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ABSTRACT OF THE DISCLOSURE

The invention provides nucleic acids encoding mutants of the acetohydroxyacid synthase (AHAS) large subunit comprising at least two mutations, for example double and triple mutants, which are useful for producing transgenic or non-transgenic plants with improved levels of tolerance to AHAS-inhibiting herbicides. The invention also provides expression vectors, cells, plants comprising the polynucleotides encoding the AHAS large subunit double and triple mutants, plants comprising two or more AHAS large subunit single mutant polypeptides, and methods for making and using the same.

AHAS MUTANTS

FIELD OF THE INVENTION

This invention relates generally to compositions and methods for increasing tolerance of plants to acetohydroxyacid synthase-inhibiting herbicides.

BACKGROUND OF THE INVENTION

Acetohydroxyacid synthase (AHAS; EC 4.1.3.18, also known as acetolactate synthase or ALS), is the first enzyme that catalyzes the biochemical synthesis of the branched chain amino acids valine, leucine and isoleucine (Singh (1999) "Biosynthesis of valine, leucine and isoleucine," in *Plant Amino Acids*, Singh, B.K., ed., Marcel Dekker Inc. New York, New York, pp. 227-247). AHAS is the site of action of four structurally diverse herbicide families including the sulfonylureas (Tan *et al.* (2005) *Pest Manag. Sci.* 61:246-57; Mallory-Smith and Retzinger (2003) *Weed Technology* 17:620–626; LaRossa and Falco (1984) *Trends Biotechnol.* 2:158-161), the imidazolinones (Shaner *et al.* (1984) *Plant Physiol.* 76:545-546), the triazolopyrimidines (Subramanian and Gerwick (1989) "Inhibition of acetolactate synthase by triazolopyrimidines," in *Biocatalysis in Agricultural Biotechnology*, Whitaker, J.R. and Sonnet, P.E.. eds., ACS Symposium Series, American Chemical Society, Washington, D.C., pp. 277-288), and the pyrimidinyloxybenzoates (Subramanian *et al.* (1990) *Plant Physiol.* 94: 239-244). Imidazolinone and sulfonylurea herbicides are widely used in modern agriculture due to their effectiveness at very low application rates and relative non-toxicity in animals. By inhibiting AHAS activity, these families of herbicides prevent further growth and development of susceptible plants including many weed species. Several examples of commercially available imidazolinone herbicides are PURSUIT® (imazethapyr), SCEPTER® (imazaquin) and ARSENAL® (imazapyr). Examples of sulfonylurea herbicides are chlorsulfuron, metsulfuron methyl, sulfometuron methyl, chlorimuron ethyl, thifensulfuron methyl, tribenuron methyl, bensulfuron methyl, nicosulfuron, ethametsulfuron methyl, rimsulfuron, triflusulfuron methyl, triasulfuron, primisulfuron methyl, cinosulfuron, amidosulfiuon, fluzasulfuron, imazosulfuron, pyrazosulfuron ethyl and halosulfuron.

Due to their high effectiveness and low-toxicity, imidazolinone herbicides are favored for application by spraying over the top of a wide area of vegetation. The ability to spray a herbicide over the top of a wide range of vegetation decreases the costs associated with plant establishment and maintenance, and decreases the need for site preparation prior to use of such chemicals. Spraying over the top of a desired tolerant species also results in the ability to achieve maximum yield potential of the desired species due to the absence of competitive species. However, the ability to use such spray-over techniques is dependent upon the presence of imidazolinone-resistant species of the desired vegetation in the spray over area.

Among the major agricultural crops, some leguminous species such as soybean are naturally resistant to imidazolinone herbicides due to their ability to rapidly metabolize the herbicide compounds (Shaner and Robinson (1985) *Weed Sci.* 33:469-471). Other crops such as corn (Newhouse *et al.* (1992) *Plant Physiol.* 100:882-886) and rice (Barrett *et al.* (1989) *Crop Safeners for Herbicides*, Academic Press, New York, pp. 195-220) are somewhat susceptible to imidazolinone herbicides. The differential sensitivity to the imidazolinone herbicides is dependent on the chemical nature of the particular herbicide and differential metabolism of the compound from a toxic to a non-toxic form in each plant (Shaner *et al.* (1984) *Plant Physiol.* 76:545-546; Brown *et al.* (1987) *Pestic. Biochem. Physiol.* 27:24-29). Other plant physiological differences such as absorption and translocation also play an important role in sensitivity (Shaner and Robinson (1985) *Weed Sci.* 33:469-471).

Plants resistant to imidazolinones, sulfonylureas, triazolopyrimidines, and pyrimidinyloxybenzoates have been successfully produced using seed, microspore, pollen, and callus mutagenesis in *Zea mays*, *Arabidopsis thaliana*, *Brassica napus* (*i.e.*, canola) *Glycine max*, *Nicotiana tabacum*, sugarbeet (*Beta vulgaris*) and *Oryza sativa* (Sebastian *et al.* (1989) *Crop Sci.* 29:1403-1408; Swanson *et al.* 1989 *Theor. Appl. Genet.* 78:525-530; Newhouse *et al.* (1991) *Theor. Appl. Genet.* 83:65-70; Sathasivan *et al.* (1991) *Plant Physiol.* 97:1044-1050; Mourand *et al.* (1993) *J. Heredity* 84:91-96; Wright and Penner (1998) *Theor. Appl. Genet.* 96:612-620; U.S. Patent No. 5,545,822). In all cases, a single, partially dominant nuclear gene conferred resistance. Four imidazolinone resistant wheat plants were also previously isolated following seed mutagenesis of *Triticum aestivum* L. cv. Fidel (Newhouse *et*

al. (1992) *Plant Physiol.* 100:882-886). Inheritance studies confirmed that a single, partially dominant gene conferred resistance. Based on allelic studies, the authors concluded that the mutations in the four identified lines were located at the same locus. One of the Fidel cultivar resistance genes was designated FS-4 (Newhouse *et al.* (1992) *Plant Physiol.* 100:882-886).

Computer-based modeling of the three dimensional conformation of the AHAS-inhibitor complex predicts several amino acids in the proposed inhibitor binding pocket as sites where induced mutations would likely confer selective resistance to imidazolinones (Ott *et al.* (1996) *J. Mol. Biol.* 263:359-368). Tobacco plants produced with some of these rationally designed mutations in the proposed binding sites of the AHAS enzyme have in fact exhibited specific resistance to a single class of herbicides (Ott *et al.* (1996) *J. Mol. Biol.* 263:359-368).

Plant resistance to imidazolinone herbicides has also been reported in a number of patents. U.S. Patent Nos. 4,761,373, 5,331,107, 5,304,732, 6,211,438, 6,211,439 and 6,222,100 generally describe the use of an altered AHAS gene to elicit herbicide resistance in plants, and specifically discloses certain imidazolinone resistant corn lines. U.S. Patent No. 5,013,659 discloses plants exhibiting herbicide resistance due to mutations in at least one amino acid in one or more conserved regions. The mutations described therein encode either cross-resistance for imidazolinones and sulfonylureas or sulfonylurea-specific resistance, but imidazolinone-specific resistance is not described. U.S. Patent No. 5,731,180 and U.S. Patent No. 5,767,361 discuss an isolated gene having a single amino acid substitution in a wild-type monocot AHAS amino acid sequence that results in imidazolinone-specific resistance. In addition, rice plants that are resistant to herbicides that interfere with AHAS have been developed by mutation breeding and tissue culture selection. *See*, U.S. Patent Nos. 5,545,822, 5,736,629, 5,773,703, 5,773,704, 5,952,553 and 6,274,796.

In plants, as in all other organisms examined, the AHAS enzyme is comprised of two subunits: a large subunit (catalytic role) and a small subunit (regulatory role) (Duggleby and Pang (2000) *J. Biochem. Mol. Biol.* 33:1-36). The AHAS large subunit (also referred to herein as AHASL) may be encoded by a single gene as in the case of *Arabidopsis*, and sugar beet or by multiple gene family members as in maize,

canola, and cotton. Specific, single-nucleotide substitutions in the large subunit confer upon the enzyme a degree of insensitivity to one or more classes of herbicides (Chang and Duggleby (1998) *Biochem J.* 333:765-777).

For example, bread wheat, *Triticum aestivum* L., contains three homoeologous acetohydroxyacid synthase large subunit genes. Each of the genes exhibit significant expression based on herbicide response and biochemical data from mutants in each of the three genes (Ascenzi *et al.* (2003) International Society of Plant Molecular Biologists Congress, Barcelona, Spain, Ref. No. S10-17). The coding sequences of all three genes share extensive homology at the nucleotide level (WO 03/014357). Through sequencing the AHASL genes from several varieties of *Triticum aestivum*, the molecular basis of herbicide tolerance in most IMI-tolerant (imidazolinone-tolerant) lines was found to be the mutation S653(*At*)N, indicating a serine to asparagine substitution at a position equivalent to the serine at amino acid 653 in *Arabidopsis thaliana* (WO 03/014357). This mutation is due to a single nucleotide polymorphism (SNP) in the DNA sequence encoding the AHASL protein.

Multiple AHASL genes are also known to occur in dicotyledonous plants species. Recently, Kolkman *et al.* ((2004) *Theor. Appl. Genet.* 109: 1147–1159) reported the identification, cloning, and sequencing for three AHASL genes (AHASL1, AHASL2, and AHASL3) from herbicide-resistant and wild type genotypes of sunflower (*Helianthus annuus* L.). Kolkman *et al.* reported that the herbicide-resistance was due either to the Pro197Leu (using the *Arabidopsis* AHASL amino acid position nomenclature) substitution or the Ala205Val substitution in the AHASL1 protein and that each of these substitutions provided resistance to both imidazolinone and sulfonylurea herbicides.

A number of single mutations in the AHAS large subunit are known that result in tolerance or resistance to herbicides (Duggleby *et al.* (2000) Journal of Biochem and Mol. Bio. 33:1-36; Jander *et al.* (2003) Plant Physiology 131:139-146). For example, an alanine to valine substitution at position 122 of *Arabidopsis* AHASL (or an alanine to threonine substitution at corresponding position 100 of Cocklebur AHASL) confers resistance to imidazolinone and sulfonylureas. A methionine to glutamic acid or isoleucine substitution at position 124 of *Arabidopsis* AHASL confers resistance to imidazolinones and sulfonylureas. A proline to serine

substitution at position 197 of *Arabidopsis* AHASL (or a proline to alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, or tyrosine substitution at corresponding position 192 of yeast AHASL) confers resistance to imidazolinones, sulfonylureas, and triazolopyrimidine. An arginine to alanine or glutamic acid substitution at position 199 of *Arabidopsis* AHASL confers imidazolinone resistance. An alanine to valine substitution at position 205 of *Arabidopsis* AHASL (or an alanine to cysteine, aspartic acid, glutamic acid, arginine, threonine, tryptophan or tyrosine substitution at corresponding position 200 of yeast AHASL) confers imidazolinones and sulfonylureas resistance. A substitution of almost any amino acid for the tryptophan at position 574 of *Arabidopsis* AHASL, corresponding to position 586 of yeast AHASL, confers resistance to imidazolinones, sulfonylureas, triazolopyrimidine, and pyrimidyl oxybenzoates. A serine to phenylalanine, asparagine, or threonine substitution at position 653 of *Arabidopsis* AHASL confers resistance to imidazolinones and pyrimidyl oxybenzoates.

U.S. Patent Nos. 5,853,973; 5,928,937; and 6,576,455 disclose structure-based modeling methods for making AHAS variants which include amino acid substitutions at specific positions that differ from the positions described above. In Mourad *et al.* (1992) *Planta* 188;491-497, it has shown that mutant lines resistant to sulfonylureas are cross-resistant to triazolopyrimidine, and mutant lines resistant to imidazolinones are cross-resistant to pyrimidyl oxybenzoates.

U.S. Patent No. 5,859,348 discloses a double mutant sugar beet AHAS large subunit having an alanine to threonine substitution at amino acid 113 and a proline to serine substitution at amino acid 188. Sugar beet plants containing the double mutant AHAS protein are described as being both imidazolinone and sulfonylurea resistant.

Mourad *et al.* (1994) *Mol. Gen. Genet.* 242:178-184, discloses an *Arabidopsis* AHAS double mutant designated *csr1-4*. The *csr1-4* mutant AHAS contained a C to T nucleotide substitution at position 589 (corresponding to a proline to serine substitution at amino acid 197 of *Arabidopsis* AHASL) and a G to A nucleotide substitution at position 1958 (corresponding to a serine to threonine substitution at amino acid 653 of *Arabidopsis* AHASL).

Lee *et al.* (1988) EMBO Journal 7:1241-1248, discloses a tobacco AHAS double mutant designated S4-Hra, which includes a Pro-Ala substitution at amino acid 196 (corresponding to the amino acid 197 of *Arabidopsis* AHASL) and a Trp-Leu substitution at amino acid 573 (corresponding to amino acid 574 of *Arabidopsis* AHASL). Transgenic lines carrying the double mutant gene show resistance to sulfonylurea herbicide.

U.S. Patent No. 7,119,256 discloses a double mutant rice AHAS large subunit having a tryptophan to leucine substitution at amino acid 548 and a serine to isoleucine substitution at amino acid 627. Transgenic rice plants expressing a polynucleotide encoding this double mutant AHAS protein were reported to have increased resistance to the pyrimidinyl carboxy herbicide, bispyribac-sodium.

Given their high effectiveness and low-toxicity, imidazolinone herbicides are favored for agricultural use. However, the ability to use imidazolinone herbicides in a particular crop production system depends upon the availability of imidazolinone-resistant varieties of the crop plant of interest. To produce such imidazolinone-resistant varieties, there remains a need for crop plants comprising mutant AHAS polypeptides which confer demonstrated improved tolerance to imidazolinones and/or other AHAS-inhibiting herbicides when compared to crop plants with existing AHAS mutants.

Although some AHAS mutants have been characterized, there remains a need for mutant AHAS polypeptides which confer, when expressed in a crop plant of interest, demonstrated improved herbicide tolerance to one or more classes of AHAS-inhibiting herbicides when compared to existing AHAS mutants in crop plants.

SUMMARY OF THE INVENTION

This invention relates to new mutant AHAS polypeptides that demonstrate tolerance to a herbicide, in particular, an imidazolinone herbicide, or sulfonylurea herbicide, or a mixture thereof. In preferred embodiments, the herbicide tolerance conferred by the mutants of the invention is improved and/or enhanced relative to that obtained using known AHAS mutants. The mutants of the invention comprise at least two amino acid substitutions in the AHAS large subunit polypeptide.

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In one embodiment, the invention provides an isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2.

In another embodiment, the invention provides an isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) amino acid-substituted polypeptide selected from:

a) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glutamic acid, valine, tryptophan, or tyrosine substitution at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

b) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a cysteine, glutamic acid, arginine, threonine, tryptophan, or tyrosine substitution at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

c) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glycine, alanine, valine, isoleucine, methionine, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, or histidine substitution at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

In yet another embodiment, the invention provides an isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) amino acid-substituted polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine substitution at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine substitution at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2.

The invention also relates to AHASL polypeptides comprising the double and triple mutants described above, expression vectors comprising the polynucleotides

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encoding the AHASL double and triple mutants described above, cells comprising the polynucleotides encoding the AHASL double and triple mutants described above, transgenic plants comprising the polynucleotides and polypeptides described above and methods of making and using transgenic plants comprising the polynucleotides encoding the AHASL double and triple mutants described above.

In yet another embodiment, the invention provides an isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) amino acid-substituted polypeptide selected from:

a) a polypeptide having glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine substitution at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

b) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a valine, cysteine, aspartic, acid, glutamic acid, arginine, threonine, tryptophan, or tyrosine substitution at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

c) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and any amino acid substitution at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2,

wherein the isolated polynucleotide is a polynucleotide of a plant selected from *Arabidopsis thaliana*, maize, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, and perennial grass.

The invention further relates to transgenic and non-transgenic plants comprising one or more polynucleotides comprising two or more mutations. In one embodiment, there is provided an isolated plant comprising a polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) polypeptide having a glutamine

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substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2.

According to yet another embodiment, there is provided an isolated plant comprising:

a) a first polynucleotide encoding a first AHASL polypeptide having a first amino acid substitution and a second polynucleotide encoding a second AHASL polypeptide having a second amino acid substitution, or

b) a polynucleotide encoding an AHASL polypeptide having both said first and second amino acid substitutions;

wherein said first and second substitutions are selected from:

i) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a glutamic acid, valine, tryptophan, or tyrosine for the amino acid at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

ii) the first substitution being glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a cysteine, glutamic acid, arginine, threonine, tryptophan, or tyrosine for the amino acid at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a glycine, alanine, valine, isoleucine, methionine, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, or histidine for the amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

According to yet another embodiment, there is provided an isolated plant comprising:

a) a first polynucleotide encoding a first AHASL polypeptide having a first amino acid substitution, and a second polynucleotide encoding a second AHASL polypeptide having second amino acid substitution and a third amino acid substitution;

b) a first polynucleotide encoding a first AHASL polypeptide having the first amino acid substitution and the second amino acid substitution, and a second

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polynucleotide encoding a second AHAASL polypeptide having the third amino acid substitution;

c) a first polynucleotide encoding a first AHASL polypeptide having the first amino acid substitution and the third amino acid substitution, and a second polynucleotide encoding a second AHASL polypeptide having the second amino acid substitution; or

d) a polynucleotide encoding an AHASL polypeptide having the first, second, and third amino acid substitutions;

wherein said first amino acid substitution is a glutamine, for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, said second amino acid substitution is a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine for the amino acid at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, and said third amino acid substitution is an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine for the amino acid at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2.

According to yet another embodiment, there is provided an isolated plant comprising:

a) a first polynucleotide encoding a first AHASL polypeptide having a first amino acid substitution and a second polynucleotide encoding a second AHASL polypeptide having a second amino acid substitution, or

b) a polynucleotide encoding an AHASL polypeptide having both said first and second amino acid substitutions;

wherein said first and second substitutions are selected from:

i) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine for the amino acid at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

ii) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a valine, cysteine, aspartic acid, glutamic acid, arginine, threonine, tryptophan, or tyrosine for the amino acid at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

iii) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being any amino acid for the amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2,

wherein the isolated plant is selected from *Arabidopsis thaliana*, maize, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, and perennial grass.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 sets forth the full length sequence of the *Arabidopsis* AHAS large subunit protein (amino acid sequence SEQ ID NO: 1; nucleic acid sequence SEQ ID NO: 31) with putative translation showing positions of mutations indicated in bold and underlined. DNA numbering is on the left and amino acid numbering on the right.

Figure 2 sets forth the sequence of the maize AHAS large subunit protein (amino acid sequence SEQ ID NO: 2; nucleic acid sequence SEQ ID NO: 32) with amino acids at positions of claimed mutations indicated in bold and underlined. DNA numbering is on the left and amino acid numbering on the right.

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Figure 3 is an alignment of the positions of correspondence of the *Arabidopsis* AHAS large subunit protein (AtAHASL, SEQ ID NO: 1) with the AHAS large subunit protein of a number of species where the double and triple mutations of the invention may be made showing the position of substitutions which correspond to the positions of substitution in SEQ ID NO: 1: *Amaranthus* sp. (AsAHASL SEQ ID NO:9), *Brassica napus* (BnAHASL1A SEQ ID NO:3, BnAHASL1C SEQ ID NO:10, BnAHASL2A SEQ ID NO:11), *Camelina microcarpa* (CmAHASL1 SEQ ID NO:12, CmAHASL2 SEQ ID NO:13), *Solanum tuberosum* (StAHASL1 SEQ ID NO:16, StAHASL2 SEQ ID NO:17), *Oryza sativa* (OsAHASL SEQ ID NO:4), *Lolium multiflorum* (LmAHASL SEQ ID NO:20), *Solanum ptychanthum* (SpAHASL SEQ ID NO:14), *Sorghum bicolor* (SbAHASL SEQ ID NO:15), *Glycine max* (GmAHASL SEQ ID NO:18), *Helianthus annuus* (HaAHASL1 SEQ ID NO:5, HaAHASL2 SEQ ID NO:6, HaAHASL3 SEQ ID NO:7), Triticum aestivum (TaAHASL1A SEQ ID NO:21, TaAHASL1B SEQ ID NO:22, TaAHASL1D SEQ ID NO:23), *Xanthium* sp. (XsAHASL SEQ ID NO:19), *Zea mays* (ZmAHASL1 SEQ ID NO:8, ZmAHASL2 SEQ ID NO:2), *Gossypium hirsutum* (GhAHASA5 SEQ ID NO:24, GhAHASA19 SEQ ID NO:25), and *E.coli* (ilvB SEQ ID NO:26, ilvG SEQ ID NO:27, ilvI SEQ ID NO:28).

Figure 4 is a map of the AE base vector used for construction of *Arabidopsis* AHASL mutants AE2-AE8 in *E. coli*, with relative positions of mutations in *Arabidopsis* AHASL indicated.

Figure 5 is a vector map of plant transformation base vector AP used for construction of vectors AP2-AP5, which differ only by the mutations indicated in Table 1.

Figure 6 is a map of base vector ZE used to study maize AHASL mutants ZE2, ZE5, ZE6, and ZE7 in *E. coli*, with relative positions of mutations indicated.

Figure 7 is a map of plant transformation vector ZP used as a base vector for construction of vectors ZP2-ZP10.

Figure 8 is a table showing the concordant amino acid positions of AHASL genes derived from different species.

Figure 9 is a table showing the protein identity percentage of the AHASL genes derived from different species. The analysis was performed in Vector NTI software suite (gap opening penalty = 10, gap extension penalty = 0.05, gap separation penalty = 8, blosum 62MT2 matrix).

Figure 10 sets forth the results of a vertical plate growth assay of seeds from several lines of *Arabidopsis* plated on media with 37.5 micromolar of imazethapyr. The seeds used were: 1) wild type ecotype Columbia 2; 2) the csr1-2 mutant (homozygous for the AtAHASL S653N mutation in the genomic copy of the AHAS large subunit gene); 3) Columbia 2 transformed with AP1; 4) Columbia 2 transformed with AP7; and 5) Columbia 2 transformed with AP2.

Figure 11 is a vector map of plant transformation base vector AUP used for construction of vectors AUP2 and AUP, which differ only by the mutations indicated in Table 3.

Figure 12 is a vector map of plant transformation vector BAP1, which comprises the coding sequence for an AtAHASL with the S653N mutation.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides polynucleotides encoding AHASL with at least two mutations, for example double and triple mutants, that demonstrate tolerance to herbicides, in particular, to imidazolinone herbicides and optionally, to sulfonylurea, triazolopyrimidine sulfoanilide, and/or pyrimidyl oxybenzoate herbicides. The AHASL mutants of the invention may be used to create transgenic plants that demonstrate levels of herbicide resistance sufficient to confer commercial levels of herbicide tolerance when present on only one parent of a hybrid cross or on one genome of a polyploid plant. The polynucleotides of the invention may also be used as selectable markers for transformation of linked genes encoding other traits, as set forth in U.S. Patent No. 6,025,541.

Although the AHASL proteins of various species differ in length by a few amino acids, the relative positions of residues subject to modification in accordance with the present invention are conserved (Figure 8). Accordingly, the mutations described herein are expressed in terms of positions corresponding to the amino acid residue numbers of the *Arabidopsis* AHASL polypeptide (SEQ ID NO: 1, Figure 1,

Figure 8) unless noted otherwise or apparent from the context. For example, residue 122 of the *Arabidopsis* AHASL corresponds to residue 90 of maize AHASL, residue 104 of *Brassica napus* AHASL 1A, residue 107 of *B. napus* AHASL 1C, residue 96 of *O. sativa* AHASL, residue 113 of *Amaranthus* AHASL, residue 26 of *Escherichia coli* ilvG, residue 117 of *Saccharomyces cerevisiae* AHASL, residue 113 of sugar beet, residue 111 of cotton, residue 120 of *Camelina microcarpa* AHASL1, residue 117 of *Camelina microcarpa* AHASL2, residue 109 of *Solanum tuberosum* AHASL1, residue 111 of *Solanum tuberosum* AHASL2, residue 92 of *Lolium multiflorum*, residue 27 of *Solanum ptychanthum*, residue 93 of *Sorghum bicolor*, residue 103 of *Glycine max*, residue 107 of *Helianthus annuus* AHASL1, residue 101 of *Helianthus annuus* AHASL2, residue 97 of *Helianthus annuus* AHASL3, residue 59 of *Triticum aestivum*, and residue 100 of *Xanthium* sp. These correspondences are well known to those of skill in the art. Based on such correspondence, the corresponding positions in AHAS large subunit sequences not specifically disclosed herein could be readily determined by the skilled artisan. Specific exemplary regions of correspondence relevant to the present invention are set forth in Figure 3.

In a preferred embodiment, the invention provides an isolated polynucleotide encoding an *Arabidopsis* AHASL double mutant selected from the group consisting of a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a

position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine at a position corresponding to position 139 of SEQ ID NO:1 or position 107 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a histidine at a position corresponding to position 269 of SEQ ID NO:1 or position 237 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a methionine at a position corresponding to position 416 of SEQ ID NO:1 or position 384 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine at a position corresponding to position 426 of SEQ ID NO:1 or position 394 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a valine at a position corresponding to position 430 of SEQ ID NO:1 or position 398 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine at a position corresponding to position 442 of SEQ ID NO:1 or position 410 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine or aspartic acid at a position corresponding to position 445 of SEQ ID NO:1 or position 413 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glutamic acid at a position corresponding to position 580 of SEQ ID NO:1 or position 548 of SEQ ID NO:2; a polypeptide having a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2 and a

phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an asparagine at a position corresponding to position 375 of SEQ ID NO:1 or position 343 of SEQ ID NO:2; a polypeptide having an alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; and a polypeptide having a valine, cysteine, aspartic acid, glutamic acid, arginine, threonine, tryptophan, or tyrosine at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2.

In another preferred embodiment, the invention provides an isolated polynucleotide encoding an *Arabidopsis* AHAS large subunit triple mutant polypeptide selected from the group consisting of: a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and

a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, an arginine at a position corresponding to position 57 of SEQ ID NO:1 and a leucine at a position corresponding to position 398 of SEQ ID NO:1 or position 366 of SEQ ID NO:2; a polypeptide having a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a leucine at a position corresponding to position 95 of SEQ ID NO:1 or position 63 of SEQ ID NO:2, a glutamic acid at a position corresponding to position 416 of SEQ ID NO:1 or position 384 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; and a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine,

valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and any amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

Other preferred embodiments include AHASL double and triple mutants from other species, wherein the double and triple mutations occur at positions corresponding to those of the specific *Arabidopsis* and maize mutants described above and in table shown in Figure 8. For example, corresponding double and triple mutants of AHASL from microorganisms such as *E. coli*, *S. cerevisiae*, *Salmonella*, *Synichocystis*; and from plants such as wheat, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, sunflower, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao are also within the scope of the present invention. Such double and triple mutants can be made using known methods, for example, *in vitro* using site-directed mutagenesis, or *in vivo* using targeted mutagenesis or similar techniques, as described in U.S. Patent Nos. 5,565,350; 5,731,181; 5756,325; 5,760,012; 5,795,972 and 5,871,984.

