al., Pest. Sci., 33, 455 (1991)), 3-isopropyl-6-(N-[2,2-
dimethylpropyl]-acetamido-1,3,5-triazine-2,4-(1H,3H)dione (Walker et al.,
Phytochem., 29, 3743 (1990)), soraphen A (Vahlenesiock et al., Curr. Genet., 25,
93 (1994)), and structural and/or functional analogs thereof. The method
comprises crossing a first corn plant, which is homozygous for an allele of Acc1
which imparts tolerance to the agent, with a second corn plant, which is
homozygous for an allele of Acc2 which imparts tolerance to the agent, so as to
yield progeny plants. The progeny plant is heterozygous for the \textit{Acc1} allele which imparts agent tolerance and heterozygous for the \textit{Acc2} allele which imparts agent tolerance.

Also provided is a method to prepare an herbicide resistant or tolerant corn plant. The method comprises crossing a first corn plant which comprises at least one herbicide resistant allele with a second corn plant which comprises at least one herbicide resistant allele which is not allelic to the herbicide resistant allele in the first plant, to yield a progeny plant which is a heterozygote for each allele.

The invention further provides a method of imparting cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance to a corn plant. The method comprises self pollinating a corn plant which comprises (i) an allele of \textit{Acc1} which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and (ii) an allele of \textit{Acc2} which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, so as to yield a progeny plant. Then a progeny plant is identified that is homozygous for the allele of \textit{Acc1} which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and is homozygous for the allele of \textit{Acc2} which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

Also provided is a method of preparing a plant which is a double heterozygote for alleles of \textit{Acc1} and \textit{Acc2} which impart cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance. The method comprises crossing a first corn plant with a second corn plant so as to yield progeny plants. The first plant is homozygous for an allele of \textit{Acc1} which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and the second plant is homozygous for an allele of \textit{Acc2} which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance. The progeny plant is heterozygous for the \textit{Acc1} allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and heterozygous for the \textit{Acc2} allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.
The invention also provides a method of preparing a plant which is a double homozygote for alleles of Acc1 and Acc2 which impart cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance. The method comprises self pollinating a corn plant which comprises (i) an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and (ii) an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, so as to yield a progeny plant. A progeny plant is identified that is homozygous for the allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and is homozygous for the allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

Also provided are progeny and seed derived from the plants prepared by the methods described herein.

Yet another embodiment of the invention is an inbred or hybrid cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerant Zea mays plant. The genome of the inbred or hybrid plant of the invention is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and is homozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

A further embodiment of the invention is an inbred or hybrid cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerant Zea mays plant, the genome of which is heterozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and is heterozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

Progeny and seed derived from the inbred plants of the invention are also provided.

As used herein, the term “cyclohexanedione herbicide” includes, but is not limited to, 1,3-cyclohexanediones which exhibit general and selective herbicidal activity against plants. One such cyclohexanedione is sethoxydim (2-
[1-(ethoxyimino)-butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one]. Sethoxydim is commercially available from BASF (Parsippany, New Jersey) under the designation POAST™.

Other herbicidal cyclohexanediones falling within the scope of the invention include clethodim, (E,E)-(±)-2-[1-[[3-chloro-2-propenyl]oxy]imino]propyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; available as SELECT™ from Chevron Chemical (Valent) (Fresno, California); cloproxydim, (E,E)-2-[1-[[3-chloro-2-propenyl]oxy]imino]butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; available as SELECTONE™ from Chevron Chemical (Valent) (Fresno, California); and tralkoxydim, 2-[1-(ethoxyimino)propyl]-3-hydroxy-5-mesitylcyclohex-2-enone, available as GRASPTM from Dow Chemical USA (Midland, Michigan), as well as other cyclohexanedione herbicides that are structurally related to the compounds described hereinabove.

As used herein, the term “aryloxyphenoxypropanoic acid herbicide” includes aryloxyphenoxypropanoic acids which exhibit general and selective herbicidal activity against plants. Such herbicides include, but are not limited to compounds wherein the aryloxy group may be phenoxy, pyridinyl oxy or quinoxaliny. One such herbicidal aryloxyphenoxypropanoic acid is haloxyfop, 2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]-propanoic acid, which is available as VERDICT™ from Dow Chemical USA (Midland, Michigan). Another is diclofop, (±)-2-[4-(2,4-dichlorophenoxy)-phenoxy]propanoic acid, available as HOELON™ from Hoechst-Roussel Agri-Vet Company (Somerville, New Jersey). Other aryloxyphenoxypropanoic acid herbicides within the scope of the invention include fenoxaprop, (±)-2-[4-[[6-chloro-2-benzoxazolyl]oxy]phenoxy]propanoic acid; available as WHIPTM from Hoechst-Roussel Agri-Vet Company (Somerville, New Jersey); fluazifop, (±)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid; available as FUSILADE™ from ICI Americas (Wilmington, Delaware); fluazifop-P, (R)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid; available as FUSILADE 2000™ from ICI Americas (Wilmington, Delaware); and
quizalofop, (±)-2-[4[(6-chloro-2-quinoxalinyloxy)phenoxy]propanoic acid; available as ASSURE™ from E. I. DuPont de Nemours (Wilmington, Delaware), as well as other herbicidal compounds which are structurally related to the compounds described herein above.

As used herein, a plant that is "resistant or tolerant to inhibition by a herbicide or agent" is a plant that grows in an amount of herbicide or agent that normally inhibits growth of a corresponding susceptible plant, as determined by methodologies known to the art. For example, a maize plant of the invention (e.g., Acc1-S2/Acc1-S2; Acc2-S5/Acc2-S5 or Acc1-S2/+; Acc2-S5/+ or Acc1-S2/Acc2-S5) grows in an amount of cyclohexanedione that inhibits the growth of a corresponding susceptible maize plant (e.g.,+/+;+/+, Acc1-S2/+;+/+ or +/+; Acc2-S5/+). In the alternative, the herbicide resistance of a homozygous backcross converted inbred plant of the invention (Inbred A (Acc1-S2/Acc1-S2; Acc2-S5/Acc2-S5)) is compared to the herbicide resistance of a recurrent inbred susceptible plant (Inbred A (+/+; +/+)). A homozygous backcross converted inbred plant of the invention is a plant which has been repeatedly crossed to the recurrent inbred parent until the backcross converted inbred plant is substantially isogenic with the recurrent inbred parent except at Acc1 and Acc2 loci, and is then self-pollinated (selfed) at least once.

As used herein, "substantially isogenic" means that the genomic DNA content of a homozygous backcross converted inbred plant is at least about 92%, preferably at least about 98%, and most preferably at least about 99%, identical to the genomic DNA content of a recurrent inbred parent of the backcross converted inbred plant.

Exemplary susceptible maize lines, e.g., lines which are sensitive to growth inhibition by cyclohexanedione or aryloxyphenoxypropanoic acid herbicides include, but are not limited to, A188, A641, A619, B73 and Zea mays var. PI 3140. Exemplary maize lines which are a source of either Acc1 or Acc2 herbicide resistance alleles include, but are not limited to, Zea mays var. DK 592SR, Zea mays var. DK 404SR, 4400SR and 7800SR.

**Brief Description of the Figures**
Figure 1 depicts the genetic map of maize chromosome 2.

Figure 2 depicts the genetic map of maize chromosome 10.

Figure 3 depicts the DNA sequence (SEQ ID NO:9) of a 7470 base pair cDNA of a maize ACCase gene.

Figure 4 depicts the predicted amino acid sequence of a complete ACCase gene of maize (SEQ ID NO:10).

Figure 5 depicts the partial nucleotide sequence of a Type A1 ACCase genomic clone (SEQ ID NO:11).

Figure 6 depicts the partial nucleotide sequence of clone 5A, a Type A ACCase genomic clone (SEQ ID NO:12).

Figure 7 depicts the partial nucleotide sequence of five Type A2 ACCase genomic clones (A-F) (SEQ ID NOs 13, 14, 15, 16 and 17).

Figure 8 depicts the partial nucleotide sequence of three Type B ACCase clones (SEQ ID NOs 18, 19 and 20).

**Detailed Description of the Invention**

It is envisioned that a variety of corn breeding programs may be employed to introduce an herbicide tolerant gene or allele into a particular genetic background. Field crops are bred through techniques that take advantage of the plant’s method of pollination. A plant is self-pollinating if pollen from one flower is transferred to the same or another flower of the same plant. A plant is cross pollinated if the pollen comes from a flower on a different plant.

Plants that have been self-pollinated and selected for type for many generations become homozygous at almost all gene loci and produce a uniform populations of true breeding progeny. A cross between two homozygous plants from differing backgrounds or two homozygous lines produce a uniform population of hybrid plants that may be heterozygous for many gene loci. A cross of two plants that are each heterozygous at a number of gene loci will produce a population of hybrid plants that differ genetically and will not be uniform.

Corn plants can be bred by both self-pollination and cross pollination techniques. Corn has male flowers, located on the tassel, and female flowers,
located on the ear, on the same plant. Natural pollination occurs in corn when wind blows pollen from the tassels to the silks that protrude from the tops of incipient ears.

The development of corn hybrids requires the development of homozygous inbred lines, the crossing of these lines, and the evaluation of the crosses. Pedigree breeding and recurrent selection breeding methods are used to develop inbred lines from breeding populations. Breeding programs combine desirable traits from two or more inbred lines or various broad-based sources into breeding pools from which new inbred lines are developed by selfing and selection of desired phenotypes. The new inbreds are crossed with other inbred lines and the hybrids from these crosses are evaluated to determine which have commercial potential.

Pedigree breeding starts with the crossing of two genotypes, each of which may have one or more desirable characteristics that is lacking in the other or which complement the other. If the two original parents do not provide all of the desired characteristics, other sources can be included in the breeding population. In the pedigree method, superior plants are selfed and selected in successive generations. In the succeeding generations, the heterozygous condition gives way to homogenous lines as a result of self-pollination and selection. Typically, in the pedigree method of breeding, five or more generations of selfing and selection is practiced.

Backcrossing can be used to improve an inbred line. Backcrossing transfers a specific desirable trait from one inbred or other source to an inbred that lacks that trait (see below).

A hybrid corn variety is the cross of two inbred lines, each of which may have one or more desirable characteristics lacked by the other or which complement the other. The hybrid progeny of the first generation is designated $F_1$. In the development of hybrids, only the $F_1$ hybrid plants are sought. The $F_1$ is more vigorous than its inbred parents.

The development of a hybrid corn variety involves three steps: (1) the selection of superior plants from various germplasm pools; (2) the selfing of the
superior plants for several generations to produce a series of inbred lines which, although different from each other, each breed true and are highly uniform; (3) crossing the selected inbred lines with unrelated inbred lines to produce hybrid progeny (F_1). During the inbreeding process, the vigor of the lines decreases. Vigor is restored in the F_1. Once the best hybrid is identified, the hybrid seed can be reproduced indefinitely as long as the homogeneity of the inbred parent is maintained.

A single cross hybrid is produced when two inbred lines are crossed to produce the F_1 progeny. A double cross hybrid is produced from four inbred lines crossed in pairs (A x B and C x D) and then the two F_1 hybrids are crossed again. Because the vigor exhibited by F_1 hybrids is lost in the next generation, seed from hybrid varieties is not used for planting stock.

Hybrid corn seed can be produced by manual detasseling. Alternative strips of two inbred varieties of corn are planted in a field, and the pollen-bearing tassels are removed from one of the inbreds. Providing that there is sufficient isolation from sources of foreign corn pollen, the ears of the detassels inbred (female) will be fertilized only by the other inbred (male), and the resulting seed is therefore hybrid and will form hybrid plants.

The laborious detasseling process can be avoided by using cytoplasmic male sterile (CMS) inbreds. Plants of a CMS inbred are fertilized with pollen from another inbred that is not male sterile. Pollen from the second inbred can contribute genes that make the hybrid plants male fertile. Such breeding methods are well known to the art. See, for example, Hallauer et al., In: Corn and Corn Improvement, pp. 463-564 (1988).

Herbicide resistant or tolerant plant variants can be obtained by several methods including, but not limited to, spontaneous variation and direct mutant selection in cultures, direct or indirect mutagenesis procedures on tissue cultures of all cell types, seeds or plants, and mutation of cloned ACCase genes by methods such as site directed mutagenesis. For example, the preparation of herbicide tolerant maize from herbicide tolerant cell lines is described in U.S. Patent No. 5,162,602, issued November 10, 1992, the disclosure of which is
incorporated by reference herein, and in Examples I-III. Briefly, partially differentiated cell cultures are grown and subcultured with continuous exposures to low herbicide levels. Herbicide concentrations are then gradually increased over several subculture intervals. Maize cells or tissues growing in the presence of normally toxic herbicide levels are repeatedly subcultured in the presence of the herbicide and characterized. Stability of the herbicide tolerance trait of the cultured cells may be evaluated by growing the selected cell lines in the absence of herbicides for various periods of time and then analyzing growth after exposing the tissue to normally toxic amounts of herbicide. Mature maize plants are then obtained from maize cell lines that are known to express the trait. Thus, this method is useful to isolate heterozygous dominant, or partially dominant, herbicide resistant mutants.

If possible, the regenerated plants are self-pollinated. Otherwise, pollen obtained from the regenerated plants is crossed to seed-grown plants of agronomically important inbred lines. Conversely, pollen from plants of these inbred lines is used to pollinate regenerated plants. The genetics of the trait are then characterized by evaluating the segregation of the trait in the first and later generation progeny. Stable inheritance of the herbicide tolerance trait is achieved if the plants maintain herbicide tolerance for at least about three to six generations.

Seed from maize plants regenerated from transformed tissue cultures is grown in the field and self-pollinated to generate true breeding plants. Progenies from these plants become true breeding lines which are evaluated for herbicide tolerance in the field under a range of environmental conditions. Herbicide tolerance must be sufficient to protect the monocot plants at the maximum labeled delivery rate under field conditions which cause herbicides to be most active. Appropriate herbicide concentrations and methods of application are those which are known and have been developed for the cyclohexanedione and/or aryloxyphenypropanoic acid herbicides disclosed herein.

The commercial value of herbicide resistant corn is greatest if many different hybrid combinations are available for sale. The farmer typically grows
more than one kind of hybrid based on such differences as maturity, standability or other agronomic traits. Additionally, hybrids adapted to one part of the corn belt are not adapted to another part because of differences in such traits as maturity, disease, and insect resistance. Because of this, it is necessary to breed herbicide tolerance into a large number of parental lines so that many hybrid combinations can be produced.

To introduce a particular herbicide resistance allele into an inbred background, a conversion process (backcrossing) is carried out by crossing the original herbicide resistant line to normal elite lines and crossing the progeny back to the normal parent. The progeny from this cross will segregate such that some plants carry the gene responsible for tolerance whereas some do not. Plants carrying such genes will be crossed again to the normal parent resulting in progeny which segregate for herbicide resistance and sensitivity once more. This is repeated until the original normal parent has been converted to an herbicide resistant line, yet possesses all other important attributes as originally found in the normal parent. A separate backcrossing program is implemented for every elite line that is to be converted to herbicide resistant line.

Subsequent to the backcrossing, the new resistant lines and the appropriate combinations of lines which make good commercial hybrids are evaluated for herbicide resistance as well as a battery of important agronomic traits. Resistant lines and hybrids are produced which are true to type of the original normal lines and hybrids. This requires evaluation under a range of environmental conditions where the lines or hybrids will generally be grown commercially. Parental lines of hybrids that perform satisfactorily are increased and used for hybrid production using standard hybrid seed corn production practices.

The plants of the invention are expected to be useful for a variety of commercial and research purposes. The plants can be created for use in traditional agriculture to possess traits beneficial to the consumer of the grain harvested from the plant (e.g., improved nutritive content in human food or animal feed). In such uses, the plants are generally grown for the use of their
grain in human or animal foods. However, other parts of the plants, including stalks, husks, vegetative parts, and the like, may also have utility, including use as part of animal silage or for ornamental purposes.

The plants may also find use in the commercial manufacture of proteins or other molecules, where the molecule of interest is extracted or purified from plant parts, seeds, and the like. Cells or tissue from the plants may also be cultured, grown in vitro, or fermented to manufacture such molecules.

The plants may also be used in commercial breeding programs, or may be crossed or bred to plants of related crop species.

The invention will be further described by the following examples.

EXAMPLE I

**Identification of Herbicide Mechanism and Site of Action**

The objective of this Example was to identify the mechanism whereby sethoxydim and/or haloxyfop inhibit fatty acid synthesis in maize. The results, reported in J. D. Burton et al., *Biochem. Biophys. Res. Comm.* 148, 1039 (November 13, 1987), show that both sethoxydim and haloxyfop inhibit acetyl-coenzyme A carboxylase (ACCase) (EC 6.4.1.2) in maize chloroplasts.

A. **Chemicals**

Buffers and cofactors were purchased from Sigma Chemical Company (St. Louis, Missouri); [2-14C]acetate was purchased from Research Products International; [2-14C]pyruvate and [14C]NaHCO3 were purchased from New England Nuclear; and [2-14C]malonyl coenzyme A was purchased from Amersham. Sethoxydim was a gift from BASF (Parsippany, New Jersey), and haloxyfop was provided by Dow Chemical USA (Midland, Michigan).

B. **Plant Growth Conditions**

Corn (*Z. mays* L., 'B37 x Oh43') seeds were germinated in darkness for 96 hours in vermiculite in an incubation chamber maintained at 30°C, 80% RH. Seedlings were then transferred to a growth chamber with a 16 hour light (25°C) and an 8 hour dark (20°C) cycle, 90% relative humidity (RH). After greening 48 hours, seedlings were returned to the dark incubation chamber for 12 hours to
deplete chloroplast starch reserves. Seedlings were harvested 6 days after planting. Pea (*P. sativum* L., 'PI 9901-C') seedlings were grown in vermiculite in a growth chamber with a 16 hour light (21°C) and 8 hour dark (16°C) cycle, 80% RH. Peas were harvested 10 to 13 days after planting. Black Mexican Sweet (BMS) corn suspension cultures were maintained in a supplemented Murashige-Skoog (MS) medium (C. E. Green, *Hort. Sci.*, 12, 7-10 (1977)), and subcultured weekly by 20-fold dilution of the suspension culture into fresh medium.

