Title: ALS INHIBITOR HERBICIDE TOLERANT B. NAPUS MUTANTS

Abstract: The present invention relates to an ALS inhibitor herbicide tolerant B. napus plant, progeny and parts thereof comprising a mutation of an acetolactate synthase I gene and an anitmutation of an acetolactate synthase III gene.
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ALS INHIBITOR HERBICIDE TOLERANT B. NAPUS MUTANTS

FIELD OF THE INVENTION

[1] This invention relates to herbicide-resistant *Brassica napus* plants, seed of such plants, parts thereof, progeny thereof as well as a method for their manufacture, and methods using such plants, and to crop protection by using ALS (acetolactate synthase; also known as AHAS (acetohydroxyacid synthase; EC 2.2.1.6; formerly EC 4.1.3.18)) inhibitor herbicides against unwanted vegetation in areas of growing such herbicide-resistant *Brassica* plants.

BACKGROUND OF THE INVENTION

[2] Since more than 40 years, herbicides are the preferred tools to control weeds in *B. napus*. The products used for this purpose, namely Metazachlor, Dimethachlor, Quinmerac, Clomazone, Metolachlor, Napropamide, Clopyralid, Propyzamide, Propaquizafop, Fluazifop and others allow suppressing weeds in *B. napus* fields without damaging the crop. Nevertheless, under adverse environmental conditions the efficacy of these products leaves room for improvements, especially if noxious weeds like *Geranium dissectum*, *Centaurea cyanus*, *Sinapis arvensis* and/or *Alopecurus myosuroides* germinate over an extended period of time.

[3] Acetohydroxyacid synthase (AHAS), also known as "acetolactate synthase" (ALS [EC 2.2.1.6; formerly EC 4.1.3.18]) 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 Acid, Singh, B.K., ed., Marcel Dekker Inc. New York, New York, pp. 227-247).


[5] ALS is the target of five structurally diverse herbicide families belonging to the class of ALS inhibitor herbicides, like (a) sulfonylurea herbicides (Beyer E.M et al. (1988), Sulfonylureas in Hericides: Chemistry, Degradation, and Mode of Action; Marcel Dekker, New York, 1988, 117-189), (b) sulfonilaminocarbonyltriadolinone herbicides (Pontzen, R., Pflanz.-Nachrichten Bayer, 2002, 55,

[6] Inhibitors of the ALS interrupt the biosynthesis of valine, leucine and isoleucine in plants. The consequence is an immediate depletion of the respective amino acid pools causing a stop of protein biosynthesis leading to a cessation of plant growth and eventually the plant dies, or - at least - is damaged.

[7] ALS inhibitor herbicides such as imidazolinone and sulfonylurea herbicides are widely used in modern agriculture due to their effectiveness at moderate application rates and relative non-toxicity in animals. By inhibiting ALS activity, these families of herbicides prevent further growth and development of susceptible plants including many weed species.

[8] Various mutants in ALS in various plants have been described that confer resistance to one or more ALS inhibitor herbicides. Plants conferring mutant ALS alleles show different levels of tolerance to ALS inhibitor herbicides, depending on the chemical structure of the ALS inhibitor herbicide and the site of the point mutation(s) in the ALS gene and the hereby encoded ALS protein.


[10] Among the artificially obtained various mutants, it has already been described that these are tolerant against various classes of ALS inhibitor herbicides, such as against certain sulfonylureas or representative compounds of the class of imidazolinones.
EP-A-0360750 describes the production of ALS inhibitor herbicide tolerant plants by producing an increased amount of the targeted ALS inside the plant. Such plants show an increased tolerance against certain sulfonyureas, like chlorosulfuron, sulfometuron-methyl, and triasulfuron.

US 5,198,599 describes sulfonylurea and imidazolinone tolerant plants that have been obtained via a selection process and which show a tolerance against chlorosulfuron, bensulfuron, chlorimuron, thifensulfuron and sulfometuron.

WO09/046334 describes mutated acetohydroxyacid synthase (AHAS) nucleic acids and the proteins encoded by the mutated nucleic acids, as well as canola plants, cells, and seeds comprising the mutated genes, whereby the plants display increased tolerance to imidazolinones and sulfonylureas.

WO09/031031 discloses herbicide-resistant Brassica plants and novel polynucleotide sequences that encode wild-type and imidazolinone-resistant Brassica acetohydroxyacid synthase large subunit proteins, seeds, and methods using such plants.

US patent application 09/0013424 describes improved imidazolinone herbicide resistant Brassica lines, including Brassica juncea, methods for generation of such lines, and methods for selection of such lines, as well as Brassica AHAS genes and sequences and a gene allele bearing a point mutation that gives rise to imidazolinone herbicide resistance.

WO08/124495 discloses 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.

WO 2010/037061 describes transgenic and non-transgenic plants with improved tolerance to AHAS-inhibiting herbicides such as an oilseed rape which is tolerant towards one specific class of ALS inhibitors, the Imidazolinone herbicides.

WO201 1/1 14232 describes herbicide-tolerant winter-type Brassica plants which express an AHAS enzyme that is tolerant to the action of one or more AHAS enzyme inhibitors.


In order to provide plants with an increased tolerance to even high concentrations of ALS inhibitor herbicides and to mixtures of herbicidal compounds that may be required for sufficient weed control, additional ALS-inhibiting herbicide-resistant breeding lines and varieties of crop plants, as well as methods and compositions for the production and use of ALS inhibiting herbicide-resistant breeding lines and varieties, are needed.
Thus, the technical problem is to comply with this need.

The present invention addresses this need and thus provides as a solution to the technical problem an herbicide tolerant Brassica napus (B. napus) plant and parts thereof according to the present invention.

By applying various breeding methods, high yielding commercial varieties highly competitive in all specific markets with the add-on of a robust ALS inhibitor herbicide tolerance can be developed subsequently by using the originally obtained mutant plants.

SUMMARY OF THE INVENTION

In one aspect, the present invention provides an ALS inhibitor herbicide tolerant B. napus plant or parts thereof comprising an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

Another embodiment refers to a B. napus plant or parts thereof according to the present invention, wherein said ALS I polypeptide is at least 90% identical to SEQ ID NO: 6 and wherein said ALS III polypeptide is at least 90% identical to SEQ ID NO: 8.

Yet another embodiment refers to a B. napus plant or parts thereof according to the present invention, wherein said ALS I polypeptide is identical to SEQ ID NO: 6 and wherein said ALS III polypeptide is encoded by the nucleotide sequence corresponding to SEQ ID NO: 5, and said ALS III protein is encoded by the nucleotide sequence corresponding to SEQ ID NO: 7.

Yet another embodiment refers to a B. napus plant or parts thereof according to the present invention, which are tolerant to one or more ALS-inhibitor herbicides belonging to the group consisting of sulfonylurea herbicides, sulfonylaminocarbonyltriazolinone herbicides, imidazolinone herbicides, triazolopyrimidine herbicides, and pyrimidinyl(thio)benzoate herbicides.

Yet another embodiment refers to a B. napus plant or parts thereof according to the present invention, characterized in that both ALS I alleles encode an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and that both ALS III alleles encode an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.
[29] Yet another embodiment refers to parts of the *B. napus* plant according to the present invention, wherein the parts are organs, tissues, cells or seeds.

[30] Another aspect refers to food, feed, or an industrial product obtainable from a plant according to the invention. Yet another aspect refers to food, feed, or an industrial product obtainable from a plant according to the invention, wherein the food or feed is oil, meal, grain, starch, flour or protein, or the industrial product is biofuel, fiber, industrial chemicals, a pharmaceutical or a nutraceutical.

[31] Yet another embodiment refers to progeny of a *B. napus* plant according to the present invention obtained by further breeding with said plant according to the present invention obtained, wherein said progeny contains an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[32] Yet another aspect refers to an Essentially Derived Variety having at least an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[33] Yet another aspect refers to a method of producing a hybrid seed, comprising crossing a parent *B. napus* plant according to the present invention with a second parent *Brassica* plant.

[34] Yet another aspect refers to a hybrid plant produced from crossing a parent *B. napus* plant according to the present invention with a second parent *Brassica* plant and harvesting a resultant hybrid seed and growing said seed, wherein said hybrid plant having at least an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[35] Another embodiment of the invention refers to a method for producing food, feed, or an industrial product, such as oil, meal, grain, starch, flour, protein, biofuel, fiber, industrial chemicals, a pharmaceutical or a nutraceutical, comprising obtaining the plant according to the present invention or a part thereof, and preparing the food, feed, or industrial product from the plant or part thereof.