The polynucleotides of the invention are provided in expression cassettes for expression in the plant of interest. The cassette will include regulatory sequences operably linked to an AHASL polynucleotide sequence of the invention. The term "regulatory element" as used herein refers to a polynucleotide that is capable of regulating the transcription of an operably linked polynucleotide. It includes, but not limited to, promoters, enhancers, introns, 5' UTRs, and 3' UTRs. By "operably linked" is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The

cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the AHASL polynucleotide sequence to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region (i.e., a promoter), an AHASL polynucleotide sequence of the invention, and a transcriptional and translational termination region (i.e., termination region) functional in plants. The promoter may be native or analogous, or foreign or heterologous, to the plant host and/or to the AHASL polynucleotide sequence of the invention. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. Where the promoter is "foreign" or "heterologous" to the plant host, it is intended that the promoter is not found in the native plant into which the promoter is introduced. Where the promoter is "foreign" or "heterologous" to the AHASL polynucleotide sequence of the invention, it is intended that the promoter is not the native or naturally occurring promoter for the operably linked AHASL polynucleotide sequence of the invention. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence.

While it may be preferable to express the AHASL polynucleotides of the invention using heterologous promoters, the native promoter sequences may be used. Such constructs would change expression levels of the AHASL protein in the plant or plant cell. Thus, the phenotype of the plant or plant cell is altered.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked AHASL sequence of interest, may be native with the plant host, or may be derived from another source (i.e., foreign or heterologous to the promoter, the AHASL polynucleotide sequence of interest, the plant host, or any combination thereof). Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet.*

262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon *et al.* (1991) *Genes Dev.* 5:141-149; Mogen *et al.* (1990) *Plant Cell* 2:1261-1272; Munroe *et al.* (1990) *Gene* 91:151-158; Ballas *et al.* (1989) *Nucleic Acids Res.* 17:7891-7903; and Joshi *et al.* (1987) *Nucleic Acid Res.* 15:9627-9639.

Where appropriate, the gene(s) may be optimized for increased expression in the transformed plant. That is, the genes can be synthesized using plant-preferred codons for improved expression. See, for example, Campbell and Gowri (1990) *Plant Physiol.* 92:1-11 for a discussion of host-preferred codon usage. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res.* 17:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

Nucleotide sequences for enhancing gene expression can also be used in the plant expression vectors. These include the introns of the maize Adh1, intron1 gene (Callis *et al. Genes and Development* 1:1183-1200, 1987), and leader sequences, (W-sequence) from the Tobacco Mosaic virus (TMV), Maize Chlorotic Mottle Virus and Alfalfa Mosaic Virus (Gallie *et al. Nucleic Acid Res.* 15:8693-8711, 1987 and Skuzeski *et al. Plant Mol. Biol.* 15:65-79, 1990). The first intron from the shrunken-1 locus of maize, has been shown to increase expression of genes in chimeric gene constructs. U.S. Pat. Nos. 5,424,412 and 5,593,874 disclose the use of specific introns in gene expression constructs, and Gallie *et al.* (*Plant Physiol.* 106:929-939, 1994) also have shown that introns are useful for regulating gene expression on a tissue specific basis. To further enhance or to optimize AHAS large subunit gene expression, the plant expression vectors of the invention may also contain DNA

sequences containing matrix attachment regions (MARs). Plant cells transformed with such modified expression systems, then, may exhibit overexpression or constitutive expression of a nucleotide sequence of the invention.

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

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, e.g., transitions and transversions, may be involved.

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

Such constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Patent No. 6,072,050; the core CaMV 35S promoter (Odell *et al.* (1985) *Nature* 313:810-812); rice actin (McElroy *et al.* (1990) *Plant Cell* 2:163-171); ubiquitin (Christensen *et al.* (1989) *Plant Mol. Biol.* 12:619-632 and Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689); pEMU (Last *et al.* (1991) *Theor. Appl. Genet.* 81:581-588); MAS (Velten *et al.* (1984) *EMBO J.* 3:2723-2730); ALS promoter (U.S. Patent No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Patent Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142; and 6,177,611.

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

In one embodiment, the nucleic acids of interest are targeted to the chloroplast for expression. In this manner, where the nucleic acid of interest is not directly inserted into the chloroplast, the expression cassette will additionally contain a chloroplast-targeting sequence comprising a nucleotide sequence that encodes a chloroplast transit peptide to direct the gene product of interest to the chloroplasts. Such transit peptides are known in the art. With respect to chloroplast-targeting sequences, "operably linked" means that the nucleic acid sequence encoding a transit peptide (*i.e.*, the chloroplast-targeting sequence) is linked to the AHASL polynucleotide of the invention such that the two sequences are contiguous and in the

same reading frame. See, for example, Von Heijne *et al.* (1991) *Plant Mol. Biol. Rep.* 9:104-126; Clark *et al.* (1989) *J. Biol. Chem.* 264:17544-17550; Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968; Romer *et al.* (1993) *Biochem. Biophys. Res. Commun.* 196:1414-1421; and Shah *et al.* (1986) *Science* 233:478-481. While the AHASL proteins of the invention include a native chloroplast transit peptide, any chloroplast transit peptide known in the art can be fused to the amino acid sequence of a mature AHASL protein of the invention by operably linking a choloroplast-targeting sequence to the 5'-end of a nucleotide sequence encoding a mature AHASL protein of the invention.

Chloroplast targeting sequences are known in the art and include the chloroplast small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) (de Castro Silva Filho *et al.* (1996) *Plant Mol. Biol.* 30:769-780; Schnell *et al.* (1991) *J. Biol. Chem.* 266(5):3335-3342); 5-(enolpyruvyl)shikimate-3-phosphate synthase (EPSPS) (Archer *et al.* (1990) *J. Bioenerg. Biomemb.* 22(6):789-810); tryptophan synthase (Zhao *et al.* (1995) *J. Biol. Chem.* 270(11):6081-6087); plastocyanin (Lawrence *et al.* (1997) *J. Biol. Chem.* 272(33):20357-20363); chorismate synthase (Schmidt *et al.* (1993) *J. Biol. Chem.* 268(36):27447-27457); and the light harvesting chlorophyll a/b binding protein (LHBP) (Lamppa *et al.* (1988) *J. Biol. Chem.* 263:14996-14999). See also Von Heijne *et al.* (1991) *Plant Mol. Biol. Rep.* 9:104-126; Clark *et al.* (1989) *J. Biol. Chem.* 264:17544-17550; Della-Cioppa *et al.* (1987) *Plant Physiol.* 84:965-968; Romer *et al.* (1993) *Biochem. Biophys. Res. Commun.* 196:1414-1421; and Shah *et al.* (1986) *Science* 233:478-481.

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

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

In particular, the present invention describes using polynucleotides encoding AHASL mutant polypeptides comprising at least two mutations to engineer plants which are herbicide tolerant. This strategy has herein been demonstrated using *Arabidopsis* AHASL mutants in *Arabidopsis thaliana* and maize AHASL2 mutants in corn, but its application is not restricted to these genes or to these plants. In preferred embodiments, the herbicide is imidazolinone and/or sulfonylurea. In other preferred embodiments, the herbicide tolerance is improved and/or enhanced compared to wild-type plants and to known AHAS mutants.

The invention also provides a method of producing a transgenic crop plant containing AHASL mutant coding nucleic acid comprising at least two mutations, wherein expression of the nucleic acid(s) in the plant results in herbicide tolerance as compared to wild-type plants or to known AHAS mutant type plants comprising: (a) introducing into a plant cell an expression vector comprising nucleic acid encoding an AHASL mutant with at least two mutations, and (b) generating from the plant cell a transgenic plant which is herbicide tolerant. The plant cell includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant. As used herein, the term "transgenic" refers to any plant, plant cell, callus, plant tissue, or plant part that contains all or part of at least one recombinant polynucleotide. In many cases, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations.

In another embodiment, the invention relates to using the mutant AHASL polypeptides of the invention as selectable markers. The invention provides a method of identifying or selecting a transformed plant cell, plant tissue, plant or part thereof comprising a) providing a transformed plant cell, plant tissue, plant or part thereof, wherein said transformed plant cell, plant tissue, plant or part thereof comprises an isolated nucleic acid encoding an AHAS large subunit double mutant polypeptide of

the invention as described above, wherein the polypeptide is used as a selection marker, and wherein said transformed plant cell, plant tissue, plant or part thereof may optionally comprise a further isolated nucleic acid of interest; b) contacting the transformed plant cell, plant tissue, plant or part thereof with at least one AHAS inhibitor or AHAS inhibiting compound; c) determining whether the plant cell, plant tissue, plant or part thereof is affected by the inhibitor or inhibiting compound; and d) identifying or selecting the transformed plant cell, plant tissue, plant or part thereof.

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

The invention further provides non-transgenic and transgenic herbicide-tolerant plants comprising one polynucleotide encoding an AHASL double mutant polypeptide, or two polynucleotides encoding AHASL single mutant polypeptides. Non-transgenic plants generated therefrom can be produced by cross-pollinating a first plant with a second plant and allowing the pollen acceptor plant (can be either the first or second plant) to produce seed from this cross pollination. Seeds and progeny plants generated thereof can have the double mutations crossed onto one single allele or two alleles. The pollen-acceptor plant can be either the first or second plant. The first plant comprises a first polynucleotide encoding a first AHASL single mutant polypeptide. The second plant comprises a second polynucleotide encoding a second AHASL single mutant polypeptide. The first and second AHASL single mutant polypeptides comprise a different single amino acid substitution relative to a wild-type AHASL polypeptide. Seeds or progeny plants arising therefrom which comprise one polynucleotide encoding the AHASL double mutant polypeptide or two polynucleotides encoding the two AHASL single mutant polypeptides can be selected. The selected progeny plants display an unexpectedly higher level of tolerance to an AHAS-inhibiting herbicide, for example an imidazolinone herbicide or sulfonylurea herbicide, than is predicted from the combination of the two AHASL single mutant polypeptides in a single plant. The progeny plants display a synergy

with respect to herbicide tolerance, whereby the level of herbicide tolerance in the progeny plants comprising the first and second mutations from the parent plants is greater than the herbicide tolerance of a plant comprising two copies of the first polynucleotide or two copies of the second polynucleotide.

When the first and second plants are homozygous for the first and second polynucleotides, respectively, each of the resulting progeny plants comprises one copy of each of the first and second polynucleotides and the selection step can be omitted. When at least one of the first and second plants is heterozygous, progeny plants comprising both polynucleotides can be selected, for example, by analyzing the DNA of progeny plants to identify progeny plants comprising both the first and second polynucleotides or by testing the progeny plants for increased herbicide tolerance. The progeny plants that comprise both the first and second polynucleotides display a level of herbicide tolerance that is greater than the herbicide tolerance of a plant comprising two copies of the first polypeptide or two copies of the second polypeptide.

In one embodiment, the plants of the invention comprise a first polynucleotide encoding a first AHASL single mutant polypeptide and a second polynucleotide encoding a second AHASL single mutant polypeptide, or an AHASL encoding polynucleotide comprising two nucleotide mutations that result in the amino acid mutations corresponding to the amino acid mutations of said first and said second AHASL single mutant polypeptides, wherein said first and said second AHASL single mutant polypeptides are selected from the group consisting of: a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or

methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having an isoleucine at a position corresponding to position 139 of SEQ ID NO:1 or position 107 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a histidine at a position corresponding to position 269 of SEQ ID NO:1 or position 237 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a methionine at a position corresponding to position 416 of SEQ ID NO:1 or position 384 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having an isoleucine at a position corresponding to position 426 of SEQ ID NO:1 or position 394 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a valine at a position corresponding to position 430 of SEQ ID NO:1 or position 398 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having an isoleucine at a position corresponding to position 442 of SEQ ID NO:1 or position 410 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a

position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having an isoleucine or aspartic acid at a position corresponding to position 445 of SEQ ID NO:1 or position 413 of SEQ ID NO:2; a first polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a second polypeptide having a glutamic acid at a position corresponding to position 580 of SEQ ID NO:1 or position 548 of SEQ ID NO:2; a first glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2 and a second polypeptide having a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a first polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a second polypeptide having a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a first polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a second polypeptide having an asparagine at a position corresponding to position 375 of SEQ ID NO:1 or position 343 of SEQ ID NO:2; a first polypeptide having an alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a second polypeptide having an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a first polypeptide having an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and a second polypeptide having a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; and a first polypeptide having a valine, cysteine, aspartic acid, glutamic acid, arginine, threonine, tryptophan, or

tyrosine at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2 and a second polypeptide having a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2. Non-transgenic plants comprising the double mutations of AHASL polynucleotides can be produced by methods other than the cross pollination described above, such as, for example but not limited to, targeted *in vivo* mutagenesis as described in Kochevenko *et al.* (Plant Phys. 132:174-184, 2003). The double mutations can be localized on a single allele, or two alleles of a plant genome.

Another embodiment of the invention relates to a transgenic plant transformed with an expression vector comprising an isolated polynucleotide, wherein the isolated polynucleotide encodes an acetohydroxyacid synthase large subunit (AHASL) double mutant polypeptide selected from the group consisting of: a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isolcucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine at a position corresponding to position 139 of

SEQ ID NO:1 or position 107 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a histidine at a position corresponding to position 269 of SEQ ID NO:1 or position 237 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a methionine at a position corresponding to position 416 of SEQ ID NO:1 or position 384 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine at a position corresponding to position 426 of SEQ ID NO:1 or position 394 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a valine at a position corresponding to position 430 of SEQ ID NO:1 or position 398 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine at a position corresponding to position 442 of SEQ ID NO:1 or position 410 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and an isoleucine or aspartic acid at a position corresponding to position 445 of SEQ ID NO:1 or position 413 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glutamic acid at a position corresponding to position 580 of SEQ ID NO:1 or position 548 of SEQ ID NO:2; a polypeptide having a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at

a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an asparagine at a position corresponding to position 375 of SEQ ID NO:1 or position 343 of SEQ ID NO:2; a polypeptide having an alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; and a polypeptide having a valine, cysteine, aspartic acid, glutamic acid, arginine, threonine, tryptophan, or tyrosine at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2.

The invention further provides non-transgenic and transgenic herbicide-tolerant plants comprising one polynucleotide encoding an AHASL triple mutant polypeptide, or one or more AHASL encoding polynucleotides comprising three mutations. For the production of a non-transgenic plant with one or more polynucleotides comprising three mutations, a progeny plant comprising one or two polynucleotides comprising said first and said second mutations described above is cross pollinated with third plant that comprises a third polynucleotide encoding a third AHASL single mutant polypeptide. The third AHASL single mutant polypeptide comprises a different single amino acid substitution relative to a wild-type AHASL polypeptide than either the first or second AHASL single mutant polypeptides. Seeds or progeny plants that comprise one or more polynucleotides comprising the three mutations are selected as described above. The selected progeny plants comprise a

level of herbicide tolerance that is greater than the additive effect of combining the three AHASL single mutant polypeptides in a single plant. Non-transgenic plants comprising the triple or multiple mutations of AHASL polynucleotides can be produced by methods other than the cross pollination described above, such as, for example but not limited to, targeted *in vivo* mutagenesis as described above. The multiple mutations can be localized on a single allele, or multiple alleles of a plant genome.

In one embodiment, plants of the invention comprise a first polynucleotide encoding a first AHASL single mutant polypeptide, a second polynucleotide encoding a second AHASL single mutant polypeptide, and a third polynucleotide encoding a third AHASL single mutant polypeptide. In another embodiment, plants of the invention comprise an AHASL encoding polynucleotide comprising three mutations, wherein the three nucleotide mutations result in the amino acid mutations corresponding to the mutations of said first, said second and said third AHASL single mutant polypeptides. In yet another embodiment, plants of the invention comprise an AHASL encoding polynucleotide comprising a single mutation and a polynucleotide comprising a double mutations, wherein the nucleotide mutations result in the amino acid mutations corresponding to the mutations of aforementioned first, second and third AHASL single mutant polypeptides, wherein said first, second, and third AHASL single mutant polypeptides are selected from the group consisting of: a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to

position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, an arginine at a position corresponding to position 57 of SEQ ID NO:1 and a leucine at a position corresponding to position 398 of SEQ ID NO:1 or position 366 of SEQ ID NO:2; a polypeptide having a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a leucine at a position corresponding to position 95 of SEQ ID NO:1 or position 63 of SEQ ID NO:2, a glutamic acid at a position corresponding to position 416 of SEQ ID NO:1 or position 384 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; and a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and any amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

Alternatively, plants comprising one or more polynucleotides encoding AHASL single mutant polypeptides are produced by transforming a plant with two or more of such polynucleotides or transforming a first plant with a first polynucleotide encoding a first AHASL single mutant polypeptide and cross pollinating the first plant with a second plant comprising a second polynucleotide encoding a second AHASL single mutant polypeptide. The second plant comprises a second polynucleotide comprising second AHASL single mutant polypeptide that is endogenous or was introduced via transformation. The first and second AHASL single mutant polypeptides comprise a different single amino acid substitution relative to a wild-type AHASL polypeptide. As necessary, seeds or progeny plants comprising both the first and second polynucleotides are selected as described above.

Yet another embodiment of the invention relates to a transgenic plant transformed with an expression vector comprising an isolated polynucleotide, wherein the isolated polynucleotide encodes an acetohydroxyacid synthase large subunit (AHASL) triple mutant polypeptide selected from the group consisting of: a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ

ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a valine, threonine, glutamine, cysteine, or methionine at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, an arginine at a position corresponding to position 57 of SEQ ID NO:1 and a leucine at a position corresponding to position 398 of SEQ ID NO:1 or position 366 of SEQ ID NO:2; a polypeptide having a glutamic acid, isoleucine, leucine, or asparagine at a position corresponding to position 124 of SEQ ID NO:1 or position 92 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2 and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2; a polypeptide having a leucine at a position corresponding to position 95 of SEQ ID NO:1 or position 63 of SEQ ID NO:2, a glutamic acid at a position corresponding to position 416 of SEQ ID NO:1 or position 384 of SEQ ID NO:2 and a phenylalanine, asparagine, threonine, glycine, valine, or tryptophan at a position corresponding to position 653 of SEQ ID NO:1 or position 621 of SEQ ID NO:2; and a polypeptide having a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2 and any amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

The present invention provides herbicide-tolerant or herbicide-resistant plants comprising a herbicide-tolerant or herbicide-resistant AHASL protein including, but not limited to, AHASL single mutant polypeptides and AHASL double and triple mutant polypeptides that are encoded by the polynucleotides of the present invention. By a "herbicide-tolerant" or "herbicide-resistant" plant, it is intended that a plant that is tolerant or resistant to at least one herbicide at a level that would normally kill, or inhibit the growth of, a normal or wild-type plant. By "herbicide-tolerant AHASL

protein" or "herbicide-resistant AHASL protein", it is intended that such an AHASL protein displays higher AHAS activity, relative to the AHAS activity of a wild-type AHASL protein, when in the presence of at least one herbicide that is known to interfere with AHAS activity and at a concentration or level of the herbicide that is known to inhibit the AHAS activity of the wild-type AHASL protein. Furthermore, the AHAS activity of such a herbicide-tolerant or herbicide-resistant AHASL protein may be referred to herein as "herbicide-tolerant" or "herbicide-resistant" AHAS activity.

For the present invention, the terms "herbicide-tolerant" and "herbicide-resistant" are used interchangeable and are intended to have an equivalent meaning and an equivalent scope. Similarly, the terms "herbicide-tolerance" and "herbicide-resistance" are used interchangeable and are intended to have an equivalent meaning and an equivalent scope. Likewise, the terms "imidazolinone-resistant" and "imidazolinone-resistance" are used interchangeable and are intended to be of an equivalent meaning and an equivalent scope as the terms "imidazolinone-tolerant" and "imidazolinone-tolerance", respectively.

The invention encompasses herbicide-resistant AHASL polynucleotides and herbicide-resistant AHASL proteins. By "herbicide-resistant AHASL polynucleotide" is intended a polynucleotide that encodes a protein comprising herbicide-resistant AHAS activity. By "herbicide-resistant AHASL protein" is intended a protein or polypeptide that comprises herbicide-resistant AHAS activity.

Further, it is recognized that a herbicide-tolerant or herbicide-resistant AHASL protein can be introduced into a plant by transforming a plant or ancestor thereof with a nucleotide sequence encoding a herbicide-tolerant or herbicide-resistant AHASL protein. Such herbicide-tolerant or herbicide-resistant AHASL proteins are encoded by the herbicide-tolerant or herbicide-resistant AHASL polynucleotides. Alternatively, a herbicide-tolerant or herbicide-resistant AHASL protein such as, for example, an AHASL single mutation polypeptide as disclosed herein, may occur in a plant as a result of a naturally occurring or induced mutation in an endogenous AHASL gene in the genome of a plant or progenitor thereof.

The present invention provides plants, plant tissues, plant cells, and host cells with increased resistance or tolerance to at least one herbicide, particularly an imidazolinone or sulfonylurea herbicide. The preferred amount or concentration of the herbicide is an "effective amount" or "effective concentration." By "effective amount" and "effective concentration" is intended an amount and concentration, respectively, that is sufficient to kill or inhibit the growth of a similar, wild-type, plant, plant tissue, plant cell, or host cell, but that said amount does not kill or inhibit as severely the growth of the herbicide-resistant plants, plant tissues, plant cells, and host cells of the present invention. Typically, the effective amount of a herbicide is an amount that is routinely used in agricultural production systems to kill weeds of interest. Such an amount is known to those of ordinary skill in the art.

By "similar, wild-type, plant, plant tissue, plant cell or host cell" is intended a plant, plant tissue, plant cell, or host cell, respectively, that lacks the herbicide-resistance characteristics and/or particular polynucleotide of the invention that are disclosed herein. The use of the term "wild-type" is not, therefore, intended to imply that a plant, plant tissue, plant cell, or other host cell lacks recombinant DNA in its genome, and/or does not possess herbicide-resistant characteristics that are different from those disclosed herein.

As used herein unless clearly indicated otherwise, the term "plant" intended to mean a plant at any developmental stage, as well as any part or parts of a plant that may be attached to or separate from a whole intact plant. Such parts of a plant include, but are not limited to, organs, tissues, and cells of a plant. Examples of particular plant parts include a stem, a leaf, a root, an inflorescence, a flower, a floret, a fruit, a pedicle, a peduncle, a stamen, an anther, a stigma, a style, an ovary, a petal, a sepal, a carpel, a root tip, a root cap, a root hair, a leaf hair, a seed hair, a pollen grain, a microspore, a cotyledon, a hypocotyl, an epicotyl, xylem, phloem, parenchyma, endosperm, a companion cell, a guard cell, and any other known organs, tissues, and cells of a plant. Furthermore, it is recognized that a seed is a plant.

The plants of the present invention include both non-transgenic plants and transgenic plants. By "non-transgenic plant" is intended to mean a plant lacking recombinant DNA in its genome. By "transgenic plant" is intended to mean a plant comprising recombinant DNA in its genome. Such a transgenic plant can be

produced by introducing recombinant DNA into the genome of the plant. When such recombinant DNA is incorporated into the genome of the transgenic plant, progeny of the plant can also comprise the recombinant DNA. A progeny plant that comprises at least a portion of the recombinant DNA of at least one progenitor transgenic plant is also a transgenic plant.

In certain embodiments, the present invention involves herbidicide-resistant plants that are produced by mutation breeding. Such plants comprise a polynucleotide encoding an AHAS large subunit single mutant polypeptide and are tolerant to one or more AHAS-inhibiting herbicides. Such methods can involve, for example, exposing the plants or seeds to a mutagen, particularly a chemical mutagen such as, for example, ethyl methanesulfonate (EMS) and selecting for plants that have enhanced tolerance to at least one AHAS-inhibiting herbicide, particularly an imidazolinone herbicide or sulfonylurea herbicide. However, the present invention is not limited to herbicide-tolerant plants that are produced by a mutagenesis method involving the chemical mutagen EMS. Any mutagenesis method known in the art may be used to produce the herbicide-resistant plants of the present invention. Such mutagenesis methods can involve, for example, the use of any one or more of the following mutagens: radiation, such as X-rays, Gamma rays (e.g., cobalt 60 or cesium 137), neutrons, (e.g., product of nuclear fission by uranium 235 in an atomic reactor), Beta radiation (e.g., emitted from radioisotopes such as phosphorus 32 or carbon 14), and ultraviolet radiation (preferably from 2500 to 2900 nm), and chemical mutagens such as base analogues (e.g., 5-bromo-uracil), related compounds (e.g., 8-ethoxy caffeine), antibiotics (e.g., streptonigrin), alkylating agents (e.g., sulfur mustards, nitrogen mustards, epoxides, ethylenamines, sulfates, sulfonates, sulfones, lactones), azide, hydroxylamine, nitrous acid, or acridines. Herbicide-resistant plants can also be produced by using tissue culture methods to select for plant cells comprising herbicide-resistance mutations and then regenerating herbicide-resistant plants therefrom. See, for example, U.S. Patent Nos. 5,773,702 and 5,859,348, both of which are herein incorporated in their entirety by reference. Further details of mutation breeding can be found in "Principals of Cultivar Development" Fehr, 1993 Macmillan Publishing Company the disclosure of which is incorporated herein by reference.

The present invention provides methods for enhancing the tolerance or resistance of a plant, plant tissue, plant cell, or other host cell to at least one herbicide that interferes with the activity of the AHAS enzyme. Preferably, such a herbicide is an imidazolinone herbicide, a sulfonylurea herbicide, a triazolopyrimidine herbicide, a pyrimidinyloxybenzoate herbicide, a sulfonylamino-carbonyltriazolinone herbicide, or mixture thereof. More preferably, such a herbicide is an imidazolinone herbicide, a sulfonylurea herbicide, or mixture thereof. For the present invention, the imidazolinone herbicides include, but are not limited to, PURSUIT® (imazethapyr), CADRE® (imazapic), RAPTOR® (imazamox), SCEPTER® (imazaquin), ASSERT® (imazethabenz), ARSENAL® (imazapyr), a derivative of any of the aforementioned herbicides, and a mixture of two or more of the aforementioned herbicides, for example, imazapyr/imazamox (ODYSSEY®). More specifically, the imidazolinone herbicide can be selected from, but is not limited to, 2- (4-isopropyl-4-methyl-5-oxo-2-imidiazolin-2-yl) -nicotinic acid, [2- (4-isopropyl)-4-] [methyl-5-oxo-2-imidazolin-2-yl)-3-quinolinecarboxylic] acid, [5-ethyl-2- (4-isopropyl-] 4-methyl-5-oxo-2-imidazolin-2-yl) -nicotinic acid, 2- (4-isopropyl-4-methyl-5-oxo-2- imidazolin-2-yl)-5- (methoxymethyl)-nicotinic acid, [2- (4-isopropyl-4-methyl-5-oxo-2-] imidazolin-2-yl)-5-methylnicotinic acid, and a mixture of methyl [6- (4-isopropyl-4-] methyl-5-oxo-2-imidazolin-2-yl) -m-toluate and methyl [2- (4-isopropyl-4-methyl-5-] oxo-2-imidazolin-2-yl) -p-toluate. The use of 5-ethyl-2- (4-isopropyl-4-methyl-5-oxo- 2-imidazolin-2-yl) -nicotinic acid and [2- (4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-] yl)-5- (methoxymethyl)-nicotinic acid is preferred. The use of [2- (4-isopropyl-4-] methyl-5-oxo-2-imidazolin-2-yl)-5- (methoxymethyl)-nicotinic acid is particularly preferred.