C. **Chloroplast Isolation**

Chloroplasts from corn and pea seedlings were isolated at 4°C (K. Cline et al., *J. Biol. Chem.*, 260, 3691-3696 (1985)). Seedlings (50 g of shoots) were homogenized in 200 ml buffer A (50 mM HEPES-NaOH pH 7.5, 330 mM sorbitol, 0.1% w/v BSA, 1 mM MgCl₂, 1 mM MnCl₂, 2 mM EDTA, 5 mM isoascorbate, 1.3 mM glutathione) in an omnimixer (five, 3-second bursts at full speed). The homogenate was filtered through six layers of cheesecloth and two layers of miracloth, and then centrifuged at 3000 g for 3 minutes with hand-braking. The pellet was gently resuspended in buffer A and layered onto a preformed linear Percoll gradient (50 mM HEPES-NaOH pH 7.5, 330 mM sorbitol, 1.9 mM isoascorbate, 1.08 mM glutathione, 0.1% w/v BSA, 50% Percoll) which was centrifuged at 3000 g for 20 minutes in a Sorvall HB-4 rotor. The lower band in the gradient, containing intact chloroplasts, was washed twice by gently resuspending it in 20 ml of buffer B (50 mM HEPES-NaOH, pH 7.5, and 330 mM sorbitol) followed by repelleting (3000 g, 5 minutes). The final pellet, consisting of intact chloroplasts, was resuspended in 2 to 3 ml of buffer B and stored on ice in the dark until use.

D. **Fatty Acid Synthesis**

[^14C]acetate and[^14C]pyruvate were used as precursors to measure fatty acid biosynthesis in isolated chloroplasts (B. Liedvogel et al., *Planta*, 169, 481-489 (1986)).[^14C]acetate incorporation was assayed in a 0.5 ml-volume containing: 50 mM HEPES-NaOH (pH 7.5), 330 mM sorbitol, 5 mM KH₂PO₄,
10 mM NaHCO₃, 1 mM MgCl₂, 1 mM ATP, 0.1 mM CoA, 0.15 mM [¹⁴C]acetate (3.33 mCi/mmole), and chloroplasts (20 to 50 μg chlorophyll). [¹⁴C]pyruvate incorporation into fatty acids was assayed in the same medium except that it included 2 mM TPP, 1 mM NAD⁺, 0.15 mM [¹⁴C]-pyruvate (1.33 mCi/mmole), but no acetate. Assay suspensions were illuminated with 1400 μE/m²-second PAR at 25°C. Assays were initiated by the addition of the labelled substrate and stopped by the addition of 0.5 ml of 40% KOH. To determine the incorporation of radiolabel into a non-polar (fatty acid) fraction, each treatment was saponified at 90°C for 30 minutes in capped vials (P. B. Hoj et al., *Carlsberg Res. Commun.*, 47, 119-141 (1982)). The vials were acidified with 0.5 ml 40% H₂SO₄, and carrier fatty acids (20 μg each of C₁₄:0, C₁₆:0, and C₁₈:0) were added. The assay mixture was extracted twice with 4 ml hexane. The extracts were combined, dried under N₂, and redissolved in 0.3 ml hexane. Aliquots (50 μl) were counted for radioactivity by liquid scintillation spectrometry.

Incorporation of [¹⁴C]malonyl-Coenzyme A into fatty acids (P. B. Hoj et al., *supra*; and J. B. Ohlrogge et al., *Proc. Natl. Acad. Sci. USA*, 76, 1194-1198 (1979)) was assayed using cell-free preparations from BMS tissue culture. Cells harvested during logarithmic growth phase were frozen in liquid nitrogen.

ground with a mortar and pestle, and thawed in a medium containing: 0.1 M HEPES-KOH, pH 7.5; 0.3 M glycerol, and 5 mM DTT (buffer:tissue, 2:1, v/w). The homogenate was centrifuged at 12,000 g for 20 minutes. The supernatant was filtered through miracloth and centrifuged (125,000 g) for 60 minutes and then filtered through miracloth and assayed. Assays were conducted at 25°C in a 0.4 ml volume containing: 1.0 mM ATP, 0.32 mM NADPH, 0.38 mM NADH, 25 μM CoA, 10 μM acetyl-CoA, 25 μg acetyl-carrier protein, and 12 μM malonyl-CoA (11.54 μCi/μmol). Reactions were initiated by addition of [¹⁴C]malonyl CoA and stopped by addition of 0.4 ml 40% KOH. Label incorporation into fatty acids was determined as above. Chlorophyll (D. I. Arnon, *Plant Physiol.*, 24, 1-15 (1949)) and protein (P. K. Smith et al., *Anal. Biochem.*, 150, 76-85 (1985)) were determined as described therein.
E. **Acetyl-Coenzyme A Carboxylase (ACCase) Activity**

Maize chloroplasts, isolated as described above, were suspended in buffer C (0.1 M Tricine-KOH, pH 8.0; 0.3 M glycerol, and 1 mM DTT) and homogenized in a glass tissue homogenizer. The disrupted chloroplast fraction was centrifuged at 16,000 g for 15 minutes. The supernatant was desalted on a Sephadex G-25 column (1.5 x 5 cm equilibrated with 0.1 M Tricine-KOH, pH 8.0; and 0.3 M glycerol) and assayed directly. ACCase activity (B. J. Nikolau et al., *Arch. Biochem. Biophys.*, 211, 605-612 (1981)) was assayed at 30°C in a 0.2 ml volume which contained 1 mM ATP, 3 mM acetyl coenzyme A, 2.5 mM MgCl₂, 50 mM KCl, 0.5 mM DTT, and 15 mM [¹⁴C]NaHCO₃ (0.17 mCi/mmol). Reactions were initiated by addition of acetyl coenzyme A and stopped by addition of 25 µl of 12 N HCl. Product formation was determined by the radioactivity found in an acid stable fraction by liquid scintillation spectrometry. Enzyme activity was linear for 15 minutes.

F. **Results**

To probe for the site of herbicidal activity of sethoxydim and haloxyfop, labelled acetate, pyruvate, and malonyl-CoA were used individually as precursors for fatty acid synthesis. Isolated chloroplasts from corn seedlings incorporated [¹⁴C]acetate and [¹⁴C]pyruvate into a non-polar fraction (fatty acids). Acetate incorporation was linear for 30 min after a 5 min lag period, and dependent upon the addition of free acetyl coenzyme A. Addition of either 10 µM sethoxydim or 1 µM haloxyfop inhibited [¹⁴C]acetate incorporation into fatty acids by 90% and 89%, respectively, as shown in Table I, below. Sethoxydim
(10 μM) and haloxyfop (1 μM) also inhibited the incorporation of [14C]pyruvate into fatty acids by 98% and 99%, respectively.

**TABLE I**

Inhibition of [14C]acetate and [14C]pyruvate incorporation into fatty acids in corn seedling chloroplasts by Sethoxydim (10 μM) and Haloxyfop (1 μM), 10 minute assay time

<table>
<thead>
<tr>
<th></th>
<th>Acetate</th>
<th>Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.4 ± 0.4†</td>
<td>10.8 ± 2.3</td>
</tr>
<tr>
<td>Sethoxydim</td>
<td>90 ± 2.5</td>
<td>98 ± 1.1</td>
</tr>
<tr>
<td>Haloxyfop</td>
<td>89 ± 3.1</td>
<td>99 ± 0.3</td>
</tr>
</tbody>
</table>

†Results are expressed as mean of two experiments ± standard error.

The effect of 10 μM sethoxydim and 1 μM haloxyfop on [14C]malonyl-CoA incorporation into fatty acids was determined using cell-free extracts from corn suspension cultures. Neither sethoxydim (10 μM) nor haloxyfop (1 μM) inhibited fatty acid synthetase activity. Thus, both herbicides inhibited fatty acid synthesis in intact chloroplasts from corn seedlings with either acetate or pyruvate as a precursor, but did not inhibit incorporation of malonyl-CoA into fatty acids. This suggests that ACCase which catalyzes the formation of malonyl-CoA is the site of action of these herbicides.

**EXAMPLE II**

**Selection and Characterization of Herbicide-tolerant Cell Lines**

A selection protocol to identify and isolate herbicide-tolerant maize cells was developed to minimize the adverse effects of high herbicide concentrations on somatic embryo development and plant regeneration capacity. The procedure involved exposing tissue to gradually increasing concentrations of herbicide beginning with a sethoxydim concentration representing 1/20th of lethal dose and doubling the herbicide concentration at approximately two-week intervals until the lethal dose (10 μM sethoxydim) was reached. In this way, the herbicide
was allowed to take effect slowly with continuous selection pressure, thus permitting herbicide-tolerant cells to accumulate over time while not affecting the potential for plant regeneration.

A. **Selection of a Sethoxydim-Tolerant Cell Line**

Many selections were carried out utilizing the selection protocol described in the preceding paragraph. The selection of one such sethoxydim-tolerant cell line that was identified and characterized is described below in detail.

Approximately 100 grams of vigorously growing, regenerable, friable, embryogenic maize callus tissue established from an F₁, immature embryo resulting from the cross A188 x B73 were transferred to agar-solidified maintenance medium (Armstrong and Green, *Planta*, 164, 207 (1985)) in petri plates containing 0.5 μM sethoxydim (BASF) (Parsippany, New Jersey). This callus line was designated 2167-9/2160-154. Forty plates were prepared and five clumps of callus tissue weighing about 0.5 grams each were placed on each plate. The 0.5 μM sethoxydim concentration was chosen from growth inhibition studies to provide less than 10-20% growth inhibition during the first two weeks of herbicide exposure. After 14 days, 0.25-0.5 gram pieces of tissue showing vigorous growth rate and retention of embryogenic morphology (i.e., presence of somatic embryos) were subcultured on fresh medium containing 1.0 μM sethoxydim. Eighty plates containing five pieces of tissue per plate were prepared. For each subsequent transfer, all callus tissue showing growth and somatic embryo forming ability was placed on fresh media containing a two-fold increased sethoxydim concentration. Therefore, callus was transferred at two-week intervals to petri plates containing 0.5, 1.0, 2.0, 5.0 and 10.0 μM sethoxydim. During the course of the selection process, the total number of lines decreased as the herbicide-mediated growth inhibition became more intense.

Cell lines exhibiting growth on 10 μM sethoxydim were designated as herbicide-tolerant and given an identification number. Two sethoxydim-tolerant lines were recovered that exhibited uninhibited growth at 10 μM sethoxydim. These lines were designated 2167-9/2160-154 S-1 and 2167-9/2160-154 S-2.
B. **Characterization of Herbicide-Tolerant Maize Cell Line 2167-9/2160-154 S-2**

Tolerant cell line 2167-9/2160-154 S-2 ("S-2") was characterized to evaluate: (1) the magnitude of sethoxydim tolerance; (2) cross-tolerance of haloxyfop; and (3) the biochemical basis for the tolerance. Callus tissue from S-2 that had been maintained on 10 μM sethoxydim was transferred to media containing up to 100 μM sethoxydim. One-half gram of S-2 tissue was plated on a 7 cm filter paper as a lawn overlaying 50 ml agar-solidified culture medium containing 0, 0.5, 1.0, 2.0, 5.0, 10.0, 50.0 and 100 μM sethoxydim, and cultured for two weeks. Control cell line 2167-9/2160-154 was plated similarly on medium containing the same levels of sethoxydim. The control cell line growth after two weeks was inhibited 50% at 1 μM sethoxydim. Growth of S-2 was not inhibited at 100 μM sethoxydim, indicating that S-2 was at least 100-fold more tolerant than the control callus line.

Growth of S-2 was inhibited with 0.65 μM haloxyfop, whereas the control cell line was inhibited 50% with 0.02 μM, indicating approximately a 30-fold increase in tolerance.

C. **Acetyl-Coenzyme A Carboxylase (ACCase) Activity of Maize Cell Line S-2**

Assays were conducted to determine if ACCase extracted from cell line S-2 was altered with respect to herbicide activity. ACCase activity of control tissue was 50% inhibited either by 1.5 μM sethoxydim, or by 0.25 μM haloxyfop. ACCase activity of S-2 tissue was inhibited 50% either by 70 μM sethoxydim, or by 1.8 μM haloxyfop, indicating at least 40-fold and 7-fold decreases in herbicide sensitivity on concentration basis, respectively.

**EXAMPLE III**

**Plant Regeneration and Production of Herbicide-Tolerant Seed**

A. **Plant Regeneration Protocol**

Sixteen ca. 150 mg clumps of S-2 callus were transferred per 25 x 100 mm petri plate containing agar-solidified N6 basal salts and 6% sucrose and incubated 7-14 days in low light (20 μE m⁻² s⁻¹). Several plates containing callus on plant regeneration medium were prepared. Callus was transferred to agar-
solidified Murashige-Skoog (MS) medium without hormones and incubated in high intensity light (200 µE m-2 s⁻¹) for shoot elongation. Developing plants (1-3 cm long) were isolated from the callus surface and transferred to magenta boxes containing agar-solidified MS salts, 2% sucrose with no hormones for two weeks of further growth. When plants reached the 2-3 leaf stage, they were transplanted to peat pots containing potting soil, and were incubated in the growth room until growing stably. Surviving plants were transferred to soil in 4” diameter plastic pots and grown in the greenhouse.

B. **Expression of Herbicide Tolerance in Plants Regenerated from S-2 Callus Tissue**

Groups of eight control (2167-9/2160-154 unselected) and eight S-2 plants were sprayed with either 0.0, 0.01, 0.05, 0.11, 0.22 or 0.44 kg/ha sethoxydim to determine whole plant sethoxydim-tolerance of greenhouse-grown plants. Control plants were killed by 0.05 kg/ha or more sethoxydim. Plants regenerated from the S-2 cell line survived the 0.44 kg/ha sethoxydim treatment, indicating that S-2 plants exhibit at least 20-fold more tolerance of sethoxydim than control. Shoot height of regenerated S-2 plants was only slightly reduced 14 days after treatment with 0.44 kg/ha sethoxydim.

C. **Seed Production from S-2 Plants**

Plants surviving sethoxydim treatments of up to 0.44 kg/ha were transplanted to the genetics plot on the University of Minnesota campus, St. Paul, Minnesota. Additional S-2 plants were transplanted to the field that had not been sprayed. Sixty-five 2167-9/2160-154 control plants and ninety-five S-2 plants were grown to maturity in the field. Plants were either self-pollinated or cross-pollinated to inbred maize lines A188, A619, A641, A661, A665, B37, B73, R806, and W153R. Control seed were produced by selfing 2167-9/2160-154 regenerated plants, or by crossing them with the inbreds listed above.

D. **Expression of Herbicide Tolerance in Progeny of Regenerated Plants**

Seeds obtained by the crossing procedure described above were viable and germinated normally. Seeds from thirty S-2 selfed plants and fifteen 2167-9/2160-154 control plants were planted in 25 x 50 cm trays of soil (28 seeds from each plant in one tray) and grown in the greenhouse. Seedlings at the 3-4
leaf stage were treated with 0.1, 0.44, and 1.1 kg/ha sethoxydim and evaluated for visual herbicide damage and shoot height. Based on visual rating of herbicide damage two weeks after treatment, selfed progeny of S-2 plants segregated approximately 1:2:1 for healthy, uninjured plants: to plants showing partial injury: to dead plants, respectively, at 0.44 and 1.1 kg/ha sethoxydim treatments. All control progeny of 2167-9/2160-154 control plants were killed by 0.1 kg/ha and greater levels of sethoxydim. These results demonstrate partially dominant expression of sethoxydim tolerance indicating that sethoxydim tolerance in S-2 plants is a heritable trait. Similar tests were conducted on progeny of S-2 plants crossed to the other inbreds. In all cases, these test cross progeny treated with 0.44 kg/ha sethoxydim segregated 1:1 for growing shoots versus dead shoots whether S-2 plants were used as male or female parents. These results confirm that sethoxydim tolerance is controlled by a single partially dominant nuclear gene. In all cases, control plants crossed to the other inbreds were killed and therefore sethoxydim-sensitive.

E. **Method for Obtaining Uniform Herbicide-Tolerant Seed**

Progeny of S-2 plants surviving sethoxydim treatments of 0.44 and 1.1 kg/ha and showing no herbicide injury were transferred to the greenhouse and grown to maturity. These plants may be selfed and their progeny evaluated for sethoxydim and haloxyfop tolerance to identify pure breeding herbicide-tolerant maize lines.

Progeny of S-2 plants crossed to inbred lines and exhibiting sethoxydim tolerance may be recurrently backcrossed to the same inbreds. Progeny of each cross may be screened for sethoxydim-tolerance, and tolerant plants grown to maturity and again crossed to the recurrent parent. After six or seven cycles of backcrossing, sethoxydim-tolerant plants may be selfed and progeny screened for tolerance to produce homozygous sethoxydim tolerant maize inbreds.

**EXAMPLE IV**

**Selection of Additional Herbicide-Tolerant Maize Cell Lines**

One primarily sethoxydim-tolerant maize cell line, 2167-9/2160-154 S-I, and two haloxyfop-tolerant maize cell lines, 2167-9/2160-154 H-I and 2167-9/2160-154 H-2, were selected and characterized as follows:
A. **Selection of Maize Cell Line 2167-9/2160-154 S-1**

Maize cell line 2167-9/2160-154 S-1 was selected from maize cell culture using the protocol described in detail above for the selection of Line 2167-9/2160-154 S-2. Approximately 70 plants were regenerated from Line 2167-9/2160-154 S-1, and either self-pollinated or cross-pollinated to the inbred maize lines A188, A619, A641, A661, A665, B37, B73, R806, and W153R.