[36] A further aspect of the present invention refers to the use of one or more ALS inhibitor herbicide(s) for controlling unwanted vegetation in *Brassica* growing area, such as *B. napus* plants, comprise an altered ALS I *Brassica*, such as *B. napus*, polypeptide comprising at a position
corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine; and an altered ALS III *Brassica*, such as *B. napus*, polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

5 One embodiment refers to the use according to the invention, wherein the ALS inhibitor herbicide(s) belong(s) to:

the group of the (sulfon)amides (group (A)) consisting of:


![Chemical Structure](image)

(1)
where $M^+$ denotes the respective salt of the compound (I), i.e. its lithium salt ($= \text{Al-40}$); its sodium salt ($= \text{Al-41}$); its potassium salt ($= \text{Al-42}$); its magnesium salt ($= \text{Al-43}$); its calcium salt ($= \text{Al-44}$); its ammonium salt ($= \text{Al-45}$); its methylammonium salt ($= \text{Al-46}$); its dimethylammonium salt ($= \text{Al-47}$); its tetramethylammonium salt ($= \text{Al-48}$); its ethylammonium salt ($= \text{Al-49}$); its diethylammonium salt ($= \text{Al-50}$); its tetraethylammonium salt ($= \text{Al-51}$); its propylammonium salt ($=\text{Al-52}$); its tetrapropylammonium salt ($= \text{Al-53}$); its isopropylammonium salt ($= \text{Al-54}$); its diisopropylammonium salt ($= \text{Al-55}$); its butylammonium salt ($= \text{Al-56}$); its tetrabutylammonium salt ($= \text{Al-57}$); its (2-hydroxyeth-1-y1)ammonium salt ($= \text{Al-58}$); its bis-N,N-(2-hydroxyeth-1-yl)ammonium salt ($= \text{Al-59}$); its tris-N,N,N-(2-hydroxyeth-1-yl)ammonium salt ($= \text{Al-60}$); its 1-phenylethylammonium salt ($= \text{Al-61}$); its 2-phenylethylammonium salt ($= \text{Al-62}$); its trimethylsulphonium salt ($= \text{Al-63}$); its trimethyloxonium salt ($= \text{Al-64}$); its pyridinium salt ($= \text{Al-65}$); its 2-methylpyridinium salt ($= \text{Al-66}$); its 4-methylpyridinium salt ($= \text{Al-67}$); its 2,4-dimethylpyridinium salt ($= \text{Al-68}$); its 2,6-dimethylpyridinium salt ($= \text{Al-69}$); its piperidinium salt ($= \text{Al-70}$); its imidazolium salt ($= \text{Al-71}$); its mofholinium salt ($= \text{Al-72}$); its 1,5-diazabicyclo[4.3.0]non-7-enium salt ($= \text{Al-73}$); its 1,8-diazabicyclo[5.4.0]undec-7-enium salt ($= \text{Al-74}$); or a compound of the formula (II) or salts thereof

![Chemical structure](image)

with $R^2$ and $R^3$ having the meaning as defined in the below table

<table>
<thead>
<tr>
<th>Compound</th>
<th>$R^2$</th>
<th>$R^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-75</td>
<td>OCH$_3$</td>
<td>OC$_2$H$_5$</td>
</tr>
<tr>
<td>Al-76</td>
<td>OCH$_3$</td>
<td>CH$_3$</td>
</tr>
<tr>
<td>Al-77</td>
<td>OCH$_3$</td>
<td>C$_2$H$_5$</td>
</tr>
<tr>
<td>Al-78</td>
<td>OCH$_3$</td>
<td>CF$_3$</td>
</tr>
<tr>
<td>Al-79</td>
<td>OCH$_3$</td>
<td>OCF$_2$H</td>
</tr>
<tr>
<td>Al-80</td>
<td>OCH$_3$</td>
<td>NHCH$_3$</td>
</tr>
<tr>
<td>Al-81</td>
<td>OCH$_3$</td>
<td>N(CH$_3$)$_2$</td>
</tr>
<tr>
<td>Al-82</td>
<td>OCH$_3$</td>
<td>Cl</td>
</tr>
<tr>
<td>Al-83</td>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
</tr>
<tr>
<td>Al-84</td>
<td>OC$_2$H$_5$</td>
<td>OC$_2$H$_5$</td>
</tr>
</tbody>
</table>
or the compound of formula (III) (= Al-87), i.e. the sodium salt of compound (Al-83)

![Chemical Structure of III](image)

or the compound of formula (IV) (=Al-88), i.e. the sodium salt of compound (Al-82)

![Chemical Structure of IV](image)

the subgroup of the sulfonanilides (subgroup (A4)), consisting of: compounds or salts thereof from the group described by the general formula (I):

![Chemical Structure of V](image)
in which
R\textsuperscript{1} is halogen, preferably fluorine or chlorine,
R\textsuperscript{2} is hydrogen and R\textsuperscript{3} is hydroxyl or
R\textsuperscript{2} and R\textsuperscript{3} together with the carbon atom to which they are attached are a carbonyl group C=O and
R\textsuperscript{4} is hydrogen or methyl;
and more especially compounds of the below given chemical structure (A4-1) to (A4-8)

\begin{align*}
\text{(A4-1)} & \quad \begin{array}{c}
\text{F} \\
\text{F} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{CH}_3 \\
\text{OCH}_3
\end{array} \\
\text{(A4-2)} & \quad \begin{array}{c}
\text{F} \\
\text{F} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{OCH}_3
\end{array} \\
\text{(A4-3)} & \quad \begin{array}{c}
\text{F} \\
\text{F} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{OCH}_3
\end{array} \\
\text{(A4-4)} & \quad \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{CH}_3 \\
\text{OCH}_3
\end{array} \\
\text{(A4-5)} & \quad \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{OCH}_3
\end{array} \\
\text{(A4-6)} & \quad \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{OCH}_3
\end{array} \\
\text{(A4-7)} & \quad \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{OCH}_3
\end{array} \\
\text{(A4-8)} & \quad \begin{array}{c}
\text{Cl} \\
\text{Cl} \\
\text{OS} \\
\text{N} \\
\text{N} \\
\text{OH} \\
\text{OCH}_3
\end{array}
\end{align*}

the group of the imidazolinones (group (B)), consisting of:

imazamethabenzmethyl [CAS RN 81405-85-8] (= Bl-1); imazamox [CAS RN 11431 1-32-9] (= Bl-2);
imazapic [CAS RN 104098-48-8] (= Bl-3); imazapyr [CAS RN 81334-34-1] (= Bl-4); imazaquin [CAS RN 81335-37-7] (= Bl-5); imazethapyr [CAS RN 81335-77-5] (= Bl-6); SYP-298 [CAS RN 557064-77-4] (= Bl-7); and SYP-300 [CAS RN 374718-10-2] (= Bl-8);
the group of the pyrimidinyl(thio)benzoates (group (C)), consisting of:

the subgroup of the pyrimidinylxybenzoacids (subgroup (CI)) consisting of: bispyribac-sodium [CAS RN 125401-92-5] (= CI-1); pyribenoxim [CAS RN 168088-61-7] (= CI-2); pyriminobac-methyl [CAS RN 136191-64-5] (= CI-3); pyribambenz-isopropyl [CAS RN 420138-41-6] (= CI-4); and pyribambenz-propyl [CAS RN 420138-40-5] (= CI-5);

the subgroup of the pyrimidinylthiobenzoeacids (subgroup (C2)), consisting of: pyrifaltalid [CAS RN 135186-78-6] (= C2-1); and pyrithiobac-sodium [CAS RN 123343-16-8] (= C2-2).

[38] Another embodiment refers to the use according to the invention, wherein the ALS inhibitor herbicide(s) belong(s) to the group consisting of: amidosulfuron [CAS RN 120923-37-7] (= Al-1); chlorimuron-ethyl [CAS RN 90982-32-4] (= Al-4); clorsulfuron [CAS RN 64902-72-3] (=Al-5); ethamsulfuron-methyl [CAS RN 97780-06-8] (= Al-8); ethoxysulfuron [CAS RN 126801-58-9] (= Al-9); flupyrsulfuron-methyl-sodium [CAS RN 144740-54-5] (= Al-12); foramsulfuron [CAS RN 173159-57-4] (= Al-13); iodosulfuron-methyl-sodium [CAS RN 144550-36-7] (= Al-16); mesosulfuron-methyl [CAS RN 208465-21-8] (= Al-17); metsulfuron-methyl [CAS RN 74223-64-6] (= Al-18); monosulfuron [CAS RN 155860-63-2] (= Al-19); nicosulfuron [CAS RN 111991-09-4] (= Al-20); rimsulfuron [CAS RN 122931-48-0] (= Al-26); sulfosulfuron [CAS RN 141776-32-1] (= Al-28); thifensulfuron-methyl [CAS RN 79277-27-3] (= Al-29); tribenuron-methyl [CAS RN 101200-48-0] (= Al-31); triflusulfuron-methyl [CAS RN 126535-15-7] (= Al-33); 2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide (= Al-39); 2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide sodium salt (= Al-41); (Al-83) or its sodium salt (=Al-87); flucarbazone-sodium [CAS RN 181274-17-9] (= A2-1); propoxycarbazone-sodium [CAS RN 181274-15-7] (= A2-2); thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3); florasulam [CAS RN 145701-23-1] (= A3-3); metosulam [CAS RN 139528-85-1] (= A3-5); pyroxysulam [CAS RN 422556-08-9] (= A3-7); (A4-1); (A4-2); (A4-3); imazamox [CAS RN 114311-32-9] (= BI-2); and bispyribac-sodium [CAS RN 125401-92-5] (= CI-1).

[39] Another embodiment refers to the use according to the present invention, wherein the ALS inhibitor herbicide(s) belong(s) to the group consisting of: amidosulfuron [CAS RN 120923-37-7] (= Al-1); foramsulfuron [CAS RN 173159-57-4] (= Al-13); sodium salt of compound of formula (I) (= Al-41); compound of formula (III) (=A1-41); thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3); imazamox [CAS RN 114311-32-9] (= BI-2); and bispyribac-sodium [CAS RN 125401-92-5] (= CI-1).

[40] Yet another embodiment refers to the use according to the present invention, wherein the Brassica plants are B. napus plants comprising an ALS I B. napus polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and wherein an ALS III B. napus polypeptide comprising at a position corresponding
to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[41] Yet another embodiment refers to the use according to the present invention, wherein the ALS inhibitor herbicide(s) are used in combination with non-ALS inhibitor herbicides (i.e. herbicides showing a mode of action that is different to the inhibition of the ALS enzyme [acetohydroxyacid synthase; EC 2.2.1.6] (group D herbicides), and wherein the non ALS inhibitor herbicide(s) is/are selected from the group consisting of: acetochlor (= D1), carbetamide (= D56), fenoxaprop-P-ethyl (= D164), fluazifop-P-butyl (= D174), haloxyfop-P-methyl (= D222), metolachlor (= D275), dimethenamid (= D132), napropamide (= D290), pethoxamid (= D317), propaquizafop (= D341), propisochlor (= D344), propyzamide (= D345), quinmerac (= D363), propachlor (D 427), clomazone (= D83), cropyralid (= D86), dimethachlor (= D130), metazachlor (= D265), picloram (= D321), and quizalofop-P-ethyl (= D368).