For the present invention, the sulfonylurea herbicides include, but are not limited to, chlorsulfuron, metsulfuron methyl, sulfometuron methyl, chlorimuron ethyl, thifensulfuron methyl, tribenuron methyl, bensulfuron methyl, nicosulfuron, ethametsulfuron methyl, rimsulfuron, triflusulfuron methyl, triasulfuron, primisulfuron methyl, cinosulfuron, amidosulfiuon, fluzasulfuron, imazosulfuron, pyrazosulfuron ethyl, halosulfuron, azimsulfuron, cyclosulfuron, ethoxysulfuron, flazasulfuron, flupyrsulfuron methyl, foramsulfuron, iodosulfuron, oxasulfuron, mesosulfuron, prosulfuron, sulfosulfuron, trifloxysulfuron, tritosulfuron, a derivative

of any of the aforementioned herbicides, and a mixture of two or more of the aforementioned herbicides. The triazolopyrimidine herbicides of the invention include, but are not limited to, cloransulam, diclosulam, florasulam, flumetsulam, metosulam, and penoxsulam. The pyrimidinyloxybenzoate (or pyrimidinyl carboxy) herbicides of the invention include, but are not limited to, bispyribac, pyrithiobac, pyriminobac, pyribenzoxim and pyriftalid. The sulfonylamino-carbonyltriazolinone herbicides include, but are not limited to, flucarbazone and propoxycarbazone.

It is recognized that pyrimidinyloxybenzoate herbicides are closely related to the pyrimidinylthiobenzoate herbicides and are generalized under the heading of the latter name by the Weed Science Society of America. Accordingly, the herbicides of the present invention further include pyrimidinylthiobenzoate herbicides, including, but not limited to, the pyrimidinyloxybenzoate herbicides described above.

The present invention provides methods for enhancing AHAS activity in a plant comprising transforming a plant with a polynucleotide construct comprising a promoter operably linked to an AHASL nucleotide sequence of the invention. The methods involve introducing a polynucleotide construct of the invention into at least one plant cell and regenerating a transformed plant therefrom. The methods involve the use of a promoter that is capable of driving gene expression in a plant cell. Preferably, such a promoter is a constitutive promoter or a tissue-preferred promoter. The methods find use in enhancing or increasing the resistance of a plant to at least one herbicide that interferes with the catalytic activity of the AHAS enzyme, particularly an imidazolinone herbicide.

The present invention provides expression cassettes for expressing the polynucleotides of the invention in plants, plant tissues, plant cells, and other host cells. The expression cassettes comprise a promoter expressible in the plant, plant tissue, plant cell, or other host cells of interest operably linked to a polynucleotide of the invention that comprises a nucleotide sequence encoding either a full-length (*i.e.* including the chloroplast transit peptide) or mature AHASL protein (*i.e.* without the chloroplast transit peptide). If expression is desired in the plastids or chloroplasts of plants or plant cells, the expression cassette may also comprise an operably linked chloroplast-targeting sequence that encodes a chloroplast transit peptide.

The expression cassettes of the invention find use in a method for enhancing the herbicide tolerance of a plant or a host cell. The method involves transforming the plant or host cell with an expression cassette of the invention, wherein the expression cassette comprises a promoter that is expressible in the plant or host cell of interest and the promoter is operably linked to a polynucleotide of the invention that comprises a nucleotide sequence encoding an imidazolinone-resistant AHASL protein of the invention.

The use of the term "polynucleotide constructs" herein is not intended to limit the present invention to polynucleotide constructs comprising DNA. Those of ordinary skill in the art will recognize that polynucleotide constructs, particularly polynucleotides and oligonucleotides, comprised of ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides may also be employed in the methods disclosed herein. Thus, the polynucleotide constructs of the present invention encompass all polynucleotide constructs that can be employed in the methods of the present invention for transforming plants including, but not limited to, those comprised of deoxyribonucleotides, ribonucleotides, and combinations thereof. Such deoxyribonucleotides and ribonucleotides include both naturally occurring molecules and synthetic analogues. The polynucleotide constructs of the invention also encompass all forms of polynucleotide constructs including, but not limited to, single-stranded forms, double-stranded forms, hairpins, stem-and-loop structures, and the like. Furthermore, it is understood by those of ordinary skill in the art that each nucleotide sequences disclosed herein also encompasses the complement of that exemplified nucleotide sequence.

Further, it is recognized that, for expression of a polynucleotide of the invention in a host cell of interest, the polynucleotide is typically operably linked to a promoter that is capable of driving gene expression in the host cell of interest. The methods of the invention for expressing the polynucleotides in host cells do not depend on particular promoter. The methods encompass the use of any promoter that is known in the art and that is capable of driving gene expression in the host cell of interest.

The present invention encompasses AHASL polynucleotide molecules and fragments and variants thereof. Polynucleotide molecules that are fragments of these nucleotide sequences are also encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence encoding an AHASL protein of the invention. Preferably, a fragment of an AHASL nucleotide sequence of the invention encodes a biologically active portion of an AHASL protein. A biologically active portion of an AHASL protein can be prepared by isolating a portion of one of the AHASL nucleotide sequences of the invention, expressing the encoded portion of the AHASL protein (e.g., by recombinant expression *in vitro*), and assessing the activity of the encoded portion of the AHASL protein. Polynucleotide molecules that are fragments of an AHASL nucleotide sequence and encode biologically active portions of AHASL proteins comprise at least about 500, 750, 1000, 1250, 1500, 1600, 1700, 1800, 1900, or 2000 nucleotides, or up to the number of nucleotides present in a full-length nucleotide sequence disclosed herein (for example, 2013 nucleotides for SEQ ID NO: 30) depending upon the intended use.

A fragment of an AHASL nucleotide sequence that encodes a biologically active portion of an AHASL protein of the invention will encode at least about 200, 300, 400, 500, 550, 650, or 650 contiguous amino acids, or up to the total number of amino acids present in a full-length AHASL protein of the invention (for example, 670 amino acids for SEQ ID NO: 1).

\ Polynucleotide molecules comprising nucleotide sequences that are variants of the nucleotide sequences disclosed herein are also encompassed by the present invention. "Variants" of the AHASL nucleotide sequences of the invention include those sequences that encode the mutant AHASL polypeptides disclosed herein but that differ conservatively because of the degeneracy of the genetic code. These naturally occurring allelic variants can be identified with the use of well-known molecular biology techniques, such as polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences that have been generated, for example, by using site-directed mutagenesis but which still encode the AHASL protein disclosed in the present invention as discussed below. Generally, polynucleotide sequence variants of the invention will have at least about 80%, 85%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a particular nucleotide sequence disclosed herein. A variant AHASL polynucleotide sequence will encode an AHASL mutant polypeptide, respectively, that has an amino acid sequence having at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of an AHASL polypeptide disclosed herein.

In addition, the skilled artisan will further appreciate that changes can be introduced by mutation into the polynucleotides sequences of the invention thereby leading to changes in the amino acid sequence of the encoded AHASL double and triple mutant polypeptides without altering the biological activity of the double and triple mutant polypeptides. Thus, an isolated polynucleotide molecule encoding an AHASL double and triple mutant polypeptide having a sequence that differs from the double and triple mutant sequences set forth in Figures 1 and 2 can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such variant nucleotide sequences are also encompassed by the present invention.

For example, preferably, conservative amino acid substitutions may be made at one or more predicted, preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of an AHASL protein (*e.g.*, the sequence of SEQ ID NO: 1) without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine,

isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif.

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

It is recognized that the polynucleotide molecules and polypeptides of the invention encompass polynucleotide molecules and polypeptides comprising a nucleotide or an amino acid sequence that is sufficiently identical to the double or triple nucleotide sequences set forth in Figures 1 and 2, or to the amino acid sequences set forth in Figures 1 and 2. The term "sufficiently identical" is used herein to refer to a first amino acid or nucleotide sequence that contains a sufficient or minimum number of identical or equivalent (e.g., with a similar side chain) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have a common structural domain and/or common functional activity. For example, amino acid or nucleotide sequences that contain a common structural domain having at least about 80% identity, preferably 85% identity, more preferably 90%, 95%, or 98% identity are defined herein as sufficiently identical.

To determine the percent identity of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, percent identity = number of identical positions/total number of positions (*e.g.*, overlapping positions) x 100). In one embodiment, the two sequences are the same length. The percent identity between two sequences can be determined using techniques similar to those described below, with or without allowing gaps. In calculating percent identity, typically exact matches are counted.

The determination of percent identity between two sequences can be accomplished using a mathematical algorithm. A preferred, nonlimiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to the polynucleotide molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-Blast can be used to perform an iterated search that detects distant relationships between molecules. *See,* Altschul *et al.* (1997) *supra.* When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. *See* http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0), which is part of the GCG sequence alignment software

package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. Alignment may also be performed manually by inspection.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the full-length sequences of the invention and using multiple alignment by mean of the algorithm Clustal W (Nucleic Acid Research, 22(22):4673-4680, 1994) using the program AlignX included in the software package Vector NTI Suite Version 9 (Invitrogen, 1600 Faraday Ave., Carlsbad, CA 92008) using the default parameters; or any equivalent program thereof. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by AlignX in the software package Vector NTI Suite Version 9.

The deletions, insertions, and substitutions of the protein sequences encompassed herein are not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays. That is, the activity can be evaluated by AHAS activity assays. *See*, for example, Singh *et al.* (1988) *Anal. Biochem.* 171:173-179, herein incorporated by reference.

As disclosed herein, the polynucleotides of the invention find use in enhancing the herbicide tolerance of plants that comprise in their genomes a gene encoding a herbicide-tolerant AHASL protein. Such a gene may be an endogenous gene or a transgene. Additionally, in certain embodiments, the polynucleotides of the present invention can be stacked with any combination of polynucleotide sequences of interest in order to create plants with a desired phenotype. For example, the polynucleotides of the present invention may be stacked with any other polynucleotides encoding polypeptides having pesticidal and/or insecticidal activity, such as, for example, the *Bacillus thuringiensis* toxin proteins (described in U.S.

Patent Nos. 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; and Geiser *et al.* (1986) *Gene* 48:109). The combinations generated can also include multiple copies of any one of the polynucleotides of interest.

While the polynucleotides of the invention find use as selectable marker genes for plant transformation, the expression cassettes of the invention can include another selectable marker gene for the selection of transformed cells. Selectable marker genes, including those of the present invention, are utilized for the selection of transformed cells or tissues. Marker genes include, but are not limited to, genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). See generally, Yarranton (1992) *Curr. Opin. Biotech.* 3:506-511; Christopherson *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:6314-6318; Yao *et al.* (1992) *Cell* 71:63-72; Reznikoff (1992) *Mol. Microbiol.* 6:2419-2422; Barkley *et al.* (1980) in *The Operon*, pp. 177-220; Hu *et al.* (1987) *Cell* 48:555-566; Brown *et al.* (1987) *Cell* 49:603-612; Figge *et al.* (1988) *Cell* 52:713-722; Deuschle *et al.* (1989) *Proc. Natl. Acad. Aci. USA* 86:5400-5404; Fuerst *et al.* (1989) *Proc. Natl. Acad. Sci. USA* 86:2549-2553; Deuschle *et al.* (1990) *Science* 248:480-483; Gossen (1993) Ph.D. Thesis, University of Heidelberg; Reines *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:1917-1921; Labow *et al.* (1990) *Mol. Cell. Biol.* 10:3343-3356; Zambretti *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:3952-3956; Baim *et al.* (1991) *Proc. Natl. Acad. Sci. USA* 88:5072-5076; Wyborski *et al.* (1991) *Nucleic Acids Res.* 19:4647-4653; Hillenand-Wissman (1989) *Topics Mol. Struc. Biol.* 10:143-162; Degenkolb *et al.* (1991) *Antimicrob. Agents Chemother.* 35:1591-1595; Kleinschnidt *et al.* (1988) *Biochemistry* 27:1094-1104; Bonin (1993) Ph.D. Thesis, University of Heidelberg; Gossen *et al.* (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Oliva *et al.* (1992) *Antimicrob. Agents Chemother.* 36:913-919; Hlavka *et al.* (1985) *Handbook of Experimental Pharmacology*, Vol. 78 (Springer-Verlag, Berlin); Gill *et al.* (1988) *Nature* 334:721-724. Such disclosures are herein incorporated by reference.

The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

The isolated polynucleotide molecules comprising nucleotide sequence that encode the AHASL proteins of the invention can be used in vectors to transform plants so that the plants created have enhanced resistant to herbicides, particularly an imidazolinone herbicide or sulfonylurea herbicide. The isolated AHASL polynucleotide molecules of the invention can be used in vectors alone or in combination with a nucleotide sequence encoding the small subunit of the AHAS (AHASS) enzyme in conferring herbicide resistance in plants. *See*, U.S. Patent No. 6,348,643; which is herein incorporated by reference.

The invention also relates to a plant expression vector comprising a promoter that drives expression in a plant operably linked to an isolated polynucleotide molecule of the invention. The isolated polynucleotide molecule comprises a nucleotide sequence encoding an AHASL protein of the invention, or a functional fragment and variant thereof. The plant expression vector of the invention does not depend on a particular promoter, only that such a promoter is capable of driving gene expression in a plant cell. Preferred promoters include constitutive promoters and tissue-preferred promoters.

The transformation vectors of the invention can be used to produce plants transformed with a gene of interest. The transformation vector will comprise a selectable marker gene of the invention and a gene of interest to be introduced and typically expressed in the transformed plant. Such a selectable marker gene comprises a polynucleotide of the invention that encodes an AHASL double or triple mutant polypeptide, wherein the polynucleotide is operably linked to a promoter that drives expression in a host cell. For use in plants and plant cells, the transformation vector comprises a selectable marker gene comprising a polynucleotide of the invention that encodes an AHASL double or triple mutant polypeptide operably linked to a promoter that drives expression in a plant cell.

The genes of interest of the invention vary depending on the desired outcome. For example, various changes in phenotype can be of interest including modifying the fatty acid composition in a plant, altering the amino acid content of a plant, altering a plant's insect and/or pathogen defense mechanisms, and the like. These results can be

achieved by providing expression of heterologous products or increased expression of endogenous products in plants. Alternatively, the results can be achieved by providing for a reduction of expression of one or more endogenous products, particularly enzymes or cofactors in the plant. These changes result in a change in phenotype of the transformed plant.

In one embodiment of the invention, the genes of interest include insect resistance genes such as, for example, *Bacillus thuringiensis* toxin protein genes (U.S. Patent Nos. 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881; and Geiser *et al.* (1986) *Gene* 48:109).

The AHASL proteins or polypeptides of the invention can be purified from, for example, sunflower plants and can be used in compositions. Also, an isolated polynucleotide molecule encoding an AHASL protein of the invention can be used to express an AHASL protein of the invention in a microbe such as *E. coli* or a yeast. The expressed AHASL protein can be purified from extracts of *E. coli* or yeast by any method known to those of ordinary skill in the art.

The polynucleotides of the invention find use in methods for enhancing the resistance of herbicide-tolerant plants. In one embodiment of the invention, the herbicide-tolerant plants that comprise a polynucleotide of the invention that encodes an AHASL double or triple mutant polypeptide. The invention further provides herbicide-tolerant plants that comprise two or more polynucleotides encoding AHASL single mutant polypeptides. Polynucleotides encoding herbicide-tolerant AHASL proteins and herbicide-tolerant plants comprising an endogenous gene that encodes a herbicide-tolerant AHASL protein include the polynucleotides and plants of the present invention and those that are known in the art. *See*, for example, U.S. Patent Nos. 5,013,659, 5,731,180, 5,767,361, 5,545,822, 5,736,629, 5,773,703, 5,773,704, 5,952,553 and 6,274,796; all of which are herein incorporated by reference. Such methods for enhancing the resistance of herbicide-tolerant plants comprise transforming a herbicide-tolerant plant with at least one polynucleotide construct comprising a promoter that drives expression in a plant cell that is operably linked to a polynucleotide of the invention.

Numerous plant transformation vectors and methods for transforming plants are available. *See*, for example, An, G. *et al.* (1986) *Plant Physiol.*, 81:301-305; Fry, J., *et al.* (1987) *Plant Cell Rep.* 6:321-325; Block, M. (1988) *Theor. Appl Genet.*76:767-774; Hinchee, *et al.* (1990) *Stadler. Genet. Symp.*203212.203-212; Cousins, *et al.* (1991) *Aust. J. Plant Physiol.* 18:481-494; Chee, P. P. and Slightom, J. L. (1992) *Gene.*118:255-260; Christou, *et al.* (1992) *Trends. Biotechnol.* 10:239-246; D'Halluin, *et al.* (1992) Bio/Technol. 10:309-314; Dhir, *et al.* (1992) *Plant Physiol.* 99:81-88; Casas *et al.* (1993) *Proc. Nat. Acad Sci. USA* 90:11212-11216; Christou, P. (1993) *In Vitro Cell. Dev. Biol.-Plant*; 29P:119-124; Davies, *et al.* (1993) *Plant Cell Rep.* 12:180-183; Dong, J. A. and Mchughen, A. (1993) *Plant Sci.* 91:139-148; Franklin, C. I. and Trieu, T. N. (1993) *Plant. Physiol.* 102:167; Golovkin, *et al.* (1993) *Plant Sci.* 90:41-52; *Guo Chin Sci. Bull.* 38:2072-2078; Asano, *et al.* (1994) *Plant Cell Rep.* 13; Ayeres N. M. and Park, W. D. (1994) *Crit. Rev. Plant. Sci.* 13:219-239; Barcelo, *et al.* (1994) *Plant. J.* 5:583-592; Becker, *et al.* (1994) *Plant. J.* 5:299-307; Borkowska *et al.* (1994) *Acta. Physiol Plant.* 16:225-230; Christou, P. (1994) *Agro. Food. Ind. Hi Tech.* 5: 17-27; Eapen *et al.* (1994) *Plant Cell Rep.* 13:582-586; Hartman, *et al.* (1994) *Bio-Technology* 12: 919923; Ritala, *et al.* (1994) *Plant. Mol. Biol.* 24:317-325; and Wan, Y. C. and Lemaux, P. G. (1994) *Plant Physiol.* 104:3748.

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

By "stable transformation" is intended that the polynucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being

(1990) *Biotechnology* 8:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology* 6:559-563 (maize); Tomes, U.S. Patent No. 5,240,855; Buising *et al.* U.S. Patent Nos. 5,322,783 and 5,324,646; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg (Springer-Verlag, Berlin) (maize); Klein *et al.* (1988) *Plant Physiol.* 91:440-444 (maize); Fromm *et al.* (1990) *Biotechnology* 8:833-839 (maize); Hooykaas-Van Slogteren *et al.* (1984) Nature (London) 311:763-764; Bowen *et al.* U.S. Patent No. 5,736,369 (cereals); Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet *et al.* (1985) in *The Experimental Manipulation of Ovule Tissues*, ed. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports* 9:415-418 and Kaeppler *et al.* (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell* 4:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford (1995) *Annals of Botany* 75:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology* 14:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

The polynucleotides of the invention may be introduced into plants by contacting plants with a virus or viral nucleic acids. Generally, such methods involve incorporating a polynucleotide construct of the invention within a viral DNA or RNA molecule. It is recognized that the AHASL protein of the invention may be initially synthesized as part of a viral polyprotein, which later may be processed by proteolysis *in vivo* or *in vitro* to produce the desired recombinant protein. Further, it is recognized that promoters of the invention also encompass promoters utilized for transcription by viral RNA polymerases. Methods for introducing polynucleotide constructs into plants and expressing a protein encoded therein, involving viral DNA or RNA molecules, are known in the art. See, for example, U.S. Patent Nos. 5,889,191, 5,889,190, 5,866,785, 5,589,367 and 5,316,931; herein incorporated by reference.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports* 5:81-84. These plants may then be grown, and either pollinated with the

same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure expression of the desired phenotypic characteristic has been achieved. In this manner, the present invention provides transformed seed (also referred to as "transgenic seed") having a polynucleotide construct of the invention, for example, an expression cassette of the invention, stably incorporated into their genome.

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

The plants of the invention are herbicide-resistant plants and thus, find use in methods for controlling weeds that involve the application of a herbicide. Thus, the present invention further provides a method for controlling weeds in the vicinity of a

herbicide-resistant plant of the invention. The method comprises applying an effective amount of a herbicide to the weeds and to the herbicide-resistant plant, wherein the plant has increased resistance to at least one AHAS-inhibiting herbicide, particularly an imidazolinone or sulfonylurea herbicide, when compared to a wild-type plant. In such a method for controlling weeds, the herbicide-resistant plants of the invention are preferably crop plants, including, but not limited to, sunflower, alfalfa, *Brassica sp.*, soybean, cotton, safflower, peanut, tobacco, tomato, potato, wheat, rice, maize, sorghum, barley, rye, millet, and sorghum.

By providing plants having increased resistance to herbicides, particularly imidazolinone and sulfonylurea herbicides, a wide variety of formulations can be employed for protecting plants from weeds, so as to enhance plant growth and reduce competition for nutrients. A herbicide can be used by itself for pre-emergence, post-emergence, pre-planting and at planting control of weeds in areas surrounding the plants described herein or an imidazolinone herbicide formulation can be used that contains other additives. The herbicide can also be used as a seed treatment. Additives found in an imidazolinone or sulfonylurea herbicide formulation include other herbicides, detergents, adjuvants, spreading agents, sticking agents, stabilizing agents, or the like. The herbicide formulation can be a wet or dry preparation and can include, but is not limited to, flowable powders, emulsifiable concentrates and liquid concentrates. The herbicide and herbicide formulations can be applied in accordance with conventional methods, for example, by spraying, irrigation, dusting, or the like.

The present invention provides non-transgenic and transgenic plants and seeds with increased tolerance to at least one herbicide, particularly an AHAS-inhibiting herbicide, more particularly imidazolinone and sulfonylurea herbicides, most particularly imidazolinone herbicides. In preferred embodiment of the invention, the plants and seeds of the invention display a higher level of herbicide tolerance that similar plants that comprise only one AHASL single mutant polypeptide. Such plants and seeds of the invention find use in improved methods for controlling weeds that allow for the application of a herbicide to the weeds and to the herbicide-resistant plant at an effective amount that comprises a higher herbicidal concentration or rate than can be used with similar plants that comprise only one AHASL single mutant

polypeptide. Accordingly, such improved methods provide superior weed control when compared to existing methods involving plants comprising only one AHASL single mutant polypeptide and the application of a lower herbicidal concentration or rate.

The present invention provides herbicide-resistant plants comprising polynucleotides encoding AHASL double or triple mutant polypeptides and herbicide-resistant plants comprising two or more polynucleotides encoding AHASL single mutant polypeptides. These herbicide-resistant plants of the present invention find use in methods for producing herbicide-resistant plants through conventional plant breeding involving sexual reproduction. The methods comprise crossing a first plant that is a herbicide-resistant plant of the invention to a second plant that is not resistant to the herbicide. The second plant can be any plant that is capable of producing viable progeny plants (i.e., seeds) when crossed with the first plant. Typically, but not necessarily, the first and second plants are of the same species. The methods can optionally involve selecting for progeny plants that comprise the polynucleotide encoding the AHASL mutant polypeptide or the two or more polynucleotides encoding AHASL single mutant polypeptides of the first plant. The methods of the invention can further involve one or more generations of backcrossing the progeny plants of the first cross to a plant of the same line or genotype as either the first or second plant. Alternatively, the progeny of the first cross or any subsequent cross can be crossed to a third plant that is of a different line or genotype than either the first or second plant.

The herbicide-resistant plants of the invention that comprise polynucleotides encoding AHASL double or triple mutant polypeptides and herbicide-resistant plants comprising two or more polynucleotides encoding AHASL single mutant polypeptides also find use in methods for increasing the herbicide-resistance of a plant through conventional plant breeding involving sexual reproduction. The methods comprise crossing a first plant that is a herbicide-resistant plant of the invention to a second plant that may or may not be resistant to the same herbicide or herbicides as the first plant or may be resistant to different herbicide or herbicides than the first plant. The second plant can be any plant that is capable of producing viable progeny plants (i.e., seeds) when crossed with the first plant. Typically, but not necessarily,

the first and second plants are of the same species. The methods can optionally involve selecting for progeny plants that comprise the polynucleotide encoding the AHASL mutant polypeptide or the two or more polynucleotides encoding AHASL single mutant polypeptides of the first plant and the herbicide resistance characteristics of the second plant. The progeny plants produced by this method of the present invention have increased resistance to a herbicide when compared to either the first or second plant or both. When the first and second plants are resistant to different herbicides, the progeny plants will have the combined herbicide tolerance characteristics of the first and second plants. The methods of the invention can further involve one or more generations of backcrossing the progeny plants of the first cross to a plant of the same line or genotype as either the first or second plant. Alternatively, the progeny of the first cross or any subsequent cross can be crossed to a third plant that is of a different line or genotype than either the first or second plant.

The present invention also provides plants, plant organs, plant tissues, plant cells, seeds, and non-human host cells that are transformed with the at least one polynucleotide molecule, expression cassette, or transformation vector of the invention. Such transformed plants, plant organs, plant tissues, plant cells, seeds, and non-human host cells have enhanced tolerance or resistance to at least one herbicide, at levels of the herbicide that kill or inhibit the growth of an untransformed plant, plant tissue, plant cell, or non-human host cell, respectively. Preferably, the transformed plants, plant tissues, plant cells, and seeds of the invention are *Arabidopsis thaliana* and crop plants.

The present invention provides methods that involve the use of at least one AHAS-inhibiting herbicide selected from the group consisting of imidazolinone herbicides, sulfonylurea herbicides, triazolopyrimidine herbicides, pyrimidinyloxybenzoate herbicides, sulfonylamino-carbonyltriazolinone herbicides, and mixtures thereof. In these methods, the AHAS-inhibiting herbicide can be applied by any method known in the art including, but not limited to, seed treatment, soil treatment, and foliar treatment.

Prior to application, the AHAS-inhibiting herbicide can be converted into the customary formulations, for example solutions, emulsions, suspensions, dusts,

powders, pastes and granules. The use form depends on the particular intended purpose; in each case, it should ensure a fine and even distribution of the compound according to the invention.