B. **Selection of Maize Cell Line 2167-9/2160-154 H-1**

Line 2167-9/2160-154 H-1 was selected from maize cell culture using a similar protocol described in detail above except maize callus tissue was selected using the herbicide haloxyfop. Maize callus tissue was initially plated on 0.01 μM haloxyfop. At two-week intervals, surviving tissue was subcultured onto 0.05, 0.10 and 0.20 μM haloxyfop. Approximately 50 plants were regenerated from Line 2167-9/2160-154 H-1, and were self-pollinated.

C. **Selection of Maize Cell Line 2167-9/2160-154 H-2**

Line 2167-9/2160-154 H-2 was selected from maize cell culture using a similar protocol described in detail for line 2167-9/2160-154 H-1. No plants have been successfully regenerated from this line.

D. **Characterization of Lines 2167-9/2160-154 S-1, H-1 and H-2**

The tolerant callus cultures were characterized to determine the magnitude of sethoxydim and haloxyfop tolerance. Callus tissue from these lines was evaluated in experiments as described above in the characterization of line 2167-9/2160-154 S-2. Table II summarizes the results of these studies. Line 2167-9/2160-154 S-1 and Line 2167-9/2160-154 H-2 showed a four-fold increase in haloxyfop tolerance, while Line 2167-9/2160-154 H-1 exhibited approximately a 60-fold increase in haloxyfop tolerance. Neither haloxyfop selected line showed a significant degree of sethoxydim tolerance, while the sethoxydim selected line S-1 exhibited approximately a 100-fold increase in sethoxydim tolerance.
TABLE II

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Haloxyfop</th>
<th>Sethoxydim</th>
</tr>
</thead>
<tbody>
<tr>
<td>2167-9/2160-154</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>S-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2167-9/2160-154</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td>H-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2167-9/2160-154</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>H-2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

'The numbers represent the fold increase in herbicide concentration that results in a 50% reduction in growth of the selected cell lines compared to the unselected control cell line 2167-9/2160-154.

E. Herbicide Inhibition of Acetyl Coenzyme A Carboxylase of Maize Cell Lines S-1, H-1 and H-2

Acetyl Coenzyme A Carboxylase (ACCase) was extracted from cell lines S-1, H-1 and H-2 and assayed as described in detail for maize cell line S-2 above. Table III below summarizes the results of these studies. The ACCase from line S-1 was more tolerant of both sethoxydim and haloxyfop, while the ACCase from line H-1 was more tolerant of haloxyfop, but not of sethoxydim. The ACCase from line H-2 showed no difference from the unselected parent line 2167-9/2160-154 in sensitivity to either herbicide.

However, cell line H-2 exhibited approximately a five-fold higher level of ACCase activity as compared to the unselected parent line 2167-9/2160-154.

Thus, selection for sethoxydim or haloxyfop tolerance resulted in a less sensitive
ACCase in cell line S-1 and H-1, as well as a higher level of ACCase activity in cell line H-2.

**TABLE III**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Haloxyfop</th>
<th>Herbicide</th>
<th>Sebothoxydim</th>
</tr>
</thead>
<tbody>
<tr>
<td>2167-9/2160-154 S-1</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>2167-9/2160-154 H-1</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2167-9/2160-154 H-2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

'The numbers represent the fold increase in herbicide concentration that inhibits ACCase activity of the selected cell lines by 50% compared to the unselected parent cell line 2167-9/2160-154.

**F. Plant Regeneration and Production of Seed**

Cell lines derived by the procedures described above which exhibit herbicide tolerance are put through a plant regeneration protocol to obtain mature plants and seed expressing the resistance trait. The plant regeneration protocol allows the development of somatic embryos and the subsequent growth of roots and shoots.

Mature plants are then obtained from cell lines that are known to express the trait. If possible, the regenerated plants are self pollinated. In addition, pollen obtained from the regenerated plants is crossed to seed grown plants of agronomically important inbred lines. In some cases, pollen from plants of these inbred lines is used to pollinate regenerated plants. The trait is genetically characterized by evaluating the segregation of the trait in first and later generation progeny. The heritability and expression in plants of traits selected in tissue culture are of particular importance if the traits are to be commercially useful.
G. **Deposit of Seeds**

Seeds from representative S-2 plants (Ex. III (B)) and H-1 plants (Ex. IV(B)) have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 USA on October 25, 1988 and assigned accession numbers ATCC 40507, and ATCC 40508, respectively.

**EXAMPLE V**

**Allelic Analysis of Herbicide Tolerant Maize Lines**

To determine whether the mutations in herbicide tolerant maize plants derived from herbicide tolerant cell lines were allelic, plants derived from the resistant cell lines were crossed. The results, reported in Marshall et al. (*Theor. Appl. Genet.*, 83, 435 (1992)), show that of the five mutations tested, all were allelic.

**Materials and Methods**

Foliar applications of the herbicides were made to greenhouse- or field-grown seedlings at the three- to five-leaf stage. Herbicides were applied in a spray volume of 187 l/ha that includes 2.3 l/ha crop oil concentrate to enhance uptake. Sethoxydim and haloxyfop were applied using commercial formulations (Poast from BASF Corp and Verdict from Dow Elanco, respectively). Visual observations were recorded 14-21 days after herbicide treatments.

For each mutant, two homozygous tolerant R₄ families were derived from two different regenerated plants and two heterozygous F₁ families were obtained from crosses of R₃ homozygous tolerant lines with the susceptible inbreds A188 and A619. Sethoxydim and haloxyfop each were applied at rates ranging from 0.0034 to 7.0 kg/ha. For each genotype a five-rate doubling series was used to determine herbicide lethality. Because of the large differences between wildtype and mutants in herbicide susceptibility, the ranges of herbicide application rates were varied. Two five plant/pot replicates of each family were evaluated for each rate. Herbicide injury symptoms were scored visually 21 days after herbicide treatment. The lethal rate was considered to be the lowest application rate resulting in death of all seedlings in the four replications of each
homozygous tolerant genotype or in the eight replications of the heterozygous combinations.

All F$_1$ combinations (no reciprocals) between S1, S2, S3, H1 and H2 homozygotes were made, and these F$_1$s were test crossed to susceptible inbred lines. The testcross progeny were grown in the greenhouse and treated with the herbicides. In Experiment 1, progeny from testcrosses with each of the ten F$_1$ combinations were treated with 0.05 kg/ha sethoxydim for combinations involving only S lines or with 0.009 kg/ha haloxyfop for combinations involving either of the H lines. In Experiment 2, a selected subset of the F$_1$ combinations was tested that used testcross families that were separately derived from those used in Experiment 1. Testcross progeny of S lines were treated with 0.03 kg/ha sethoxydim, and H1 and H2 progeny were treated with 0.009 kg/ha haloxyfop. In each experiment, known susceptible and heterozygous plants were included as controls.

For each mutant, homozygous tolerant and homozygous susceptible families derived from R$_0$ (heterozygous) plants were classified by evaluating plant responses to the herbicides. Enzyme extracts from these families were assayed for ACCase activity (% of no herbicide control) at herbicide concentrations near or greater than the susceptible wildtype $I_{50}$ value. Activities in tolerant and susceptible extracts included in the same experiments were compared. ACCase activity was considered tolerant if when assayed in the presence of herbicide, it was at least 20% greater than the inhibited wildtype ACCase activity. For each of the mutant lines, tolerant lines tracing back to at least two different R$_0$ plants were evaluated.

**Results**

Homozygous seedlings of S2 and S3 exhibited only slightly bleached leaves and a slight reduction in plant height, but were not killed at 7 kg/ha sethoxydim, which is at least a 127-fold increase over the lethal rate for wildtype plants (Table IV). S2 and S3 lines also exhibited cross tolerance to haloxyfop.

Homozygous S1 seedlings exhibited herbicide tolerance similar to that of S2 and S3. Heterozygotes derived from crosses of the S lines with the wildtype
exhibited a tolerance to both herbicides that was intermediate between wildtype and homozygous tolerant seedlings.

In contrast to the S lines, a second distinctive phenotype was represented by H1 homozygous seedlings that exhibited little or no sethoxydim tolerance but significant haloxyfop tolerance. Heterozygotes with the H1 lines exhibited haloxyfop tolerance that was similar to that of the homozygous H1 seedlings. H2 seedlings represented the third phenotype, which exhibited about 16-fold increases in tolerance to sethoxydim and haloxyfop compared to the wildtype. The H2 heterozygotes exhibited a tolerance to both herbicides that was intermediate between wildtype and homozygous tolerant seedlings.

Table IV

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Lethal rates (kg/ha)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sethoxydim</td>
<td>Haloxyfop</td>
</tr>
<tr>
<td>Wildtype&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.055</td>
<td>0.014</td>
</tr>
<tr>
<td>Homozygous tolerant&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2</td>
<td>&gt; 7.0</td>
<td>0.22</td>
</tr>
<tr>
<td>S3</td>
<td>&gt; 7.0</td>
<td>0.22</td>
</tr>
<tr>
<td>H1</td>
<td>0.055</td>
<td>0.11</td>
</tr>
<tr>
<td>H2</td>
<td>0.88</td>
<td>0.22</td>
</tr>
<tr>
<td>Inbred x S2</td>
<td>≥ 1.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Inbred x S3</td>
<td>≥ 1.8</td>
<td>0.11</td>
</tr>
<tr>
<td>Inbred x H1</td>
<td>0.055</td>
<td>0.11</td>
</tr>
<tr>
<td>Inbred x H2</td>
<td>0.22</td>
<td>0.11</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average response of A188 and A619

<sup>b</sup> Average response of two families per mutant line

<sup>c</sup> Average response of A188- and A619-derived F<sub>1</sub> families

The two families of each mutant, derived from different R<sub>0</sub> plants and representing independent derivations from the parental A188 x B73 cross, had
similar responses to the herbicides. Herbicide lethality of heterozygotes of each mutant from the A188 cross was similar to that of the A619 cross. This suggested that background effects did not play a large role in modifying herbicide tolerance.

To determine allelic relationships among the mutations, F<sub>1</sub>s made between the different homozygous mutants were crossed to herbicide-susceptible inbreds. The testcross progeny were treated with rates of herbicides intended to kill wildtype plants but allow heterozygous plants to survive. If the mutant alleles from the different mutant lines were at the same locus, then all testcross progeny would have had one or the other mutant allele contributed by the F<sub>1</sub> parent and would have been tolerant to the herbicide rates used. No susceptible plants were found in a total of 260 testcross progeny from crosses among S1, S2 and S3. Interpretation of these results was straightforward because all S line heterozygous plants included as controls in these tests survived the herbicide treatments as expected, and all inbred wildtype plants died when treated with sethoxydim. Additionally, no haloxyfop-susceptible plants were found in a total of 190 testcross progeny from H1 combinations with S1, S2 or S3, indicating that the H1 mutation is allelic to S1, S2 and S3 mutations.

Testcrosses involving H1 and H2 alleles were more difficult to interpret. The heterozygous controls for H1 and H2 were injured, and some were killed with the haloxyfop rate (0.009 kg/ha) that killed most, but not all, wildtype control plants. Four susceptible plants were found in a total of 310 testcross progeny from combinations of H2 and the S lines. This frequency was clearly different from the 77 susceptible progeny expected if the H2 allele was at an independent locus. It seems most likely that these deaths occurred due to variability in response of the H2 heterozygous plants, but the possibility that the haloxyfop-susceptible plants represented recombination between two closely linked loci cannot be ruled out.

The 5 haloxyfop-susceptible plants among 102 testcross progeny from the H1 and H2 combination also represented a clear difference from the 25 susceptible expected for independent loci, and again may be explained by a
variable response of H2 or H1 heterozygous plants. In the testcrosses involving H2, all of the plants that died were from crosses to the inbred A188. A188 was slightly more susceptible than other inbreds, which may have resulted in a slightly lower tolerance in heterozygotes derived from A188. The cause of rare deaths cannot be conclusively interpreted. However, the most likely interpretation is that the S1, S2, S3 and H1 mutations are allelic and that H2 either is allelic or linked (≤ 4.4 map units, if all 9 plants that died were due to recombination).

Summary

Herbicide tolerance for five maize (Zea mays L.) mutants regenerated from tissue cultures selected for tolerance to the ACCase-inhibiting herbicides, sethoxydim and haloxyfop was determined. Herbicide tolerance in each mutant was inherited as a partially dominant, nuclear mutation. Allelism tests indicated that the five mutations were allelic. Three distinguishable herbicide tolerance phenotypes were differentiated among the five mutants. Seedling tolerance to herbicide treatments cosegregated with reduced inhibition of seedling leaf ACCase activity by sethoxydim and haloxyfop demonstrating that alterations of ACCase conferred herbicide tolerance. Five putative new alleles of the maize ACCase structural gene (Acc1) were identified based on their differential response to sethoxydim and haloxyfop. The group represented by Acc1-S1, Acc1-S2 and Acc1-S3 alleles, which had similar phenotypes, exhibited tolerance to high rates of sethoxydim and haloxyfop. The Acc1-H1 allele lacked sethoxydim tolerance but was tolerant to haloxyfop, whereas the Acc1-H2 allele had intermediate tolerance to sethoxydim but was tolerant to haloxyfop.

Differences in tolerance to the two herbicides among mutants homozygous for different Acc1 alleles suggested that sites on ACCase that interact with the different herbicides do not completely overlap.

EXAMPLE VI

Identification and Cloning of Maize cDNA and Genomic Clones Encoding ACCase

Maize cDNA clones encoding a portion of the ACCase gene were identified by screening a DNA library generated from maize. The cDNA clones
were used to identify the sequence of the ACCase gene and to identify the
genomic DNA fragments encoding the gene or genes for ACCase.

A λ gt11 cDNA library from maize inbred A188 seedlings was prepared
by standard method for oligo-dT priming, as described for pea cDNA. (Gantt
library were screened with maize ACCase antiserum (Egli et al., *Plant Physiol.*, 101, 499 (1993)) to identify plaques expressing ACCase-like proteins, as
described by Sambrook et al., cited supra. (1989). The initial screen of 800,000 plaques yielded 120 positives. Rescreening and plaque purification reduced the
number of positives to 14. All 14 clones bound ACCase antibodies that, when
eluted from plaque lifts (J. Hammarback et al., *J. Biol. Chem.*, 265:12763
(1990)), recognized a 227-kD biotinylated polypeptide on SDS-PAGE western
blots of embryo and leaf crude extracts. The strongest western blot reaction was
obtained with cDNA clone #15-14. The six best clones were digested with
EcoRI to excise maize cDNA inserts. Total insert sizes ranged from 1.2 to 5.1
kb indicating the clones most likely did not contain the full coding sequences for
the mature 219-kD and 227-kD ACCase polypeptides (minimum estimates of 6.1
and 6.3 kb, respectively).

Clone #15-14 contained three EcoRI fragments of 2.0, 1.2 and 0.23 kb
Southern blots showed that the 1.2 and 2.0-kb fragments of clone #15-14 each
hybridized to different fragments in the other five clones, with the exception of
clone #4-4 which only contained a 1.2-kb fragment. The six maize cDNA clones
contained EcoRI fragments that hybridized to a large transcript (ca. 8.3 kb) on
Northern blots of total RNA from maize leaves, embryos and endosperm. BMS
cell culture RNA also contained a 7.8 kb transcript. The relative abundance of
the 7.8-kb transcript in embryos was higher than the other sources which is
consistent with their ACCase activity.

The three EcoRI fragments were subcloned from cDNA clone #15-14
into BlueScript vector and sequenced by the dideoxy chain termination method
(Sequenase 2.0 USB) initially using T3 and T7 primers and then oligonucleotide
primers based on insert sequence. A clone #16-6 was also sequenced in a similar
manner. Clone #16-6 included three EcoRI fragments of 3.1 kb, 1.2 kb, and 0.23 kb and had additional sequence located upstream from that of clone #15-14. After comparing the sequence and determining that the sequence was the same, the additional 1.2 kb sequence at the 5' end was sequenced.

Clone #18-5 was sequenced in a similar manner. Clone #18-5 included 3.9 kb, 1.2 kb, and 0.23 kb EcoRI fragments and contains an additional 1.9 kb 5' sequence upstream from clone #15-14. Subclone #18-51 (3.9 kb EcoRI fragment) has been deposited with the American Type Culture Collection and given Accession No. 69236.

GenBank, PIR-29, and Swiss-Prot 19 data banks have been searched for amino acid homology with the corresponding amino acid sequences of the three subclones of clone #18-5. Peptide sequences corresponding to the maize cDNA subclones had higher similarity to chicken, rat, yeast and other plant and animal ACCases than to any other peptide sequence in the data banks. A comparison of the relative organization of the 3.9, 1.2 and 0.23-kb EcoRI fragments of clone #18-5, their co-linearity and extent of amino acid identity with chicken ACCase cDNA sequence shows that the maize clone #18-5 has a large region near the 3' end with high amino acid identity (40 to 61%) to chicken ACCase; a longer region with 23% identity in the middle of the 3.9-kb sequence, and a short region with 52% identity near the 5' of the 3.9 kb sequence.