[42] Yet another embodiment refers to the use according to the present invention, wherein the ALS inhibitor herbicide(s) are used in combination with non-ALS inhibitor herbicide(s) is/are selected from the group consisting of: clomazone (= D83), cropyralid (= D86), dimethachlor (= D130), metazachlor (= D265), picloram (= D321), and quizalofop-P-ethyl (= D368).

[43] Another aspect of the present invention refers to a method for controlling unwanted vegetation in Brassica, such as B. napus, plant growing areas by applying one or more ALS inhibitor herbicide(s) alone or in combination with one or more herbicide(s) that do(es) not belong to the class of ALS inhibitor herbicides for weed control in Brassica growing areas, such as B. napus growing areas, which Brassica plants, such as B. napus plants comprise an altered ALS I Brassica, such as B. napus, polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine; and an altered ALS III Brassica, such as B. napus, polypeptide polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[44] One embodiment refers to a method according to the present invention for controlling unwanted vegetation, and wherein the ALS inhibitor herbicide(s) are taken from the groups as defined in [37].

[45] One embodiment refers to a method according to the present invention, and wherein the ALS inhibitor herbicide(s) are taken from the groups as defined in [38].

[46] One embodiment refers to a method according to the present invention, and wherein the non ALS inhibitor herbicide(s) are taken from the group as defined in [41].

[47] One embodiment refers to a method according to the present invention, and wherein the non ALS inhibitor herbicide(s) are taken from the group as defined in [42].
BRIEF DESCRIPTION OF THE DRAWINGS

[48] Figure 1: Alignment of SEQ ID NOs: 9, 5, 1, 3, 7

[49] Figure 2: Alignment of SEQ ID NOs: 10, 2, 6, 4, 8

[50] Figure 3: ALS enzyme activity in leaves of plants with different mutant AHAS alleles. A: inhibition of ALS enzyme activity by Foramsulfuron; B: inhibition of ALS enzyme activity by Thiencarbazone methyl. Concentrations of the respective herbicides are indicated in µM. I: plants homozygous for both HETO 108 and HETO121; II: plants homozygous for HETO108; III: plants homozygous for HETO121; IV: wild-type plants not comprising HETO108 and HETO121. Dark-coloured wells are the result of high ALS activity, whereas the lower the color in the wells, the lower the ALS activity.

DETAILED DESCRIPTION

General definitions

[51] It must be noted that as used herein, the terms "a", "an", and "the", include singular and plural references unless the context clearly indicates otherwise, i.e., such terms may refer to "one", "one or more" or "at least one". Thus, for example, reference to "a reagent" includes one or more of such different reagents and reference to "the method" includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

[52] All publications and patents cited in this disclosure are incorporated by reference in their entirety. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

[53] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

[54] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step.
When used herein the term "Brassica napus" is abbreviated as "B. napus". Furthermore, the term "oilseed rape" is used herein. Said three terms are interchangeably used and should be understood to fully comprise the cultivated forms of B. napus, e.g., as defined in Tang et al., Plant Breeding, Volume 116, Issue 5, pages 471-474, October 1997 and Jesske et al., Tagung der Vereinigung der Pflanenzüchter und Saatgutkaufleute Österreichs, 2009, 171-172, ISBN: 978-3-902559-37-1). Similarly, for example, the term "Arabidopsis thaliana" is abbreviated as "A. thaliana". Both terms are interchangeably used herein.

The term "wild-type" as used herein refers to a plant, a nucleic acid molecule or protein that can be found in nature as distinct from being artificially produced or mutated by man. Thus, in one embodiment, a "wild type" B. napus plant does not produce or comprise ALS proteins with an amino acid different from proline97 (P197) or tryptophane574 (W574 (the numbers behind the amino acids indicate the positions corresponding to these positions of SEQ ID NO: 10, which is the ALS protein as derived from A. thaliana).

In one embodiment, a "wild-type" B. napus plant refers to a B. napus plant having at least one ALS nucleic acid sequence containing at least 60%, or 70%, or 80%, or 90%, or 95%, or 97%, or 98%, or 99% sequence identity, or is identical to SEQ ID NO: 1 and at least one ALS nucleic acid sequence containing at least 60%, or 70%, or 80%, or 90%, or 95%, or 97%, or 98%, or 99% sequence identity, or is identical to SEQ ID NO: 3, provided that said plant does not carry an ALS I gene carrying a mutation in the Pro197 codon yielding an amino acid different from Pro, and does not carry an ALS III gene carrying a mutation in the Trp574 codon yielding an amino acid different from Trp, wherein the amino acid position referred to is the position in the reference A. thaliana sequence (SEQ ID NO: 10). The use of the term "wild-type" is not intended to necessarily 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.

Due to the fact that the B. napus plants of the present invention which are herbicide resistant were generated by "random evolution", i.e., methods preferably leading to fertile B. napus plants having two point mutation as described herein in more detail without exogenous genetic manipulation, they are non-transgenic as far as the ALS gene in its endogenous gene locus is concerned.

Mutant ALS I and ALS III alleles according to the invention can also be provided to plant cells as transgene. Accordingly, plants may contain a mutant ALS I gene according to the invention, or a mutant ALS III gene according to the invention, or both a mutant ALS I gene according to the invention and a mutant ALS III gene according to the invention as transgene.
Moreover, the plants of the present invention and their offspring are fertile and thus useful for breeding purposes in order to generate B. napus varieties conferring agronomically useful levels of tolerance to ALS inhibitor herbicides, thus allowing innovative weed control measures in B. napus growing areas.

The term "Brassica plant" as used herein refers to the genus of plants in the mustard family (Brassicaceae). The members of the genus may be collectively known either as cabbages, or as mustards. The genus "Brassica" encompasses, e.g., B. carinata, B. elongata, B. fruticulosa, B. juncea, B. napus, B. narinoso, B. nigra, B. oleracea, B. perviridis, B. rapa, B. rupestris, B. septiceps, and B. tournefortii. The skilled person will understand that the term not only encompasses B. napus but also other hybrids which have at least one parent plant of the genus "Brassica".

As used herein unless clearly indicated otherwise, the term "plant" intends to mean a plant at any developmental stage. Moreover, the term also encompasses "parts of a plant". The term "plant" encompasses a plant as described herein, or progeny of the plants which retain the distinguishing characteristics of the parents, such as seed obtained by selfing or crossing, e.g. hybrid seed (obtained by crossing two inbred parental lines), hybrid plants and plant parts derived therefrom are encompassed herein, unless otherwise indicated.

Parts of (a) plant(s) may be attached to or separate from a whole intact plant. Such parts of a plant include, but are not limited to, cells of a plant, tissues or organs, seeds, severed parts such as roots, leaves, flowers, pollen, etc.

The obtained plants according to the invention can be used in a conventional breeding scheme to produce more plants with the same characteristics or to introduce the ALS alleles according to the invention in other varieties of the same or related plant species, or in hybrid plants. The obtained plants can further be used for creating propagating material. Plants according to the invention can further be used to produce gametes, seeds (including crushed seeds and seed cakes), seed oil, embryos, either zygotic or somatic, progeny or hybrids of plants obtained by methods of the invention.

"Creating propagating material", as used herein, relates to any means known in the art to produce further plants, plant parts or seeds and includes inter alia vegetative reproduction methods (e.g. air or ground layering, division, (bud) grafting, micropropagation, striking or cutting), sexual reproduction (crossing with another plant) and asexual reproduction (e.g. apomixis, somatic hybridization).

In one embodiment, a B. napus plant of the invention comprises an ALS I protein wherein Pro at a position corresponding to position 182 of SEQ ID NO: 2 is substituted by Ser and an ALS III protein wherein Trp at a position corresponding to position 556 of SEQ ID NO: 4 is substituted by Leu.
In a further embodiment, a B. napus plant of the invention comprises an ALS I protein wherein Pro at a position corresponding to position 182 of SEQ ID NO: 2 is substituted by Ser and an ALS III protein wherein Trp at a position corresponding to position 556 of SEQ ID NO: 4 is substituted by Leu, and does neither comprise a wild type ALS I protein nor a wild type ALS III protein.

In one embodiment, a B. napus plant of the invention comprises an ALS I gene of SEQ ID NO: 5 and an ALS III gene of SEQ ID NO: 7.

In one embodiment, a plant in accordance with the present invention is obtainable from or derivable from or can be obtained from or derived from seeds deposited with the NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB 21 9YA UK, under the Budapest Treaty on December 15, 2011, under accession number NCIMB 41912. In one embodiment, said plant obtainable from or derivable from or can be obtained from or derived from seeds deposited with the NCIMB under Number 41912 is a plant directly grown or regenerated from one of said deposited seeds or a plant comprising both mutant alleles described herein, i.e., an ALS I allele coding for an ALS I protein having a mutation at a position corresponding to position 182 of SEQ ID NO: 2 as described herein and an ALS III allele coding for an ALS III protein having a mutation at a position corresponding to position 556 of SEQ ID NO: 4 as described herein. In one embodiment, such a plant obtainable from or derivable from or can be obtained from or derived from seeds deposited with the NCIMB under Number 41912 encompasses also a first, second, third, fourth or higher generation progeny of a plant directly grown or regenerated from said deposited seed or a first, second, third, fourth or higher generation progeny of a plant having at least one ALS I allele decoding for an ALS I protein having a mutation at a position corresponding to position 182 of SEQ ID NO: 2 as described herein and at least one ALS III allele decoding for an ALS III protein having a mutation at a position corresponding to position 556 of SEQ ID NO: 4 as described herein. In one embodiment, such a plant is homozygous regarding its ALS I and ALS III alleles. In a further embodiment, a plant in accordance with the present invention is provided which comprises an ALS I allele coding for an ALS I protein having a mutation at a position corresponding to position 182 of SEQ ID NO: 2 an ALS III allele coding for an ALS III protein having a mutation at a position corresponding to position 556 of SEQ ID NO: 4 as present in reference seeds deposited with the NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB 21 9YA UK, under the Budapest Treaty on December 15, 2011, under accession number NCIMB 41912.