The formulations are prepared in a known manner (see e.g. for review US 3,060,084, EP-A 707 445 (for liquid concentrates), Browning, "Agglomeration", Chemical Engineering, Dec. 4, 1967, 147-48, Perry's Chemical Engineer's Handbook, 4th Ed., McGraw-Hill, New York, 1963, pages 8-57 and et seq. WO 91/13546, US 4,172,714, US 4,144,050, US 3,920,442, US 5,180,587, US 5,232,701, US 5,208,030, GB 2,095,558, US 3,299,566, Klingman, Weed Control as a Science, John Wiley and Sons, Inc., New York, 1961, Hance *et al.* Weed Control Handbook, 8th Ed., Blackwell Scientific Publications, Oxford, 1989 and Mollet, H., Grubemann, A., Formulation technology, Wiley VCH Verlag GmbH, Weinheim (Germany), 2001, 2. D. A. Knowles, Chemistry and Technology of Agrochemical Formulations, Kluwer Academic Publishers, Dordrecht, 1998 (ISBN 0-7514-0443-8), for example by extending the active compound with auxiliaries suitable for the formulation of agrochemicals, such as solvents and/or carriers, if desired emulsifiers, surfactants and dispersants, preservatives, antifoaming agents, anti-freezing agents, for seed treatment formulation also optionally colorants and/or binders and/or gelling agents.

Examples of suitable solvents are water, aromatic solvents (for example Solvesso products, xylene), paraffins (for example mineral oil fractions), alcohols (for example methanol, butanol, pentanol, benzyl alcohol), ketones (for example cyclohexanone, gamma-butyrolactone), pyrrolidones (NMP, NOP), acetates (glycol diacetate), glycols, fatty acid dimethylamides, fatty acids and fatty acid esters. In principle, solvent mixtures may also be used.

Examples of suitable carriers are ground natural minerals (for example kaolins, clays, talc, chalk) and ground synthetic minerals (for example highly disperse silica, silicates).

Suitable emulsifiers are nonionic and anionic emulsifiers (for example polyoxyethylene fatty alcohol ethers, alkylsulfonates and arylsulfonates).

Examples of dispersants are lignin-sulfite waste liquors and methylcellulose.

Suitable surfactants used are alkali metal, alkaline earth metal and ammonium salts of lignosulfonic acid, naphthalenesulfonic acid, phenolsulfonic acid, dibutylnaphthalenesulfonic acid, alkylarylsulfonates, alkyl sulfates, alkylsulfonates, fatty alcohol sulfates, fatty acids and sulfated fatty alcohol glycol ethers, furthermore condensates of sulfonated naphthalene and naphthalene derivatives with formaldehyde, condensates of naphthalene or of naphthalenesulfonic acid with phenol and formaldehyde, polyoxyethylene octylphenol ether, ethoxylated isooctylphenol, octylphenol, nonylphenol, alkylphenol polyglycol ethers, tributylphenyl polyglycol ether, tristearylphenyl polyglycol ether, alkylaryl polyether alcohols, alcohol and fatty alcohol ethylene oxide condensates, ethoxylated castor oil, polyoxyethylene alkyl ethers, ethoxylated polyoxypropylene, lauryl alcohol polyglycol ether acetal, sorbitol esters, lignosulfite waste liquors and methylcellulose.

Substances which are suitable for the preparation of directly sprayable solutions, emulsions, pastes or oil dispersions are mineral oil fractions of medium to high boiling point, such as kerosene or diesel oil, furthermore coal tar oils and oils of vegetable or animal origin, aliphatic, cyclic and aromatic hydrocarbons, for example toluene, xylene, paraffin, tetrahydronaphthalene, alkylated naphthalenes or their derivatives, methanol, ethanol, propanol, butanol, cyclohexanol, cyclohexanone, isophorone, highly polar solvents, for example dimethyl sulfoxide, N-methylpyrrolidone or water.

Also anti-freezing agents such as glycerin, ethylene glycol, propylene glycol and bactericides such as can be added to the formulation.

Suitable antifoaming agents are for example antifoaming agents based on silicon or magnesium stearate.

Suitable preservatives are, for example, dichlorophenol and benzylalcoholhemiformaldehyde.

Seed Treatment formulations may additionally comprise binders and optionally colorants.

Binders can be added to improve the adhesion of the active materials on the seeds after treatment. Suitable binders are block copolymers EO/PO surfactants but also polyvinylalcoholsl, polyvinylpyrrolidones, polyacrylates, polymethacrylates,

polybutenes, polyisobutylenes, polystyrene, polyethyleneamines, polyethyleneamides, polyethyleneimines (Lupasol®, Polymin®), polyethers, polyurethans, polyvinylacetate, tylose and copolymers derived from these polymers.

Optionally, also colorants can be included in the formulation. Suitable colorants or dyes for seed treatment formulations are Rhodamin B, C.I. Pigment Red 112, C.I. Solvent Red 1, pigment blue 15:4, pigment blue 15:3, pigment blue 15:2, pigment blue 15:1, pigment blue 80, pigment yellow 1, pigment yellow 13, pigment red 112, pigment red 48:2, pigment red 48:1, pigment red 57:1, pigment red 53:1, pigment orange 43, pigment orange 34, pigment orange 5, pigment green 36, pigment green 7, pigment white 6, pigment brown 25, basic violet 10, basic violet 49, acid red 51, acid red 52, acid red 14, acid blue 9, acid yellow 23, basic red 10, basic red 108.

An example of a suitable gelling agent is carrageen (Satiagel®).

Powders, materials for spreading, and dustable products can be prepared by mixing or concomitantly grinding the active substances with a solid carrier.

Granules, for example coated granules, impregnated granules and homogeneous granules, can be prepared by binding the active compounds to solid carriers. Examples of solid carriers are mineral earths such as silica gels, silicates, talc, kaolin, attaclay, limestone, lime, chalk, bole, loess, clay, dolomite, diatomaceous earth, calcium sulfate, magnesium sulfate, magnesium oxide, ground synthetic materials, fertilizers, such as, for example, ammonium sulfate, ammonium phosphate, ammonium nitrate, ureas, and products of vegetable origin, such as cereal meal, tree bark meal, wood meal and nutshell meal, cellulose powders and other solid carriers.

In general, the formulations comprise from 0.01 to 95% by weight, preferably from 0.1 to 90% by weight, of the AHAS-inhibiting herbicide. In this case, the AHAS-inhibiting herbicides are employed in a purity of from 90% to 100% by weight, preferably 95% to 100% by weight (according to NMR spectrum). For seed treatment purposes, respective formulations can be diluted 2-10 fold leading to concentrations in the ready to use preparations of 0.01 to 60% by weight active compound by weight, preferably 0.1 to 40% by weight.

The AHAS-inhibiting herbicide can be used as such, in the form of their formulations or the use forms prepared therefrom, for example in the form of directly sprayable solutions, powders, suspensions or dispersions, emulsions, oil dispersions,

pastes, dustable products, materials for spreading, or granules, by means of spraying, atomizing, dusting, spreading or pouring. The use forms depend entirely on the intended purposes; they are intended to ensure in each case the finest possible distribution of the AHAS-inhibiting herbicide according to the invention.

Aqueous use forms can be prepared from emulsion concentrates, pastes or wettable powders (sprayable powders, oil dispersions) by adding water. To prepare emulsions, pastes or oil dispersions, the substances, as such or dissolved in an oil or solvent, can be homogenized in water by means of a wetter, tackifier, dispersant or emulsifier. However, it is also possible to prepare concentrates composed of active substance, wetter, tackifier, dispersant or emulsifier and, if appropriate, solvent or oil, and such concentrates are suitable for dilution with water.

The active compound concentrations in the ready-to-use preparations can be varied within relatively wide ranges. In general, they are from 0.0001 to 10%, preferably from 0.01 to 1% per weight.

The AHAS-inhibiting herbicide may also be used successfully in the ultra-low-volume process (ULV), it being possible to apply formulations comprising over 95% by weight of active compound, or even to apply the active compound without additives.

The following are examples of formulations:

1. Products for dilution with water for foliar applications. For seed treatment purposes, such products may be applied to the seed diluted or undiluted.

A) Water-soluble concentrates (SL, LS)

Ten parts by weight of the AHAS-inhibiting herbicide are dissolved in 90 parts by weight of water or a water-soluble solvent. As an alternative, wetters or other auxiliaries are added. The AHAS-inhibiting herbicide dissolves upon dilution with water, whereby a formulation with 10 % (w/w) of AHAS-inhibiting herbicide is obtained.

B) Dispersible concentrates (DC)

Twenty parts by weight of the AHAS-inhibiting herbicide are dissolved in 70 parts by weight of cyclohexanone with addition of 10 parts by weight of a dispersant, for example polyvinylpyrrolidone. Dilution with water gives a dispersion, whereby a formulation with 20% (w/w) of AHAS-inhibiting herbicide is obtained.

C) Emulsifiable concentrates (EC)

Fifteen parts by weight of the AHAS-inhibiting herbicide are dissolved in 7 parts by weight of xylene with addition of calcium dodecylbenzenesulfonate and castor oil ethoxylate (in each case 5 parts by weight). Dilution with water gives an emulsion, whereby a formulation with 15% (w/w) of AHAS-inhibiting herbicide is obtained.

D) Emulsions (EW, EO, ES)

Twenty-five parts by weight of the AHAS-inhibiting herbicide are dissolved in 35 parts by weight of xylene with addition of calcium dodecylbenzenesulfonate and castor oil ethoxylate (in each case 5 parts by weight). This mixture is introduced into 30 parts by weight of water by means of an emulsifier machine (e.g. Ultraturrax) and made into a homogeneous emulsion. Dilution with water gives an emulsion, whereby a formulation with 25% (w/w) of AHAS-inhibiting herbicide is obtained.

E) Suspensions (SC, OD, FS)

In an agitated ball mill, 20 parts by weight of the AHAS-inhibiting herbicide are comminuted with addition of 10 parts by weight of dispersants, wetters and 70 parts by weight of water or of an organic solvent to give a fine AHAS-inhibiting herbicide suspension. Dilution with water gives a stable suspension of the AHAS-inhibiting herbicide, whereby a formulation with 20% (w/w) of AHAS-inhibiting herbicide is obtained.

F) Water-dispersible granules and water-soluble granules (WG, SG)

Fifty parts by weight of the AHAS-inhibiting herbicide are ground finely with addition of 50 parts by weight of dispersants and wetters and made as water-dispersible or water-soluble granules by means of technical appliances (for example extrusion, spray tower, fluidized bed). Dilution with water gives a stable dispersion or solution of the AHAS-inhibiting herbicide, whereby a formulation with 50% (w/w) of AHAS-inhibiting herbicide is obtained.

G) Water-dispersible powders and water-soluble powders (WP, SP, SS, WS)

Seventy-five parts by weight of the AHAS-inhibiting herbicide are ground in a rotor-stator mill with addition of 25 parts by weight of dispersants, wetters and silica gel. Dilution with water gives a stable dispersion or solution of the AHAS-inhibiting herbicide, whereby a formulation with 75% (w/w) of AHAS-inhibiting herbicide is obtained.

H) Gel-Formulation (GF)

In an agitated ball mill, 20 parts by weight of the AHAS-inhibiting herbicide are comminuted with addition of 10 parts by weight of dispersants, 1 part by weight of a gelling agent wetters and 70 parts by weight of water or of an organic solvent to give a fine AHAS-inhibiting herbicide suspension. Dilution with water gives a stable suspension of the AHAS-inhibiting herbicide, whereby a formulation with 20% (w/w) of AHAS-inhibiting herbicide is obtained. This gel formulation is suitable for us as a seed treatment.

2. Products to be applied undiluted for foliar applications. For seed treatment purposes, such products may be applied to the seed diluted.

A) Dustable powders (DP, DS)

Five parts by weight of the AHAS-inhibiting herbicide are ground finely and mixed intimately with 95 parts by weight of finely divided kaolin. This gives a dustable product having 5% (w/w) of AHAS-inhibiting herbicide.

B) Granules (GR, FG, GG, MG)

One-half part by weight of the AHAS-inhibiting herbicide is ground finely and associated with 95.5 parts by weight of carriers, whereby a formulation with 0.5% (w/w) of AHAS-inhibiting herbicide is obtained. Current methods are extrusion, spray-drying or the fluidized bed. This gives granules to be applied undiluted for foliar use.

Conventional seed treatment formulations include for example flowable concentrates FS, solutions LS, powders for dry treatment DS, water dispersible powders for slurry treatment WS, water-soluble powders SS and emulsion ES and EC and gel formulation GF. These formulations can be applied to the seed diluted or undiluted. Application to the seeds is carried out before sowing, or either directly on the seeds.

In a preferred embodiment a FS formulation is used for seed treatment. Typcially, an FS formulation may comprise 1-800 g/l of active ingredient, 1-200 g/l Surfactant, 0 to 200 g/l antifreezing agent, 0 to 400 g/l of binder, 0 to 200 g/l of a pigment and up to 1 liter of a solvent, preferably water.

For seed treatment, seeds of the herbicide resistant plants according of the present invention are treated with herbicides, preferably herbicides selected from the group consisting of AHAS-inhibiting herbicides such as amidosulfuron, azimsulfuron, bensulfuron, chlorimuron, chlorsulfuron, cinosulfuron, cyclosulfamuron, ethametsulfuron, ethoxysulfuron, flazasulfuron, flupyrsulfuron, foramsulfuron, halosulfuron, imazosulfuron, iodosulfuron, mesosulfuron, metsulfuron, nicosulfuron, oxasulfuron, primisulfuron, prosulfuron, pyrazosulfuron, rimsulfuron, sulfometuron, sulfosulfuron, thifensulfuron, triasulfuron, tribenuron, trifloxysulfuron, triflusulfuron, tritosulfuron, imazamethabenz, imazamox, imazapic, imazapyr, imazaquin, imazethapyr, cloransulam, diclosulam, florasulam, flumetsulam, metosulam,

penoxsulam, bispyribac, pyriminobac, propoxycarbazone, flucarbazone, pyribenzoxim, pyriftalid, pyrithiobac, and mixtures thereof, or with a formulation comprising a AHAS-inhibiting herbicide.

The term seed treatment comprises all suitable seed treatment techniques known in the art, such as seed dressing, seed coating, seed dusting, seed soaking, and seed pelleting.

In accordance with one variant of the present invention, a further subject of the invention is a method of treating soil by the application, in particular into the seed drill: either of a granular formulation containing the AHAS-inhibiting herbicide as a composition/formulation (e.g .a granular formulation, with optionally one or more solid or liquid, agriculturally acceptable carriers and/or optionally with one or more agriculturally acceptable surfactants. This method is advantageously employed, for example, in seedbeds of cereals, maize, cotton, and sunflower.

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

The term seed embraces seeds and plant propagules of all kinds including but not limited to true seeds, seed pieces, suckers, corms, bulbs, fruit, tubers, grains, cuttings, cut shoots and the like and means in a preferred embodiment true seeds.

The term "coated with and/or containing" generally signifies that the active ingredient is for the most part on the surface of the propagation product at the time of

application, although a greater or lesser part of the ingredient may penetrate into the propagation product, depending on the method of application. When the said propagation product is (re)planted, it may absorb the active ingredient.

The seed treatment application with the AHAS-inhibiting herbicide or with a formulation comprising the AHAS-inhibiting herbicide is carried out by spraying or dusting the seeds before sowing of the plants and before emergence of the plants.

In the treatment of seeds, the corresponding formulations are applied by treating the seeds with an effective amount of the AHAS-inhibiting herbicide or a formulation comprising the AHAS-inhibiting herbicide. Herein, the application rates are generally from 0.1 g to 10 kg of the a.i. (or of the mixture of a.i. or of the formulation) per 100 kg of seed, preferably from 1 g to 5 kg per 100 kg of seed, in particular from 1 g to 2.5 kg per 100 kg of seed. For specific crops such as lettuce the rate can be higher.

The present invention provides a method for combating undesired vegetation or controlling weeds comprising contacting the seeds of the resistant plants according to the present invention before sowing and/or after pregermination with an AHAS-inhibiting herbicide. The method can further comprise sowing the seeds, for example, in soil in a field or in a potting medium in greenhouse. The method finds particular use in combating undesired vegetation or controlling weeds in the immediate vicinity of the seed.

The control of undesired vegetation is understood as meaning the killing of weeds and/or otherwise retarding or inhibiting the normal growth of the weeds. Weeds, in the broadest sense, are understood as meaning all those plants which grow in locations where they are undesired.

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

Echinochloa, Setaria, Panicum, Digitaria, Phleum, Poa, Festuca, Eleusine, Brachiaria, Lolium, Bromus, Avena, Cyperus, Sorghum, Agropyron, Cynodon, Monochoria, Fimbristyslis, Sagittaria, Eleocharis, Scirpus, Paspalum, Ischaemum, Sphenoclea, Dactyloctenium, Agrostis, Alopecurus, and Apera.

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

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

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

The following examples are offered by way of illustration and not by way of limitation. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

Example 1

Vectors containing *Arabidopsis* AHASL mutant genes

The entire XbaI fragment of *Arabidopsis thaliana* genomic DNA that contains the entire AHAS large subunit gene with some additional DNA, inclusive of the XbaI sites at the 5' and 3' ends is set forth in SEQ ID NO: 34 (AtAHASL). Bases 2484 to 4496 of SEQ ID NO: 34 encompass the coding sequence of the *Arabidopsis thaliana* AHAS large subunit gene serine 653 to threonine mutant allele, inclusive of the stop codon shown in SEQ ID NO: 30. A smaller genomic fragment of the *Arabidopsis thaliana* AHAS large subunit gene serine 653 to threonine mutant allele shown in SEQ ID NO: 33, encompassed in bases 2484 to 5717 of SEQ ID NO: 34, includes the coding sequence and the 3' end, up to and including the 3' end XbaI site, with the first two bases of the NcoI site found at the start codon of AtAHASL left off for clarity.

The DNA fragment of SEQ ID NO: 33 encoding the full-length *Arabidopsis* AHASL single mutation S653N and 3' untranslated region was cloned into pKK233-2 to yield the vector designated AE1 for expression and testing in *E. coli*. (pKK233-2, bacterial expression vector, Pharmacia, GenBank Accession No. X70478). Vectors AE2 through AE9 were generated from AE1 by mutagenesis and standard cloning procedures. Figure 4 shows the map of the AE1 base vector, with positions of mutations indicated.

Vector AP1 (Figure 5) is a plant transformation vector that includes a genomic fragment of *Arabidopsis thaliana* DNA that includes the AtAHASL gene with the single S653N mutation (SEQ ID NO:34). The DNA fragment as shown in SEQ ID NO: 34 was cloned into AP1 in the reverse-complement orientation. Vectors AP2-AP7 were generated from AP1 and the AE plasmids using standard cloning procedures and differ only by mutations as indicated in Table 1. For convenience in cloning, different fragments were used to generate AP6 and AP7, compared to AP2-AP5. Thus, AP6 and AP7 are 47 base pairs shorter than AP1-AP5 . This difference is in the plasmid backbone and not the *Arabidopsis thaliana* genomic fragment.

Vectors AE10 through AE24 were made as follows. The wild type *Arabidopsis thaliana* AHAS large subunit gene was amplified under mutagenic conditions using the Genemorph II random mutagenesis kit (Stratagene, La Jolla, California), resulting in randomly mutagenized amplified DNA fragments of this gene. This mutant DNA was then cloned back into AE7, replacing the wild type *A. thaliana* large subunit gene (between the unique SacII and AgeI sites on AE7) with the mutagenized forms. This DNA was transformed into *E. coli* strain TOP10 and selected on LB agar medium in such a fashion as to have a large number of unique transformants, each with independent, mutagenized AHAS genes. These colonies were scraped together and plasmid DNA was prepared from this entire primary library. This DNA was transformed into AHAS minus *E. coli* and again selected on LB agar media with carbenicillin. Plasmid positive colonies from this step were replica plated using velveteen onto minimal agar medium without branched chain amino acids and containing 30-micromolar imazethapyr. Those colonies that grew on this selective media possessed a functional *A. thaliana* AHAS mutant gene that was also imidazolinone tolerant.

The DNA sequence of the *A. thaliana* AHAS large subunit gene was determined for each of the growth positive colonies. No effort was made to determine the sequence of the *A. thaliana* AHAS large subunit genes that did not confer growth on the selective media. Because the AHAS function and imidazolinone tolerance screen was on a secondary library, replicates of the same mutations were found, as determined by DNA sequence analysis. Only one clone of each was advanced for testing on increasing imidazolinone concentrations and inclusion in Table 1.

Table 1

E. coli Plasmid*	*Arabidopsis* Transformation Vector*	Mutations*	*E. coli* Imazethapyr Tolerance Score**	Transgenic Plant Tolerance: X-fold improvement over AP1@(approximate)
AE1	AP1	S653N	+	NA
AE2	AP2	A122T & S653N	++	16
AE3	AP3	P197S & S653N	+	2
AE4	AP8	A122T, R199A, & S653N	NA	16
AE5	AP4	R199A, & S653N	+++	1.5
AE6	AP5	A122T, P197S, & S653N	NA	8
AE7		Wild type	- (IN)	NA
AE8	AP6	A122T and R199A	+++	2
AE9	AP7	A122T and P197S	NA	8
AE10		A122T, S57R and S398L	+++	NA
AE11		A122T and V139I	++	NA
AE12		A122T and Q269H	+	NA
AE13		A122T and K416M	++	NA
AE14		A122T and L426I	+++	NA
AE15		A122T and A430V	+++	NA
AE16		A122T and N442I	++	NA
AE17		A122T and N445I	++	NA
AE18		A122T and N445D	+++	NA
AE19		A122T and K580E	+++	NA
AE20		A122T, V439G, D595G, and S653N	+	NA
AE21		P197S and D375N	+++	NA
AE22		D375N	untested[†]	NA
AE23		D375N, K83R, V254I, M277I, and D315Y	+	NA
AE24		Q95L, K416E, and S653N	+	NA

* List of vectors for expression of AtAHASL2 in *E. coli* (AE plasmids) and for plant transformation plasmids (AP plasmids). Mutations in each vector are indicated relative to SEQ ID NO: 1.

** A simple single, double or triple plus system, +, ++, or +++ for respectively increasing colony size, was used to visually score the tolerance of the *Arabidopsis* AHAS function in AHAS minus *E. coli* containing the AE plasmids in the presence of the AHAS inhibitor imazethapyr. A "-", indicates there was no growth, meaning the mutation combination caused an inactive protein or there was no tolerance for imazethapyr at the selected rate. IN means inactive protein, while NT means not imidazolinone tolerant. NA means no data available (not tested).

† Not tested compared to S653N, fact of tolerance determined by screening protocol.

@ For transgenic plants comprising the AP1 vector, 18.75 μM imazethapyr was the highest concentration which allowed good growth of the plants in the microtiter format plates. This concentration was used as the basis for determining X-fold improvement over AP1.

Example 2

Vectors containing *Zea mays* AHASL mutant genes

The *Zea mays* AHASL2 gene (SEQ ID NO: 29) was cloned into the bacterial expression vector pTrcHis A (Invitrogen Corporation, Carlsbad, CA), fused to the vector tag and translational start site, beginning with base 160 of SEQ ID NO: 29. Mutagenesis and subcloning procedures were utilized to create vectors ZE2, ZE5, ZE6, and ZE7 using ZE1 as a base vector. Subcloning procedures were used to make ZE3 from ZE1, which is the maize AHASL2 gene fused to the vector tag and translational start site of pTrcHis A, beginning with base 121 of SEQ ID NO: 29. Since no functional difference was noted in *E. coli* between ZE1 or ZE3, standard mutagenesis and subcloning procedures were utilized to create vectors ZE4 and ZE8 through ZE22 using ZE3 as a base vector.

A plant transformation vector with an expression cassette comprising the maize ubiquitin promoter in combination with a polynucleotide encoding the maize AHASL2 S653N mutant was prepared using standard methods and designated ZP1 (Figure 7). To produce plant transformation vectors for expression of the other

AHASL mutants, standard cloning techniques were used to replace polynucleotide segments of ZP1 with polynucleotide fragments of the ZE vectors encoding the mutations.

Vectors ZE23 through ZE38 were made as follows. Vector ZE3 was subjected to saturating site directed mutagenesis using the QuikChange® Multi Site Directed Mutagenesis Kit (Stratagene, La Jolla, California) following the "General Guidelines for Creating Engineered Mutant Clone™ Collections" appendix protocol. Mutagenic oligonucleotides that would generate all possible codons at the critical sites of the maize AHAS large subunit were used in various combinations to create a collection of mutants with substitutions at residues A90, M92, P165, R167, S621, and G622. The mutant collection plasmids were transformed into AHAS deficient *E. coli* and plated on LB agar medium supplemented with 100 ug per liter of carbenicillin. Colonies from this were picked into M9 salts at 1x concentration (for an isotonic buffer) in microtiter plates and then replica plated on minimal agar medium without branched chain amino acids and containing 150 micromolar imazethapyr. Those colonies that grew on this selective media possessed a functional maize AHAS mutant gene that was also imidazolinone tolerant.

The DNA sequence of the maize AHAS large subunit gene was determined for each of the growth positive colonies. No effort was made to determine the sequence of the maize AHAS large subunit genes that did not confer growth on the selective media.

Table 2

E. coli Plasmid*	Maize Transformation Vector*	Mutations*	*E. coli* Imidazolinone Tolerance Score**	Maize Whole Plant Tolerance Score@
ZE1	-	S621N	+	NA
ZE2	-	wild type	- (NT)	NA
ZE3	-	wild type	- (NT)	NA
ZE4	ZP1	S621N	+	+
ZE5	-	W542L, S621N	- (IN)	NA
ZE6	ZP4	P165S, S621N	+	+
ZE7	-	W542L	- (NT)	NA
ZE8	-	M92E, S621N	- (IN)	NA
ZE9	ZP5	R167S, S621N	+++	+++
ZE10	ZP2	A90T, S621N	+++	+++
ZE11	ZP3	A90T, R167S, S621N	+++	+++
ZE12	ZP9	M92I, S621N	+++	+++
ZE13	-	R167A, S621N	NA	NA
ZE14	-	A173V, S621N	++	NA
ZE15	ZP8	A90T, M92I	+++	+++
ZE16	ZP10	A90T, M92E	NA	NA
ZE17	ZP6	A90T, R167A	+++	+++
ZE18		P165S	- (NT)	NA
ZE19	-	P165S, R167A	- (NT)	NA
ZE20	-	T171I, S621N	+	NA
ZE21	ZP7	A90T	++	+++
ZE22	-	A90T, P165S	+++	NA
ZE23	ZP11	A90Q	+++	+++
ZE24	ZP12	A90Q, M92L	++	+++
ZE25	-	A90Q, M92I	+++	NA
ZE26	-	A90C	++	NA
ZE27	-	A90M, M92I	+	NA
ZE28	-	P165E, R167F	- (NT)	NA
ZE29	-	P165V, R167A	- (NT)	NA
ZE30	-	P165E, R167T	+	+
ZE31	-	P165I, R167D	- (IN)	NA
ZE32	-	P165I, R167E	+	NA
ZE33	-	M92I, P165E, R167A	- (NT)	NA
ZE34	-	A90M, P165R, R167C	- (IN)	NA
ZE35	-	M92N, S621G	+++	NA
ZE36	-	P165S, R167N, S621V, G622D	+++	NA
ZE37	-	S621W	+++	NA
ZE38	-	P165S, R167C, W542M	+++	NA

* List of vectors for expression of ZmAHASL2 in E. coli (ZE plasmids) and for plant transformation plasmids (ZP plasmids). Mutations in each vector are indicated relative to SEQ ID NO: 2.