Portions of the sequence of the #18-51 subclone have been identified as encoding domains of the ACCase enzyme of functional significance. Those functional regions include a fragment that spans the presumed transcarboxylase
active site in the enzyme having the following presumed sequence SEQ ID NO: 1:

```
1112-856
5  GTT CCT GCA AAC ATT GGT GGA CCT CTT CCT ATT ACC AAA CCT CTG GAC
   CCT CCA GAC AGA CTT GGT GCT TAC ATC CCT CTT GAG AAC ACA TGC GAT CCA
   GGT GCA GCT ATC TGT GGT GTA GAT GAC AGC CAA GGG AAA TGG TGG GGT
   GGT ATG TTT GAC AAA GAC AGC TTT GTG GAG ACA TTT GAA GGA TGG GCA
   AAA ACA GTG GTT ACT GGC AGA GCA AAG CTT GGA GGA ATT CCT GTG GGC
 10 GTG ATG CTT GTG GAG ACA
```

This functional domain is contained in the sequence 1112 to 856 base pair from
the 3' stop codon or carboxy terminus region of the ACCase coding sequence of
maize. This transcarboxylase active sequence is also present in clone #15-14.

Another functional region that has been identified spans the 12 base pair
sequence encoding the biotin binding site having the following peptide sequence
SEQ ID NO: 2:

```
5' GTT ATG AAG ATG 3'
Val Met Lys Met
```

The biotin binding site is encoded approximately 30% in from the 5' (N-
terminus) end of rat, chicken and yeast ACCase genes. These functional
domains are useful in mapping and further identifying other cDNA and/or
genomic fragments encoding ACCase genes.

The cDNA clones encoding portions of the acetyl CoA carboxylase genes
are useful to identify the sequence of the gene or genes and are useful as probes
to locate the genomic copies of the gene or genes. Because the ACCase
antibodies used to screen the λ gt11 library recognize both the 219 and 227 kD
ACCase polypeptides, it has not been determined which polypeptide is encoded
by these less than full length clones. It is likely that the majority of the clones
encode the 227 kD polypeptide since that polypeptide is more abundant in the
leaf tissue source of the DNA library and the antibodies have a higher affinity for
the 227 kD ACCase polypeptide.
The maize genome was analyzed to identify copy number and location of the genomic copies of ACCase gene or genes. Four distinct types of maize ACCase genomic clones have been identified, termed A1, A2, B1 and B2 (see below).

To obtain genomic copies of ACCase genes, a maize B73 genomic library (Clontech, Palo Alto, CA) was screened with the 2 kb subclone from #15-14 and several clones of about 15 kb were identified as having homology to the ACCase cDNA. Restriction mapping and partial sequence analysis revealed two types of genomic clones (Type A and Type B) that differed in restriction sites and in their position relative to the ACCase partial cDNA sequence.

The 2.5 kb EcoRI-SalI fragment (#16) from the Type A genomic clone and the 3.0 kb EcoRI-EcoRI fragment (#34) from the Type B genomic clone were shown to hybridize to the 3.9 kb probe from #18-5 and were subcloned into the Bluescript vector and sequenced. Approximately 1.5 kb of DNA sequence from the genomic Type A 2.5 kb fragment were 100% identical to coding sequence from the 3.9 kb cDNA subclone #18-51 described hereinabove; the remaining sequence exhibited no identity with the cDNA clone and presumably represents a noncoding intron sequence. A 350 nucleotide sequence derived from the genomic Type B 3.0 kb fragment was about 95% identical to the cDNA clone indicating that its coding sequence differs from that of genomic Type A. These results also indicate that the maize genome encodes at least two different genes encoding a polypeptide having acetyl CoA carboxylase activity.

To identify and clone the remainder of the gene representing the aminoterminus of maize ACCase, additional regions from the Type A genomic clone have been subcloned and sequenced. To synthesize the remaining coding region between the end of the cDNA clone #18-5 and the start of transcription, two oligonucleotide primers were synthesized. Primer 1 is complementary to the DNA sequence: (SEQ ID NO:3)

5' GCCAGATTC ACCAAAGCAT ATATCC 3'
near the 5' end of cDNA subclone #18-51 and was used as a primer for synthesis of cDNA molecules from maize seedling, leaf or embryo RNA. Several independent clones were sequenced and their sequences compared to the known sequence of the Type A genomic clone to determine the exact coding sequence corresponding to that maize gene for ACCase. A similar strategy can be used to obtain the complete coding sequence for genomic Type B ACCase.

The remaining cDNA sequence was obtained by three successive rounds of RT-PCR using oligonucleotide primers based on genomic apparent exon (5') and known cDNA (3') sequences. The primers used to amplify nucleotides 1-240 of the cDNA were 28sst-a5+ (SEQ ID NO:4) and 28sst-6at3+ (SEQ ID NO:5), nucleotides 217-610 of the cDNA were 28sst-5+ (SEQ ID NO:6) and 28-2t3+ (SEQ ID NO:7), and nucleotides 537-2094 of the cDNA were ACCPCR5' (SEQ ID NO:8) and 155' (SEQ ID NO:3) (Table V). PCR products corresponding to nucleotides 1-240, 217-610, and 537-2094 of the final sequence were cloned into PCR-script (Stratagene).
<table>
<thead>
<tr>
<th>cDNA Position</th>
<th>5' primer designation</th>
<th>5' primer sequence</th>
<th>3' primer designation</th>
<th>3' primer sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>nt-1-240</td>
<td>28sst-a5+</td>
<td>GGTCTTCAATTGTGCTGTCTGG (SEQ ID NO:4)</td>
<td>28sst-6at3+</td>
<td>CCTGACGAACAGACTGGCTGTGC (SEQ ID NO:5)</td>
</tr>
<tr>
<td>nt 217-610</td>
<td>28sst-5+</td>
<td>CACAGCCAGTCTGGTCAGG (SEQ ID NO:6)</td>
<td>28-2t3+</td>
<td>CCTCTACGTTATTGGTCAGC (SEQ ID NO:7)</td>
</tr>
<tr>
<td>nt 537-2094</td>
<td>ACCPCR5'</td>
<td>CATAGCTATGGCAACTCCGG (SEQ ID NO:8)</td>
<td>I55</td>
<td>GGATATATGCTTTGGTGGAATCTGGC (SEQ ID NO:3)</td>
</tr>
</tbody>
</table>
The original 5.4-kb cDNA clone #18-5 and PCR products from at least three individual PCR per oligonucleotide pair were sequenced in both directions by the dideoxy chain-termination method, using either Sequenase II (U.S. Biochemicals) or ABI 373 (Applied Biosystems, Inc.) protocols. No sequence differences were found in regions of clone overlaps. The complete sequence of the cDNA of maize ACCase (nucleotides 1-7470; SEQ ID NO:9) and its corresponding amino acid sequence (amino acids 1-2325; SEQ ID NO:10) are shown in Figures 3 and 4. The 7470 bp cDNA includes a 459 nucleotide 3' untranslated region and 36 nucleotides of 5' untranslated sequences.

The first Met codon in the cDNA (nucleotides 37-39) was identified as the start codon based on its similarity to consensus initiation sequences (Kozak, J. Cell. Biol., 108, 229 (1989); Lutcke et al., Embo. J., 6, 43 (1987)). An in-frame stop was found in the genomic sequence 6 nucleotides upstream of the sequenced cDNA, and RT-PCR analysis of this region suggested that the in-frame stop codon was also present in the cDNA. The 3' end of the coding sequence was defined by two stop codons present in the large open reading frame after nucleotide 7011. The translated coding sequence predicted a polypeptide of 2325 amino acids (257 kD; SEQ ID NO:10) which was 79 to 81% identical to the multifunctional (MF) ACCases from alfalfa (Shorrosh et al., Proc. Nat'l Acad. Sci., 91, 4323 (1994)) and wheat (Gornicki et al., Proc. Nat'l Acad. Sci., 91, 6860 (1994)), and to a 118-amino acid predicted polypeptide of a rice expressed sequence tag (Genbank accession # D39099, T. Sasaki), but only 53 to 55% identical to ACCase from other eukaryotes.

In a pileup alignment of plant ACCases (Genetics Computer Group, Madison, Wisconsin), Met 1 of both maize and Brassica napus ACCases was located about 130 amino acids upstream of the conserved sequence VDEFCKALGG, compared to only 25 amino acids upstream for other plant ACCases. The predicted 2325 amino acids of maize ACCase contains a biotinylation site at position 806, within the conserved VMKM motif (Ton et al., Eur. J. Biochem., 215, 687 (1993)). The arrangement and amino acid sequence of binding sites (Shorrosh et al., Proc. Nat'l Acad. Sci., 91, 4323 (1994)) for
ATP (amino acids 318-333), biotin (amino acids 799-811; biotin at 806),
acetyl-CoA (amino acids 1952-1961), and carboxybiotin (amino acids
1662-1711) were highly conserved among all Mt ACCases.

EXAMPLE VII

Characterization of Maize ACCase Genomic Clones

The initial restriction fragment length polymorphism (RFLP) analysis of
EcoRI-digested total DNA from three maize inbred lines showed one band when
probed with the 2 kb subclone from #15-14 (internal to gene) and two bands
when probed with the 1.2 kb subclone (near the 3' end of the gene). Fragments
homologous to the 2 kb probe were monomorphic and the more intense of the
two bands hybridizing with the 1.2 kb probe was dimorphic. As discussed
above, these results support the view that maize contains at least two
distinguishable ACCase genes and that they may be quite similar for much of the
coding region. Additional genomic Southern blots of a set of recombinant
inbred lines were used to map polymorphisms for the ACCase probes to maize
chromosomes. One polymorphism was mapped to the short arm of chromosome
2; other polymorphisms were not evident in these initial tests to identify a
chromosomal location for other maize ACCase genes.

The isolation and restriction mapping of additional genomic clones from
a B73 genomic library (Clontech) resulted in the identification of four different
types of clones termed A1, A2, B1 and B2 which had 96% nucleotide sequence
identity. Types A and B correspond to previously published pA3 and pA4
cDNAs (Ashton et al., Plant Mol. Biol., 24, 35 (1994)) and differ from pA3 and
pA4 by ~ 4% in their coding sequences.

Type A and B genomic clones have linear sequence homology except for
an insertion in an intron of the Type B genes about 1400 bp 3' of the A1(SEQ ID
NO:9) translation start site. Analysis of the insert boundaries revealed a 3-bp
target site duplication and a 6-bp direct repeat, and further sequence analysis
showed the presence of two new and unique LINE elements (Long Interspersed
Nuclear Elements) in B1 and B2. Mammalian LINE elements are highly
abundant (10^4 to 10^5 copies), 6 to 7 kb long, and have frequent 5'-end deletions
and an A-rich 3' terminus. They are flanked by short direct repeats, and contain two ORFs, one encoding a reverse transcriptase. Three LINE elements (Cin4, 50-100 copies in maize; del2, 250,000 copies in lily; BNR1, 2-5% of genome in sugarbeet) have been described in plants (Leeton et al., Mol. Gen. Genet., 237, 97 (1993); Schmidt et al., Chromo. Res., 3, 335 (1995); Schwarz-Sommer et al., EMBO J., 6, 3873 (1987)). Maize ACCase B1 has one unique LINE element and B2 has two. The two B2 LINE elements were characterized by differences in their reverse transcriptase sequence. The B genomic clone inserts have characteristic LINE features including cysteine motifs and a possible polyA tail, and high abundance. The LINE insert also has been found in an intron of the maize Shrunken-2 gene (Hannah et al., Plant Physiol., 98, 1214 (1992)).

The nucleotide sequence (3544 nucleotides) of a Type A1 ACCase genomic clone is shown in Figure 5 (SEQ ID NO:11).

The nucleotide sequence of another Type A clone is shown in Figure 6 (SEQ ID NO:12). The sequence is all 5' untranslated sequence and contains two non-identical 7 nucleotide inserts, a CTP (i.e., it can be amplified with primers 28sst-97F and 28sst-6t3+) and at least the first 1/3 of an ACCase coding sequence. The CTP of SEQ ID NO:12 is identical to the CTP of A1 clones.

The partial nucleotide sequence of five Type A2 clones is shown in Figure 7 (SEQ ID NOs 13, 14, 15, 16 and 17, respectively).

A limited sequence comparison of SEQ ID NO:12 and A2 clones showed that SEQ ID NO:12 and A2 clones were more closely related than SEQ ID NO:12 and A1 clones.

The partial nucleotide sequence (231, 207 and 180 nucleotides) of three Type B clones is shown in Figure 8 (SEQ ID NOs 18, 19 and 20, respectively).

The cDNAs corresponding to genomic clones A2, B1 and B2 are cloned and sequenced in a manner similar to that described above. The derived amino acid sequences are aligned with known ACCase sequences. If putative CTP sequences are identified, functionality is tested as described below. Also if the tissue specificity and developmental timing of expression differ for different ACCase genes, the sequences of the promoter regions of the corresponding
genomic clones are compared. Gene-specific probes for specific ACCase genes can provide more information on their roles in lipid synthesis (plastid and cytoplasmic isoforms), secondary metabolism (cytoplasmic isoforms), and herbicide resistance (likely plastid isoforms).

Only one plastidic ACCase polypeptide was identified by SDS-PAGE of maize leaf extracts, although 2-D gel analyses might provide evidence for a second, highly similar isoform. Of the two ACCase isoforms, only ACCase I shows altered herbicide inhibition in Acc1-S2 mutants, and most of the ACCase activity in leaves and developing embryos is herbicide-resistant and thus attributed to the Acc1-S2 gene product.

Although a 3' ACCase probe has been mapped both to chromosome 10 near Acc2-S5 and to chromosome 2, the conserved sequence of ACCase genes and lack of polymorphism in multiple bands complicates identification of genes encoded at these loci. The Type A1 ACCase gene is probably located on chromosome 2, since (i) 5' untranslated and chloroplast transit peptide probes from Type A1 hybridize to two bands (dark and light) in maize inbreds, and (ii) analysis of maize-oat addition lines carrying maize chromosomes 2 through 9 indicates the dark band is on chromosome 2 and the light band is on chromosome 1 or 10.

Type B ACCase genes are likely to encode cytosolic isoforms. Given that cytosolic malonyl-CoA is a precursor in the synthesis of many secondary metabolites including flavonoids (e.g. maysin, a corn silk component associated with corn earworm resistance), these cytosolic ACCases can have agronomic utility.

Northern blot analysis of total maize RNA with an ACCase probe (nucleotides 3400-5932) showed a single 8.3 kilobase band. To determine whether the expression of ACCase RNAs was developmentally regulated, blots of total RNA from 16 to 42 DAP (days after pollination) embryos were probed with an ACCase cDNA fragment. Transcript abundance peaked about 23 DAP and the steady state pattern was similar to in vitro ACCase enzyme activities and protein measured from developing embryos. Type A- and B- specific 32P-CTP-
labeled antisense transcripts were 780 nt long (662 nt of ACCase sequence + 118 nt of vector/promoter sequence) and were identical except for 15 base mismatches scattered along their length. Each antisense transcript was hybridized to total RNA from embryos at 16, 20, 23, and 42 DAP and digested with RNAse A/TR mixture to yield a 662-base fragment specific to the probe used. The results showed that the Type A transcript was more abundant than Type B at all tested stages, and that only Type A remained high in older embryos. Types A and B had similar expression patterns and peaked around 20-23 DAP. The ratio of Type A:B mRNA in leaves was about 2:1, similar to its relative abundance in cDNA expression libraries.

**EXAMPLE VIII**

**Identification of a Second Herbicide Resistance Locus on Chromosome 10**

To determine if sethoxydim and haloxyfop mutations segregated, all possible pairwise combinations of crosses were made between the sethoxydim (designated S1, S2, S3, S4, S5) or haloxyfop (designated H1, H2) tolerant mutant lines. The resulting F1 plants were test-crossed to wild-type susceptible plants. The testcross progeny were grown in the greenhouse and sprayed with Poast at a rate of 0.4 pounds/acre. If the two parents of the original cross had tolerance mutations in the same gene, then all testcross plants were expected to be heterozygous for a tolerance gene and would not segregate for wild-type susceptible plants. If the two parents had tolerance mutations in two different genes, then the testcross plants were expected to segregate for wild-type susceptible plants. Table VI below summarizes the results from these testcross progeny.
Table VI

<table>
<thead>
<tr>
<th>PARENT 2</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
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<tr>
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<td>H1</td>
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</tbody>
</table>

RES = all resistant testcross progeny
SEG = segregation for wildtype susceptible progeny at an approximate frequency of 25%

Subsequent to these analyses, another Acc resistance allele, S6, was identified. S6 is allelic to S5. S5 is a mutant Acc allele, which maps to chromosome 10, identified by Van Dee (an allele referred to as “M” in Van Dee, M.S. Thesis, University of Minnesota, 1994)).

These testcross progeny data clearly showed that the mutant line designated S5 was not allelic to any of the other sethoxydim-tolerant mutants. The tolerance gene, Acc2, was mapped to chromosome 10, flanked by umc155 and umc146 by a distance of 5.9 and 4.1 centimorgans (cM), respectively, by analyzing standard RFLP markers in an F2 population segregating for tolerance and susceptibility (VanDee, M.S. Thesis, University of Minnesota (1994)). The Acc2 locus is about 10 map units from gl, which is a “golden plant” phenotypic marker on the long arm of chromosome 10. The Acc2 mutant is designated Acc2-S5.

The testcross data also showed that all the mutant lines other than S5 have mutations in the same gene (Acc1) because their testcross progeny did not segregate. Thus, tissue culture selection for sethoxydim or haloxyfop resistance
resulted in 7 independently isolated mutations representing 2 different, unlinked genes.

As described above, genomic clones representing at least 4 different ACCase genes have been isolated from a genomic library of the inbred line B73. The complete coding sequence for one gene (A1) was determined and, where determined, the coding sequences for the other clones were highly identical to that of A1. A1 has a functional chloroplast transit peptide sequence as expected for an ACCase that is localized in plastids. Another genomic clone (designated 5A) from the 5' end of the gene also contained a transit peptide sequence and other 5' sequences that differed slightly from A1. These results indicated that corn has at least 2 genes for plastidic ACCase. The 5' sequence differences between A1 and 5A (Type A2) genomic clones are useful in designing PCR primers that would be specific for either A1 or 5A (Type A2) genes. Restriction site differences in the upstream 5' regions for Type A1 and A2 genes may also be useful for RFLP mapping with gene-specific ACCase probes.