Moreover, also plant cells are obtainable from or are derivable from or are obtained from or are derived from said deposited seeds; or plant cells are obtainable from or are derivable from or are obtained from or are derived from plants which were grown from said deposited seeds.

Accordingly, one embodiment of the present invention is also directed to reference seeds comprising both mutant alleles described herein having been deposited under Number NCIMB 41912.
One embodiment of the present invention refers to progeny of an ALS inhibitor herbicide tolerant *B. napus* plant or parts thereof comprising an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

"Progeny" as used herein refers to plants derived from an ALS inhibitor herbicide tolerant *Brassica napus* plant or parts thereof comprising an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine, e.g., a plant obtainable from or derivable from or obtained from or derived from seeds deposited with the NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB 2 1 9YA UK, under the Budapest Treaty on December 15, 2011, under accession number NCIMB 41912. Progeny may be derived by regeneration of cell or tissue culture or parts of a plant in accordance with the present invention or selfing of a plant in accordance with the present invention or by growing seeds of a plant in accordance with the present invention. In further embodiments, progeny may also encompass plants derived from crossing of at least a plant in accordance with the present invention with another *B. napus* or *Brassica* plant, backcrossing, inserting of a locus into a plant or further mutation(s). In one embodiment, a progeny is, e.g., a first generation plant such as a hybrid plant (F1) of a crossing of a plant according to the present invention with another *B. napus* or *Brassica* plant, or a progeny is regenerated from a plant part of a plant according to the present invention or is the result of self pollination. In another embodiment, a progeny is, e.g., a first, second, third, fourth, fifth, or sixth or higher generation plant derived from, derivable from, obtained from or obtainable from a *B. napus* plant in accordance with the present invention.

An "Essentially Derived Variety" (EDV) shall be deemed to be essentially derived from another variety, "the initial variety", under the following circumstances and in the case that the Initial Variety is a plant which is derived from seeds deposited with the NCIMB, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB 2 1 9YA UK, under the Budapest Treaty on December 15, 2011, under accession number NCIMB 41912: (i) it is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety, comprising an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine; (ii) it is clearly distinguishable from the initial variety (e.g., by its phenotype or genotype); and (iii) except for the differences which result from the act
of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety. Thus, an EDV may be obtained for example by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing, or transformation by genetic engineering.

[75] "Plant line" is for example a breeding line which can be used to develop one or more varieties. One embodiment of the present invention refers to a B. napus plant line comprising an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[76] A "variety" is used herein in conformity with the UPOV convention and refers to a plant grouping within a single botanical taxon of the lowest known rank, which grouping can be defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, can be distinguished from any other plant grouping by the expression of at least one of the said characteristics and is considered as a unit with regard to its suitability for being propagated unchanged (stable).

[77] "Hybrid" refers to the seeds harvested from crossing one plant line or variety with another plant line or variety.

[78] "Fi Hybrid" refers to the first generation progeny of the cross of two genetically divergent plants. In one embodiment, such a Fi Hybrid is homozygous in the essential feature, i.e., said Fi Hybrid comprising ALS I alleles encoding an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine and comprising ALS III alleles encoding an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

[79] "Crossing" refers to the mating of two parent plants.

[80] "Backcrossing" refers to a process in which a breeder repeatedly crosses hybrid progeny, for example a first generation hybrid (Fi), back to one of the parents of the hybrid progeny. Backcrossing can be used to introduce one or more single locus conversions from one genetic background into another.

[81] "Cross-pollination" refers to fertilization by the union of two gametes from different plants.
"Regeneration" refers to the development of a plant from tissue culture.

"Selfing" refers to self-pollination of a plant, i.e., the transfer of pollen from the anther to the stigma of the same plant.

Single Locus Converted (Conversion) Plant: Plants which are developed by a plant breeding technique called backcrossing, wherein essentially all of the desired morphological and physiological characteristics of a oilseed rape variety are recovered in addition to the characteristics of the single locus transferred into the variety via the backcrossing technique and/or by genetic transformation.

Plants of the present invention can be identified using any genotypic analysis method. Genotypic evaluation of the plants includes using techniques such as Isozyme Electrophoresis, Restriction Fragment Length Polymorphisms (RFLPs), Randomly Amplified Polymorphic DNAs (RAPDs), Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), Allele-specific PCR (AS-PCR), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) which are also referred to as "Microsatellites". Additional compositions and methods for analyzing the genotype of the plants provided herein include those methods disclosed in U.S. Publication No. 2004/0171027, U.S. Publication No. 2005/02080506, and U.S. Publication No. 2005/0283858.

Sequences/Position

The term "sequence" when used herein relates to nucleotide sequence(s), polynucleotide(s), nucleic acid sequence(s), nucleic acid(s), nucleic acid molecule, peptides, polypeptides and proteins, according to the context in which the term "sequence" is used.

Generally, the skilled person knows, because of his common general knowledge and the context when the terms ALS, ALSL, AHAS or AHASL are used herein as to whether the nucleotide sequence or nucleic acid, or the amino acid sequence or polypeptide, respectively, is meant.

The term B. napus "ALS" or "AHAS" gene refers to B. napus nucleotide sequences which are at least 60, 70, 80, 90, 95, 97, 98, 99% or 100% identical to the B. napus ALS nucleotide sequence of SEQ ID NO: 1 or 3.

The term "ALS I" gene refers to a B. napus ALS gene present on the C genome, wherein the sequence of said gene is at least 60, 70, 80, 90, 95, 97, 98, 99% or 100% identical to the nucleotide sequence of SEQ ID NO: 1.
The term "ALS III" gene refers to a \textit{B. napus} ALS gene present on the A genome, wherein the sequence of said gene is at least 60, 70, 80, 90, 95, 97, 98, 99\% or 100\% identical to the nucleotide sequence of SEQ ID NO: 3.

The term \textit{B. napus} "ALS" or "AHAS" polypeptide refers to amino acid sequences which are at least 90, 95, 97, 98, 99\% or 100\% identical to the ALS amino acid sequence of SEQ ID NO: 2 or 4. Said X\% identical amino acid sequences retain the activity of ALS as described herein, more preferably the ALS polypeptide is tolerant to ALS inhibitor herbicides as described herein. However, such "ALS" or "AHAS" polypeptides still show ALS enzymatic activity at a level of at least 20\%, 30\%, 40\%, 50\%, 60\%, 70\%, 80\%, 90\%, compared to the level of the ALS enzymatic activity of a protein having the SEQ ID NO: 2 (when referring to an ALS I protein) or 4 (when referring to an ALS III protein).

The term "ALS I" protein refers to the protein encoded by the ALS I gene, wherein said ALS I protein contains at least 90, 95, 97, 98, 99 or 100\% sequence identity to the ALS amino acid sequence of SEQ ID NO: 2.

The term "ALS III" protein refers to the protein encoded by the ALS III gene, wherein said ALS III protein contains at least 90, 95, 97, 98, 99\% or 100\% sequence identity to the ALS amino acid sequence of SEQ ID NO: 4.

The term "position" when used in accordance with the present invention means the position of either an amino acid within an amino acid sequence depicted herein or the position of a nucleotide within a nucleotide sequence depicted herein. The term "corresponding" as used herein also includes that a position is not only determined by the number of the preceding nucleotides/amino acids.

The position of a given nucleotide in accordance with the present invention which may be substituted may vary due to deletions or additional nucleotides elsewhere in the ALS 5\'-untranslated region (UTR) including the promoter and/or any other regulatory sequences or gene (including exons and introns). Similarly, the position of a given amino acid in accordance with the present invention which may be substituted may vary due to deletion or addition of amino acids elsewhere in the ALS polypeptide.

Thus, under a "corresponding position" or "a position corresponding to position" in accordance with the present invention it is to be understood that nucleotides/amino acids may differ in the indicated number but may still have similar neighbouring nucleotides/amino acids. Said nucleotides/amino acids which may be exchanged, deleted or added are also comprised by the term "corresponding position".

In order to determine whether a nucleotide residue or amino acid residue in a given ALS nucleotide/amino acid sequence corresponds to a certain position in the nucleotide sequence of SEQ ID NO: 1, 3 or 9, respectively, or their corresponding amino acid sequences of SEQ ID NO: 2, 4 or 10.
respectively, the skilled person can use means and methods well-known in the art, e.g., alignments, either manually or by using computer programs such as BLAST (Altschul et al. (1990), Journal of Molecular Biology, 215, 403-410), which stands for Basic Local Alignment Search Tool or ClustalW (Thompson et al. (1994), Nucleic Acid Res., 22, 4673-4680) or any other suitable program which is suitable to generate sequence alignments.

[98] SEQ ID NO: 1 is the nucleotide sequence encoding a B. napus wild type ALS I, whereas SEQ ID NO: 2 is the B. napus amino acid sequence derived from SEQ ID NO: 1. Accordingly, the codon at position 544-546 of the nucleotide sequence of SEQ ID NO: 1 encodes the amino acid at position 182 of SEQ ID NO: 2 (this position, again, corresponds to position 197 of SEQ ID NO: 10). In other words, the amino acid proline ("Pro" (three letter code) or "P" (one letter code)) of SEQ ID NO: 2 is encoded by the codon at positions 544-546 of the nucleotide sequence of SEQ ID NO: 1.