** A simple single, double or triple plus system, +, ++, or +++ for respectively increasing colony size, was used to visually score the tolerance of the maize AHAS function in AHAS minus E. coli containing the ZE plasmids in the presence of the AHAS inhibitor imazethapyr. A "-", indicates there was no growth, meaning the mutation combination caused an inactive protein or there was no tolerance for imazethapyr at the selected rate. IN means inactive protein, while NT means not imidazolinone tolerant. NA means no data available (not tested).

@ The maize whole plant tolerance scores are based on combined results from tests conducted in the greenhouse and at multiple field sites over several growing seasons. The scoring system for the maize whole plant tolerance was the same as described above for the *E. coli* imidazolinone tolerance. Note that all ZP constructs with +++ scores are tolerant to more than three thousand grams imazamox per hectare, which represents the highest tested spray rate.

Example 3

E. coli Complementation Assay

E. coli strain JMC1 (genotype [*ilvB1201 ilvHI2202 rbs-221 ara thi delta(pro-lac) recA56 srlC300::Tn10*], *DE(hsdR)::Cat*) is a knockout for all copies of *ilvG* of the native *E. coli* AHASL gene. This strain can only grow on minimal growth medium lacking leucine, isoleucine, and valine if AHASL is complemented by an exogenous AHASL gene (see Singh, *et al.* (1992) *Plant Physiol.* **99**, 812-816; Smith, *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4179-4183). This *E. coli* complementation assay was used to screen for AHASL enzyme activity and herbicide tolerance encoded by the AE and ZE vectors in the absence and presence of the imidazolinone herbicide Pursuit® (imazethapyr, BASF Corporation, Florham Park, NJ).

Example 4

Biochemical Characterization

Based on growth during complementation testing or simple activity tests, certain of the ZE series of vectors were used for AHAS biochemical assay inhibition testing in crude E. coli lysates. A 2-4 ml culture of LB containing 50 μg/ml carbenicillin (LB-carb) was inoculated with a single colony of JMC1 transformed with the ZE vector to be tested and incubated overnight at 37°C with shaking. The following morning, 0.5-1 ml of overnight culture was used to inoculate 20 ml of LB-carb, which was incubated at 37°C with shaking until the culture optical density (OD) at 600 nm was approximately 0.6 to 0.8 OD units. Isopropyl-l -thio-beta D-galactopyranoside (IPTG) was added to a concentration of 0.1 mM and the cultures incubated with shaking for 1-1.5 hours. The culture was centrifuged to pellet the cells and the supernatant discarded. The cell pellet was lysed with AHAS assay buffer (as in Singh *et al.* (1988) *Anal. Biochem.* 171:173-179) supplemented with 10 mg/ml lysozyme and subjected to brief sonication. The insoluble fraction was pelleted by centrifugation and the supernatant used in an assay for AHAS activity. At each concentration of imazethapyr inhibitor used, the activity was compared to an uninhibited control of the same ZE mutant. This results in a "percent of control" measurement.

Example 5

Plant Transformation

The AP vectors were transformed into *A. thaliana* ecotype Col-2. The T1 seeds were selected for transformation on plates with 100 nM Pursuit® as the selective agent. T2 seeds from approximately twenty independent transformation events (lines) were plated on MS agar with increasing Pursuit® concentrations, to score increases in tolerance compared to AP1. The vectors were scored by comparison of the highest concentrations of Pursuit® having uninhibited growth of seedlings by visual examination. The results of the *Arabidopsis* transformation experiments are shown in Table 1.

Seeds from several lines of *Arabidopsis* were tested by a vertical plate growth assay. A plate with standard Murashige and Skoog semisolid media containing 37.5 micromolar Pursuit (imazethapyr) was spotted with several seeds in 0.1% agarose. The plate was held vertically, so that the roots would grow along the agar surface. The seeds used were: 1) wild type ecotype Columbia 2; 2) the csr1-2 mutant (homozygous for the AtAHASL S653N mutation in the genomic copy of the AHAS large subunit gene); 3) Columbia 2 transformed with AP1; 4) Columbia 2 transformed with AP7; 5) Columbia 2 transformed with AP2. Note that numbers 2 and 3 are roughly equivalent in terms of probable tolerance, as the AP1 plants are transformed with a clone of the genomic XbaI fragment of csr1-2 (SEQ ID NO: 34). At this concentration of imazethapyr, the wild type seedlings failed to germinate, the single mutant plants (csr1-2 and AP1 transformants) barely germinated. AP7 and AP2 produced good tolerant growth, although the AP7 plants appear to have slightly less root growth. Note that all lines germinated and grew well on media without imazethapyr. The results of the vertical plate growth assay are depicted in Figure 10.

ZP constructs were introduced into maize immature embryos via *Agrobacterium*-mediated transformation. Transformed cells were selected on selection media supplemented with 0.75 μM Pursuit® for 3-4 weeks. Transgenic plantlets were regenerated on plant regeneration media supplemented with 0.75 μM Pursuit®. Transgenic plantlets were rooted in the presence of 0.5 μM Pursuit®. Transgenic plants were subjected to TaqMan analysis for the presence of the transgene before being transplanted to potting mixture and grown to maturity in greenhouse. The results of the maize transformation experiments are shown in Table 2. Maize plants transformed with the ZP constructs were sprayed with varying rates of imazamox, in several field locations and in the greenhouse. The relative ratings of the ZP constructs' whole plant test data are summarized in Table 2.

Example 6

Expression of AtAHASL Mutant Genes in Soybean

Vectors were prepared for expressing the AtAHASL genes in transformed soybean plants. Vectors AUP2 and AUP3 were made by cloning a polymerase chain reaction product of the parsley ubiquitin promoter, amplified to incorporate sites for the Asp718 and NcoI restriction enzymes, digested and ligated into the same sites of AP2 and AP3 by standard cloning techniques (see, Figures 11 and 12). AUP2 encodes an AtAHASL protein with the A122T and S653N mutations, and AUP3 encodes an AtAHASL protein with the A122T and S653N mutations. Vector BAP1 was made by cloning the entire promoter, coding sequence and 3'-untranslated region sequence of AP1 into a standard dicot transformation backbone containing the BAR selectable marker expression cassette, by standard blunt-ended cloning techniques.

Constructs AP2, AUP2, and AUP3 were introduced into soybean's axillary meristem cells at the primary node of seedling explants via *Agrobacterium*-mediated transformation. After inoculation and co-cultivation with *Agrobacteria*, the explants were transferred to shoot induction media without selection for one week. The explants were subsequently transferred to a shoot induction medium with 5 μM imazapyr (Arsenal) for 3 weeks to select for transformed cells. Explants with healthy callus/shoot pads at the primary node were then transferred to shoot elongation medium containing 3 μM imazapyr until a shoot elongated or the explant died. Transgenic plantlets were rooted, transplanted to potting mixture, subjected to TaqMan analysis for the presence of the trangene, and then grown to maturity in greenhouse. Construct BAP1 was used to produce transformed soybean plants in a like manner, except that the selection agent was BASTA.

The transformed soybean plants were tested for herbicide tolerance in both greenhouse and field studies. For the field study, imazapyr was applied at a rate of 300 g ai/ha at V3 stage. For the greenhouse study, imazapyr was applied at about the V2 stage. The results of these studies are summarized in Table 3.

Table 3

Transformation Vector, Native *Arabidopsis* promoter	Transformation Vector, Parsley Ubiquitin promoter	Mutations	Maximum Greenhouse Tolerance (grams Imazapyr per hectare)	Field Tolerance (grams Imazapyr per hectare)
	BAP1*	S653N	500	NA
AP2	AUP2	A122T & S653N	AP2 - 1000, AUP2 - 1500	AP2 – NA AUP2 - 300
-	AUP3	P197S & S653N	NA	300**

* BAP1 (figure 12) was transformed using the BAR gene for selection, as imazapyr selection in soybeans with the S653N mutation alone has not been optimized.

** Some injury compared to AUP2

Example 7

Transformant Selection

The polynucleotides generated by the invention may be used as selectable markers for plant transformation. The polynucleotides generated by the invention may be used as selectable markers to identify and/or select transformed plants which may comprise additional genes of interest. Plants or plant cells transformed with vectors containing the multiple mutant forms of the AHAS large subunit genes can be selected from non-transformed plants or plant cells by plating on minimal media, such as MS media, which incorporate AHAS inhibitors or AHAS inhibiting herbicides, such as imidazolinones. The transformed plants or tissues will be able to continue growing in the presence of these inhibitors, while the untransformed plants or tissues will die. In the case of transformed tissues, since the non-transformed tissues may receive branched chain amino acids from the transformed tissues, the actively growing tissues are removed from the slower growing or dying tissues and replated on selective media.

Whole plants may also be selected by planting the seeds and waiting for germination and seedling growth, followed by spraying the seedlings with AHAS inhibitors or AHAS inhibiting herbicides, such as imidazolinones. The transformed plants will survive while the untransformed plants will be killed.

5

Example 8

Field Trials with Transformed Maize

Field trials were conducted to assess whether or not maize plants transformed with one of the vectors comprising AHASL double and triple mutants displayed any gross physiological or reproductive affects with and without an imazamox application.

Materials and Methods

Source of Test Material

The genetically modified organism was produced by transforming corn inbred J553. F1 hybrid seed from 8 vector constructs were produced using TR5753 as an inbred male tester. The vector constructs are described in Table 4. Seed for the trial were produced in an isolated crossing block on the island of Kauai, Hawaii. USA during the 2006-2007 contraseason. Subsamples of each F1 hybrid produced were analyzed for the presence of the correct vector construct and absence of adventitious presence of other AHASL contructs.

Nontransformed commercial hybrids were purchased from Midwest USA corn seed companies and analyzed to confirm the absence of any adventitious presence of other AHASL contructs.

Table 4

Construct	pZm UBI + I::c-ZmAHAS L2::t-ZmAHAS L2 Mutations (at Designation)
1	P197S
2	A122T, R199A
3	A122T
4	A122T, M124I
5	M124I, S653N
6	A122T, S653N
7	A122T, R199S, S653N
8	S653N

Trial Methodology

Trial design was a Split Plot in a Randomized Complete Block Design, with the main plot being an herbicide treatment, and the sub plot being an F1 hybrid entry. The herbicide treatments included 1) untreated and 2) imazamox applied at 150 gai/ha. The F1 hybrid entries included 29 events from 8 vector constructs and 4 non-transformed commercial hybrids (3395IR from Pioneer Hi-Bred International, Inc, Johnston, IA, USA; and 8342GLS/IT, 8546IT, and 8590IT from Garst Seed Co., Inc., Slater, IA. USA). Plot size was 2 rows; row width 2.5 feet; row length 20 feet. Each treatment combination had 4 replications. The trial was planted at three locations. These locations were: Ames, IA, USA; Estherville, IA, USA; and York, NE, USA. All trials were planted during May 2007.

Location conditions

The Ames, IA location was in a corn-after-corn rotation which may have had some impact on uniformity of emergence and early growth as significant amounts of corn residue were present at planting. No major influences on the crop due to

weather, disease or insects were noted. The Estherville, IA site received heavy rain driven by 70 mph wind gusts and sustained winds of around 40 mph on July 16. Root lodging was observed in essentially every plot. No major influences on the crop due to disease or insects were noted. The York, NE site received above-normal rainfall in May, July and August and no major insect or disease issues were noted.

Results and Discussion

Data analyses combined across three locations resulted in one vector construct with a significant yield decrease ($p \leq 0.05$) when comparing yield with or without the imazamox herbicide application (Table 5). One vector construct had a significant yield increase when treated with the imazamox application. Other agronomic characteristics were also collected from the three trial locations, and no significant differences were detected within a construct when treated or untreated with imazamox for the traits plant height, ear height, stalk lodging and root lodging (data not shown).

The objective of the trial was to identify if an herbicide application of imazamox applied to vector constructs that have been optimized to provide improved herbicide tolerance to imazamox would result in gross, or obvious, physiological or reproductive affects, primarily yield. Only one vector construct (Construct 1, single mutant, P197L) had a significant ($p \leq 0.05$) negative response for grain yield when treated with imazamox. The remaining seven vector constructs exhibited no adverse physiological or reproductive affects in the presence or absence of the herbicide imazamox. The results of these field trials demonstrate the excellent agronomic potential of maize plants transformed with a vector comprising either an AHASL double or triple mutant.

Table 5

		Yield (bu/A)					
Description		Herbicide (H)		Non-herbicide (NH)			
Construct	# Event	Mean	α= 0.05	Mean	α= 0.05	(H/NH)%	p-value
1	4	160.68	B	175.58	BC	91.51	0.003
2	4	176.72	AB	177.05	ABC	99.81	0.94
3	4	180.59	A	179.02	AB	100.87	0.74
4	4	173.57	AB	163.79	C	105.97	0.11
5	4	173.87	AB	180.84	AB	96.14	0.10
6	4	188.02	A	178.65	AB	105.24	0.04
7	1	187.45	AB	189.03	AB	99.16	0.88
8	4	183.14	A	177.54	ABC	103.15	0.27
3395IR		168.03	AB	176.89	ABC	94.99	0.09
8590IT		181.92	AB	197.57	A	92.08	0.04
G8546IT		174.94	AB	186.74	AB	93.68	0.40
G8342GLS/IT		180.14	AB	177.53	ABC	101.47	0.84

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

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

inherited by progeny thereof. By "transient transformation" is intended that a polynucleotide construct introduced into a plant does not integrate into the genome of the plant.

For the transformation of plants and plant cells, the nucleotide sequences of the invention are inserted using standard techniques into any vector known in the art that is suitable for expression of the nucleotide sequences in a plant or plant cell. The selection of the vector depends on the preferred transformation technique and the target plant species to be transformed. In an embodiment of the invention, an AHASL nucleotide sequence is operably linked to a plant promoter that is known for high-level expression in a plant cell, and this construct is then introduced into a plant that is susceptible to an imidazolinone or sulfonylurea herbicide and a transformed plant is regenerated. The transformed plant is tolerant to exposure to a level of an imidazolinone or sulfonylurea herbicide that would kill or significantly injure an untransformed plant. This method can be applied to any plant species; however, it is most beneficial when applied to crop plants.

Methodologies for constructing plant expression cassettes and introducing foreign nucleic acids into plants are generally known in the art and have been previously described. For example, foreign DNA can be introduced into plants, using tumor-inducing (Ti) plasmid vectors. Agrobacterium based transformation techniques are well known in the art. The Agrobacterium strain (e.g., *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*) comprises a plasmid (Ti or Ri plasmid) and a T-DNA element which is transferred to the plant following infection with Agrobacterium. The T-DNA (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the Agrobacterium-mediated transformation are described, for example, in Horsch RB *et al.* (1985) *Science* 225:1229f. The Agrobacterium-mediated transformation can be used in both dicotyledonous plants and monocotyledonous plants. The transformation of plants by *Agrobacteria* is described in White FF, *Vectors for Gene Transfer in Higher Plants*; Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B *et al.* (1993) *Techniques for Gene Transfer, in: Transgenic Plants*, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, pp.

128-143; Potrykus (1991) *Annu Rev Plant Physiol Plant Molec Biol* 42:205- 225. Other methods utilized for foreign DNA delivery involve the use of PEG mediated protoplast transformation, electroporation, microinjection whiskers, and biolistics or microprojectile bombardment for direct DNA uptake. Such methods are known in the art. (U.S. Pat. No. 5,405,765 to Vasil *et al.*; Bilang *et al.* (1991) *Gene* 100: 247-250; Scheid *et al.* (1991) *Mol. Gen. Genet.*, 228: 104-112; Guerche *et al.* (1987) *Plant Science* 52: 111-116; Neuhause *et al.* (1987) *Theor. Appl Genet.* 75: 30-36; Klein *et al.* (1987) *Nature* 327: 70-73; Howell *et al.* (1980) *Science* 208:1265; Horsch *et al.* (1985) *Science* 227: 1229-1231; DeBlock *et al.* (1989) *Plant Physiology* 91: 694-701; *Methods for Plant Molecular Biology* (Weissbach and Weissbach, eds.) Academic Press, Inc. (1988) and *Methods in Plant Molecular Biology* (Schuler and Zielinski, eds.) Academic Press, Inc. (1989). The method of transformation depends upon the plant cell to be transformed, stability of vectors used, expression level of gene products and other parameters.

Other suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection as Crossway *et al.* (1986) *Biotechniques* 4:320-334, electroporation as described by Riggs *et al.* (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606, *Agrobacterium*-mediated transformation as described by Townsend *et al.* U.S. Patent No. 5,563,055, Zhao *et al.* U.S. Patent No. 5,981,840, direct gene transfer as described by Paszkowski *et al.* (1984) *EMBO J.* 3:2717-2722, and ballistic particle acceleration as described in, for example, Sanford *et al.* U.S. Patent No. 4,945,050; Tomes *et al.* U.S. Patent No. 5,879,918; Tomes *et al.* U.S. Patent No. 5,886,244; Bidney *et al.* U.S. Patent No. 5,932,782; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); McCabe *et al.* (1988) *Biotechnology* 6:923-926); and Lec1 transformation (WO 00/28058). *Also see*, Weissinger *et al.* (1988) *Ann. Rev. Genet.* 22:421-477; Sanford *et al.* (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou *et al.* (1988) *Plant Physiol.* 87:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology* 6:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh *et al.* (1998) *Theor. Appl. Genet.* 96:319-324 (soybean); Datta *et al.*

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2.

2. An isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) amino acid-substituted polypeptide selected from:

a) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glutamic acid, valine, tryptophan, or tyrosine substitution at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

b) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a cysteine, glutamic acid, arginine, threonine, tryptophan, or tyrosine substitution at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

c) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a glycine, alanine, valine, isoleucine, methionine, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, or histidine substitution at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

3. An isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) amino acid-substituted polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine substitution at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, and an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine substitution at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2.

4. The isolated polynucleotide of any one of claims 1 to 3, wherein said polynucleotide is a polynucleotide of a plant selected from *Arabidopsis thaliana*, maize, wheat, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, sunflower, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, perennial grass, and forage crops.

5. An isolated polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) amino acid-substituted polypeptide selected from:

a) a polypeptide having glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine substitution at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

b) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and a valine, cysteine, aspartic, acid, glutamic acid, arginine, threonine, tryptophan, or tyrosine substitution at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

c) a polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and any amino acid substitution at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2,

wherein the isolated polynucleotide is a polynucleotide of a plant selected from *Arabidopsis thaliana*, maize, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, and perennial grass.

6. An expression vector comprising the polynucleotide of any one of claims 1-5.

7. A plant transformed with the expression vector of claim 6.

8. The plant of claim 7, wherein the plant is a monocot.

9. The plant of claim 7, wherein the plant is a dicot.

10. The plant of claim 7, wherein the plant is selected from *Arabidopsis thaliana,* maize, wheat, rye, oat, triticale, rice, barley, sorghum, millet, sugarcane, soybean, sugar beet, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, sunflower, squash, pepper, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, perennial grass, and forage crops.

11. The plant of any one of claims 7-10, wherein expression of the polynucleotide in the plant results in tolerance to an herbicide selected from the imidazolinones, the sulfonylureas, the triazolopyrimidines, and the pyrimidinyloxybenzoates.

12. A seed of the plant of any one of claims 7-11, wherein the seed comprises the polynucleotide.

13. A purified AHASL protein encoded by the polynucleotide of any one of claims 1-5.

14. An isolated plant comprising a polynucleotide encoding an acetohydroxyacid synthase large subunit (AHASL) polypeptide having a glutamine substitution at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2.

15. An isolated plant comprising:

a) a first polynucleotide encoding a first AHASL polypeptide having a first amino acid substitution and a second polynucleotide encoding a second AHASL polypeptide having a second amino acid substitution, or

b) a polynucleotide encoding an AHASL polypeptide having both said first and second amino acid substitutions;

wherein said first and second substitutions are selected from:

i) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a glutamic acid, valine, tryptophan, or tyrosine for the amino acid at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

ii) the first substitution being glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a cysteine, glutamic acid, arginine, threonine, tryptophan, or tyrosine for the amino acid at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

iii) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a glycine, alanine, valine, isoleucine, methionine, phenylalanine, proline, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, or histidine for the amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2.

16. An isolated plant comprising:

a) a first polynucleotide encoding a first AHASL polypeptide having a first amino acid substitution, and a second polynucleotide encoding a second AHASL polypeptide having second amino acid substitution and a third amino acid substitution;

b) a first polynucleotide encoding a first AHASL polypeptide having the first amino acid substitution and the second amino acid substitution, and a second polynucleotide encoding a second AHAASL polypeptide having the third amino acid substitution;

c) a first polynucleotide encoding a first AHASL polypeptide having the first amino acid substitution and the third amino acid substitution, and a second polynucleotide encoding a second AHASL polypeptide having the second amino acid substitution; or

d) a polynucleotide encoding an AHASL polypeptide having the first, second, and third amino acid substitutions;

wherein said first amino acid substitution is a glutamine, for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2, said second amino acid substitution is a serine, alanine, glutamic acid, leucine, glutamine,

arginine, valine, tryptophan, tyrosine, or isoleucine for the amino acid at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2, and said third amino acid substitution is an alanine, glutamic acid, serine, phenylalanine, threonine, aspartic acid, cysteine, or asparagine for the amino acid at a position corresponding to position 199 of SEQ ID NO:1 or position 167 of SEQ ID NO:2.

17. The isolated plant of any one of claims 14-16, wherein the plant is a monocot.

18. The isolated plant of any one of claims 14-16, wherein the plant is a dicot.

19. The isolated plant of any one of claims 14-16, wherein said plant is selected from *Arabidopsis thaliana*, maize, wheat, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, sunflower, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, perennial grass, and forage crops.

20. An isolated plant comprising:

a) a first polynucleotide encoding a first AHASL polypeptide having a first amino acid substitution and a second polynucleotide encoding a second AHASL polypeptide having a second amino acid substitution, or

b) a polynucleotide encoding an AHASL polypeptide having both said first and second amino acid substitutions;

wherein said first and second substitutions are selected from:

i) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a serine, alanine, glutamic acid, leucine, glutamine, arginine, valine, tryptophan, tyrosine, or isoleucine for the amino acid at a position corresponding to position 197 of SEQ ID NO:1 or position 165 of SEQ ID NO:2;

ii) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being a valine, cysteine, aspartic acid, glutamic acid, arginine,

threonine, tryptophan, or tyrosine for the amino acid at a position corresponding to position 205 of SEQ ID NO:1 or position 173 of SEQ ID NO:2; or

iii) the first substitution being a glutamine for the amino acid at a position corresponding to position 122 of SEQ ID NO:1 or position 90 of SEQ ID NO:2 and the second substitution being any amino acid for the amino acid at a position corresponding to position 574 of SEQ ID NO:1 or position 542 of SEQ ID NO:2,

wherein the isolated plant is selected from *Arabidopsis thaliana*, maize, rye, oat, triticale, rice, barley, sorghum, millet, sugar beet, sugarcane, soybean, peanut, cotton, rapeseed, canola, *Brassica* species, manihot, melon, squash, pepper, tagetes, solanaceous plants, potato, sweet potato, tobacco, eggplant, tomato, *Vicia* species, pea, alfalfa, coffee, cacao, tea, *Salix* species, oil palm, coconut, and perennial grass.

21. A seed of the isolated plant of any one of claims 14-20, wherein the seed comprises the AHASL-encoding polynucleotide(s).

22. A method for controlling weeds in a field, said method comprising:

growing, in a field, the isolated plant of any one of claims 7-11 and 14-20; and

contacting said plant and weeds in the field with an effective amount of an AHAS-inhibiting herbicide to which the plant is tolerant, thereby controlling the weeds.

23. The method of claim 22, wherein the AHAS-inhibiting herbicide comprises one or more of an imidazolinone herbicide, a sulfonylurea herbicide, a triazolopyrimidine herbicide, and a pyrimidinyloxybenzoate herbicide.

24. The method of claim 23, wherein the AHAS-inhibiting herbicide comprises an imidazolinone herbicide.

25. The method of claim 24, wherein the imidazolinone herbicide comprises one or more of imazethapyr, imazapic, imazamox, and imazapyr.

26. A method of producing a transgenic plant comprising the steps of:

transforming a plant cell with the expression vector of claim 6; and

regenerating from the plant cell a plant that expresses the AHASL polypeptide.

27. A method of identifying or selecting a plant cell, plant tissue, plant seed, or plant or part thereof comprising:

providing a plant cell, plant tissue, plant seed, or plant or part thereof, wherein said plant cell, plant tissue, plant seed, or plant or part thereof comprises the polynucleotide of any one of claims 1-5;

contacting the plant cell, plant tissue, plant seed, or plant or part thereof with at least one AHAS-inhibiting compound; and

determining whether the plant cell, plant tissue, plant seed, or plant or part thereof is affected by the AHAS-inhibiting compound, thereby identifying or selecting the plant cell, plant tissue, plant seed, or plant or part thereof.

28. The method of claim 27, wherein the AHAS-inhibiting compound comprises one or more of an imidazolinone herbicide, a sulfonylurea herbicide, a triazolopyrimidine herbicide, and a pyrimidinyloxybenzoate herbicide.

29. The method of claim 28, wherein the AHAS-inhibiting compound comprises an imidazolinone herbicide.

30. The method of claim 29, wherein the imidazolinone herbicide comprises one or more of imazethapyr, imazapic, imazamox, and imazapyr.

31. A method for producing a herbicide-tolerant plant comprising crossing a first plant that is resistant to a herbicide to a second plant that is not resistant to the herbicide, wherein the first plant is the plant of any one of claims any one of claims 7-11 and 14-20.

32. The method of claim 31 further comprising selecting for a progeny plant that is resistant to the herbicide.

33. A seed of the plant of any one of claims 7-11 and 14-20, wherein said seed is treated with a seed treatment formulation.

34. The seed of claim 33, wherein the seed treatment formulation comprises an AHAS-inhibiting herbicide.

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35. The seed of claim 34, wherein the AHAS-inhibiting herbicide comprises one or more of an imidazolinone herbicide, a sulfonylurea herbicide, a triazolopyrimidine herbicide, and a pyrimidinyloxybenzoate herbicide.

36. The seed of claim 35, wherein the AHAS-inhibiting herbicide comprises an imidazolinone herbicide.

37. The seed of claim 36, wherein the imidazolinone herbicide comprises one or more of imazethapyr, imazapic, imazamox, and imazapyr.