A 3' Type A1 ACCase cDNA probe mapped to chromosome 2 (Egli et al., *Maize Genetics Newsletter*, 68, 92 (1994)) and to chromosome 10 (Caffrey et al., *Maize Gen. Coop.*, 69, 3 (1995)). Two 5' Type A1 cDNA probes which span the transit peptide mapped to chromosome 2 in the same location as the 3' probe (see maize genetic map, 1996 version, Maize Genomic Database). Similar results were observed when the 5' or 3' probe was used to analyze total genomic DNA from some inbred lines relative to oat-corn additional lines. Both probes hybridized to two different fragments in total maize DNA but to only one fragment in oat-corn lines containing maize chromosome 2 and chromosome 3, 4, 5, 6, 7, 8 or 9 (Rines et al., In: *Modification of Gene Expression and NonMendelian Inheritance*, pp. 235-251 (1995)). Thus, A1 and A2, described herein above, appear to encode plastic ACCases that correspond to the *Acc1* and *Acc2* loci, respectively.

To identify segregating *Acc1-S3* tolerance genes, a pair of PCR primers (28sst-97F, CCTTTTTATGGCACCTGTGCG, SEQ ID NO:21) and 28sst-6t3+, CATCGTAGCCTATATGAGGACG, SEQ ID NO:22) were identified that
amplify the 5' end of the A1 gene sequence from B73, which segregates with the resistance trait, but not from A188 or A641. These PCR primers are located in non-coding regions of A1 that span the chloroplast transit peptide. A control reaction employed a nearby 5' primer (28sst-a5+, SEQ ID NO:4) and 28sst-6t3+. The amplification reactions comprised 1 µl sense primer (from a 2.5 µM 10X stock solution), 1 µl anti-sense primer (from a 2.5 µM stock solution), 0.5-1.0 µl genomic DNA (1 µg/µl), 2.5 µl 10X Taq buffer (Promega), 2.5 µl MgCl₂ (Promega, stock solution is 25 mM), 0.5 µl of a dNTP stock solution (Promega, 10 mM stock solution), 0.25 µl Taq polymerase, and 24.75 µl water. DMSO at 5% can be added to the amplification reaction to improve specificity.

The following cycling parameters were used: 94°C for 2 minutes for 1 cycle; 94°C for 1 minute for 35 cycles; 54°C for 1 minute and 74°C for 70 seconds, and then 4°C. Amplified products were analyzed by gel electrophoresis. A nearby 5' primer (28sst-a5+, SEQ ID NO:4), when employed with 28sst-6t3+, amplified all genotypes and functioned as a positive control. In an F2 family segregating for the S3 source of sethoxydim tolerance, amplification of the A1 PCR product (528 bp which includes nucleotides corresponding to nucleotides 1-238 of SEQ ID NO:11, 29 bp of noncoding 5' sequence and 261 bp of 3' intronic sequence) was always associated with sethoxydim tolerance (29 tolerant plants were PCR+). The B73 PCR product was not detected in DNA from 15 of 17 susceptible plants (died after spraying), but was in 2 susceptible plants. If these plants died only from herbicide exposure and not from an unrelated cause, then the results indicate that the A1 PCR marker maps approximately 12 cM from the resistance locus. These results showed that the B73 chromosome was the donor of the Acc1-S3 tolerance gene in the selected tissue cultures and suggests that the Acc1 gene is at least closely linked to the A1 genomic clone.

RFLP mapping was also employed to determine whether the Acc1 gene cosegregates with the herbicide tolerance locus on the short arm of chromosome 2. A probe, a 1.2 kb 3' fragment of Acc1 (22J), was labeled and used to probe
EcoRI digested genomic DNA isolated from eighty progeny of a cross between S3 x A641. DNA from all herbicide resistant progeny had the B73 Acc1 polymorphism while DNA from all herbicide sensitive progeny did not show the polymorphism. These results provide further support that the Acc1 gene is, or is very closely linked to, the locus conferring herbicide tolerance. Moreover, a monomorphic hybridizing fragment was observed in this mapping population which may represent the Acc2 locus on the long arm of chromosome 10.

**EXAMPLE IX**

**Methods to Prepare a Two-Gene Heterozygous Herbicide Tolerant Hybrid**

A preferred embodiment of the invention is an herbicide resistant inbred double homozygous maize plant (e.g., Inbred A (Acc1-S3/Acc1-S3;Acc2-S5/Acc2-S5)) that can be crossed to any normal susceptible inbred line. The resulting F1 contains one dominant allele for herbicide tolerance from each tolerance gene. The F1, which is grown by farmers, has herbicide tolerance equivalent to that given by single homozygous hybrids.

One method to prepare a two-gene heterozygous herbicide tolerant hybrid is to incorporate both tolerance genes at the end of inbred parent development. For example, cross Inbred A separately to S2 and to S5 homozygous mutant lines as shown:

20  Inbred A x S2
    +/-; +/+ x Acc1-S2/Acc1-S2

25  Inbred A x S5
    +/-; +/+ x Acc2-S5/Acc2-S5

This results in two genotypes each heterozygous for a different herbicide tolerance allele.

25  Acc1-S2/+; +/-
    +/-; Acc2-S5/+  

These genotypes are then backcrossed to Inbred A for the desired number of generations to recover the Inbred A parent. The susceptible plants segregating in each backcross generation can be eliminated by spraying with Poast.

Segregating backcross progeny include:

30  Inbred A x Acc1-S2/+; +/-
    +/+; +/- x Acc1-S2/+; +/+

30  Inbred A x +/-; Acc2-S5/+  
    +/+; +/- x +/+; Acc2-S5/+
+/+; +/+ (discard)  
\(\textit{Acc1-S2}+/+; \textit{Acc2-S5}/+\) (repeat cross)  
At end of backcrossing process, self pollinate to recover Inbred A with the homozygous mutant gene.

5

\text{Inbred A (\textit{Acc1-S2}/\textit{Acc1-S2};} +/+) \quad \text{Inbred A (}+/+; \textit{Acc2-S5}/\textit{Acc2-S5})

The two mutant versions of Inbred A are then crossed to produce double heterozygous mutant version of Inbred A:

\text{Inbred A (}\textit{Acc1-S2}/+; \textit{Acc2-S5}/+)\)

Self pollinate to produce F2 generation segregating for the following genotypes:

1/16  \textit{Acc1-S2}/\textit{Acc1-S2}; \textit{Acc2-S5}/\textit{Acc2-S5} identify homozygotes
2/16  \textit{Acc1-S2}/\textit{Acc1-S2}; \textit{Acc2-S5}/+
1/16  \textit{Acc1-S2}/\textit{Acc1-S2}; +/+/+  
2/16  \textit{Acc1-S2}/+; \textit{Acc2-S5}/\textit{Acc2-S5}

4/16  \textit{Acc1-S2}/+; \textit{Acc2-S5}/+
2/16  \textit{Acc1-S2}/+; +/+/+
1/16  +/+/+; +/+/+; \textit{Acc2-S5}/\textit{Acc2-S5}
2/16  +/+/+; +/+/+; \textit{Acc2-S5}/+
1/16  +/+/+; +/+/+; +/+/+

15

One sixteenth of the F2 plants are double homozygotes in Inbred A background. These F2 plants can be identified by:

a) Crossing F2 plants to susceptible plants and testing the progeny for sethoxydim tolerance. Plants homozygous for both genes produce nonsegregating double heterozygous testcross progeny that do not exhibit herbicide damage symptoms typical of single heterozygous mutant testcross genotypes.

b) Using RFLP molecular markers to identify chromosome regions that flank the \textit{Acc1} and \textit{Acc2} loci. Preferred RFLP markers flank and are tightly linked to, the ACCase coding sequences of \textit{Acc1} or \textit{Acc2}.

30 For instance, RFLP markers umc131 and umc2, or umc131 and uox, for the \textit{Acc1} region on chromosome 2 and umc155 and umc146, or umc155 and ncsu2, for
the \textit{Acc2} region on chromosome 10 can be used to identify F2 plants homozygous for both sets of flanking polymorphisms from the tolerant mutant donor parents. It is also envisioned that other more closely, or distantly, linked flanking RFLP markers or genotype-specific ACCase probes can be used to identify the desired progeny.

c) Using PCR amplification which employs genotype-specific primers to detect the presence of a particular allele of either \textit{Acc1} or \textit{Acc2}. For example, a primer that hybridizes to a region in the 5' untranslated portion of \textit{Acc1} and another primer that hybridizes to a region in the first intron of \textit{Acc1} are useful in an amplification reaction to detect the presence or absence of the fragment in progeny plants.

Another method to prepare a two-gene heterozygous herbicide tolerant hybrid is to incorporate both tolerance genes at the start of inbred parent development. For example, the two sources of tolerance (i.e., S2 and S5) are crossed to produce a double heterozygous mutant F1 genotype. This genotype is the equivalent of the Inbred A genotype indicated above (\textit{Acc1-S2}/+;\textit{Accs-S5}/+) except that the tolerance genes are not yet in the Inbred A background. The double heterozygous mutant F1 genotype is crossed to Inbred A and the resulting progeny plants analyzed for presence of the RFLP flanking markers for both \textit{Acc1} and \textit{Acc2} tolerance genes. Plants containing both sets of markers are used for the next cycle of backcrossing to Inbred A. The backcrossing and RFLP analysis are repeated for appropriate number of cycles to recover the Inbred A genotype. The final steps in developing the double homozygous mutant Inbred A genotype are the same as the final two steps in the incorporation of both tolerance genes at the end of inbred parent development (see above). The crossing of the inbred plant to a wild-type (susceptible) plant then results in a plant which is a hybrid double heterozygote.

A third method to prepare a two-gene heterozygous herbicide tolerant hybrid is to incorporate one tolerance gene into one inbred line (e.g., (Inbred A \textit{Acc1-S3}/\textit{Acc1-S3};+/+) and incorporate another tolerance gene into another
inbred line (e.g., Inbred B (+/+; Acc2-S5/Acc2-S5)). These two inbred lines are then crossed to produce a two-gene heterozygous herbicide tolerant hybrid.

**EXAMPLE X**

**Field Tests of Herbicide Tolerant Double Heterozygotes**

A double heterozygous hybrid was produced by crossing the following parent lines each homozygous for one of the sethoxydim tolerance mutations: Acc1-S2/Acc1-S2; +/+ x +/+; Acc2-S5/Acc2-S5, where + represents the normal alleles for sethoxydim susceptibility. The resultant F1 (Acc1-S2/+; Acc2-S5/+)

10 contained one copy of each sethoxydim-tolerant allele. Similar F1’s were produced between S3 and S5 mutant lines.

Several double heterozygous F1 hybrids were tested for tolerance to sethoxydim (Poast) applications in the field in 1996. Poast was applied at the 6 to 8 leaf stage at a rate of 0.8 pounds/acre. Six days after application, visual ratings of the double heterozygous F1 hybrids unexpectedly indicated either no herbicide injury symptoms or slight symptoms on a few plants (< 10%) as evidenced by transitory yellowing of leaves emerging from the whorl of the main stalk or from side tillers. These symptoms disappeared thirteen days after herbicide application. Similar results were obtained with the single mutant homozygous parent lines S2, S3 and S5. In contrast, single heterozygous F1 hybrids, obtained by crossing S2, S3 and S5 homozygous parent lines with normal susceptible lines, exhibited herbicide injury symptoms in most of the plants. These symptoms, especially yellowing of the tillers, persisted through flowering. Plants of normal susceptible lines were killed by this application.

Twenty-three days after the first application of herbicide, when tassels were emerging from most plants, another application of herbicide was performed (1.6 pounds Poast/acre). The double heterozygous F1 plants and the single homozygous parent lines showed little visible herbicide injury after the second treatment of the same plants. These herbicide applications showed that the herbicide tolerance of the double heterozygous F1 hybrids was equivalent to that of the single mutant homozygous parent lines. The response to herbicide treatment of hybrids with one mutant allele from each of the chromosome 2 and
the chromosome 10 genes (i.e., \textit{Acc1-S2+/+; Acc2-S5+/+}) was indistinguishable from the response of plants containing two mutant alleles from either gene (i.e., \textit{Acc1-S2/Acc1-S2; +/+} or \textit{+/-}; \textit{Acc2-S5/Acc2-S5}).

**EXAMPLE XI**

**Greenhouse Trials of Herbicide Tolerant Double Heterozygotes**

Two greenhouse trials were conducted to assess relative heterozygous sethoxydim resistant corn safety to 1/8X, 1/4X, 1/2X, 1X, 2X, and 4X use rates of POAST PLUS® in comparison to homozygous sethoxydim resistant corn and to sethoxydim susceptible corn (commercial Pioneer hybrid). Tolerance to sethoxydim was compared between double heterozygotes that contained the chromosome 2 resistance gene plus the chromosome 10 resistance gene, e.g., (\textit{Acc1-S3+/+; Acc2-S5+/+}) to heterozygotes with only the chromosome 2 gene, e.g., (\textit{Acc1-S3+/+; +/+}) or the chromosome 10 gene (+/+; \textit{Acc2-S5+/+}).

The following plant genotypes and growth stages were employed in the greenhouse trials: 1) commercial homozygous sethoxydim resistant corn hybrid (\textit{Zea mays}, var. DK 592 \textit{sr}), growth stage-14 (4 leaves expanded); 2) commercial non-sethoxydim resistant corn hybrid (\textit{Zea mays}, var. PI 3140), growth stage-14, 3) heterozygous sethoxydim resistant corn (\textit{Zea mays}), growth stage-14.

Sethoxydim (POAST PLUS, 120 g/l) was applied at 0.02 (1/8X), 0.04 1/4X), 0.09 (1/2X), 0.18 (1X), 0.36 (2X), and 0.71 (4X) lb ai/A (active ingredient per acre). The spray volume was 20 GPA (gallons per acre) and an adjuvant, ATPLUS® 411 F, was added at 1.25% v/v COC (crop oil concentrate).

Metro 360 soilless potting media was used. Osmocote 14-14-14 was applied at planting. STEM (Soluble Trace Element Mixture) and Peters 20-20-20 was applied within 3 days of germination and again 1 day after treatment at normal greenhouse use rates. Plants were watered overtop prior to application then subirrigated as needed. Plants were not exposed to UV light after application to maximize sethoxydim activity and potential injury. Corn plants were evaluated for percent injury at 7-8 and 14-15 days after treatment (DAT).

Results
GH Trial 067/96: Germination rates for the heterozygous sethoxydim resistant corn hybrids varied from 3 to 94 percent. Heterozygotes with the following line designations had germination rates of 80% or better and were included in GH trial 067/96: 4163/4142-2, 4142-2/4163, 4142-8/4158, 4158/4142-20, 4143-3/4160, 4143-1/4160. Heterozygotes with the following line designations did not have sufficient germination to test, most likely due to seed age (the seed was several years old): 4145-19/4160, 4144-12/4160, and 4142-7/4163. The heterozygotes in this trial all contained both chromosome 2 plus chromosome 10 resistance genes (homozygous chromosome 2 plants were crossed with homozygous chromosome 10 plants resulting in the same heterozygous genotype as results from a two gene homozygote crossed to a wild type). Homozygous mutant sources used in the crosses were S2 and S3 for the chromosome 2 gene location, and S5 for the chromosome 10 gene location.

Four of the six 2 + 10 double heterozygotes tested demonstrated less then 5% injury to the 2X rate (0.367 lb al/A) of POAST PLUS (see Table VIII). Only 9% initial injury 7 DAT was observed to the 4X rate of POAST PLUS with one of the 2 + 10 heterozygotes (4163/4142-2). This degree of tolerance was almost as high as the chromosome 2 homozygous commercial hybrid DK 592sr. The susceptible Pioneer Hybrid 3140 was severely injured (22%) at the 1/8X use rate (0.02 lb ai/A) of POAST PLUS.

GH Trial 126/96: Chromosome 2 and chromosome 10 single heterozygotes were employed in a second comparative trial testing the best 2 +10 double heterozygotes from trial 067/96 plus three new double heterozygotes produced in 1995, to these single heterozygotes. The chromosome 10 single heterozygotes demonstrated slight tolerance to sethoxydim in comparison to the susceptible Pioneer hybrid, through the plants were still injured 15 to 19% at 0.02 lb al/A, 8 DAT (see Table IX). The chromosome 2 single heterozygotes demonstrated more tolerance than the chromosome 10 single heterozygotes but still demonstrated a range of 17 to 48% injury at 0.09 to 0.18 lb al/A. All of the 2 +10 double heterozygotes demonstrated excellent tolerance (0% injury) at 0.36 lb al/A (2X rate) of POAST PLUS. Five of the seven double heterozygotes
demonstrated less than 10% injury to POAST PLUS at 0.76 lb al/ha. This was better tolerance than was observed with the chromosome 10 single homozygous line, and almost equivalent to the tolerance observed with the chromosome 2 single homozygous commercial hybrid, DK 592sr.