[99] SEQ ID NO: 3 is the nucleotide sequence encoding a B. napus wild type ALS III, whereas SEQ ID NO: 4 is the B. napus amino acid sequence derived from SEQ ID NO: 3. Accordingly, the codon at position 1666-1668 of the nucleotide sequence of SEQ ID NO: 3 encodes the amino acid at position 556 of SEQ ID NO: 4 (this position, again, corresponds to position 574 of SEQ ID NO: 10). In other words, the amino acid tryptophan ("Trp" (three letter code) or "W" (one letter code)) of SEQ ID NO: 4 is encoded by the codon at positions 1666-1668 of the nucleotide sequence of SEQ ID NO: 3.

[100] In the alternative to determine whether a nucleotide residue or amino acid residue in a given ALS nucleotide/amino acid sequence corresponds to a certain position in the nucleotide sequence of SEQ ID NO: 1, 3, 5 or 7, respectively, the nucleotide sequence encoding A. thaliana wild type ALS shown in SEQ ID NO: 9 can be used. SEQ ID NO: 10 is the A. thaliana amino acid sequence derived from SEQ ID NO: 9.

[101] The codons at position 589-591 and 1720-1722, respectively, of the nucleotide sequence of SEQ ID NO: 9 encodes the amino acid at position 197 and 574 of SEQ ID NO: 10, whereby position 197 of SEQ ID NO: 10 corresponds to position 182 of SEQ ID NOs: 2 and 6, and position 574 of SEQ ID NO: 10 corresponds to position 556 of SEQ ID NOs: 4 and 8.

[102] If the A. thaliana wild type ALS nucleotide sequence shown in SEQ ID NO: 9 is used as reference sequence (as it is done in most of the relevant literature and, therefore, is used to enable an easier comparison to such known sequences), the codon encoding a serine instead of a proline at position 182 of SEQ ID NO: 2 is at a position 544-546 of SEQ ID NO: 1 which corresponds to position 589-591 of SEQ ID NO: 9 and the codon encoding a leucine instead of a tryptophan at a position 556 of SEQ ID NO: 4 is at a position 1666-1668 of SEQ ID NO: 3 which corresponds to position 1720-1722 of SEQ ID NO: 9.
However, SEQ ID NO: 1 is preferred as the reference nucleotide sequence for mutated ALS I protein encoding sequences such as SEQ ID NO: 5, and SEQ ID NO: 2 is preferred as the reference amino acid sequence for mutated sequences such as SEQ ID NO: 6 in all of the subsequent disclosures.

Similarity, SEQ ID NO: 3 is preferred as the reference nucleotide sequence for mutated ALS III protein encoding sequences such as SEQ ID NO: 7 and SEQ ID NO: 4 is preferred as the reference amino acid sequence for mutated sequences such as SEQ ID NO: 8 in all of the subsequent disclosures.

Thus, in any event, the equivalent position can still be determined through alignment with a reference sequence, such as SEQ ID NO: 1 or 3 (nucleotide sequence) or SEQ ID NO: 2 or 4 (amino acid sequence). Alignments of the various sequences listed above are given in figures 1 and 2.

In view of the difference between the B. napus wild-type ALS genes (ALS I and III gene) and the mutated ALS genes comprised by a B. napus plant of the present invention or progeny thereof, the ALS genes (or polynucleotides or nucleotide sequences) comprised by a B. napus plant of the present invention or progeny thereof may also be regarded as a "mutant ALS gene", "mutant ALS allele", "mutant ALS polynucleotide" or the like. Thus, throughout the specification, the terms "mutant allele", "mutant ALS allele", "mutant ALS gene" or "mutant ALS polynucleotide" are used interchangeably.

Unless indicated otherwise herein, these terms refer to a nucleotide sequence encoding an ALS I protein that comprises a codon at a position which corresponds to position 544-546 of SEQ ID NO: 1 and said codon encodes a serine instead of a proline; and to a second nucleotide sequence encoding for an ALS III protein that comprises a codon at a position which corresponds to position 1666-1668 of SEQ ID NO: 3 and said codon of said second nucleotide sequence encodes a leucine instead of a tryptophan.

The term "P197S mutation" in ALS I refers to a mutation in the codon corresponding to nt 589-591 in A. thaliana (SEQ ID NO 9) or in the codon corresponding to nt 544-546 of B. napus ALS I (SEQ ID NO: 1) leading to a substitution of the amino acid proline by a serine.

The term "W574L mutation" in ALS III refers to a mutation in the codon corresponding to nt 1720-1722 in A. thaliana (SEQ ID NO 9) or in the codon corresponding to nt 1666-1668 of B. napus ALS III (SEQ ID NO: 3) leading to a substitution of the amino acid tryptophan by a leucine.

The terms "nucleotide sequence(s)", "polynucleotide(s)", "nucleic acid sequence(s)", "nucleic acid(s)", "nucleic acid molecule" are used interchangeably herein and refer to nucleotides, either ribonucleotides or deoxyribonucleotides or a combination of both, in a polymeric unbranched form of any length. Nucleic acid sequences include DNA, cDNA, genomic DNA, RNA, synthetic forms and mixed polymers, both sense and antisense strands, or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art.
Homology/identity

[111] In order to determine whether a nucleic acid sequence has a certain degree of identity to the nucleotide sequences of the present invention, the skilled person can use means and methods well-known in the art, e.g., alignments, either manually or by using computer programs such as those mentioned further down below in connection with the definition of the term "hybridization" and degrees of homology.

[112] For the purpose of this invention, the "sequence identity" or "sequence homology" (the terms are used interchangeably herein) of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e., a position in an alignment where a residue is present in one sequence but not in the other, is regarded as a position with non-identical residues. The "optimal alignment" of two sequences is found by aligning the two sequences over the entire length according to the Needleman and Wunsch global alignment algorithm (Needleman and Wunsch, 1970, J Mol Biol 48(3):443-53) in The European Molecular Biology Open Software Suite (EMBOSS, Rice et al., 2000, Trends in Genetics 16(6): 276—277; see e.g. http://www.ebi.ac.uk/emboss/align/index.html) using default settings (gap opening penalty = 10 (for nucleotides) / 10 (for proteins) and gap extension penalty = 0.5 (for nucleotides) / 0.5 (for proteins)). For nucleotides the default scoring matrix used is EDNAFULL and for proteins the default scoring matrix is EBLOSUM62.

[113] The term B. napus "ALS" or "AHAS" gene also includes B. napus nucleotide sequences which are at least 60, 70, 80, 90, 95, 97, 98, 99% or 100% identical to the B. napus ALS nucleotide sequence of SEQ ID NO: 1 or 3, wherein these 60, 70, 80, 90, 95, 97, 98, 99, or 100% identical nucleotide sequences comprise at a position corresponding to position 544-546 of the nucleotide sequence of SEQ ID NO: 1 a codon encoding Ser instead of Pro (at position 182 of SEQ ID NO: 2) or at a position corresponding to position 1666-1668 of the nucleotide sequence of SEQ ID NO: 3 a codon encoding Leu instead of Thr (at position 556 of SEQ ID NO: 4).

[114] Likewise, these at least 60, 70, 80, 90, 95, 97, 98, 99, or 100% identical nucleotide sequences include sequences encoding an ALS polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 Ser instead of Pro, or at a position corresponding to position 556 of SEQ ID NO: 4 Leu instead of Thr. Of course, these nucleotide sequences encode for ALS proteins which retain the activity as described herein, more preferably the thus-encoded ALS polypeptide is tolerant to one or more ALS inhibitor herbicides as described herein. Said term also includes allelic variants and homologs encoding an ALS polypeptide which is preferably tolerant to one or more ALS inhibitor herbicides as described herein.
When used herein, the term "polypeptide" or "protein" (both terms are used interchangeably herein) means a peptide, a protein, or a polypeptide which encompasses amino acid chains of a given length, wherein the amino acid residues are linked by covalent peptide bonds. However, peptidomimetics of such proteins/polypeptides wherein amino acid(s) and/or peptide bond(s) have been replaced by functional analogs are also encompassed by the invention as well as other than the 20 gene-encoded amino acids, such as selenocysteine. Peptides, oligopeptides and proteins may be termed polypeptides. The term polypeptide also refers to, and does not exclude, modifications of the polypeptide, e.g., glycosylation, acetylation, phosphorylation and the like. Such modifications are well described in basic texts and in more detailed monographs, as well as in the research literature. The polypeptide (or protein) that are preferably meant herein have an amino acid sequence that comprises the mutated *B. napus* ALS I and III polypeptides (or ALS I and III proteins) of SEQ ID NO: 6 and 8, respectively.

The term *B. napus* "ALS" or "AHAS" polypeptide also includes amino acid sequences which comprise an amino acid sequences which is at least 90, 95, 97, 98, 99% or 100% identical to the ALS amino acid sequence of SEQ ID NO: 2 or 4, wherein these at least 90, 95, 97, 98, 99 or 100% identical amino acid sequences comprising at a position corresponding to position 182 of SEQ ID NO: 2 a serine instead of a proline, and at a position corresponding to position 556 of SEQ ID NO: 4 a leucine instead of a tryptophan. Said X% identical amino acid sequences retain the activity of ALS as described herein, more preferably the ALS polypeptide is tolerant to ALS inhibitor herbicides as described herein.

However, such "ALS" or "AHAS" polypeptides still show ALS activity of at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% compared to ALS activity of an protein having the SEQ ID NO: 2 (when referring to an ALS I protein)or 4 (when referring to an ALS III protein).

The same techniques, e.g., BLAST, as described above for the alignment of nucleic acid sequences can be used for alignments of protein sequences as well. For Example, a BLAST search can be performed from those skilled in the art using ExPASy (see world wide net: http://expasy.org/tools/).