38. A method for combating undesired vegetation comprising contacting, with an AHAS-inhibiting herbicide, a seed of the plant of any one of claims 7-10 and 14-20 before sowing and/or after pregermination.

39. The method of claim 38, wherein the AHAS-inhibiting herbicide comprises one or more of an imidazolinone herbicide, a sulfonylurea herbicide, a triazolopyrimidine herbicide, and a pyrimidinyloxybenzoate herbicide.

40. The method of claim 40, wherein the AHAS-inhibiting herbicide comprises an imidazolinone herbicide.

41. The method of claim 41, wherein the imidazolinone herbicide comprises one or more of imazethapyr, imazapic, imazamox, and imazapyr.

42. An agricultural product produced from the plant of any one of claims 7-10 and 14-20 or the seed of any one of claims 12 and 21.

43. The agricultural product of claim 42, wherein the product is seed oil.

44. A method for identifying a plant according to any one of claims 7-10 and 14-20, said method comprising:

(A) providing biological material from a plant,

(B) performing PCR or hybridization testing of the AHASL genes in said biological material to determine if the biological material comprises the polynucleotide(s) of the plant of any one of claims 7-10 and 14-20, and

(C) identifying, based on the results of step (B), that the plant of step (A) is a plant according to any one of claims 7-10 and 14-20

45. The method of claim 44, wherein said biological material is a plant seed.

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FIGURE 1 (Sheet 1 of 2)

Full length arabidopsis AHAS with conceptual translation and relevant amino acids highlighted. DNA numbering on the left, amino acid numbering on the right.

```
      MetAlaAlaAlaTh rThrThrThrThrTh rSerSerSerIleSe rPheSerThrLysPr oSerProSerSerS er   25
    1 ATGGCGGCGGCAAC AACAACAACAACAAC ATCTTCTTCGATCTC CTTCTCCAGCAAACC ATCTCCTTCCTCCTC C

      LysSerProLeuPr oIleSerArgPheSe rLeuProPheSerLe uAsnProAsnLysSe rSerSerSerSerAr g   50
   76 AAATCACCATTACC AATCTCCAGATTCTC CCTCCCATTCTCCCT AAACCCCAACAAATC ATCCTCCTCCTCCC GC

      ArgArgGlyIleLy sSerSerSerProSe rSerIleSerAlaVa lLeuAsnThrThrTh rAsnValThrThrTh r   75
  151 CGCCGCGGTATCAA ATCCAGCTCTCCCTC CTCCATCTCCGCCGT GCTCAACACAACCAC CAATGTCACAACCAC T

      ProSerProThrLy sProThrLys ProGluThrPheIleSerAr gPheAlaProAspG lnProArgLysGlyA la  100
  226 CCCTCTCCAACCAA ACCTACCAAACCCGA AACATTCATCTCCGG ATTCGCTCCAGATC AACCCCGCAAAGGCG CT
                      K83                                 Q95

      AspIleLeuValGl uAlaLeuGluArgGl nGlyValGluThrVa lPheAlaTyrProGl yGlyAlaSerMetGlu  125
  301 GATATCCTCGTCGA AGCTTTAGAACGTCA AGGCGTAGAAACCGT ATTCGCTTACCCTGG AGGTGCATCAATGGAG
                                                                      A122  M124

      IleHisGlnAlaLe uThrArgSerSerSe rIleArgAsnVal LeuProArgHisGluG lnGlyGlyValPheA la  150
  376 ATTCACCAAGCCT TAACCCGCTCTTCCT CAATCCGTAAC GTCCTTCCTCGTCACGAAC AAGGAGGTGTATTCG CA
                                          V139

      AlaGluGlyTyrAl aArgSerSerGlyLy sProGlyIleCysIleAlaThrSerGlyPr oGlyAlaThrAsnLe u  175
  451 GCAGAAGGATACGC TCGATCCTCAGGTAA ACCAGGTATCTGTA TAGCCACTTCAGGTC CCGGAGCTACAAATC TC

      ValSerGlyLeuAl aAspAlaLeuLeuAs pSerValProLeuVa lAlaIleThrGlyGl nValProArgArgMet  200
  526 GTTAGCCGATTAGC CGATGCGTTGTTAGA TAGTGTTCCTCTTGT AGCAATCACAGGACA AGTCCGTCGTCGTATG
                                                                      P197  R199

      IleGlyThrAspAlaPheGlnGluThrP roIleValGluValT hrArgSerIleThrL ysHisAsnTyrLeuV al  225
  601 ATTGGTACAGAT GCGTTTCAAGAGACTC CGATTGTTGAGGTAA CGCGTTCGATTACGA AGCATAACTATCTTG TG

      MetAspValGluAs pIleProArgIleIl eGluGluAlaPhePh eLeuAlaThrSerGl yArgProGlyProVa l  250
  676 ATGGATGTTGAAGA TATCCCTAGGATTAT TGAGGAAGCTTTCTT TTTAGCTACTTCTGG TAGACCTGGACCTGT T

      LeuValAspVal ProLysAspIleGlnG lnGlnLeuAlaIleP roAsnTrpGluGln AlaMetArgLeuProG ly  275
  751 TTGGTTGAT GTTCCTAAAGATATTCAAC AACAGCTTGCGATTC CTAATTGGGAA CAGGCTATGAGATTACCTG GT
            V254                                          Q269

      TyrMetSerArgMe tProLysProProGl uAspSerHisLeuGl uGlnIleValArgLe uIleSerGluSerLy s  300
  826 TATATGTCTAGGAT GCCTAAACCTCCGGA AGATTCTCATTTGGA GCAGATTGTTAGGT TGATTTCTGAGTCTA AG
        M277

      LysProValLeuTy rValGlyGlyGlyCy sLeuAsnSerSerA spGluLeuGlyArgP heValGluLeuThrG ly  325
  901 AAGCCTGTGTTGTA TGTTGGTGGTGGTTG TTTGAATTCTAGC GATGAATTGGGTAGGT TTGTTGAGCTTACGG GG
                                          D315

      IleProValAlaSe rThrLeuMetGlyLe uGlySerTyrProCy sAspAspGluLeuSe rLeuHisMetLeuGl y  350
  976 ATCCCTGTTGCGAG TACGTTGATGGGGCT GGGATCTTATCCTTG TGATGATGAGTTGTC GTTACATATGCTTGG A

      MetHisGlyThrVa lTyrAlaAsnTyrAl aValGluHisSerAs pLeuLeuLeuAlaPh eGlyValArgPheAsp  375
 1051 ATGCATGGGACTGT GTATGCAAATTACGC TGTGGAGCATAGTGA TTTGTTGTTGGCGTT TGGGGTAAGGTTT GAT
                                                                      D375

      AspArgValThrGl yLysLeuGluAlaPh eAlaSerArgAlaLy sIleValHisIleAs pIleAspSerAlaGlu  400
 1126 GATCGTGTCACGGG TAAGCTTGAGGCTTT TGCTAGTAGGGCTAA GATTGTTCATATTGA TATTGACTCGGCTGAG
                                                                      S398

      IleGlyLysAsnLy sThrProHisValSe rValCysGlyAspVa lLysLeuAlaLeuG lnGlyMetAsnLysVal  425
 1201 ATTGGGAAGAATA AGACTCCTCATGTGT CTGTGTGTGGTGATG TTAAGCTGGCTTTGC AAGGGATGAATAAGG TT
                                          K416
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FIGURE 1 (Sheet 2 of 2)

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      LeuGluAsnArgAlaGluGluLeuLysLeuAspPheGlyValTrpArgAsnGluLeuAsnValGlnLysGlnLys 450
1276  CTTGAGAACCGAGCGGAGGAGCTTAAGCTTGATTTTGGAGTTTGGAGGAATGAGTTGAACGTACAGAAACAGAAG
      L426          A430                          V439       N442       N445

      ProLeuSerPheLysThrPheGlyGluAlaIleProProGlnTyrAlaIleLysValLeuAspGluLeuThr 475
1351  TTTCCGTTGAGCTTTAAGACGTTTGGGGAAGCTATTCCTCCACAGTATGCGATTAAGGTCCTTGATGAGTTGACT

      AspGlyLysAlaIleIleSerThrGlyValGlyGlnHisGlnMetTrpAlaAlaGlnPheTyrAsnTyrLysLys 500
1426  GATGGAAAAGCCATAATAAGTACTGGTGTCGGGCAACATCAAATGTGGGCGGCGCAGTTCTACAATTACAAGAAA

      ProArgGlnTrpLeuSerSerGlyLeuGlyAlaMetGlyPheGlyLeuProAlaAlaIleGlyAlaSerVal 525
1501  CCAAGGCAGTGGCTATCATCAGGAGGCCTTGGAGCTATGGGATTTGGACTTCCTGCTGCGATTGGAGCGTCTGTT

      AlaAsnProAspAlaIleValValAspIleAspGlyAspGlySerPheIleMetAsnValGlnGluLeuAlaThr 550
1576  GCTAACCCTGATGCGATAGTTGTGGATATTGACGGAGATGGAAGCTTTATAATGAATGTGCAAGAGCTAGCCACT

      IleArgValGluAsnLeuProValLysValLeuLeuLeuAsnAsnGlnHisLeuGlyMetValMetGlnTrpGlu 575
1651  ATTCGTGTAGAGAACTTCCCAGTGAAGGTACTTTACTAAACAACCAGCATCTTGGCATGGTTATGCAATGGGAA
                                                                  W574

      AspArgPheTyrLysAlaAsnArgAlaHisThrPheLeuGlyAspProAlaGlnGluAspGluIlePheProAsn 600
1726  GATCGGTTCTACAAAGCTAACCGAGCTCACACATTTCTCGGGGATCCGGCTCAGGAGGACGAGATATTCCCGAAC
            K580                                          D595

      MetLeuLeuPheAlaAlaAlaCysGlyIleProAlaAlaArgValThrLysLysAlaAspLeuArgGluAlaIle 625
1801  ATGTTGCTGTTTGCAGCAGCTTGCGGGATTCCAGCCGGCGAGGGTGACAAAGAAAGCAGATCTCCGAGAAGCTATT

      GlnThrMetLeuAspThrProGlyProTyrLeuLeuAspValIleCysProHisGlnGluHisValLeuProMet 650
1876  CAGACAATGCTGGATACACCAGGACCTTACCTGTTGGATGTGATTTGTCCGCACCAAGAACATGTGTTGCCGATG

      IleProSerGlyGlyThrPheAsnAspValIleThrGluGlyAspGlyArgIleLysTyr***              670
1951  ATCCCGAGTGGTGGCACTTTCAACGATGTCATAACGGAAGGAGATGGCCGGATTAAATACTGA
            S653/G654
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FIGURE 2 (Sheet 1 of 2)

Full length maize AHAS with conceptual translation and relevant amino acids highlighted. DNA numbering on the left, amino acid numbering on the right

```
     MetAlaThrAlaAlaAl aAlaSerThrAlaLe uThrGlyAlaThrTh rAlaAlaProLysAl aArgArgArgAla   25
   1 ATGGCCACCGCCGCCGC CGCGTCTACCGCGCT CACTGGCGCCACTAC CGCTGCGCCCAAGGC GAGGCGCCGGGCG

     MisLeuLeuAlaThrArgArgAlaLeuAlaAl aProIleArgCysSe rAlaAlaSerProAl aMetProMetAla   50
  76 CACCTCCTGGCCACCCG CCGCGCCCTCGCCGC GCCCATCAGGTGCTC AGCGGCGTCACCCGC CATGCCGATGGCT

     ProProAlaThrProLe uArgProTrpGlyPr oThrAspProArgLysGlyAlaAspIleLe uValGluSerLeu   75
 151 CCCCCGGCCACCCCGCT CCGCCCGTGGGGCCC CACCGATCCCCGCAA GGGCGCCGACATCCT CGTCGAGTCCCTC
     P51                                D63

     GluArgCysGlyValAr gAspValPheAlaTy rProGlyGlyAlaSe rMetGluIleHisGl nAlaLeuThrArg  100
 226 GAGCGCTGCGGCGTCCG CGACGTCTTCGCCTA CCCCGGCGGCGCGTC CATGGAGATCCACCA GGCACTCACCCGC
                                       A90   N92

     SerProValIleAlaAsnHisLeuPheArgHi sGluGlnGlyGluAl aPheAlaAlaSerGl yTyrAlaArgSer  125
 301 TCCCCCGTCATCGCCAACCACCTCTTCCGCCA CGAGCAAGGGGAGGC CTTTGCGGCCTCCGG CTACGCGCCGCTCC
                 H107

     SerGlyArgValGlyVa lCysIleAlaThrSe rGlyProGlyAlaTh rAsnLeuValSerAl aLeuAlaAspAla  150
 376 TCGGGCCGCGTCGGCGT CTGCATCGCCACCTC CGGCCCCGGCGCCAC CAACCTTGTCTCCGC GCTCGCCGACGCG

     LeuLeuAspSerValPr oMetValAlaIleTh rGlyGlnValProAr gArgMetIleGlyTh rAspAlaPheGln  175
 451 CTGCTCGATTCCGTCCC CATGGTCGCCATCAC GGGACAGGTGCCGCG ACGCATGATTGGCAC CGACGCCTTCCAG
                                       P165  R167            A173

     GluThrProIleValGl uValThrArgSerIl eThrLysHisAsnTy rLeuValLeuAspVa lAspAspIlePro  200
 526 GAGACGCCCATCGTCGA GGTCACCCGCTCCAT CACCAAGCACAACTA CCTGGTCCTCGACGT CGACGACATCCCC

     ArgValValGlnGluAl aPhePheLeuAlaSe rSerGlyArgProGl yProValLeuValAs pIleProLysAsp  225
 601 CGCGTCGTGCAGGAGGC TTTCTTCCTCGCCTC CTCTGGTCGACCGGG GCCGGTGCTTGTCGA CATCCCCAAGGAC
                                                      I222

     IleGlnGlnGlnMetAl aValProValTrpAs pLysProMetSerLe uProGlyTyrIleAlaArgLeuProLys  250
 676 ATCCAGCAGCAGATGGC GGTGCCTGTCTGGGA CAAGCCCATGAGTCT GCCTGGGTACATTGCGCGCCTTCCCAAG
                                       K237            I245

     ProProAlaThrGluLe uLeuGluGlnValLe uArgLeuValGlyGl uSerArgArgProVa lLeuTyrValGly  275
 751 CCCCCTGCGACTGAGTT GCTTGAGCAGGTGCT GCGTCTTGTTGGTGA ATCCCGGCGCCCTGT TCTTTATGTTGGC

     GlyGlyCysAlaAlaSe rGlyGluGluLeuArgArgPheValGluLe uThrGlyIleProVa lThrThrThrLeu  300
 826 GGTGGCTGCGCAGCATC TGGTGAGGAGTTGCG ACGCTTTGTGGAGCT GACTGGAATCCCGGT CACAACTACTCTT
                 E283

     MetGlyLeuGlyAsnPh eProSerAspAspPr oLeuSerLeuArgMe tLeuGlyMetHisGl yThrValTyrAla  325
 901 ATGGGCCTCGGCAACTT CCCCAGCGACGACCC ACTGTCTCTGCGCAT GCTAGGTATGCATGG CACGGTGTATGCA

     AsnTyrAlaValAspLys AlaAspLeuLeuLeu AlaLeuGlyValArg PheAspAspArgVa lThrGlyLysIle  350
 976 AATTATGCAGTGGATAA GGCCGATCTGTTGCT TGCACTTGGTGTGCG GTTTGATGATCGTGT GACAGGGAAGATT
                                       D343

     GluAlaPheAlaSerAr gAlaLysIleValHi sValAspIleAspPr oAlaGluIleGlyLy sAsnLysGlnPro  375
1051 GAGGCTTTTGCAAGCAG GGCTAAGATTGTGCA CGTTGATATTGAT CCGGCTGAGATTGGCAA GAACAAGCAGCCA
                                       P366

     HisValSerIleCysAl aAspValLysLeuAla LeuGlnGlyMetAsn AlaLeuLeuGluGlySerThrSerLys  400
1126 CATGTGTCCATCTGTGC AGATGTTAAGCTTGCT TTGCAGGGCATGAAT GCTCTTCTTGAAGGAAGCACATCAAAG
                 K384                                 L394          T398