Thus, double chromosome 2 plus chromosome 10 heterozygous resistant corn demonstrated greatly improved tolerance to sethoxydim as compared to chromosome 2 heterozygous material.
<table>
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<th>Growth Stage</th>
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Spray Volume = 20 GPA
The invention is not limited to the exact details shown and described, for it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention defined by the claims.
SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Regents of the University of Minnesota

(ii) TITLE OF THE INVENTION: GENE COMBINATIONS FOR HERBICIDE TOLERANCE IN CORN

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Schwegman, Lundberg, Woessner & Kluth, P.A.
(B) STREET: P.O. Box 2938
(C) CITY: Minneapolis
(D) STATE: MN
(E) COUNTRY: USA
(F) ZIP: 55402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: Unknown
(B) FILING DATE: 29-AUG-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US96/04625
(B) FILING DATE: 04-APR-1996

(A) APPLICATION NUMBER: 08/679,826
(B) FILING DATE: 30-AUG-1996

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Woessner, Warren D
(B) REGISTRATION NUMBER: 30,440
(C) REFERENCE/DOCKET NUMBER: 600.370WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 612-359-3260
(B) TELEFAX: 612-359-3263
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 258 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(xii) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTTCCCTGCAA ACATTGGTGGA CCCTCTCCCT ATTCACCAACAC CTCTGGACCCTTCCAGACAGA
CCTGTGCTTT ACATCCCTGA GAACACATGC GATCCACGTTG CAGCTATCTTG TGCTGTAGAT
GAGACCCCGGG GAAATGGTTT GTTGGGTATG TTTCACAAAG ACGCTTTTGTTT GAGACATTGTT
GAAGGGATGGG CAAACACAGT GTTTACTGCGC AGACCAAGAC TTGGAGGAATTCCTCTGGGCC
GTCATAGCTGTGGAGACA

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide
(v) FRAGMENT TYPE: internal

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

Val Met Lys Met

1

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(xii) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGATATATGC TTTGGTGGGAC TCTGGC

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(xii) SEQUENCE DESCRIPTION: SEQ ID NO:4:
GGTCTTCAAT TGTGCTGTCT GG

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 24 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

CCTTGACGAA CAGACTGGCT GTGC

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 23 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

CACAGCCAGT CTGTTGTCGA AGG

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 20 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

CCTCTACGTA ATGGTGCAGC

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 20 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7470 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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

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| TTCTCATCAT | AGTACATAGT | GGAGCGCTCA | AAAAGAAGA | GAAAACCAAC | TGTGTCACCT | 180 |
| CTGATGCTGA | GAACTGGGCT | GATGCTGACT | TATGATGC | GCACATTTG | TGTACCTGAC | 240 |
| CGTGCTGGCA | AACCATTTG | GCACTCTCAG | TTGCTCAGAT | AGATGGACG | GCACCTGGCA | 300 |
| TCTGAGGACT | AGAAGAAGA | CAGGTATGCT | TATGACATA | ATGAAAAAG | CACACTTAA | 360 |
| CAAATGAGGA | GCCTACCTTC | ATGTCCTCAG | TGTGTTGATA | TTGCTCCAGAT | ATGCATGCT | 420 |
| AAAACACCAA | TTCCATGAGT | ATTAGCTG | ACCAGTGGGA | CAGAATGAG | TGCCACGAG | 480 |
| AGGAGTGGCC | GGAAGCATG | TAATGTTAAC | CTTACATGCT | AGAAGGATG | AGAATCCCG | 540 |
| GCTTGAAGGA | AGTTCTGGTT | CGCTCAGTAG | AAgatatgA | CTAGACAG | TGCTGACCA | 600 |
| CCCAGAACG | TCTCGGTT | AGCTAACAAT | CCATCTACGG | ATCAGTATCC | CCGCTAAG | 660 |
| GGGATTTGCG | TTTTGACACT | TTATGACCTG | GTTGGAGGCT | CTGAGGCT | CGGCCTGG | 720 |
| AACATGGTG | AACACACTG | ATTGCTTGC | GACATCGTG | AGCCTGACG | CGACCTGCT | 780 |
| AGATTCAGA | CTAGTTGTC | GCCCGTGCA | GCATCTAGC | TGCGTTTCGC | ATGACCCAG | 840 |
| CTATCCTGA | AGAAGAAGA | AGGACACG | TCTCCGTA | TTAGTTGAA | AGGACGCG | 900 |
| TGTGGGCTG | CAGAATGAG | TTATGACCTG | ATGAGCTG | AGATGGATG | AGAATCCCG | 960 |
| AGTTCTGGTT | CTTGCTGTCA | ATGTTGCA | CTTGCTGCT | AGATGGAGG | GGTTGTTAAA | 1020 |
| CAAATGAGGA | AGGAGTGGCC | GCCTCAGTAG | AAgatatgA | CTAGACAG | TGCTGACCA | 1080 |
| AGAATGACG | AGTTCTGGTT | CTTGCTGTCA | ATGTTGCA | CTTGCTGCT | AGATGGAGG | 1140 |
| GGCCTCCTG | TTTTGACACT | TTATGACCTG | GTTGGAGGCT | CTGAGGCT | CGGCCTGG | 1200 |
| TGCAACAGC | AACACACTG | ATTGCTTGC | GACATCGTG | AGCCTGACG | CGACCTGCT | 1260 |
| AGCTGCTAG | TTTTGACACT | TTATGACCTG | GTTGGAGGCT | CTGAGGCT | CGGCCTGG | 1320 |
| GCTGCTAG | TTTTGACACT | TTATGACCTG | GTTGGAGGCT | CTGAGGCT | CGGCCTGG | 1380 |
| AACATGGTG | AGAAGAAGA | AGGACACG | TCTCCGTA | TTAGTTGAA | AGGACGCG | 1440 |
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| TGCAACAGC | AACACACTG | ATTGCTTGC | GACATCGTG | AGCCTGACG | CGACCTGCT | 1560 |
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| AGAATGACG | AGTTCTGGTT | CTTGCTGTCA | ATGTTGCA | CTTGCTGCT | AGATGGAGG | 1740 |
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| AGAATGACG | AGTTCTGGTT | CTTGCTGTCA | ATGTTGCA | CTTGCTGCT | AGATGGAGG | 1860 |
| AGAATGACG | AGTTCTGGTT | CTTGCTGTCA | ATGTTGCA | CTTGCTGCT | AGATGGAGG | 1920 |
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| TCTTACAGCT | GGATGGAAAT | TATGCTGCTA | TCAAGAAGA | GTTCAAGCAA | GCATTTCAAG | 2820 |
| AGTGGATTGA | AGGGAAATTA | CAAGGAAATC | AAGTGAATTT | TTAACATGAG | AAAAAGGCA | 2880 |
| GACTTCCAT | CAGAATGTGA | AAGCTGCTGGA | ATCTTTTATA | ATGTTGCAAG | 2940 |
| AAGGAAGAGG |CTTCCATAGGA |GAGTCTGTTT | GAGCCCTTTA | TGAACCTATC | GAGTCTATAT | 3000 |
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| ACAGTGGAAG | AACTTTTATAG | TGAAGGCTAT | CAGGCTGACG | TGAAGGAAC | 3120 |
| CAACAGCTTA | AAGACCTGCTA | GAAGGTGTTA | GACATTTGGA | TGTCTCAAGA | 3180 |
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| CTAAAGAGCA | GTGAGCTTCT | TGAACAAACC | AAAACTAATG | AACAGCGTAC | 3360 |
| AGAAGGCTTCTT | GCCAGTGCCG | GACATATGAA | GAGGAAAGTA | GTAATTAGAGA | 3420 |
| GATTTGCTCTT | CGGCCCATTT | ACCTGTTGAA | GATGCTGCTA | TTTCTTGGTT | 3480 |
| GATGCAAGCTA | TTCCAGCAGA | AGATGATGAG | ACACTATAT | TGGATGCTGA | 3540 |
| CTTGGAAGAT | ATGAGTCCCTA | AATGAAAATT | AGGAGAACTG | GTGACATCTA | 3660 |
| TTATATGCAA | GACAGTCTGA | GCCATTTTGA | GAGCAGTGG | TCAATTTTG | 3660 |
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| ATGGAAAGGC | TTGAGGAAGAT | ACTGAGAGAT | ACTAGGGTG | CAAAGGATCT | 3900 |
| GGTGGTGAAG | TTATAAGTCTG | TCAAGGATGT | GAGAATGAG | CTGGCTGCC | 3960 |
| ACATCTTCTT | GGGGCTGGA | GAAGGTTGTT | CTAACGTTA | AAGCATACAG | 4020 |
| GAGGCCCCCA | TCTCCTACCTT | TCCAGATGTT | TTAATGGAAT | AGAGCAATG | 4080 |
| ATGGAAGATT | TCTCCTGCCG | TGGACCCGCA | TCCGATATCT | ACAACATGAA | 4140 |
| AACCACCAAA | CTTCGCTGAT | AGTCCAGGTC | CAGGCTAT | AAATGCTCT | 4200 |
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| CATGATCGT | GGTGATGCCTA | AACATGGCAA | TTTCAACAT | AGCTGGAGAT | 4620 |
| AGTGGTACCT | GAGAGTGGT | AACTAACTA | TATTTCTGTG | ACACGTCGC | 4620 |
| TACCAGAGG | TGGGAGGAA | AGAACTCAG | AGTATAGTG | ACCATCGGC | 4740 |
| GCGGATGAC | TGGTGATGCT | TGGCTAGAAT | AATCTTCTAT | AATCTTGGT | 4800 |
| CTAAAGGCTG | CATTGCTCT | GAAACAAGC | ACAACAATT | CTTAGTATG | 4860 |
| TTTGAGACTG | CAGCAGAGT | TTTTGCGGCA | TAAATGCGT | TGAAGCCAT | 4920 |
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| TGGGGATCCTC | TTATAAACCT | GAGGAAAGAA | CTTGCTGCGG | TCAAGGCAAT | 5040 |
| GGCTGATGAT | GGATGGAGATC | AAGCAGGAT | GGAATTAAT | TATTTTGGA | 5100 |
| GCAAACTGTA | TCAATTCAG | GAGTGGATCA | TTTGCGCAGA | GGAGAAGTGC | 5160 |
| ACTGCTACAA | CTCTGCTTTG | GGAAGGAAAA | CTCTCTTCT | TATATCTGGA | 5220 |
| GGTTGAGACG | TGGGAGATG | TATGAGATT | AAATCTTGCT | TCCTGCTG | 5280 |
| GAAGGCTATC | CTCAAGGAGG | GCTTCGTGAT | ACTCTATGCA | TCTTAGGAGA | 5340 |
| ATTGACCTTG | TTGTGGCGCA | GAAGAGGTTG | CTGTTGATGC | AAGAATAC | 5400 |
| GCATCTGCCA | TGCCATATTC | TGGGATGAT | GAAGAGGAT | TATTTGCTG | 5460 |
| GGGCCGACTG | TAGAATAGGG | AGCTTATTTT | GCTGCTGTT | GATACAGGTT | 5520 |
| CTTGAGACCG | CATATTATT | ACAAGGTTTT | TCGCTGCCGA | ACAAGCGTT | 5580 |
| GTGTTGACGT | CCCACAGCAA | GCTGCTGTT | CAACTAGCA | TGGCGCAGA | 5640 |
| CACTCTGAGT | TCCAGGATG | CTTGAGTTG | TTCCTACTACA | TAATGGAAGG | 5700 |
| STTGCTGCAA | ACATGCTGGG | ACCTCTCTCTT | ATACCACAC | CTTGCGACC | 5760 |
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CACCACATTG CAATGGACGT CATCAGACAG TGATACCTTG CTTCTGAGCC CACAAACAGGA 6780
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AAGGGGACTA TTCACAAGCT TAGGGCTCAA AAAGTTGCTC ATTCGCTCTG TGATCTGTGC 6900
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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2325 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

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20      25      30
Ser Leu Ser Arg Pro Leu Asn Arg Arg Lys Ser His Thr Arg Ser Leu
35      40      45
Arg Asp Gly Gly Asp Gly Val Ser Asp Ala Lys His Ser Gln Ser
50      55      60
Val Arg Gln Leu Ala Gly Ile Ile Asp Leu Pro Ser Glu Ala Pro
65      70      75      80
Ser Glu Val Asp Ile Ser His Gly Ser Glu Asp Pro Arg Gly Pro Thr
85      90      95
Asp Ser Tyr Gln Met Asn Gly Ile Ile Asn Glu Thr His Asn Gly Arg
Lys Val Lys Glu Ile Ser Phe Lys Ser Lys Pro Asn Val Trp Ala Tyr 545 550 555 560
Phe Ser Val Lys Ser Gly Gly Gly Ile His Glu Phe Ala Asp Ser Gln 565 570 575
Phe Gly His Ala Phe Ala Tyr Gly Leu Ser Arg Pro Ala Ala Ile Thr 580 585 590
Asn Met Ser Leu Ala Leu Lys Glu Ile Gln Ile Arg Gly Glu Ile His 595 600 605
Ser Asn Val Asp Tyr Thr Val Asp Leu Asn Ala Ser Asp Phe Arg 610 615 620
Glu Asn Lys Ile His Thr Gly Trp Leu Asp Thr Arg Ile Ala Met Arg 625 630 635 640
Val Gln Ala Glu Arg Pro Pro Trp Tyr Ile Ser Val Val Gly Gly Ala 645 650 655
Leu Tyr Lys Thr Val Thr Thr Asn Ala Ala Thr Val Ser Glu Tyr Val 660 665 670
Ser Tyr Leu Thr Lys Gly His Ile Pro Pro Lys His Ile Ser Leu Val 675 680 685
Asn Ser Thr Val Asn Leu Asn Ile Glu Gly Ser Lys Tyr Thr Ile Glu 690 695 700
Thr Val Arg Thr Gly His Gly Ser Tyr Arg Leu Arg Met Asn Asp Ser 705 710 715 720
Thr Val Glu Ala Asn Val Gln Ser Leu Cys Asp Gly Gly Leu Leu Met 725 730 735
Gln Leu Asp Gly Asn Ser His Val Ile Tyr Ala Glu Glu Glu Ala Gly 740 745 750
Gly Thr Arg Leu Gln Ile Asp Gly Lys Thr Cys Leu Leu Glu Asn Asp 755 760 765
His Asp Pro Ser Lys Leu Leu Ala Glu Thr Pro Cys Lys Leu Leu Arg 770 775 780
Phe Leu Val Ala Asp Gly Ala His Val Asp Ala Asp Val Pro Tyr Ala 785 790 795 800
Glu Val Glu Val Met Lys Met Cys Met Pro Leu Leu Ser Pro Ala Ser 805 810 815
Gly Val Ile His Cys Met Ser Glu Gly Gln Ala Leu Glu Ala Gly 820 825 830
Asp Leu Ala Asp Leu Leu Asp Asp Asp Ser Ala Val Lys Arg 835 840 845
Ala Glu Pro Phe Asp Gly Ile Phe Pro Gln Met Glu Leu Pro Val Ala 850 855 860
Val Ser Ser Gln Val His Lys Arg Tyr Ala Ala Ser Leu Asn Ala Ala 865 870 875 880
Arg Met Val Leu Ala Gly Tyr Glu His Asn Ile Asn Glu Val Val Gln 885 890 895
Asp Leu Val Cys Cys Leu Asp Asn Pro Glu Leu Pro Phe Leu Gln Trp 900 905 910
Asp Glu Leu Met Ser Val Leu Ala Thr Arg Leu Pro Arg Asn Leu Lys 915 920 925
Ser Glu Leu Glu Asp Lys Tyr Lys Glu Tyr Lys Leu Asn Phe Tyr His 930 935 940
Gly Lys Asn Glu Asp Phe Pro Ser Lys Leu Arg Asp Ile Ile Glu 945 950 955 960
Glu Asn Leu Ser Tyr Gly Ser Glu Lys Glu Lys Ala Thr Asn Glu Arg 965 970 975
Leu Val Glu Pro Leu Met Asn Leu Leu Lys Ser Tyr Glu Gly Gly Arg
Glu Ser His Ala His Phe Val Val Ser Leu Phe Glu Glu Tyr Leu
980
985
990
Thr Val Glu Glu Leu Phe Ser Asp Gly Ile Gln Ser Asp Val Ile Glu
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1000
1005
1010
1015
1020
Thr Leu Arg His Glu His Ser Lys Asp Leu Gln Lys Val Val Asp Ile
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Val Leu Ser His Gln Gly Val Arg Asn Lys Ala Lys Leu Val Thr Ala
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Leu Met Glu Lys Leu Val Tyr Pro Asn Pro Gly Gly Tyr Arg Asp Leu
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Ser Thr Ile Val Asp Val Gly Gln Asp Glu Ala Thr Ala Cys Ser Leu
Leu Ser Lys Met Ala Leu Lys Ile His Glu Leu Val Gly Ala Arg Met
His His Leu Ser Val Cys Gln Trp Glu Val Lys Leu Lys Ala Arg Cys
Asp Gly Pro Ala Ser Gly Thr Trp Arg Val Val Thr Thr Asn Val Thr
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Gly His Thr Cys Thr Ile Asp Ile Tyr Arg Glu Val Gly Glu Ile Glu
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Phe Pro Leu Ala Phe Glu Thr Ala Leu Gln Lys Ser Trp Gln Ser Asn
Gly Ser Thr Val Ser Glu Gly Asn Glu Asn Ser Lys Ser Tyr Val Lys
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Ala Thr Glu Leu Val Phe Ala Glu Lys His Gly Ser Trp Gly Thr Pro
Ile Ile Pro Met Glu Arg Pro Ala Gly Leu Asn Asp Ile Gly Met Val
1600
Ala Thr Glu Leu Val Phe Ala Glu Lys His Gly Ser Trp Gly Thr Pro
Ile Ile Pro Met Glu Arg Pro Ala Gly Leu Asn Asp Ile Gly Met Val
1650
Ile Ile Val Val Ala Asn Asp Ile Thr Phe Arg Ala Gly Ser Phe Gly
Pro Arg Glu Asp Ala Phe Glu Thr Val Thr Asn Leu Ala Cys Glu
Arg Lys Leu Pro Leu Ile Tyr Leu Ala Ala Asn Ser Gly Ala Arg Ile
Gly Ile Ala Asp Glu Val Lys Ser Cys Phe Arg Val Gly Trp Ser Asp
Gly Ile Ala Asp Glu Val Lys Ser Cys Phe Arg Val Gly Trp Ser Asp
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Asp Tyr Ala Arg Ile Ser Ser Ser Val Ile Ala His Lys Leu Glu Leu
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Asp Ser Gly Glu Ile Arg Trp Ile Ile Asp Ser Val Val Gly Lys Glu
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Asp Gly Leu Gly Val Glu Asn Ile His Gly Ser Ala Ala Ile Ala Ser
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Ala Tyr Ser Arg Ala Tyr Glu Glu Thr Phe Thr Leu Thr Phe Val Thr
1950
Gly Gly Thr Val Gly Ile Gly Ala Tyr Leu Ala Arg Leu Gly Ile Arg
Gly Gly Thr Val Gly Ile Gly Ala Tyr Leu Ala Arg Leu Gly Ile Arg
2000
Cys Ile Gln Arg Leu Asp Gin Pro Ile Ile Leu Thr Gly Phe Ser Ala
Cys Ile Gln Arg Leu Asp Gin Pro Ile Ile Leu Thr Gly Phe Ser Ala
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Leu Asn Lys Leu Leu Gly Arg Glu Val Tyr Ser His Met Gin Leu
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2310
2315
2320
Lys Lys Val Leu Asp
2325