**Isolated/purified**

An "isolated" nucleic acid sequence (or DNA) is used herein to refer to a nucleic acid sequence (or DNA) that is no longer in its natural environment, for example in an in vitro or in a recombinant bacterial or plant host cell. In some embodiments, an "isolated" nucleic acid is free of nucleotide sequences (preferably protein encoding sequences) that naturally flank the nucleic acid (i.e., sequences located at the 5′ and 3′ ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For purposes of the invention, "isolated" when used to refer to nucleic acid molecules excludes isolated chromosomes. For example, in various embodiments, the isolated nucleic acid molecule encoding an ALS protein can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences that naturally flank the nucleic acid molecule in genomic DNA of the
cell from which the nucleic acid is derived. An ALS protein that is substantially free of cellular material includes preparations of protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of non-ALS protein (also referred to herein as a "contaminating protein").

Amino Acid Substitution

[119] Amino acid substitutions encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring amino acid residue. Such substitutions may be classified as 'conservative', in which an amino acid residue contained in the wild-type ALS protein is replaced with another naturally-occurring amino acid of similar character, for example Ala<->Val, Trp<->Leu, Gly<->Asp, Gly<->Ala, Val<->Ile<->Leu, Asp<->Glu, Lys<->Arg, Asn<->Gln or Phe<->Trp<->Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in the wild-type ALS protein is substituted with an amino acid with different properties, such as a naturally-occurring amino acid from a different group. In one embodiment, a plant comprises mutations of its endogenous acetolactate synthase (ALS) genes, whereby an ALS I gene encodes an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine and whereby an ALS III gene encodes an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine. In another embodiment, altered gene sequences of ALS I gene sequence SEQ ID NO: 1 and/or ALS III gene sequence SEQ ID NO: 3 may contain at least one further mutation.

[120] "Similar amino acids", as used herein, refers to amino acids that have similar amino acid side chains, i.e. amino acids that have polar, non-polar or practically neutral side chains. "Non-similar amino acids", as used herein, refers to amino acids that have different amino acid side chains, for example an amino acid with a polar side chain is non-similar to an amino acid with a non-polar side chain. Polar side chains usually tend to be present on the surface of a protein where they can interact with the aqueous environment found in cells ("hydrophilic" amino acids). On the other hand, "non-polar" amino acids tend to reside within the center of the protein where they can interact with similar non-polar neighbours ("hydrophobic" amino acids"). Examples of amino acids that have polar side chains are arginine, asparagine, aspartate, cysteine, glutamine, glutamate, histidine, lysine, serine, and threonine (all hydrophilic, except for cysteine which is hydrophobic). Examples of amino acids that have non-polar side chains are alanine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, and tryptophan (all hydrophobic, except for glycine which is neutral).

Genes/Alleles

[121] Unless indicated otherwise, the terms "wild-type allele," "wild-type ALS allele," "wild-type ALS gene" or "wild-type ALS polynucleotide" refer to a nucleotide sequence containing at least 60%, or
70%, or 80%, or 90%, or 95%, or 97%, or 98%, or 99% sequence identity, or is identical to SEQ ID NO: 1 and or an ALS nucleic acid sequence containing at least 60%, or 70%, or 80%, or 90%, or 95%, or 97%, or 98%, or 99% sequence identity, or is identical to SEQ ID NO: 3, provided that the ALS I gene does not carry a mutation in the Pro 197 codon yielding an amino acid different from Pro, and the ALS III gene does not carry a mutation in the Trp 574 codon yielding an amino acid different from Trp, wherein the amino acid position referred to is the position in the reference A. thaliana sequence (SEQ ID NO: 10).

[122] The terms "wild-type ALS I allele," "wild-type ALS I allele", "wild-type ALS I gene" or "wild-type ALS I polynucleotide" refer to a nucleotide sequence containing at least 60%, or 70%, or 80%, or 90%, or 95%, or 97%, or 98%, or 99% sequence identity, or is identical to SEQ ID NO: 1, provided that it does not carry a mutation in the Pro 197 codon yielding an amino acid different from Pro, wherein the amino acid position referred to is the position in the reference A. thaliana sequence (SEQ ID NO: 10).

[123] The terms "wild-type ALS III allele," "wild-type ALS III allele", "wild-type ALS III gene" or "wild-type ALS III polynucleotide" refer to a nucleotide sequence containing at least 60%, or 70%, or 80%, or 90%, or 95%, or 97%, or 98%, or 99% sequence identity, or is identical to SEQ ID NO: 3, provided that it does not carry a mutation in the Trp 574 codon yielding an amino acid different from Trp, wherein the amino acid position referred to is the position in the reference A. thaliana sequence (SEQ ID NO: 10).

[124] The term "wild type ALS I" protein refers to the protein encoded by the ALS I gene, wherein said ALS I protein contains at least 90, 95, 97, 98, 99, or 100% sequence identity to the ALS amino acid sequence of SEQ ID NO: 2, provided that the amino acid at the position corresponding to position 197 of SEQ ID NO: 10 is a Pro.

[125] The term "wild type ALS III" protein refers to the protein encoded by the ALS III gene, wherein said ALS III protein contains at least 90, 95, 97, 98, 99% or 100% sequence identity to the ALS amino acid sequence of SEQ ID NO: 4, provided that the amino acid at the position corresponding to position 574 of SEQ ID NO: 10 is a Trp.

[126] Such a "wild-type allele", "wild-type ALS allele", "wild-type ALS gene" or "wild-type ALS polynucleotide" may, or may not, comprise mutations, other than the mutation mentioned above. However, SEQ ID NO: 1 and SEQ ID NO: 3 are in any case "wild-type alleles" which can be used as a reference.

[127] The term "gene" when used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. The term includes double- and single-stranded DNA and RNA. It also includes known types of modifications, for example, methylation, "caps", substitutions
of one or more of the naturally occurring nucleotides with an analog. Preferably, a gene comprises a coding sequence encoding the herein defined polypeptide. A "coding sequence" is a nucleotide sequence which, when transcribed into mRNA, can be translated into a polypeptide. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleic acid sequences or genomic DNA, while introns may be present as well under certain circumstances.

[128] In essence, the difference between a wild-type B. napus plant, and a B. napus plant of the present invention is that at least an ALS I gene comprises a codon - corresponding to position 544-546 of SEQ ID NO: 1 - encodes a Ser instead of Pro; and that at least an ALS III gene comprises a codon - corresponding to position 1666-1668 of the SEQ ID NO: 3 - encodes Leu instead of Trp.

[129] In one embodiment, these codons encode an amino acid as specified herein elsewhere. However, as mentioned above, further differences such as additional mutations may be present between wild-type and the mutant ALS allele as specified herein. Yet, these further differences are not relevant as long as the difference explained before is present.

[130] In one embodiment, a plant according to the present invention comprises an ALS I gene which encodes an ALS I protein comprising Ser instead of Pro at a position 182 when comparing said ALS I protein with the wild type amino acid sequence SEQ ID NO: 2; and comprises an ALS III gene which encodes an ALS III protein comprising Leu instead of Trp at a position 556 when comparing said ALS III protein with the wild type amino acid sequence SEQ ID NO: 4. The skilled person will understand that such mutated ALS I and ALS III genes may comprise further mutations such as one, two or three further mutations.

[131] Consequently, the Pro97Ser and Trp574Leu substitutions (when the A. thaliana ALS amino acid sequence of SEQ ID NO: 10 is used as reference) are a result of an alteration of codons at a position corresponding to position 613-615 and 1720-1722 of the nucleotide sequence shown in SEQ ID NO: 9.

[132] In one embodiment, the substitution at position 197 (when the A. thaliana ALS amino acid sequence of SEQ ID NO: 10 is used as reference) is a P→S substitution, wherein "S" is encoded by any of the codons "TCT", "TCC", "TCA", "TCG", "AGT" or "AGC" and the substitution at position 574 (when the A. thaliana ALS amino acid sequence of SEQ ID NO: 10 is used as reference) is a W→L substitution, wherein "L" is encoded by any of the codons "CTT", "CTC", "CTA", "CTG", "TTA", "TTG".
Hence, in one embodiment, the present invention provides a B. napus plant comprising in the nucleotide sequence of an ALS I gene in its endogenous gene locus, at least a codon encoding Ser instead of Pro, at a position corresponding to position 589-591 of the A. thaliana ALS nucleic acid sequence of SEQ ID NO: 9 and comprising in the nucleotide sequence of an ALS III gene in its endogenous gene locus, at least a codon encoding Leu instead of Trp at a position corresponding to position 1720-1722 of the A. thaliana ALS nucleic acid sequence of SEQ ID NO: 9.

ALS alleles according to the invention or plants comprising ALS alleles according to the invention can be identified or detected by method known in the art, such as direct sequencing, PCR based assays or hybridization based assays. Alternatively, methods can also be developed using the specific ALS allele specific sequence information provided herein. Such alternative detection methods include linear signal amplification detection methods based on invasive cleavage of particular nucleic acid structures, also known as Invader™ technology, (as described e.g. in US patent 5,985,557 "Invasive Cleavage of Nucleic Acids", 6,001,567 "Detection of Nucleic Acid sequences by Invader Directed Cleavage, incorporated herein by reference), RT-PCR-based detection methods, such as Taqman, or other detection methods, such as SNPlex. Briefly, in the Invader™ technology, the target mutation sequence may e.g. be hybridized with a labeled first nucleic acid oligonucleotide comprising the nucleotide sequence of the mutation sequence or a sequence spanning the joining region between the 5’ flanking region and the mutation region and with a second nucleic acid oligonucleotide comprising the 3’ flanking sequence immediately downstream and adjacent to the mutation sequence, wherein the first and second oligonucleotide overlap by at least one nucleotide. The duplex or triplex structure that is produced by this hybridization allows selective probe cleavage with an enzyme (Cleavase®) leaving the target sequence intact. The cleaved labeled probe is subsequently detected, potentially via an intermediate step resulting in further signal amplification.