     LysSerPheAspPheGl ySerTrpAsnAspGluLeuAspGlnGlnLy sArgGluPheProLe uGlyTyrLysThr  425
1201 AAGAGCTTTGACTTTGG CTCATGGAACGATGAGTTGGATCAGCAGAA GAGGGAATTCCCCCT TGGGTATAAAACA
                 S407      D410      D413
```

FIGURE 2 (Sheet 2 of 2)

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     SerAsnGluGluIleGl nProGlnTyrAlaIl eGlnValLeuAspGl uLeuThrLysGlyGl uAlaIleIleGly  450
1276 TCTAATGAGGAG ATCCAGCCACAATAT GCTATTCAGGTTCTT GATGAGCTGACGAAA GGCGAGGCCATCATC GGC

     ThrGlyValGlyGlnHi sGlnMetTrpAlaAl aGlnTyrTyrThrTy rLysArgProArgGl nTrpLeuSerSer  475
1351 ACAGGTGTTGGGCAGCA CCAGATGTGGGCGGC ACAGTACTACACTTA CAAGCGGCCAAGGCA GTGGTTGTCTTCA

     AlaGlyLeuGlyAlaMe tGlyPheGlyLeuPr oAlaAlaAlaGlyAl aSerValAlaAsnPr oGlyValThrVal  500
1426 GCTGGTCTTGGGGCTAT GGGATTTGGTTTGCC GGCTGCTGCTGGTGC TTCTGTGGCCAACCC AGGTGTTACTGTT

     ValAspIleAspGlyAs pGlySerPheLeuMe tAsnValGlnGluLe uAlaMetIleArgIl eGluAsnLeuPro  525
1501 GTTGACATCGATGGAGA TGGTAGCTTTCTCAT GAACGTTCAGGAGCT AGCTATGATCCGAAT TGAGAACCTCCCG

     ValLysValPheValLe uAsnAsnGlnHisLe uGlyMetValValGl nTrpGluAspArgPh eTyrLysAlaAsn  550
1576 GTGAAGGTCTTTGTGCT AAACAACCAGCACCT GGGGATGGTGGTGCA GTGGGAGGACAGGTT CTATAAGGCCAAC
                                                  W542                K548

     ArgAlaHisThrTyrLe uGlyAsnProGluAs nGluSerGluIleTyrProAspPheValTh rIleAlaLysGly  575
1651 AGACCGCACACATACTT GGGAAACCCAGAGAA TGAAAGTGAGATATA TCCAGATTTCGTGAC GATCGCCAAAGGG
                                    S563

     PheAsnIleProAlaVa lArgValThrLysLy sAsnGluValArgAl aAlaIleLysLysMe tLeuGluThrPro  600
1726 TTCAACATTCCAGCGGT CCGTGTGACAAAGAA GAACGAAGTCCGCGC AGCGATAAAGAAGAT GCTCGAGACTCCA

     GlyProTyrLeuLeuAs pIleIleValProHi sGlnGluHisValLeu ProMetIleProSerGlyGlyAlaPhe  625
1801 GGGCCGTACCTCTTGGA TATAATCGTCCCACA CCAGGAGCATGTGTT GCCTATGATCCCTAG TGGTGGGGCTTTC
                                                                S621/G622

     LysAspMetIleLeuAs pGlyAspGlyArgTh rValTyr***
1876 AAGGATATGATCCTGGA TGGTGATGGCAGGAC TGTGTACTGA
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FIGURE 3 (Sheet 1 of 7)

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   1-AtAHASL  .......MAA ATTTTTTSSS ISFSTKPSPS SSKSPLPISR FSLPFSLNPN
   2-ZmAHASL2 .........M ATAAAASTAL TGATTAAP.. .......... .KARRRAHLL
  3-BnAHASL1A .......... .MAAATSSSP ISLTAKPS.. .SKSPLPISR FSLPFSLTPQ
   4-OsAHASL  ........MA TTAAAAAAAL SAAATAKTG. .......... RKNHQRHHVL
   5-HaAHASL1 ........MA APPNPSISFK PPSPAAALP. ..PRSAFLPR FALPITSTTQ
   6-HaAHASL2 ......MAAI HPPHPSITAK PPPSSAAA.. ....VALPPH FAFSITSTSH
   7-HaAHASL3 .........M AVPLTFISGK PPFSATPS.. .....QLTTN LSLPLTLLPI
   8-ZmAHASL1 .........M ATAATAAAAL TGATTATP.. .......... .KSRRRAHHL
   9-AsAHASL  MVTLNHISSF TKPNKTYLQS SIYAIPFS.. ....NSLKPT SSSSILAALF
 10-BnAHASL1C .......... .MAAATSSSP ISLTAKPS.. .SKSPLPISR FSLPFSLTPQ
 11-BnAHASL2A .......... ......MASF SFFGTIPS.. ...SPTKASV FSLPVSVTTL
  12-CmAHASL1 .........M AAATTTSSSS IPFSTKHS.. SSKSPLPISR FTLPFSLNPN
  13-CmAHASL2 .........M AAATTTSSSS IPFSTKPS.. SSKSPLPISR FTLPFSLNPN
   14-SpAHASL .......... .......... .......... .......... ..........
   15-SbAHASL ........MA TTAAAAAAAL AGATTAAP.. .......... .KARRRAHLL
  16-StAHASL1 .......MAA ASPSPCFSKN LPPSSSKS.. ....SILLPK STFTFHNHPK
  17-StAHASL2 ......MAAA ASPSPCFSKT LPPSSSKS.. ....STILPR STFPFHNHPQ
   18-GmAHASL .......... ..MAATASRT TRFSSSSS.. HPTFPKRITR STLPLSHQTL
   19-XsAHASL ......MAAI PHTNPSITTK PPSSPPRP.. .....TFLAR FTFPITSTSH
   20-LmAHASL .........M ATATSTAVAF SGATATLP.. .......... KPRTLPRHLL
 21-TaAHASL1A .......... .......... .......... .......... ..........
 22-TaAHASL1B .......... .......... .......... .......... ..........
 23-TaAHASL1D .......... .......... .......... .......... ..........
  24-GHAHASA5 .......... MAAATANSAL PKLSTLTS.. SFKSSIPISK SSLPFSTTPQ
 25-GHAHASA19 .......... MAAATSNSAL PKLSTLTS.. SFKSSIPISK SSLPFSTTPQ
      26-IlvB .......... .......... .......... .......... ..........
      27-IlvG .......... .......... .......... .......... ..........
      28-IlvI .......... .......... .......... .......... ..........
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                                                           83
   1-AtAHASL  KSSSSS.RRR GIKSSSPSSI SAVLNTTTNV TTT.PSPT.. KPTKPETFIS
   2-ZmAHASL2 ATR....... .......RAL AAPIRCSAAS PAMP...... .MAPPATPLR
  3-BnAHASL1A KPSS...... ..RLHRPLAI SAVLNSPVNV AP.......E KTDKIKTFIS
   4-OsAHASL  PARG...... .......RVG AAAVRCSAVS PVTPP..... SPAPPATPLR
   5-HaAHASL1 KRHR...... ....LHISNV LSDSK....S TTTTT.TTTQ RPLPVQPFVS
   6-HaAHASL2 KRHR...... ....LHISNV LSDST....T TTGAT..... .THPPPPFVS
   7-HaAHASL3 IPSK...... ....THVSKH LIITN....A IAKHS..... .HSHKAFVVS
   8-ZmAHASL1 ATR....... .......RAL AAPIRCSALS RATP...... .TAPPATPLR
   9-AsAHASL  KSHH...... ....LLLQSP KPKPP....S ATITQSPSSL TDDKPSSFVS
 10-BnAHASL1C KDSS...... ..RLHRPLAI SAVLNSPVNV APP.SP...E KTIKMKTFVS
 11-BnAHASL2A PSFP...... ...RRRATRV SVSANSKKDQ DRT.AS.... RRFNPSTFSS
  12-CmAHASL1 KSSSSSSKRR GIKSTALS.I SAVLNTTTNV STT.TPQSKP TKPPKKKFVS
  13-CmAHASL2 KSSS...RRR GIKSTSLS.I SAVLNTTTNV STT.TPPSKP TKPPKKKFVS
   14-SpAHASL .......... .......... .......... .......... ..........
   15-SbAHASL AAR....... .......RAL AAPIRC.... .SAAPPATLT VTAPPATPLR
  16-StAHASL1 KTSP...... ....LHLTHT QHHSR.FTVS NVILSTTTHN DVSPPEIFVS
  17-StAHASL2 KASP...... ....LHLTHT HHHRRGFAVS NVVISTTTHN DVSPPETFVS
   18-GmAHASL TKPN...... .....HALKI KCSIS..... KPPTAAPFTK EAPRTEPFVS
   19-XsAHASL KRHR...... ....LHISNV LSDSK..... .....PTITH SPLPTESFIS
   20-LmAHASL PSSR...... .......RAL AAPIRC.... ...SAVSPSP SPAPPATALR
 21-TaAHASL1A .......... ........LP ARVVRC.... ..CAASPAAT SAAPPATALR
 22-TaAHASL1B .......... ........LP ARIVRC.... ..CAASPAAT SAAPPATALR
 23-TaAHASL1D .......... ........LP ARIVRC.... ..CAASPAAT SAAPPATALR
  24-GHAHASA5 KPTP...... ....YRSFDV SCSLSHASSN PRSAATSVTP KNAPPHDFIS
 25-GHAHASA19 KPTP...... ....YRSFDV SCSLSHASSN PRNPAASVTQ KTAPPHYFIS
      26-IlvB .......... .......... .......... .......... .....MASSG
      27-IlvG .......... .......... .......... .......... ..........
      28-IlvI .......... .......... .......... .......... ..........
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FIGURE 3 (Sheet 2 of 7)

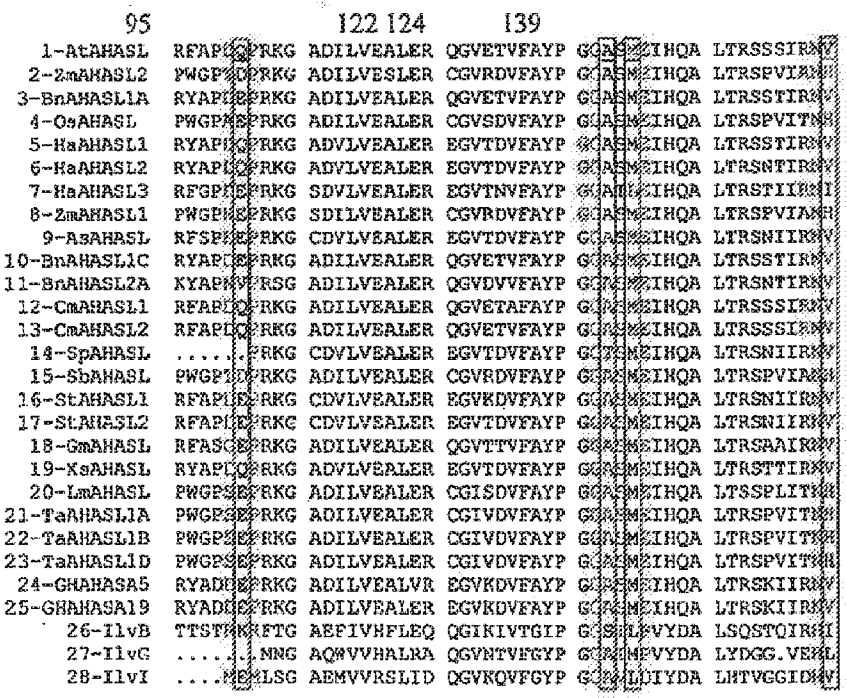

FIGURE 3 (Sheet 3 of 7)

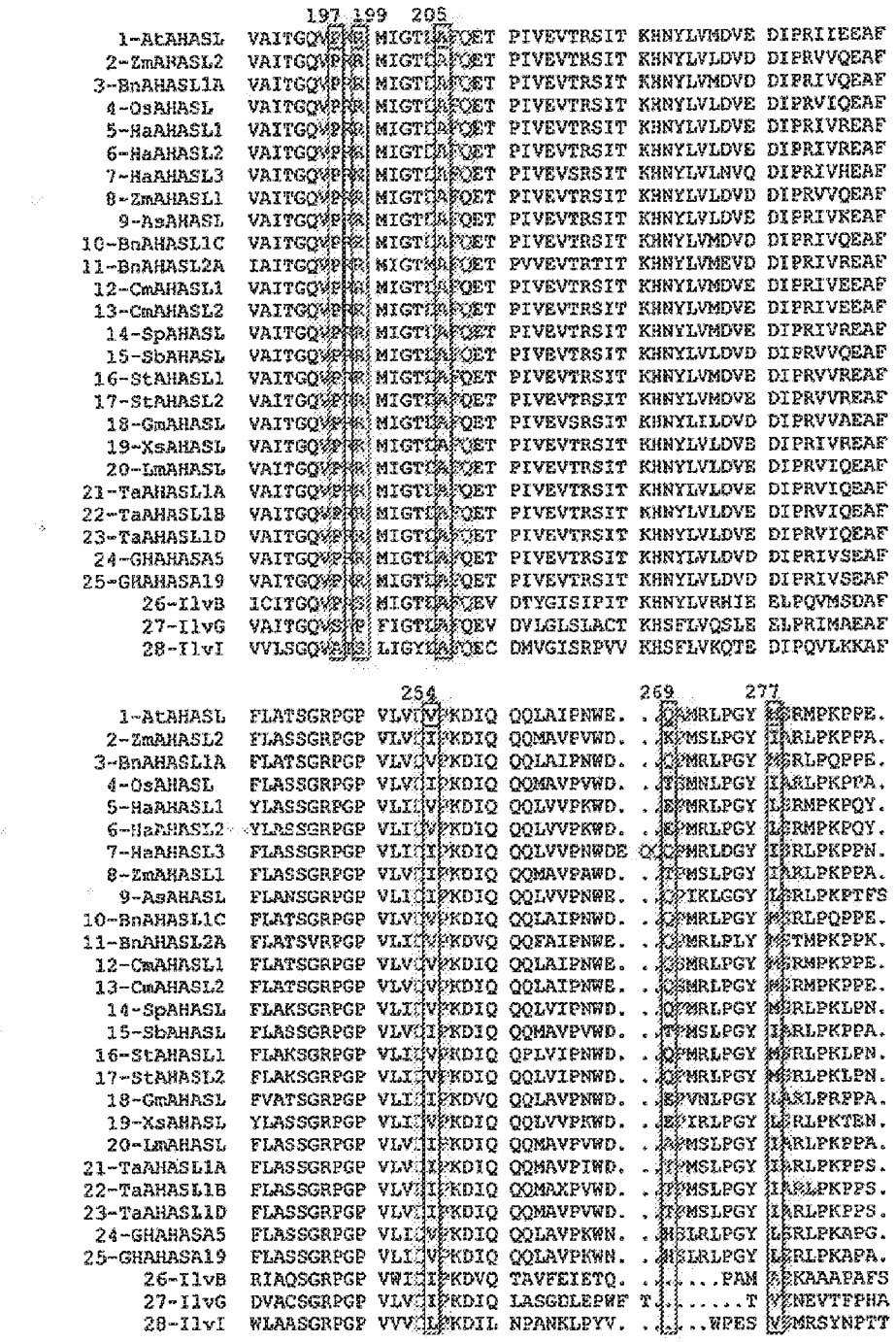

FIGURE 3 (Sheet 4 of 7)

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                                                                  313
 1-AtAHASL     ...DSHLEQI VRLISESKKP VLYVGGGC.. LNSSDELGRF VELTGIPVAS
 2-ZmAHASL2    ...TELLEQV LRLVGESRRP VLYVGGGC.. AASGEELRRF VELTGIPVTT
 3-BnAHASL1A   ...VSQLGQI VRLISESKRP VLYVGGGS.. LNSSEELGRF VELTGIPVAS
 4-OsAHASL     ...TELLEQV LRLVGESRRP ILYVGGGC.. SASGDELRWF VELTGIPVTT
 5-HaAHASL1    ...DGHLEQI VRLVGEAKRP VLYVGGGC.. LNSCDELRRF VELTGIPVAS
 6-HaAHASL2    ...DGHLEQI VRLVGEAKRP VLYVGGGC.. LNSCDELRRF VELTGIPVAS
 7-HaAHASL3    ...ETHLRQI VRFIKESKRP VLYVGGGC.. MNSSDELGRF VELTGIPVAN
 8-ZmAHASL1    ...TEFLEQV LRLVGESRRP VLYVGGGC.. AASGEELCRF VELTGIPVTT
 9-AsAHASL     ANEEGLLDQI VRLVGESKRP VLYTGGGC.. LNSSEELRKF VELTGIPVAS
10-BnAHASL1C   ...VSQLGQI VRLISESKRP VLYVGGGS.. LNSSEELGRF VELTGIPVAS
11-BnAHASL2A   ...VSHLEQI LRLVSESKRP VLYVGGGC.. LNSSEELRRF VELTGIPVAS
12-CmAHASL1    ...DSHLEQI VRLISESKKP VLYVGGGC.. LNSSEELGRF VELTGIPVAS
13-CmAHASL2    ...DSHLEQI VRLISESKKP VLYVGGGC.. LNSSEELGRF VELTGIPVAS
14-SpAHASL     ...EMLLEQI VRLISESKKP VLYVGGGC.. SQSSEELRRF VELTGIPVAS
15-SbAHASL     ...TELLEQV LRLVGESRRP VLYVGGGC.. AASGEELRRF VEMTGIPVTT
16-StAHASL1    ...EMLLEQI VRLISESKKP VLYVGGGC.. TQSSEELRRF VELTGIPVAS
17-StAHASL2    ...EMLLEQI IRLISESKKP VLYVGGGC.. LQSSEELRRF VELTGISVAS
18-GmAHASL     ...EAQLEHI VRLIMEAQKP VLYVGGGS.. LNSSAELRRF VELTGIPVAS
19-XsAHASL     ...NGQLEHI VRLVSEAKRP VLYVGGGC.. LNSGDELRRF VELTGIPVAS
20-LmAHASL     ...TELLEQV LRLVGEERRP ILYVGGGC.. SASGEEVRRF VELTGIPVTT
21-TaAHASL1A   ...TESLEQV LRLVGESRRP ILYVGGGC.. AASGEELRRF VELTGIPVTT
22-TaAHASL1B   ...TESLEQV LRLVGESRRP ILYVGGGC.. AASGEELRRF VELTGIPVTT
23-TaAHASL1D   ...TESLEQV LRLVGESRRP ILYVGGGC.. AASGEELRRF VELTGIPVTT
24-GHAHASA5    ...EAHLEQI VRLVSESKKP VLYVGGGC.. LNSSEELKRF VELTGIPVAS
25-GHAHASA19   ...EAHLEQI VRLVSESKKP VLYVGGGC.. LNSSEELKRF VELTGIPVAS
26-IlvB        ...EESIRDA AAMINAAKRP VLYLGGGV.. INAAARVREL AEKAQLPTTM
27-IlvG        .....EVEQA RQMLAKAQKP MLYVGGGVGM AQASPELREF LAATKMPATC
28-IlvI        TGHKGQIKRA LQTLVAAKKP VVYVGGGAIT AGCGQELKET VEALNLPVVC

                                                                375
 1-AtAHASL     TLMGLGSYPC D.DELSLHML GMHGTVYANY AVEHSDLLLA FGVRFDDRVT
 2-ZmAHASL2    TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA LGVRFDDRVT
 3-BnAHASL1A   TLMGLGSYPC N.DELSLQML GMHGTVYANY AVEHSDLLLA FGVRFDDRVT
 4-OsAHASL     TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
 5-HaAHASL1    TLMGLGAYPA S.SDLSLHML GMHGTVYANY AVDKSDLLLA FGVRFDDRVT
 6-HaAHASL2    TLMGLGAYPA S.SDLSLHML GMHGTVYANY AVDKSDLLLA FGVRFDDRVT
 7-HaAHASL3    TLMGLGTYPG S.HDLSLHML GMHGTVYANY AIDKSDLLLA FGVRFDDRVT
 8-ZmAHASL1    TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
 9-AsAHASL     TLMGLGAFPC T.DDLSLQML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
10-BnAHASL1C   TLMGLGSYPC N.DELSLQML GMHGTVYANY AVEHSDLLLA FGVRFDDRVT
11-BnAHASL2A   TFMGLGSYPC DDEEFSLQML GMHGTVYANY AVEYSDLLLA FGVRFDDRVT
12-CmAHASL1    TLMGLGAYPC D.DELSLHML GMHGTVYANY SVEHSDLLLA FGVRFDDRVT
13-CmAHASL2    TLMGLGAYPC D.DELSLHML GMHGTVYANY SVEHSDLLLA FGVRFDDRVT
14-SpAHASL     TLMGLGAFPT G.DELSLQML GMHGTVYANY AVDSSDLLLA FGVRFCDRVT
15-SbAHASL     TLMGLGNFPG D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
16-StAHASL1    TLMGLGAFPV G.DELSLQML GMHGTVYANY AVDSSDLLLA FGVRFDDRVT
17-StAHASL2    TLMGLGAFPT G.DELSLQML GMHGTVYANY AVDGSDLLLA FGVRFDDRVT
18-GmAHASL     TLMGLGTFPI G.DEYSLQML GMHGTVYANY AVDNSDLLLA FGVRFDDRVT
19-XsAHASL     TLMGLGAYPA S.SDLSLHML GMHGTVYANY AVDKSDLLLA FGVRFDDRVT
20-LmAHASL     TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
21-TaAHASL1A   TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
22-TaAHASL1B   TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
23-TaAHASL1D   TLMGLGNFPS D.DPLSLRML GMHGTVYANY AVDKADLLLA FGVRFDDRVT
24-GHAHASA5    TLMGLGAFPI S.DDLSLQML GMHGTVYANY AVDKSDLLLA FGVRFDDRVT
25-GHAHASA19   TLMGLGAFPI S.DELSLQML GMHGTVYANY AVDKSDLLLA FGVRFDDRVT
26-IlvB        TLMALGMLPK A.HPLSLGML GMHGVRSTNY ILQEADLLIV LGARFDDRAI
27-IlvG        TLKGLGAVEA D.YPYYLGML GMHGTKAANF AVQECDLLIA VGARFDDRVT
28-IlvI        SLMGLGAFPA T.HRQALGML GMHGTYEANM THHNADVIFA VGVRFDDRTT
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FIGURE 3 (Sheet 5 of 7)

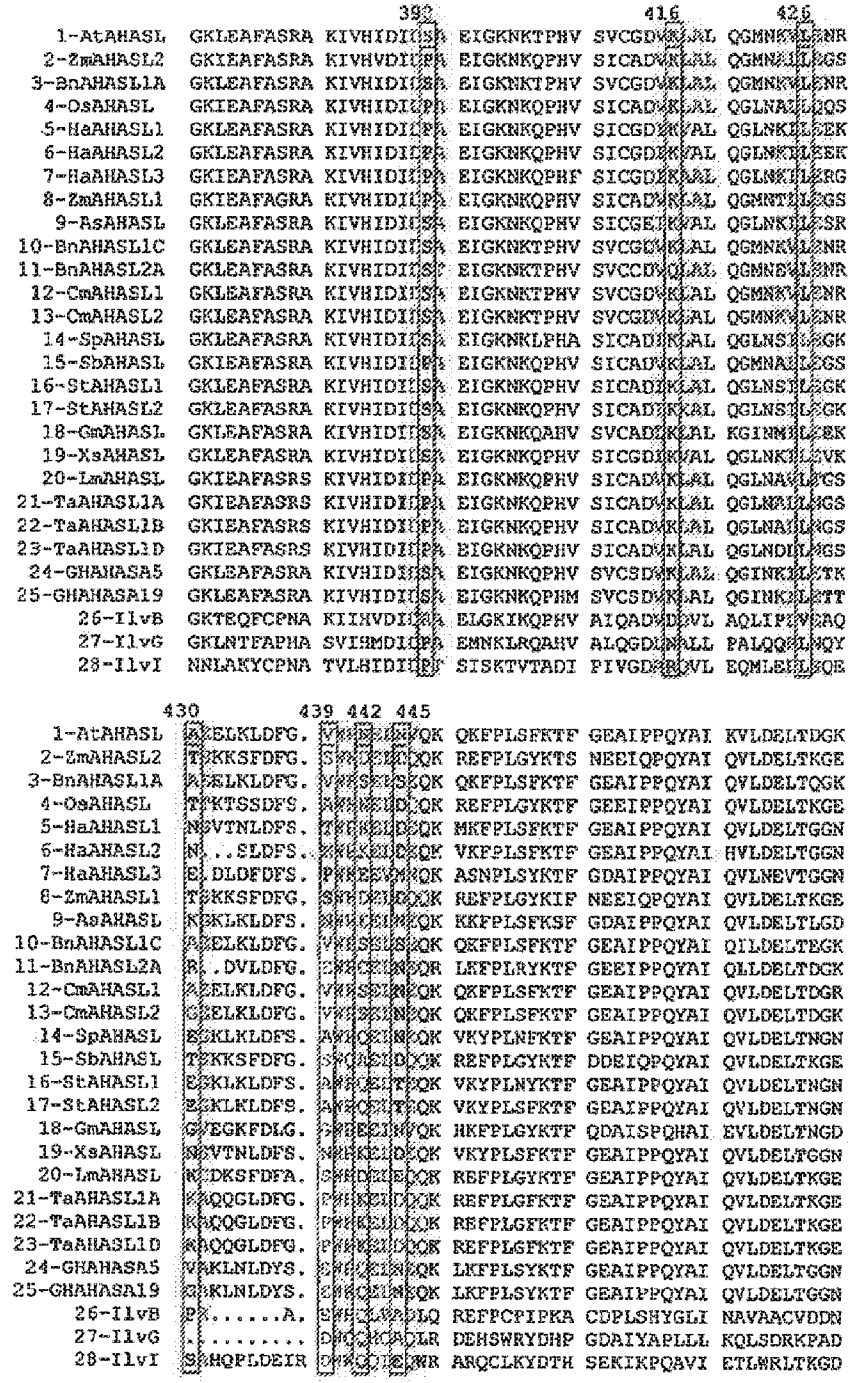

FIGURE 3 (Sheet 6 of 7)

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 2-ZmAHASL2 AIIGTGVGQH QMWAAQYYTY KRPRQWLSSA GLGAMGFGLP AAAGASVANP
 3-BnAHASL1A AIISTGVGQH QMWAAQFYKY RKPRQWLSSS GLGAMGFGLP AAIGASVANP
 4-OsAHASL  AIIATGVGQH QMWAAQYYTY KRPRQWLSSA GLGAMGFGLP AAAGASVANP
 5-HaAHASL1 AIISTGVGQH QMWAAQFYKY NKPRQWLTSG GLGAMGFGLP AAIGAAVARP
 6-HaAHASL2 AIISTGVGQH QMWAAQFYKY NKPRQWLTSG GLGAMGFGLP AAIGAAVARP
 7-HaAHASL3 AIITTGVGQH QMWSAQFYKY NRPRQWLTSA GLGAMGFGLP AAIGAAVARP
 8-ZmAHASL1 AIIATGVGQH QMWAAQYYTY KRPRQWLSSA GLGAMGFGLP AAAGAAVANP
 9-AsAHASL  AIVSTGVGQH QMWAAQFYKY RNPRQWLTSG GLGAMGFGLP ACYGAAVARP