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3544 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

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

AAGCTTGGTA TGAGTTCGTC AGCCCGAACG CGGGGTTTGG CATGCGCCCG ACTGGGAARCS 60
GAATTCCTGG AGGGGCGTAC RGCATAGGCA TCCCGGATCAG TCTGGGTGTT AGCTGAAAGG 120
CTCTGGTTGCTG GGGGGCAGCC TGTGGCCTGCT TGCCTGGTTT GGAGATGCTA TCTGGGTGTT 180
CAGTGGCGCAG GCCTGGCTGC AGGCTATATC TGCCTGGGTC TGGGGCTGAC 240
TGTGGGTGTTT GAGCTGCATC CTCTGGTTG CTGAGCTTGC TGGGGCTGAC 300
GCTGTTGGTTA TCTGCTGCTG TGGGGCTGAC TGGGGCTGAC 360
AGCAGACCTCT TGGGCTGCTG TGGGGCTGAC TGGGGCTGAC 420
TGCTGGGTGTT TGGGGCTGAC TGGGGCTGAC 480
TGGGGCTGAC TGGGGCTGAC 540
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TGGGGCTGAC TGGGGCTGAC 900
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TTTAGAGCGT GGGATATGACT GCTGCTGCGT TATATAAATGAGGTG TACGCTGAGATG 2400
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TCTTGTGATTT CTGAGGCTCCT CAAAGAGGAA TTAAAACTTTTCTAGACAGGA 2520
TAAATTGAGAA ATATAATACAG GACCCAGGGT TATGGTTGAC TTTGATATTTT TTGATACCTC 2580
ATGCACTGTT GTTCTCTCTT GCTACAGATC TATATACATTTT TTCTCTCTGT TTCTCTCTGT 2640
GATCTGAGGAA GCTTCTGCTGTT GCAAGAAATG CAGTTCTCTT TACTGTTGAGGAA 2700
CACATATGCT TCGATGTGCTT CAAATAAGC TATCTGTGTATT ATGCTGAGGAA 2760
GCCAAATACCA AAATCAGATG TATATAGTGG GCAAAATAGG AATGGCAAGCA GCAAATATTTA 2820
TGAGGATGCC TGGCAGATGC TCTCTTTTTC AAAAAATGATGG AAATGAGGAA 2880
TAGCTATGGA TACCATGCGGA GACATGGGAA TATACAGGAA ATGCTGAGGAA 2940
AATTCTGAGT GCTTCTGCTGTT TATGACTGAC TATCTGTGTATT ATGCTGAGGAA 2960
AGTTGAGGTTT AGCTTCTGCTGTT TATGACTGAC TATCTGTGTATT ATGCTGAGGAA 2960
TTGTTATGTT ATGACTGAC TATCTGTGTATT ATGCTGAGGAA 2960
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(GCTTCTGCTGTT TACAGATGCT TATCTGTGTATT ATGCTGAGGAA 3120)

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2166 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION:

GAATTCCCTG TGGAACTGTAC GCCAACAGGGT TACTGCGGTG GCTGAGATGT 60
CTCGGCTCTAC GCAATTGTTT TGGCAGACGT TGGAGGCTTAA ATGTAAGGTTG TCTCTTGCTT 120
CAGCTGCGTTA GGGATGGGTT CCGCTCTGCT CTGAGGCTTGA TCTCTTGCTT 180
GGTGGTATTAT CAGGGATGCC CCGCTCTGCT CTGAGGCTTGA TCTCTTGCTT 240
TGGTAGGGTT CAGGAGCTTT TATGAGGATA ATGCTGAGGAA TACAGATGCT TATCTGTGTATT 300
GTTTACCCCT CTTCTCTTCC TTTGCTTTGTT GCTACAGAGA TCTCTTGCTT 360
GCTTAGCTTTT GTTCTGCTTTT CAGTTCTCTC ATGTTCTGCTT 420
TGGTCTGATTT CTGAGGCTTGA TCTCTTGCTT 480
TGACTTCTCTT TTATGATCAA TATGATATATA TCTCTTGCTT 540
AACAAATTTT TATCCAGTCAA CAGAGGTTTT ATGCTGAGGAA 600
TTTTTTTTGCA AGAAAAAACCA AACCTTCTCA AAAAGGATGTT TATATGATTAA 660
TTGATTAATTT TTCTTCTCAGA AAAAAAAAAC AAAAAAAAAC TATCTGTGTATT 720
CAGGAAATT ATGTTCTGATTT TATGCTCTTCC AAGCCCTCTA TATGCTGAGGAA 780
TAAAAAACCC AAAAGGAGTTA TATGATAGAT GAGAAAAAAA ATGCTGAGGAA 840
CTGTTGTTGT ATCTTTATATT TTCCCTTGTCA AAAAAAAATTG GCTGAGGAA 900
TTGCTTCTTCT GCTTCTTCTT TATGATAGAT GAGAAAAADD TATGAGGAGAAT 960
CGTATTACAA AAAAAAATG TATGATAGAT GAGAAAAAAD TATGAGGAGAAT 1020
AGGTCTTGCA TATGCTGAGGAA TATGAGGAGAAT TATGAGGAGAAT 1080
AGTTCTGCTT GCGGAGGTGA TATGAGGAGAAT TATGAGGAGAAT 1140
AGTTCTGCTT GCGGAGGTGA TATGAGGAGAAT TATGAGGAGAAT 1200
(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 484 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(x) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAATTCTGT GGGTGTGATA CTCTGTTGAGA CACAGACC ATGCGAGATC CTGTAATCT  60
TCAAAATCTT AAGCCGACAA ACTTGAATCA TGTGATGCA AATGGGAATG CAGATGTTCA  120
GAGAAGGATT CTCAATAACG ACAAAATACCA AGAGATATGA AGCGAGAGG TGCCCGGGTG  180
GTTATCCCGT GGTGCGGCA AGACCAAGC TGTGCTATCT GACGGTGACG CGTCCCACAC  240
GAGGAGGATT CCAATCCGAC CCTCTTAAAG GTGGCAACGG CCTCCCTGG TACCGGGTTG  300
TTGCTTGTGT TTTTTGCTAC CCACTGCCG GGAACTGGCG CAGTGGCGG GCTGCAGGCC  360
TTCAAGAAAC TTGCAGAACG TTTCTGGGAG CAGGGAAAGG ATGACACACT CAGACAAGC  420
ACAAGATACG CAGTCAGATC GCCACAATAA ACCAAAGACT TGAGCTCAAG TGACTAGCAC  480
ACTT  484

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 531 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(x) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGGAAGGTGT GTATGGGCCG TAGCTTTATY TRGGAGATGA GGGATTTAATTACATTG  60
TGACTGGCGG GACTGAGAGA TAGAAATTAT CTGGTACTGAT GTATGATACGG TGATACAGS  120
KTTYGACGAG CATTATTTAT AACACAGTTT TCTGGCCCTGA ACAAATCCTT GGGCGGGAAG  180
TGTCAGCTTC
CCCATGCGAC
CTTGTGTCGG
CTAAGACATC
GGCGAACCAG
GTTGTGTCGC
240
ACCTCAGTCG
TCCAGATGAC
CTTGAAGGTG
TTCCCAATAT
ATGGAAGTGG
TCACGCTATG
300
TTCCTGCAAA
CTTGGTGGGA
CCTCTTCCTA
TTACAAACAA
TCTGACACCT
CCAGACAGAC
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CTGTGTGCTT
CATCCCTGAG
AACACATCGG
ATCCAGCTGG
AGCTATCTGT
GTTGTAAGTG
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ACGACCAAGG
GAAAATTGTTG
GGTGGTATGT
TTCGAAACAAG
CAGCTTGTGG
GAGCAGATTT
480
AAGGATCGGC
AAAACAGTGG
GTTACCGGCA
GACGAAAGCT
TGGAGGAATT
CT
531

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 882 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTCCCAATAT
TGTCATGAGG
CTTGCATCCC
AGGTTAGTTT
TTTTTCTTCT
CTGAATTTTA
60
TATCTTCCA
CTTTTGCACCT
TTAGTTATCC
TTGTATTTTC
TTGGAAGCTC
ATCTGATGCA
120
TTATTGCCAA
ATGCACTATA
GGTCATCATC
TTGGSAKAGW
YAASTATKCT
MTCTTAATTG
180
ATGGKWACTM
TTGAMWATG
SRAGSTXRGA
GCARKTRRYK
WAYASTTST
TAAATAAA
240
ACATGCATT
CTTAGAGTTG
GACTAAGCTT
TCTCTAGATG
GAAGTGCCCAT
GTITTTTACAT
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GCCATTGTG
GTCATTTTTAC
AGTGGGTAATC
ATGGAAAGGT
TTGCTAATAG
GCTGGGAGA
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AACACACAT
CTTGTGTTTTC
AACACCTTGT
GGAGAAGATG
TTTTACCTTT
TTTCTCTAAA
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TACTAAATTG
TACTAAATTT
ACAATATCTT
CCTAAATTCT
GTAACATCTGC
480
TGTTTCCA
CGCCCAAAC
ATATTTCTCA
ACTCTTACACT
TTATATTTTT
AGATGGAGAC
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CTGGAATTTG
GCTCTGTTAT
CTGTAGCTATC
GCAATTATATTG
TGCTCTTAAA
TCYTATCTCT
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TTATTGAGAR
TCSGCATCTT
GAAGTTCAGT
TCCTTTGTTGA
TCAATATGCT
AAPGACACAC
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CACATCACAG
TCTGGTATGC
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GCGCAACACA
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ATGATTTTGG
TTTACCAAT
TATTCTCACA
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ATTACACATT
TGCTACAAAC
GATTATGGAA
GAAGTTCAGG
TTACTGTCG
840
TNCCTCGTGA
GACAGTTTAA
GCACTTGGAC
AGCAGCAAGG
AG
882

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 867 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTGCACTCTGC
AGGTCACCGG
ATCTTAGGGG
GCCCAACAGAT
CTTTATCAA
TGAATGGCGAT
60
TATCAATAAGA
ACAAATAATTG
GAAGCACTGC
CTCAGTGTCC
AAGGTTGGTG
AAATTCTGTC
120
GGCACTAGTG
GGCAAACAC
CAATTCAGAG
TATAATTGG
GCCAACAAAG
GAATGTCAGC
180
AGGAARATT
ATGGAGGGTT
TCCGGACATG
GCTATAAGAT
ACCTTTGGAG
CTCGAGAGGC
240
AATTCACACT
ATAGCTATGG
CAACTCCCGA
GAGCTAGGAG
ATAAATAGCC
AACCRACTTAG
300
AATTGGCAGC
CAATCTTAGA
ARGTGGCTGG
TGGAAACAAC
AATTAAACT
ACGCAAAATG
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TCAACTCATA
GTGGAGGTTA
GCTTGGTCAA
TCTGTATTTG
TACTAGCTTG
CTCGTGTTTC
420
CTTTAATTGT
TGTAAATAGA
TTGACATATT
TAAGTAGAGA
AATTTATATT
TCTTCTCTGC
480
(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 723 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GAATAAATCTG CTCGTGAGCTC CAGTTGCTCT TGGGATGGGC ATACCTCTTT GGCAGATTCC
AGTTAAATAC CAAATTCACA TCTTTATTTAG TCTCATTCTG TTTATTTCTA TAAATTTTCA
CTATGATAG TCTCGGCTAT GAGGGATTTG GATATATATG GACGGTGGG TTTTTTTTTA
GGAAAAACAG GCCGGCTTTG TAAATTACCTG ATGGTTATTGG TTTTTATTCT TAATTTTTTA
AGGGCAGATT TGTTAGCAGTT AGAATTACTA GAGAGAGACC AGATAGATGCT TCAAGACC
CTGTGTGGAA AGTTGAGAGAT GATTTTGTAG AAGTTAGAT GAGGTTAC TCCAGCAAG
TTAATAGCT ACCGCTAGTT TTTTATTTTA TTTTTATTCT TCAAGGACAT AAATTTAAA
GGAAGCTAAA AGTTGCTGGC TCTTTTACCT CAGAATTACCA TAAAGGTACC TACGTTATTAG
TCATATTAG GTTACATGG TTTTTTATTG GGTCTCTTCT CTTTTTATTA AACAAGGACA
TGGTTCTTTG TAAATTATAT TGTCTCTCAA TTTTTGAATA TTTGCTTCTCT GGTACAGGAC
ADG

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 231 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AATTCCTGTT GGGTTTATAG CTGTGGAGAC ACAGACCATG ATCGAGCTCA TCCCTGCTGA
TCCAGTCAAA ACACAGATG TTTTTTCTGG GCTGAAAGAC TGGTTTCTCC
AGAAGCTGCA ACCAACAGC TCGAGGAAAA ATTAGAGCTC AACCAGAAAG GATGACTTCT
GTTCACTTTG GTCAACAGT GGAGGTCTCT TCGGGGACAG AGAGATCTCT T

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 207 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

AATTCATGCA TCTTAATAAA CACAGTGGCA CCTTTAAGCA AGTGAACTTC TTGAACAAAC 60
CAAACCTAAGT GAACCTCTGT CCACAGTGGCA AGAGACCTT TCAGATCGG GGAATGCATAA 120
GGGAGAAATG ACTATTAAGG ATAGCAGTGA AGATTTACG TCCTGCACAT TGCCCTGTTGA 180
AGATGCTCTT ATTTCTTGTG TTGATTA 207

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 180 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Genomic DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATAGACCTGTA CGCATACTAC CCTGAGAAACA CATGCGATCC GGCCTGCAGCC ATCCGTTGGNG 60
TAGATGAGAC CCAAGCCAAA TGGAGGCTGT GATATTTTGA CAAAGACAGC TTTGTGGAGA 120
CATTGAAGG ATGGGCAAAA ACAGGTTGTA CTGTTAGAGC AAAAGCTTGA GGAAGGAATT 180

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CCTTTTPATG GCACGTGGCG

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
CATCGTAGCC TATATGAGGA CG
WHAT IS CLAIMED IS:

1. A method of imparting cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance to a corn plant comprising:
   crossing a first corn plant with a second corn plant so as to yield a progeny plant, wherein the first plant is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, wherein the second plant is homozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and wherein the progeny plant is heterozygous for the Acc1 allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and heterozygous for the Acc2 allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

2. A method of imparting cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance to a corn plant comprising:
   (a) self pollinating a corn plant which comprises (i) an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and (ii) an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, so as to yield a progeny plant; and
   (b) identifying a progeny plant that is homozygous for the allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and is homozygous for the allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.
3. A method of preparing a plant which is a double heterozygote for alleles of Acc1 and Acc2 which impart cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, comprising:

crossing a first corn plant with a second corn plant so as to yield a progeny plant, wherein the first plant is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, wherein the second plant is homozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and wherein the progeny plant is heterozygous for the Acc1 allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and heterozygous for the Acc2 allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

4. A method of preparing a plant which is a double homozygote for alleles of Acc1 and Acc2 which impart cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance comprising:

(a) self pollinating a corn plant which comprises (i) an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and (ii) an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, so as to yield a progeny plant; and

(b) identifying a progeny plant that is homozygous for the allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and is homozygous for the allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.
5. The method of claim 2 or 4 wherein an inbred plant is self pollinated in step (a).

6. The method of claim 1 or 3 wherein the first and second plant are substantially isogenic.

7. The method of claim 1 or 3 further comprising crossing the progeny plant to an inbred plant so as to yield a further progeny plant.

8. The method of claim 7 wherein the further progeny plant is crossed to an inbred so as to yield a progeny plant that is substantially isogenic with the inbred plant.

9. The method of claim 2 or 4 wherein the progeny plant is identified by RFLP analysis.

10. The method of claim 2 or 4 wherein the Acc1 alleles of the progeny plant are identified by an amplification reaction employing a polymerase chain reaction.

11. The method of claim 1, 2, 3, or 4 wherein the progeny plant is tolerant to levels of a cyclohexanedione or an aryloxyphenoxypropanoic acid herbicide which inhibit a corresponding susceptible plant.

12. The method of claim 1, 2, 3, or 4 wherein the herbicide is selected from the group consisting of sethoxydim, haloxyfop, and mixtures thereof.

13. The method of claim 1, 2, 3, or 4 wherein the Acc1 allele imparts cyclohexanedione herbicide tolerance.
14. The method of claim 13 wherein the \textit{Acc1} allele is selected from the group consisting of \textit{Acc1-S1}, \textit{Acc1-S2}, \textit{Acc1-S3} and \textit{Acc1-S4}.