The present invention also relates to the combination of ALS alleles according to the invention in one plant, and to the transfer of ALS alleles according to the invention from one plant to another plant.

**ALS activity tolerance**

For the present invention, the terms "herbicide-tolerant" and "herbicide-resistant" are used interchangeably and are intended to have an equivalent meaning and an equivalent scope. Similarly, the terms "herbicide-tolerance" and "herbicide-resistance" are used interchangeably and are intended to have an equivalent meaning and an equivalent scope.
It is preferred that the *B. napus* plants of the present invention are less sensitive to an ALS inhibitor, such as at least 5 times, or 10 times, or 50 times, or 100 times, or 500 times, or 1000 times, or 2000 times less sensitive as compared to wild type plants, such as wild type plants comprising ALS I polypeptides of SEQ ID NO: 2 and ALS III polypeptides of SEQ ID NO: 4, i.e., wild type plants having not the substitutions of the present invention. Wild type plants wherein all ALS I alleles are alleles of SEQ ID NO: 1 and all ALS III alleles are alleles of SEQ ID NO: 3 are preferred references when comparing ALS sensitivity. Less sensitive when used herein may, vice versa, be seen as "more tolerable" or "more resistant". Similarly, "more tolerable" or "more resistant" may, vice versa, be seen as "less sensitive".

For example, the *B. napus* plants of the present invention and in particular the *B. napus* plant described in the appended Examples are/is at least sensitive to a combination of the ALS inhibitor herbicides foramsulfuron (a member of the ALS inhibitor subclass "sulfonylurea herbicides") and thiencarbazone-methyl (a member of the ALS inhibitor subclass "sulfonylaminocarboxyltriazolinone herbicides") compared to the wild type enzyme.

An "herbicide-tolerant" or "herbicide-resistant" plant refers to a plant that is tolerant or resistant to at least one AHAS -inhibiting herbicide at a level that would normally kill, or inhibit the growth of, a wild-type plant lacking a mutated AHAS nucleic acid molecule. By "herbicide-resistant AHAS nucleic acid molecule" is intended a nucleic acid molecule comprising one or more mutations that results in one or more amino acid substitutions relative to the non-mutated AHAS protein, where the mutations result in the expression of an herbicide-resistant AHAS protein. By "herbicide-tolerant AHAS protein" or "herbicide-resistant AHAS protein", it is intended that such an AHAS protein displays higher AHAS activity, relative to the AHAS activity of a wild-type AHAS 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 to known to inhibit the AHAS activity of the wild-type AHAS protein. Furthermore, the AHAS activity of such an herbicide-tolerant or herbicide-resistant AHAS protein may be referred herein as "herbicide-tolerant" or "herbicide-resistant" AHAS activity.

Preferably, the *B. napus* plants of the present invention are less sensitive to various members of ALS inhibitor herbicides, like sulfonylurea herbicides, sulfonylamino-carboxyltriazolinone herbicides, and imidazolinone herbicides. Sulfonylurea herbicides and sulfonylaminocarboxyltriazolinone herbicides against which said plants are less sensitive are preferably selected. In a particular preferred embodiment, the *B. napus* plants of the present invention are less sensitive to the ALS inhibitor herbicide foramsulfuron (sulfonylurea herbicide) either alone or in combination with one or more further ALS inhibitor herbicides either from the subclass of the sulfonyurea-herbicides or any other sub-class of the ALS inhibitor herbicides, e.g. a compound of formula (I):
Hence, the *B. napus* plants of the present invention which are preferably less sensitive to an ALS inhibitor herbicide can likewise also be characterized to be "more tolerant" to an ALS inhibitor (i.e. an ALS inhibitor tolerant plant).

Thus, an "ALS inhibitor tolerant" plant refers to a plant, preferably a *B. napus* plant according to the present invention or any of its progenies that is more tolerant to at least one ALS inhibitor herbicide at a level that would normally inhibit the growth of a wild-type plant, preferably the ALS inhibitor herbicide controls a wild-type plant. Said wild-type plant does not comprise in the nucleotide sequence of any allele of the endogenous ALS I gene, a codon encoding Ser instead of Pro at a position corresponding to position 544-546 of SEQ ID NO: 1 and does not comprise in the nucleotide sequence of any allele of the endogenous ALS III gene, a codon encoding Leu instead of Trp at a position corresponding to position 1666-1668 of SEQ ID NO: 3.

Said nucleotide sequences may generally also be characterized to be "ALS inhibitor herbicide tolerant" nucleotide sequences. By "ALS inhibitor herbicide tolerant nucleotide sequence" is intended a nucleic acid molecule comprising nucleotide sequences encoding for a ALS I protein having at least a Ser instead of Pro a position corresponding to position 182 of SEQ ID NO: 2 and/or nucleotide sequences encoding for a ALS III protein having at least a Leu instead of Trp at a position corresponding to position 556 of SEQ ID NO: 4, wherein said at least one mutation results in the expression of a less sensitive to an ALS inhibitor herbicide ALS protein.

By "herbicide-tolerant ALS protein", it is intended that such an ALS protein displays higher ALS activity, relative to the ALS activity of a wild-type ALS protein, in the presence of at least one ALS inhibitor herbicide that is known to interfere with ALS activity and at a concentration or level of said herbicide that is known to inhibit the ALS activity of the wild-type ALS protein.

Similarly, the terms "ALS-inhibitor herbicide(s)" or simply "ALS-inhibitor(s)" are used interchangeably. As used herein, an "ALS -inhibitor herbicide" or an "ALS inhibitor" is not meant to be limited to single herbicide that interferes with the activity of the ALS enzyme. Thus, unless otherwise stated or evident from the context, an "ALS-inhibitor herbicide" or an "ALS inhibitor" can be a one
herbicide or a mixture of two, three, four, or more herbicides known in the art, preferably as specified herein, each of which interferes with the activity of the ALS enzyme.

[146] "Herbicide resistance" or "herbicide tolerance" can be measured as described in the present application or, e.g., it can be measured by comparison of AHAS activity obtained from cell extracts from plants containing the mutagenized AHAS sequence and from plants lacking the mutagenized AHAS sequence in the presence of an AHAS inhibitor, such as foramsulfuron or imazamox, using the methods disclosed in Singh, et al. Anal. Biochem., (1988), 171: 173-179. In one embodiment, resistant or tolerant plants demonstrate greater than 25% uninhibition using the methods disclosed in Singh et al (1988) when assayed, e.g., using 10 µM foramsulfuron or 10µM imazamox.

[147] The activity of specific ALS proteins such as ALS I or ALS III proteins can be measured according to the following method: The coding sequences of B. napus wild-type and P197S-mutant ALS I or W574L-mutant ALS III genes can be cloned into Novagen pET-32a(+) vectors and the vectors transformed into Escherichia coli AD494 according to the instructions of the manufacturer. Bacteria are grown at 37°C in LB-medium containing 100 mg/l carbenicillin and 25 mg/l canamycin, induced with 1 mM isopropyl -P-D-thiogalactopyranoside at an ODeoo of 0.6, cultivated for 16 hours at 18°C and harvested by, e.g., centrifugation. Bacterial pellets are resuspended in 100 mM sodium phosphate buffer pH 7.0 containing 0.1 mM thiamine-pyrophosphate, 1 mM MgCl₂, and 1 µM FAD at a concentration of 1 gram wet weight per 25 ml of buffer and disrupted by, e.g., sonification. The crude protein extract obtained after centrifugation is used for ALS activity measurements.

[148] ALS protein can be extracted from B. napus leaves or B. napus tissue cultures as described by Ray (Plant Physiol., 1984, 75:827-831). An ALS assays can be carried out in 96-well microtiter plates using a modification of the procedure described by Ray (1984): The reaction mixture contains 20 mM potassium phosphate buffer pH 7.0, 20 mM sodium pyruvate, 0.45 mM thiamine-pyrophosphate, 0.45 mM MgCl₂, 9 µM FAD. ALS enzyme and various concentrations of ALS inhibitors can be mixed in a final volume of 90 µl. Assays can be initiated by adding enzyme and the assays can be terminated after 75 min incubation at 30°C by the addition of 40 µl 0.5 M H₂SO₄. After 60 min at room temperature 80 µl of a solution of 1.4% a-naphtol and 0.14% creatine in 0.7 M NaOH can be added and after an additional 45 min incubation at room temperature the absorbance can be determined at 540 nm. p50-values for inhibition of ALS can be determined as described by Ray (1984), using the XLFit Excel add-in version 4.3.1 curve fitting program of ID Business Solutions Limited.

[149] The ALS nucleotide sequences referred to herein encoding ALS polypeptides preferably confer tolerance to one or more ALS inhibitor herbicides (or, vice versa, less sensitivity to an ALS inhibitor herbicide) as described herein. This is because of the point mutation leading to an amino acid substitution as described herein. In one embodiment, the plants of the present invention show tolerance against a compound of formula (I), e.g., plants according to the invention show essentially no injury
(injury below 5%, 1% or even 0%) when 15 g a.i. / ha are applied whereas injury of wild type is above 90%.

Tolerance

[150] Surprisingly, it was found that the presence of the P197S mutation in ALS I, or, to a higher extent, of the W574L mutation in ALS III increases herbicide tolerance to ALS inhibitor herbicides of Brassica plants, and that the combination of these two mutations increases the tolerance even further, particularly if homozygocity is established. Compared to herbicide tolerant B. napus plants of the same genetic background in which the same mutations are only heterozygously present, the herbicide tolerant B. napus plants which are homozygous for the mutation revealed a higher level and/or a better agronomical level of ALS inhibitor herbicide tolerance.