10-BnAHASL1C AIISTGVGQH QMWAAQFYKY RKPRQWLSSS GLGAMGFGLP AAIGASVANP
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12-CmAHASL1 AIISTGVGQH QMWAAQFYKY KKPRQWLSSA GLGAMGFGLP AAIGASVANP
13-CmAHASL2 AIISTGVGQH QMWAAQFYKY KKPRQWLSSA GLGAMGFGLP AAIGASVANP
14-SpAHASL  AIISTGVGQH QMWAAQFYKY KKPRQWLTSG GLGAMGFGLP AAMGAAVGRP
15-SbAHASL  AIIATGVGQH QMWAAQYYTY KRPRQWLSSA GLGAMGFGLP AAAGAAVANP
16-StAHASL1 AIISTGVGQH QMWAAQYYKY KKPRQWLTSG GLGAMGFGLP AAIGAAVGRP
17-StAHASL2 AIISTGVGQH QMWAAQYYKY KKPRQWLTSG GLGAMGFGLP AAIGAAVGRP
18-GmAHASL  AIVSTGVGQH QMWAAQFYKY KRPRQWLTSG GLGAMGFGLP AAIGAAVANP
19-XsAHASL  AIISTGVGQH QMWAAQFYKY NKPRQWLTSG GLGAMGFGLP AAIGAAVARP
20-LmAHASL  AIIATGVGQH QMWAAQYYTY KRPRQVLSSA GLGAMGFGLP AAAGTAVANP
21-TaAHASL1A AIIATGVGQH QMWAAQYYTY KRPRQWLSSS GLGAMGFGLP AAAGAAVANP
22-TaAHASL1B AIIATGVGQH QMWAAQYYTY KRPRQWLSSS GLGAMGFGLP AAAGAAVANP
23-TaAHASL1D AIIATGVGQH QMWAAQYYTY KRPRQWLSSS GLGAMGFGLP AAAGAAVANP
24-GHAHASA5 AIISTGVGQH QMWAAQFYKY KKPRQWLTSG GLGAMGFGLP AAIGAAVANP
25-GHAHASA19 AIISTGVGQH QMWAAQFYKY KKPRQWLTSG GLGAMGFGLP AAIGAAVANP
    26-IlvB AIITTDVGQH QMWTAQAYPL NRPRQWLTSG GLGTMGFGLP AAIGAALANP
    27-IlvG CVVTTDVGQH QMWAAQHIAH TRPENFITSS GLGTMGFGLP AAVGAQVARP
    28-IlvI AYVTSDVGQH QMFAALYYPF DKPRRWINSG GLGTMGFGLP AALGVKMALP

                                                        S74
 1-AtAHASL  DAIVVDIDGD GSFIMNVQEL ATIRVENLPV KVLLLNNQHL GMVMQWEDRF
 2-ZmAHASL2 GVTVVDIDGD GSFLMNVQEL AMIRIENLPV KVFVLNNQHL GMVVQWEDRF
 3-BnAHASL1A DAIVVDIDGD GSFIMNVQEL ATIRVENLPV KILLLNNQHL GMVMQWEDRF
 4-OsAHASL  GVTVVDIDGD GSFLMNIQEL ALIRIENLPV KVMVLNNQHL GMVVQWEDRF
 5-HaAHASL1 DAVVVDIDGD GSFMMNVQEL ATIRVENLPV KILLLNNQHL GMVVQWEDRF
 6-HaAHASL2 DAVVVDIDGD GSFMMNVQEL ATIRVENLPV KILLLNNQHL GMVVQWEDRF
 7-HaAHASL3 DAVVVDIDGD GSFMMNVQEL ATIRVENLPV KMKVLNNQHL GMVVQWEDRF
 8-ZmAHASL1 GVTVVDIDGD GSFLMNIQEL AMIRIENLPV KVFVLNNQHL GMVVQWEDRF
 9-AsAHASL  DAVVVDIDGD GSFIMNVQEL ATIRVENLPV KIMLLNNQHL GMVVQLEDRF
10-BnAHASL1C DAIVVDIDGD GSFIMNVQEL ATIRVENLPV KILLLNNQHL GMVMQWEDRF
11-BnAHASL2A GAVVVDIDGD GSFIMNIQEL ATIRVENLPV KVLLINNQHL GMVLQWEDHF
12-CmAHASL1 DSIVVDIDGD GSFIMNVQEL ATIRVENLPV KILILNNQHL GMVMQWEDRF
13-CmAHASL2 DAVVVDIDGD GSFIMNVQEL ATIRVENLPV KILILNNQHL GMVMQWEDRF
14-SpAHASL  GEIVVDIDGD GSFMMNVQEL ATIKVENLPV KIMLLNNQHL GMVVQWEDRF
15-SbAHASL  GITVVDIDGD GSFLMNIQEL AMIRIENLPV KVFVLNNQHL GMVVQWEDRF
16-StAHASL1 GEIVVDIDGD GSFIMNVQEL ATIKVENLPV KIMLLNNQHL GMVVQWEDRF
17-StAHASL2 GEIVVDIDGD GSFIMNVQEL ATIKVENLPV KIMLLNNQHL GMVVQWEDRF
18-GmAHASL  GAVVVDIDGD GSFIMNVQEL ATIRVENLPV KILLLNNQHL GMVVQWEDRF
19-XsAHASL  DAVVVDIDGD GSFIMSVQEL ATIRVENLPV KILLLNNQHL GMVVQLEDRF
20-LmAHASL  GVTVVDIDGD GSFLMNIQEL ALIRIENLPV KVMILNNQHL GMVVQWEDRF
21-TaAHASL1A GVTVVDIDGD GSFLMNIQEL ALIRIENLPV KVMILNNQHL GMVVQWEDRF
22-TaAHASL1B GVTVVDIDGD GSFLMNIQEL ALIRIENLPV KVMILNNQHL GMVVQWEDRF
23-TaAHASL1D GVTVVDIDGD GSFLMNIQEL ALIRIENLPV KVMILNNQHL GMVVQWEDRF
24-GHAHASA5 EAVVVDIDGD GSFIMNVQEL ATIRVENLPV KILLLNNQHL GMVVQWEDRF
25-GHAHASA19 EAVVVDIDGD GSFIMNVQEL ATIRVENLPV KILLLNNQHL GMVVQWEDRF
    26-IlvB DRKVLCFSGD GSLMMNIQEN ATASENQLDV KIILMNNEAL GLVHQEQSLF
    27-IlvG NDTVVCISGD GSFMMNVQEL GTVKRKQLPL KIVLLDNQRL GMVRQWQQLF
    28-IlvI EETVVCVTGD GSIQMNIQEL STALQYELPV LVVNLNNRYL GMVKQWQDMI
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FIGURE 3 (Sheet 7 of 7)

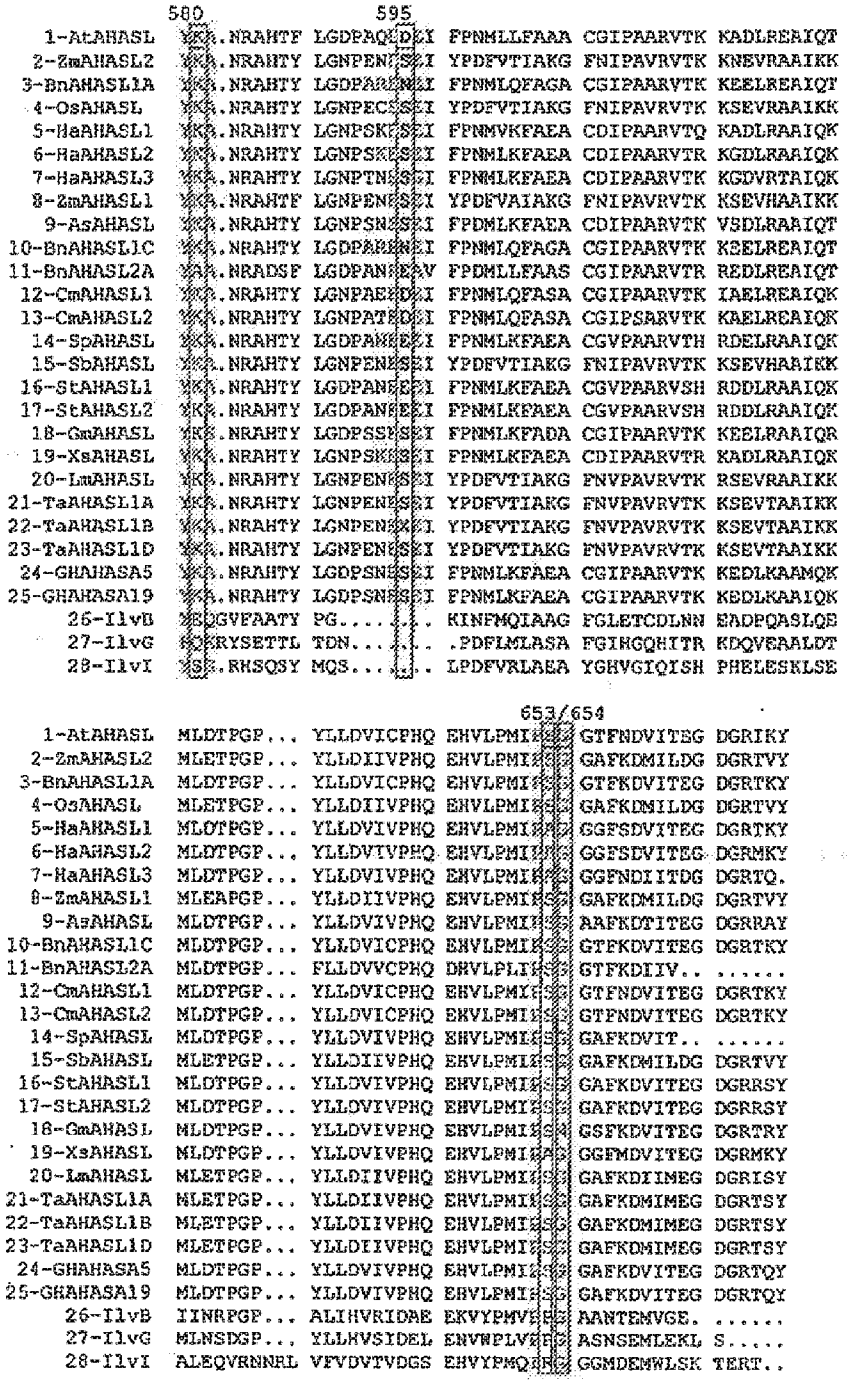


FIGURE 4

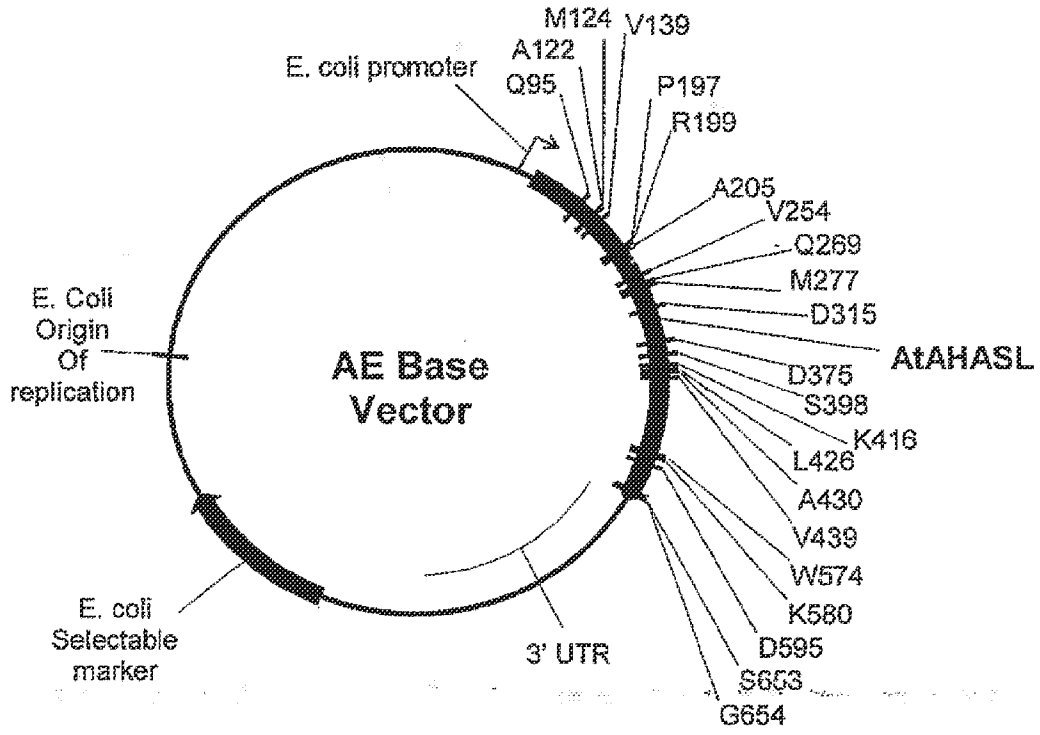
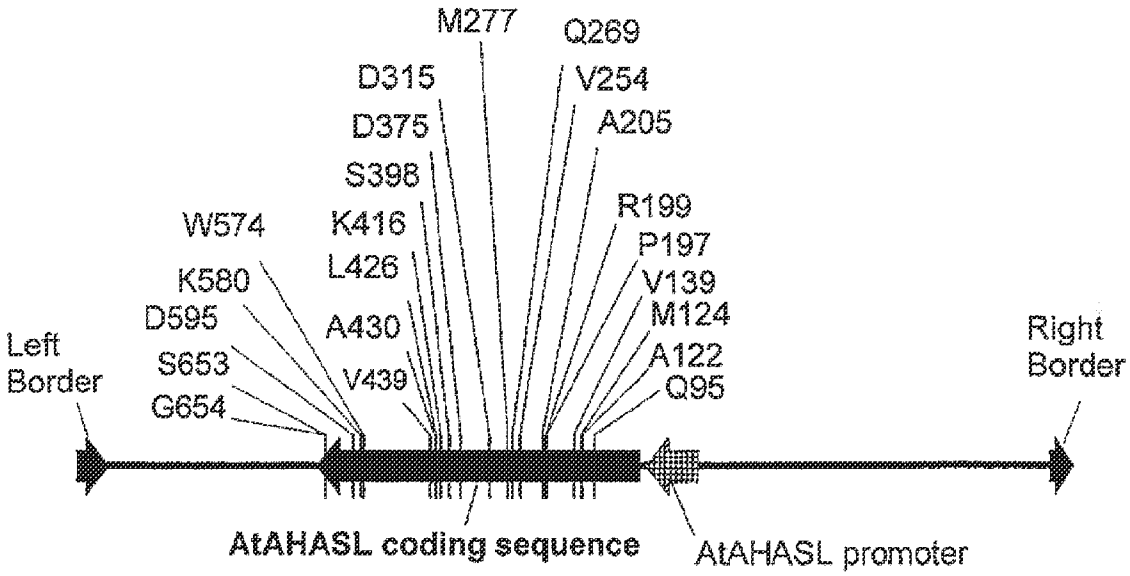


FIGURE 5



AP Base Vector
T-DNA

FIGURE 6

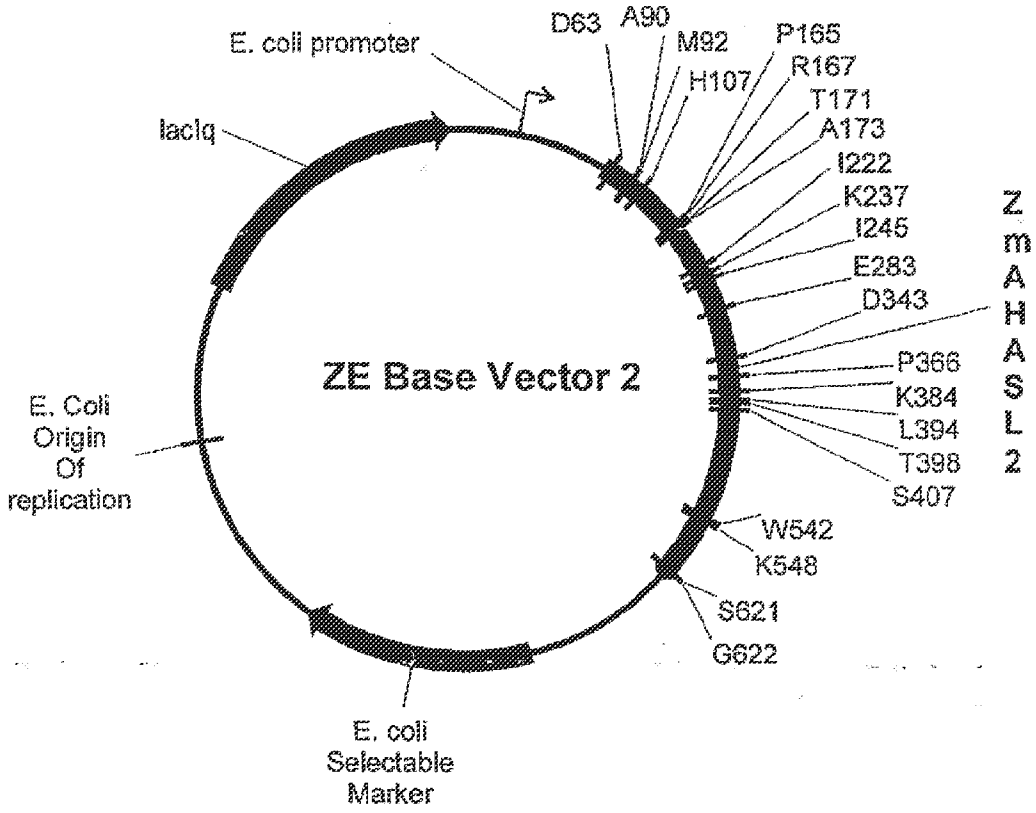
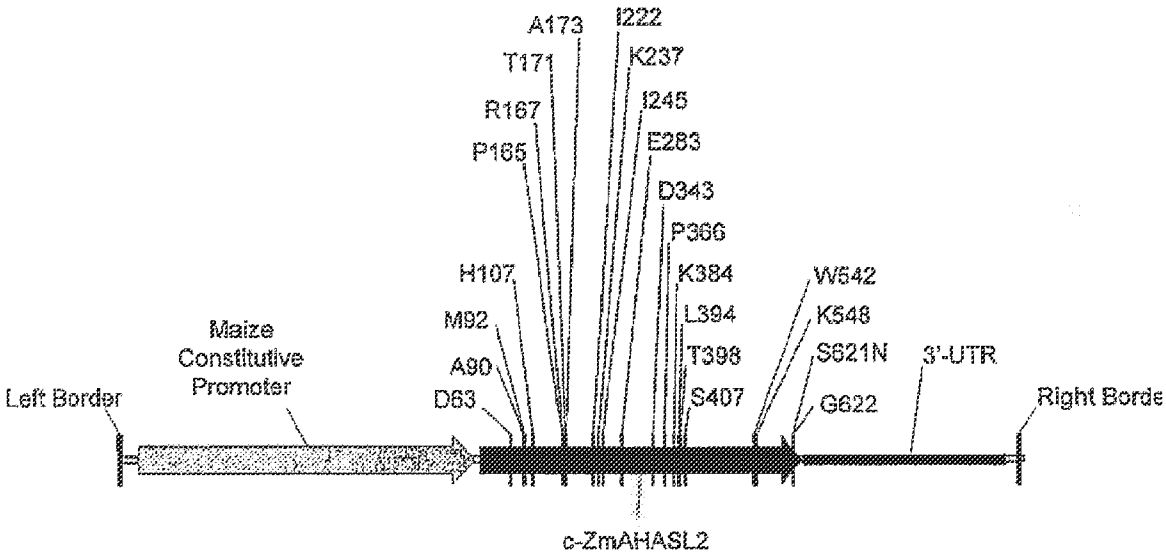


FIGURE 7



New ZP Base Vector T-DNA Map

5417 bp

FIGURE 8 (Sheet 1 of 4)

SeqID	Specific AHAS	AHAS Residues						
1	AtAHASL	K83	Q95	A122	M124	V139	P197	R199
2	ZmAHASL2	P51	D63	A90	M92	H107	P165	R167
3	BnAHASL1A	K65	E77	A104	M106	V121	P179	R181
4	OsAHASL	P57	E69	A96	M98	H113	P171	R173
5	HaAHASL1	P68	Q80	A107	M109	V124	P182	R184
6	HaAHASL2	H62	Q74	A101	M103	V118	P176	R178
7	HaAHASL3	H58	E70	A97	L99	I114	P172	N174
8	ZmAHASL1	P51	E63	A90	M92	H107	P165	R167
9	AsAHASL	K74	E86	A113	M115	V130	P188	R190
10	BnAHASL1C	K68	E80	A107	M109	V124	P182	R184
11	BnAHASL2A	N59	V71	A98	M100	V115	P173	R175
12	CmAHASL1	E81	Q93	A120	M122	V137	P195	R197
13	CmAHASL2	E78	Q90	A117	M119	V134	P192	R194
14	SpAHASL Partial	?	?	A27	M29	V44	P102	R104
15	SbAHASL	P54	D66	A93	M95	H110	P168	R170
16	StAHASL1	E70	E82	A109	M111	V126	P184	R186
17	StAHASL2	E72	E84	A111	M113	V128	P186	R188
18	GmAHASL	T64	E76	A103	M105	V120	P178	R180
19	XsAHASL	P61	Q73	A100	M102	V117	P175	R177
20	LmAHASL	P53	E65	A92	M94	H109	P167	R169
21	TaAHASL1A Partial	P20	E32	A59	M61	H76	P134	R136
22	TaAHASL1B Partial	P20	E32	A59	M61	H76	P134	R136
23	TaAHASL1D Partial	P20	E32	A59	M61	H76	P134	R136
24	GHAHASA5	P72	E84	A111	M113	V128	P186	R188
25	GHAHASA19	P72	E84	A111	M113	V128	P186	R188
26	IlvB	nd	K11	S38	L40	I55	P113	S115
27	IlvG	nd	-	A26	M28	L42	S100	P102
28	IlvI	nd	E2	A29	L31	V46	S104	S106

FIGURE 8 (Sheet 2 of 4)

SeqID	Specific AHAS	AHAS Residues						
1	AtAHASL	A205	V254	Q269	M277	D315	D375	S398
2	ZmAHASL2	A173	I222	K237	I245	E283	D343	P366
3	BnAHASL1A	A187	V236	Q251	M259	E297	D357	S380
4	OsAHASL	A179	I228	T243	I251	D289	D349	P372
5	HaAHASL1	A190	V239	E254	L262	D300	D360	P383
6	HaAHASL2	A184	V233	E248	L256	D294	D354	P377
7	HaAHASL3	A180	I229	Q247	I255	D293	D353	P376
8	ZmAHASL1	A173	I222	T237	I245	E283	D343	P366
9	AsAHASL	A196	I245	Q260	L268	E310	D370	S393
10	BnAHASL1C	A190	V239	Q254	M262	E300	D360	S383
11	BnAHASL2A	A181	V230	Q245	M253	E291	D352	S375
12	CmAHASL1	A203	V252	Q267	M275	E313	D373	S396
13	CmAHASL2	A200	V249	Q264	M272	E310	D370	S393
14	SpAHASL Partial	A110	V159	Q174	M182	E220	D280	S303
15	SbAHASL	A176	I225	T240	I248	E286	D346	P369
16	StAHASL1	A192	V241	Q256	M264	E302	D362	S385
17	StAHASL2	A194	V243	Q258	M266	E304	D364	S387
18	GmAHASL	A186	I235	E250	L258	A296	D356	S379
19	XsAHASL	A183	V232	E247	L255	D293	D353	S376
20	LmAHASL	A175	I224	A239	I247	E285	D345	P368
21	TaAHASL1A Partial	A142	I191	T206	I214	E252	D312	P335
22	TaAHASL1B Partial	A142	I191	T206	I214	E252	D312	P335
23	TaAHASL1D Partial	A142	I191	T206	I214	E252	D312	P335
24	GHAHASA5	A194	V243	H258	L266	E304	D364	S387
25	GHAHASA19	A194	V243	H258	L266	E304	D364	S387
26	IlvB	A121	I170	nd	A188	A227	D287	R310
27	IlvG	A106	I157	nd	V175	P214	D274	P297
28	IlvI	A112	L161	nd	V180	Q224	D284	P307

FIGURE 8 (Sheet 3 of 4)

SeqID	Specific AHAS	AHAS Residues						
1	AtAHASL	K416	L426	A430	V439	N442	N445	W574
2	ZmAHASL2	K384	L394	T398	S407	D410	D413	W542
3	BnAHASL1A	K398	L408	A412	V421	S424	S427	W556
4	OsAHASL	K390	L400	T404	A413	N416	D419	W548
5	HaAHASL1	K401	L411	N415	T424	K427	D430	W559
6	HaAHASL2	K395	L405	N409	N415	K418	D421	W550
7	HaAHASL3	K394	L404	E408	P416	E419	M422	W551
8	ZmAHASL1	K384	L394	T398	S407	D410	D413	W542
9	AsAHASL	K411	L421	K425	N434	E437	N440	W569
10	BnAHASL1C	K401	L411	A415	V424	S427	S430	W559
11	BnAHASL2A	Q393	L403	R407	E414	C417	N420	W549
12	CmAHASL1	K414	L424	A428	V437	S440	N443	W572
13	CmAHASL2	K411	L421	G425	V434	S437	N440	W569
14	SpAHASL Partial	K321	L331	E335	A344	Q347	N350	W479
15	SbAHASL	K387	L397	T401	S410	A413	D416	W545
16	StAHASL1	K403	L413	E417	A426	Q429	T432	W561
17	StAHASL2	K405	L415	E419	A428	Q431	T434	W563
18	GmAHASL	K397	L407	G411	G420	E423	N426	W555
19	XsAHASL	K394	L404	N408	N417	K420	D423	W552
20	LmAHASL	K386	L396	K400	S409	D412	E415	W544
21	TaAHASL1A Partial	K363	L363	K367	T376	K379	D382	W511
22	TaAHASL1B Partial	K353	L363	K367	T376	K379	D382	W511
23	TaAHASL1D Partial	K353	L363	K367	T376	K379	D382	W511
24	GHAHASA5	K405	L415	V419	E428	Q431	N434	W563
25	GHAHASA19	K405	L415	G419	E428	Q431	N434	W563
26	IlvB	D328	V338	P342	E349	Q348	A351	Q480
27	IlvG	N315	L325	Y328	D349	Q352	A332	W464
28	IlvI	R325	L335	S339	E345	Q352	E355	W484

FIGURE 8 (Sheet 4 of 4)

SeqID	Specific AHAS	AHAS Residues			
1	AtAHASL	K580	D595	S653	G654
2	ZmAHASL2	K548	S563	S621	G622
3	BnAHASL1A	K562	N577	S635	G635
4	OsAHASL	K554	S569	S627	G628
5	HaAHASL1	K565	S580	A638	G638
6	HaAHASL2	K556	S571	A629	G630
7	HaAHASL3	K557	S572	A630	G631
8	ZmAHASL1	K548	S563	S621	G622
9	AsAHASL	K575	S590	S648	G649
10	BnAHASL1C	K565	N580	S638	G638
11	BnAHASL2A	A555	E570	S628	G629
12	CmAHASL1	K578	D593	S651	G652
13	CmAHASL2	K575	D590	S648	G649
14	SpAHASL Partial	K485	E500	S558	G559
15	SbAHASL	K551	S566	S624	G625
16	StAHASL1	K567	E582	S640	G641
17	StAHASL2	K569	E584	S642	G642
18	GmAHASL	K561	S576	S634	N635
19	XsAHASL	K558	S573	A631	G632
20	LmAHASL	K550	S565	S623	G632
21	TaAHASL1A Partial	K517	S532	S590	G591
22	TaAHASL1B Partial	K517	S532	S590	G591
23	TaAHASL1D Partial	K517	S532	S590	G591
24	GHAHASA5	K569	S584	S642	G643
25	GHAHASA19	K569	S584	S642	G643
26	IlvB	E486	nd	P552	G553
27	IlvG	Q470	nd	P536	G537
28	IlvI	S490	nd	R559	G560

FIGURE 9

	SEQ ID NO:																										
Sequence Name (SEQ ID NO:)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
AtAHASL (SEQ ID NO:1)	100	67	68	66	76	76	72	67	72	68	76	92	92	72	67	76	76	74	75	66	67	67	67	78	76	38	39	35
ZmAHASL2 (SEQ ID NO:2)		100	70	90	70	71	69	95	68	70	65	68	68	70	94	70	70	69	69	87	85	85	85	71	71	43	44	40
BnAHASL1A (SEQ ID NO:3)			100	69	77	76	73	70	73	99	77	88	88	76	69	76	77	77	76	68	69	69	69	81	81	40	41	38
OsAHASL (SEQ ID NO:4)				100	71	71	69	90	68	69	65	67	68	70	90	70	70	68	69	88	87	87	87	70	71	42	43	40
HaAHASL1 (SEQ ID NO:5)					100	93	79	70	78	77	70	76	77	76	70	79	80	76	91	70	70	70	70	80	80	41	42	38
HaAHASL2 (SEQ ID NO:6)						100	79	70	78	76	70	75	76	77	70	80	80	75	91	69	70	70	70	79	79	42	43	39
HaAHASL3 (SEQ ID NO:7)							100	70	73	73	66	72	73	74	69	75	75	72	78	69	68	68	68	75	75	42	42	38
ZmAHASL1 (SEQ ID NO:8)								100	67	69	66	67	68	70	93	69	69	69	68	87	86	85	86	71	71	43	43	40
AsAHASL (SEQ ID NO:9)									100	73	67	72	73	75	68	78	78	73	78	68	69	68	69	76	75	40	40	36
BnAHASL1C (SEQ ID NO:10)										100	77	86	88	75	69	76	77	77	76	68	69	69	69	81	81	40	40	38
BnAHASL2A (SEQ ID NO:11)											100	74	75	72	65	70	70	69	69	64	65	65	65	73	73	41	41	38
CmAHASL1 (SEQ ID NO:12)												100	98	74	67	76	76	74	75	67	67	67	67	79	78	38	39	36
CmAHASL2 (SEQ ID NO:13)													100	74	68	77	77	75	76	68	68	68	68	80	80	39	40	36
SpAHASL (SEQ ID NO:14)														100	70	85	84	74	77	70	76	76	76	76	76	51	54	49
SbAHASL (SEQ ID NO:15)															100	70	69	70	70	88	87	87	87	71	71	43	43	40
SlAHASL1 (SEQ ID NO:16)																100	96	76	80	70	70	70	70	80	80	41	41	39
SlAHASL2 (SEQ ID NO:17)																	100	76	80	70	70	70	70	80	79	40	41	38
GmAHASL (SEQ ID NO:18)																		100	75	66	69	68	69	79	79	41	43	38
XsAHASL (SEQ ID NO:19)																			100	70	70	70	70	80	79	42	42	38
LmAHASL (SEQ ID NO:20)																				100	89	89	89	70	70	43	43	40
TaAHASL1A (SEQ ID NO:21)																					100	99	100	70	70	48	48	45
TaAHASL1B (SEQ ID NO:22)																						100	100	70	70	48	48	45
TaAHASL1D (SEQ ID NO:23)																							100	70	70	48	48	45
GHAHASA5 (SEQ ID NO:24)																								100	96	40	40	38
GHAHASA19 (SEQ ID NO:25)																									100	40	40	38
IlvB (SEQ ID NO:26)																										100	55	47
IlvG (SEQ ID NO:27)																											100	52
IlvM (SEQ ID NO:28)																												100

FIGURE 10

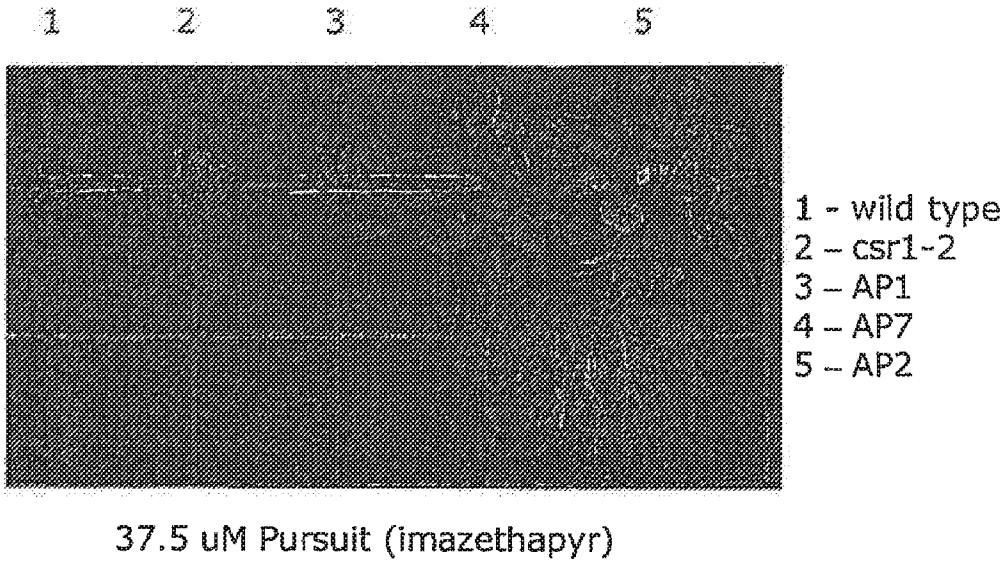


FIGURE 11

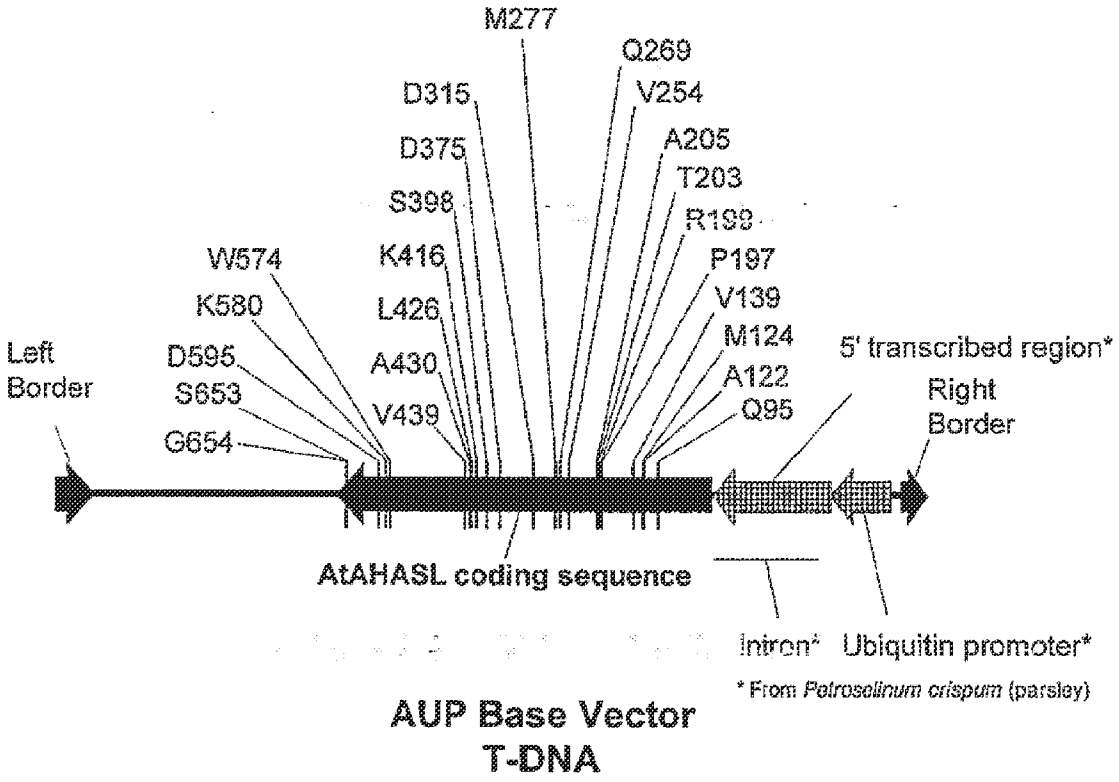
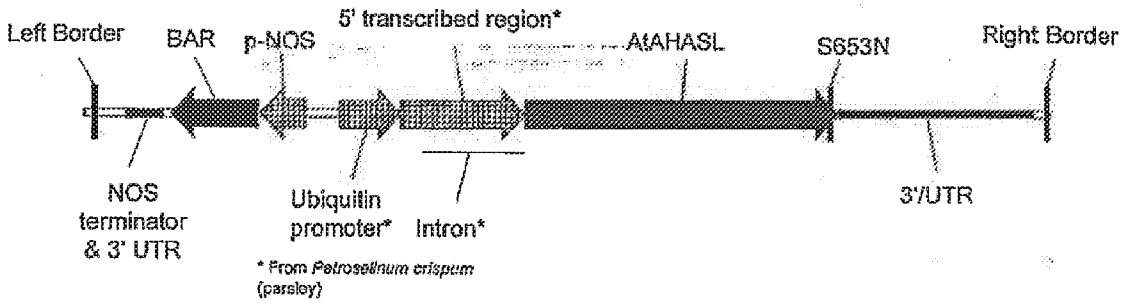


FIGURE 12



BAP1 T-DNA