15. The method of claim 1, 2, 3, or 4 wherein the \textit{Acc2} allele imparts cyclohexanedione herbicide tolerance.

16. The method of claim 17 wherein the \textit{Acc2} allele is selected from the group consisting of \textit{Acc2-S5} and \textit{Acc2-S6}.

17. The method of claim 1, 2, 3, or 4 comprising obtaining seed from said progeny and obtaining further progeny plants.

18. The method of claim 17 wherein the progeny obtained are crossed back to a corn plant, to obtain further progeny.

19. The method of claim 18 wherein seeds are obtained from further said progeny plants and plants are recovered from said seed.

20. The method of claim 17 wherein said further progeny are crossed back to a corn plant, and progeny are obtained.

21. A progeny plant prepared by the method of claim 1, 2, 3, or 4.

22. The progeny plant of claim 21 which is an inbred plant.

23. A method of obtaining a hybrid herbicide resistant plant which is a double heterozygote for \textit{Acc1} and \textit{Acc2} comprising crossing the progeny plant of claim 22 with a member of another inbred corn line so as to yield a hybrid corn plant.

25. An inbred cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerant *Zea mays* plant, the genome of which is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and is homozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

26. An inbred cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerant *Zea mays* plant, the genome of which is heterozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and is heterozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

27. The plant of claim 25 or 26 wherein the plant is resistant or tolerant to an amount of herbicide that is toxic to a corresponding susceptible *Zea mays* plant.

28. The plant of claim 25 or 26 which is resistant or tolerant to an herbicide is selected from the group consisting of sethoxydim, haloxyfop, and mixtures thereof.

29. The plant of claim 25 or 26 wherein the Acc1 allele is selected from the group consisting of Acc1-S1, Acc1-S2, Acc1-S3 and Acc1-S4.

30. The plant of claim 25 or 26 wherein the Acc2 allele is selected from the group consisting of Acc2-S5 and Acc2-S6.

31. Seed derived from the plant of claim 25 or 26.

32. A progeny plant derived from the plant of claim 25 or 26.
33. A hybrid plant derived from the plant of claim 25 or 26.

34. A seed of the plant of claim 32.

35. A method to prepare an herbicide resistant or tolerant corn plant, comprising: crossing a first corn plant to a second corn plant so as to yield progeny plant, wherein the first corn plant comprises at least one herbicide resistant allele and the second plant comprises at least one herbicide resistant allele which is not allelic to the herbicide resistant allele present in the first plant.

36. A method of imparting cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance to a hybrid corn plant comprising: crossing a first inbred corn plant with a second inbred corn plant so as to yield a progeny hybrid plant, wherein the first plant is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, wherein the second plant is homozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and wherein the progeny plant is heterozygous for the Acc1 allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance and heterozygous for the Acc2 allele which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

37. A method of preparing a hybrid plant which is a double heterozygote for alleles of Acc1 and Acc2 which impart cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, comprising: crossing a first inbred corn plant with a second inbred corn plant so as to yield a progeny hybrid plant, wherein the first plant is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, wherein the second
plant is homozygous for an allele of *Acc2* which imparts
cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance,
and wherein the progeny plant is heterozygous for the *Acc1* allele which
imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide
tolerance and heterozygous for the *Acc2* allele which imparts
cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

38. The method of claim 35, 36 or 37 wherein the progeny plant is tolerant to
levels of a cyclohexanedione or an aryloxyphenoxypropanoic acid
herbicide which inhibit a corresponding susceptible plant.

39. The method of claim 35, 36 or 37 wherein the herbicide is selected from
the group consisting of sethoxydim, haloxyfop, and mixtures thereof.

40. The method of claim 36 or 37 wherein the *Acc1* allele imparts
cyclohexanedione herbicide tolerance.

41. The method of claim 35 wherein the herbicide resistant allele in the first
plant is selected from the group consisting of *Acc1-S1, Acc1-S2, Acc1-S3*
and *Acc1-S4*.

42. The method of claim 35 wherein the herbicide resistant allele in the
second plant is selected from the group consisting of *Acc2-S5* and *Acc2-
S6*.

43. The method of claim 36 or 37 wherein the *Acc1* allele imparts
cyclohexanedione herbicide tolerance.

44. The method of claim 43 wherein the *Acc1* allele is selected from the
group consisting of *Acc1-S1, Acc1-S2, Acc1-S3* and *Acc1-S4*. 
45. The method of claim 36 or 37 wherein the Acc2 allele imparts cyclohexanedione herbicide tolerance.

46. The method of claim 45 wherein the Acc2 allele is selected from the group consisting of Acc2-S5 and Acc2-S6.

47. An hybrid cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerant Zea mays plant, the genome of which is homozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and is homozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

48. An hybrid cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerant Zea mays plant, the genome of which is heterozygous for an allele of Acc1 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance, and is heterozygous for an allele of Acc2 which imparts cyclohexanedione or aryloxyphenoxypropanoic acid herbicide tolerance.

49. The plant of claim 47 or 48 wherein the plant is resistant or tolerant to an amount of herbicide that is toxic to a corresponding susceptible Zea mays plant.

50. The plant of claim 47 or 48 which is resistant or tolerant to an herbicide is selected from the group consisting of sethoxydim, haloxyfop, and mixtures thereof.

51. The plant of claim 47 or 48 wherein the Acc1 allele is selected from the group consisting of Acc1-S1, Acc1-S2, Acc1-S3 and Acc1-S4.
52. The plant of claim 47 or 48 wherein the Acc2 allele is selected from the group consisting of Acc2-S5 and Acc2-S6.

53. A method of imparting tolerance to a corn plant to an agent which inhibits acetyl CoA carboxylase, comprising:
   crossing a first corn plant with a second corn plant so as to yield a progeny plant, wherein the first plant is homozygous for an allele of Acc1 which imparts tolerance to the agent, wherein the second plant is homozygous for an allele of Acc2 which imparts tolerance to the agent, wherein the progeny plant is heterozygous for the Acc1 allele which imparts tolerance to the agent and heterozygous for the Acc2 allele which imparts tolerance to the agent, and wherein the agent is selected from the group consisting of 3-(2,4-dichlorophenyl)-perhydrolindolizine-2,4-dione, 3-isopropyl-6-(N-[2,2-dimethylpropyl]-acetamido-1,3,5-triazine-2,4-(1H,3H)dione, soraphen A, and structural analogs thereof.

54. A method of imparting tolerance to a corn plant to an agent which inhibits acetyl CoA carboxylase comprising:
   (a) self pollinating a corn plant which comprises (i) an allele of Acc1 which imparts tolerance to the agent and (ii) an allele of Acc2 which imparts tolerance to the agent, so as to yield a progeny plant, wherein the agent is selected from the group consisting of 3-(2,4-dichlorophenyl)-perhydrolindolizine-2,4-dione, 3-isopropyl-6-(N-[2,2-dimethylpropyl]-acetamido-1,3,5-triazine-2,4-(1H,3H)dione, soraphen A, and structural analogs thereof; and
   (b) identifying a progeny plant that is homozygous for the allele of Acc1 which imparts tolerance to the agent and is
homozygous for the allele of Acc2 which imparts tolerance to the agent.
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FIG. 1C
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**FIG. 2B**
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FIG. 3A  7/25
SUBSTITUTE SHEET (RULE 28)
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TTCTATGAAA TGAGCATTGG AGGAGGCTAT GACATTTGGA GGAAAACAGC
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FIG. 3B  8/25
SUBSTITUTE SHEET (RULE 28)
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6001 GATTCCCATG AGCGATCTGT CCTCTGTGCT GGACAAGTGT GGTCCCAGA
6051 TTCTGCAACC AAGACGGCTC AGGCATTATT AGACTTCAAC GTGGAAGGAT
6101 TGCCCTGTGT CATCCCTGGT CATTGGAGAG GCTTCTCCTG TGGACAAAGA
6151 GATCTCTTTTG AAGGAAATCT TCAGGCTGGG TCAAACATTG TCGAGAACCCT
6201 TAGGAACATAT AATCAGCCTGT CTTTTGTGTA CATTCCTATG GCTGGAAGGC
6251 TTCTGGGAGG AGCCTGGGTT GTGGTGCGTA GCAAATAAA TCCAGACCAG
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7401 ATGGTTTCTTT TAAGCATAATG TACCCCTACCT CTACGTGAAA TAAAGTTTGT
7451 GAATTACGAT TCGAAAAAAA

FIG. 3E

SUBSTITUTE SHEET (RULE 28)
FIG. 4B
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ACTGGaARcS GAATTCCGgTg AgCCCTGTAc rrCaATGGCA ACCC0AsGGT
TACTggGGTG GCTGAATGGT CTCsGCTTAC GCAATTTGT TGGCAgCwG
CGTGGGCTAA ATGTARgTTG TCTCTTTgTG CACTGCArGA TGGATGGgTA
gCCTCTGGGC CGCCTCTGCT ArTGCTCTArC gTTGCTGAC TGTGGTTTA.
tCAgGGATGc CCATgcCCAT GcTAgATTGA tAgGTgCCAt TCTAAAtgTA
GTTGcGCgGTA AGGTTTATTA AgCTGTgAgTA TCagTAgGTA ACCTCATGAA
tCAgGGTTTA aGCACACcCt TTCTCTTTgTG TgGgGTgCATA AgGAAtGCAC
TTGGCCTCgT TCCCTgATAg TCTTTgCTC TA TgTgTCATTc TACCAAgTgG
GTTAAdgTAA CATgGACACTC TATGATGGTT GGTGGTgTTg CATCCTTTgTg
CTtCCCTGG TtGgTCATAA CCTGCATgTA ACTGATGACC ttCTTTTTATG
TATCATATAg ATTCATACcT TTGTgGTAC ATCTCAATTc TGAAAAACCA
ATGTTTTGCA TTCTTAGGCg TCTGTGCaCA AgGaAAGaAg gGTtTTACCT
GCAAcTTTTT TTTTCgAGAA AAAACAAAAC TTTCTGAAAg GCAGTGATCA
TTTAGATAA AAGAAATTgT ATTTAACCTT CTTGAGAGAA ATATKCCAAr
CAAAACATTc TTCTACTGTC TGACGCAcGA AATTTgATCT TGATCTTACT
TTCAACAAGGC ACATGaAGGC tTAATCATGCg TCgTgATAAAa AAgCcaaaTa
GGgGATtcAt aGAAgGag aAAAGAcCTg TTgCCaTTTG GGGCCCTgTGT
TGTTACTCA tTAcCCcCCC TGCTCAGgTT GaGGTtTTTc TTTgCCaCtGC
CACCCcTTGG CCCCTcCTTA tAcAaACATC TCCATTGAAa aAGATTTTGc
ACTAACATgTG GCCcGATgATG aCaaaaAGG aAAAtAAaC TaAAgCAGCAG
AAAACATAgTA TaATTATAgG TAAAAGgTTTc TGCCAAgGTgG aGATGAGaGA

FIG. 5A
SUBSTITUTE SHEET (RULE 26)
FIG. 5C  16/25

SUBSTITUTE SHEET (RULE 26)
1  GAATTCCTGTA AGCCCTGTAC GGCAATGGCA ACCACAGGTT TACTGCGGGTG
51  GCTGAATGGT CTCGCTTTAC GCAATTGTGT GTGCCAGCTG CGTGGGGCTAA
101  ATGTAGGTTG TCTCTTGTGG CACTGCGAGA TGGATGAGTA GCCTCTGGGC
151  CGCCTCTGCT AGTGTCAGAC GTTGCTGACT GTGTTTTATT CAGGGATGCC
201  ATGcCCATGC TAGATTGATA GGTCATAGGT GCCATTCTAA TGGTAGGTTG
251  CGGTAAAGTT TATTAAGGTT TCTGTAACAGT AGGTAAACCTC ATGAATCAGG
301  GTTTAAGCCC ACCTTCTCCT TTGGTTGGGT GCAAAAGGAAG TGCACCTTGGC
351  TTCGCTCCCT GCTAGCTCCT GTCTCATGTTG CATTCTACCA AGTGTTCCAC
401  TGTAACATTG CACTCCTAGA TGGTTGGTTG TTGGCATCTC TTTGCTTCC
451  CCTGGTTTGC TAATACTCGt ATGTAACtGA TGACcTTCTT TTATGTATCA
501  TATAGATtAc ATcCTTTTGt TGtACATcTC AATTCTGAAA AaCAATGTTT
551  TGCATTCTTA GCGcTCTGTG CACAAGGAAA AGGaGTTTTT ACCTGCAAcT
601  TTTTTTTTTcG AgAAAAAACCA AACCTTTTCTG AAAgGCAGTG ATCATTTAgT
651  ATAAAgAAAA TTTGATTTAC TTTCTtCAGa ArAgAATATt CCAAAACAAC
701  aATTTTTCTTA CAgTCCTGAGC CACGAAATTT GATCTTGATC TTACCtTTCaC
751  AAGCCACATG AAGCTTATTC ATCGCTCTGA TAAAAAACCC AAATAGGTTGA
801  TTTGATAAGA GAGAAAGAAAC ACCTGTTGCC ATTTTGGGAC TTTGTTTGTG
851  ACTCATTATc CCCCCCTGCTC AGGTTGAAGGT TT.CCTTGCC ACTGCCACCC
901  CTTGCGCCCTT TCCTTATACAA CCACTCCCAT TGGAAAAAG TTTGCACTAC
951  ATTTGGGcTT cGTATACAcA AAAAGGaaAAA TaAAAaCTAAA CAGCAGAaAC

FIG. 6A  18/25
SUBSTITUTE SHEET (RULE 26)
2051 CTGGCATTAT CGACCTCCCA AGTGAGGCAC CTTCCGAAGT GGATAATTTCA
2101 CAGTAAGGAAG TACAGTATTT TGCGTACGT TGT'TTGGAA AAAGAAATAT
2151 TCI'CAGCTTA TTTAAT

FIG. 6C
FIG. 7A

FIG. 7B
CTCCCAATAT TGTGATGAGG CTTGACATCC AGGTTAGTTT TTTTCTTTT
CTGAAATTTT TATATCATAC TTTTTCACCT TTAGTTATCC TTGTATTTTT
TGGGAGCTTC ATCTGATGCA TTATTGACAA ATGCACCTAT GGTGATCATA
TTTGAkAgw yAAsATAtkT mTCTTaattg aTGkwACtm TTgAmwATGA
srATgsTkrA GCAkrTrryk wAyAstTTTT TaATAAAAAA AcatGCaTTT
cTAgGAgtTG GACtAGctT TTCTTAGtAT GAAgtGcCAT GttTTAcATg
GTCCATTTGT GTCAATTTAC AGTCTgGTACG ATGGaaAGGt TGTCATAATG
GcTGGAAGAgA AACAACAcAT CTTGtTTTCTC AACAcTTGTg GGAGAAGAtG
TTTTAaCTTT TTTcCTAAAA TTACTTTTTG TACTAAATTG TATAAaTTTT
cCAaAaTATCT cCAATGATTAT TGAACCTCTGC TGTGTCAAAA CAGCCAAAAC
ATGTTTcCAT ACTTTACAACC TTATTATTAT AGATGGAAgC CTGGAATTGT
GCTCTGTATAT CTGTAaTCaT GCATaTATat TGACTTTAAA tCyTatTCTC
TATTGTGAR tCsaGCaTCTT GAAgTTCAGT TGCTTTGTA GCAaATaGTT
AATGTAGCAa GACTTCACAG TCGTGTATTTGC AGTGTGCAAC GGCAGCACCA
GAaGGTCUGC cCTCCACCCA CCCAGCCATA AACACGAAaGT TTATAGAAACC
ATGTATTTTG TTATGCAATA TATTTCTCAA TTGTAGTCCT CTTACaATT
TGCTCACAACA GATTATTTGA GAAGTGTCAG TTACTGTtTGC TnCCTCGTGa
GACaGTTAAA GCaCTTGAGC AGCaGCAaGG AG

FIG. 7C

SUBSTITUTE SHEET (RULE 20)
FIG. 7D
FIG. 7E
1  AATTCCTGTG GGTGTTATAG CTGTGGAGAC ACAGACCCATG ATGCAGCTCA  
51  TCCCTGCTGA TCCAGGTCAA CTTGATTCCC ATGAGCGATG TGTTCCTCGG  
101  GCTGGACAAG TGTGGTTCAC AGATNCTGCA ACCAAGCAG CTCAGGCATT  
151  ATTAGACTTC AACCGTAGAA GATTTGCTCTT GTTCATCTGT GCTAACTGGA  
201  GAGGCTCTCT TGGGGgACAG AGAGATCTCT T

FIG. 8A

1  AATTCATGCA TCTAATAAAA CACAGTTGGC CCTAAAGCA AGTGAACCTTC  
51  TTTAAACAAAC CAAAATCAAGT GAACTCTGTG CCAAGCTTGTC AACGAAGCCTT  
101  TCAGATCTGG GGAATGCATAA GGGAGAAATG ACTATTAAGG ATAGCAATGGA  
151  AGATTAGTCT CTTGNCCTCC GTGCGTTGGA AGATGCTCTT ATTTCTTGT  
201  TTGATTA

FIG. 8B

1  ATAGACCTGT CGCATACTC CCTGAGAACA CATGCAGATCC GCCTGCGAGCC  
51  ATCCGTTGGnG TAGATGACAG CCAAGGGAAA TGGTTGGTTC GTATGTTTGA  
101  CAAAGACAGC TTTGTGGGAA CATTGGAAGG ATGGGCAAAA ACAGTGGTTA  
151  CTGGTAGAGC AAAGCTTGGG GGAAGGAATT

FIG. 8C
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6  C12N15/82  //A01H1/00, A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6  C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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Date of the actual completion of the international search

29 December 1997

Date of mailing of the international search report

14.01.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel: (+31-70) 340-2040, Tx: 31 651 eipo nl, Fax: (+31-70) 340-3016

Authorized officer

De Kok, A

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