[151] One embodiment of the present invention refers to B. napus plants and parts thereof and progeny thereof which are heterozygous for the mutations described herein. Thus, also covered by the present invention are plants comprising at least in one allele of the ALS I gene in its endogenous gene locus a codon encoding Ser instead of Pro, at a position corresponding to position 544-546 of SEQ ID NO: 1, and comprising one or more further ALS I alleles encoding independently from each other Pro at a position corresponding to position 544-546 of SEQ ID NO: 1 wherein said further allele optionally comprise independently from each other at least one, two or three further mutations; and comprising in at least one allele of the ALS III gene in its endogenous gene locus a codon encoding Leu instead of Trp at a position corresponding to position 1666-1668 of SEQ ID NO: 3, and comprising one or more further ALS III allele(s) having independently from each other a codon at a position corresponding to position 1666-1668 of SEQ ID NO: 3 encoding Trp wherein said further ALS III alleles optionally comprise independently from each other at least one, two or three further mutations.

[152] However, one embodiment of the invention refers to B. napus plants and parts thereof which are homozygous regarding the point mutation of ALS I genes at a position corresponding to position 182 of SEQ ID NO: 1; and the point mutation of ALS III genes at a position corresponding to position 556 of SEQ ID NO: 3 leading to Ser instead of Pro, and Leu instead of Trp, respectively.

[153] As used herein, the term "homozygous" means a genetic condition existing when (at least) two different alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell. In other words, (at least) two different ALS I alleles and (at least) two different ALS III alleles, respectively, reside at specific loci but are positioned individually on corresponding pairs of homologous chromosomes in the cell.
Conversely, as used herein, the term "homozygous" means a genetic condition existing when two (all) identical alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell.

As used herein, the term "locus" (loci plural) means a specific place or places or a site on a chromosome where, e.g., a gene or genetic marker is found.

As mentioned herein, the *B. napus* plant of the present invention comprises in the nucleotide sequence of at least one ALS I gene in its endogenous gene locus a codon encoding Ser instead of Pro at a position as specified herein and in the nucleotide sequence of at least one ALS III gene in its endogenous gene locus a codon encoding Leu instead of Trp at a position as specified herein. By ALS genes in its "endogenous locus" it is meant that the ALS genes comprised by the *B. napus* plant of the present invention is - when compared to a wild-type *B. napus* plant - located in the same locus, i.e., the ALS genes are positioned (located) on the same chromosome in the same chromosomal context (organization) as they are positioned in a wild-type plant (i.e., without there being any human intervention so as to transfer or re-locate the ALS genes comprised by the *B. napus* plant of the present invention to another location such as to another chromosome or genomic locus (position) different from that where the ALS genes are naturally located). Accordingly, the identical genome-specific satellite markers which surround a wild-type ALS gene also surround an ALS gene comprised by the *B. napus* plant of the present invention.

"Positioned in the same chromosomal context (organization)" means that an ALS gene of the *B. napus* plant of the present invention is located on the same chromosome as it is in a wild-type *B. napus* plant. Accordingly, the same genes as in a wild-type *B. napus* plant are adjacent to the 5'- and 3'-end of an ALS gene comprised by the *B. napus* plant of the present invention. Hence, the same nucleotide sequences which are adjacent to the 5'- and 3'-end of the wild-type ALS gene are adjacent to the 5'- and 3'-end of an ALS gene comprised by the *B. napus* plant of the present invention. The similarity of the chromosomal context between an ALS gene comprised by the *B. napus* plant of the present invention and that of an ALS gene of a wild-type *B. napus* plant can, for example, be tested as follows:

Genome-specific satellite markers which surround a wild-type ALS gene and an ALS gene of the present invention can be used together with sequences from the *B. napus* ALS gene (preferably except for the codon at the position as specified herein which is different between the wild-type ALS gene and an ALS gene comprised by the *B. napus* plant of the present invention) for primer design and subsequent nucleic acid amplification, whereby the amplification product will be identical between a wild-type *B. napus* plant and the *B. napus* plant of the present invention. These genome-specific satellite markers can also be used for a fluorescent in situ hybridization (FISH) in order to check the location of the ALS gene (see Schmidt and Heslop-Harrison (1996), Proc. Natl. Acad. Sci.93:8761-8765 for a FISH protocol of *B. napus*).
[159] In view of the fact that mutated endogenous ALS I and III genes of the present invention are located at the same chromosome at the same specific location, respectively, the "staining pattern" in FISH of the chromosome on which the wild-type B. napus ALS I and III genes are located will be identical to the staining pattern in FISH of the chromosome on which the B. napus ALS I and III genes of the present invention are located.

[160] Of course, foreign genes can be transferred to the plant either by genetic engineering or by conventional methods such as crossing. Said genes can be genes conferring herbicide tolerances, preferably conferring herbicide tolerances different from ALS inhibitor herbicide tolerances, genes improving yield, genes improving resistances to biological organisms, and/or genes concerning content modifications.

[161] The plants according to the invention form the basis for the development of commercial varieties including F1 hybrids following procedures known in the breeding community supported by molecular breeding techniques (like marker assisted breeding or marker assisted selection) for speeding up the processes and to secure the correct selection of plants to either obtain the mutation in its homozygous form or in case of comprising one or more mutations at various locations of the ALS encoding endogenous gene to perform the correct selection of heterozygous plants wherein at least at one of the alleles of ALS I comprises the Pro97Ser mutation (when referring to SEQ ID NO: 10) according to present invention and at least one of the alleles of ALS III comprises the Trp574Leu mutation (when referring to SEQ ID NO: 10) according to the present invention.

[162] Calli are obtained by means and methods commonly known in the art, e.g., Alexander Dovzenko, PhD Thesis, Title: "Towards plastid transformation in rapeseed (Brassica napus L.) and sugarbeet (Beta vulgaris L.)", Ludwig-Maximilians-Universitat Miinchen, Germany, 2001:

[163] B. napus seeds can be immersed for 60 seconds in 70% ethanol, then rinsed twice in sterile water with 0,01 % detergent and then incubated for 1-4 hours in 1% NaOCl bleach. After washing with sterile ¾ 0 at 4°C, the embryos can be isolated using, e.g., forceps and scalpel.

[164] The freshly prepared embryos can be immersed in 0.5% NaOCl for 30 min and then washed in sterile ¾0. After the last washing step they can be placed on hormone free MS agar medium (Murashige and Skoog (1962), Physiol. Plantarum, 15, 473-497). Those embryos which developed into sterile seedlings can be used for the initiation of regenerable B. napus cell cultures.

[165] Cotyledons as well as hypocotyls can be cut into 2-5 mm long segments and then cultivated on agar (0.8%) solidified MS agar medium containing either 1 mg/l Benzylaminopurin (BAP) or 0.25 mg/l Thidiazuron (TDZ). 4 weeks later the developing shoot cultures can be transferred onto fresh MS
agar medium of the same composition and then sub-cultured in monthly intervals. The cultures can be kept at 25°C under dim light at a 12 h/12 h light/dark cycle.

[166] After 7-10 days, subcultures the shoot cultures which were grown on the thidiazuron containing medium formed a distinct callus type, which was fast growing, soft and friable. The colour of this callus type is typically yellowish to light green. Some of these friable calli consistently produced chlorophyll containing shoot primordia from embryo-like structures. These fast growing regenerable calli can be used for the selection of ALS inhibitor herbicide tolerant *B. napus* mutants.

**Use**

[167] The present invention further relates to the use of one or more ALS inhibitor herbicide(s) in *B. napus* mutants according to the invention comprising mutations of its endogenous acetolactate synthase (ALS) genes, wherein an ALS I gene encodes an ALS I polypeptide containing serine instead of proline at a position 182 of said ALS I polypeptide and wherein an ALS III gene encodes an ALS III polypeptide leucine instead of tryptophan at a position 559 of said ALS III polypeptide and wherein the ALS inhibitor herbicide(s) belong to:

the group of the (sulfon)amides (group (A)) consisting of:

the subgroup (A1) of the sulfonylureas, consisting of:

- amidosulfuron [CAS RN 120923-37-7] (= A1-1);
- azimsulfuron [CAS RN 120162-55-2] (= A1-2);
- bensulfuron-methyl [CAS RN 83055-99-6] (= A1-3);
- chlorimuron-ethyl [CAS RN 90982-32-4] (= A1-4);
- clorsulfuron [CAS RN 64902-72-3] (= A1-5);
- cinosulfuron [CAS RN 94593-91-6] (= A1-6);
- cyclosulfamuron [CAS RN 136849-1 5-5] (= A1-7);
- ethametsulfuron-methyl [CAS RN 97780-06-8] (= A1-8);
- ethoxysulfuron [CAS RN 126801-58-9] (= A1-9);
- flazasulfuron [CAS RN 104040-78-0] (= A1-10);
- flucetosulfuron [CAS RN 412928-75-7] (= A1-11);
- flupyrsulfuron-methyl-sodium [CAS RN 144740-54-5] (= A1-12);
- foramsulfuron [CAS RN 173159-57-4] (= A1-13);
- halosulfuron-methyl [CAS RN 100784-20-1] (= A1-14);
- imazosulfuron [CAS RN 122548-33-8] (= A1-15);
- iodosulfuron-methyl-sodium [CAS RN 144550-36-7] (= A1-16);
- mesosulfuron-methyl [CAS RN 208465-21-8] (= A1-17);
- metsulfuron-methyl [CAS RN 74223-64-6] (= A1-18);
- monosulfuron [CAS RN 155860-63-2] (= A1-19);
nicosulfuron [CAS RN 111991-09-4] (= Al-20);
orthosulfamuron [CAS RN 213464-77-8] (= Al-21);
oxasulfuron [CAS RN 144651-06-9] (= Al-22);
primisulfuron-methyl [CAS RN 86209-51-0] (= Al-23);
prosulfuron [CAS RN 94125-34-5] (= Al-24);
pyrazosulfuron-ethyl [CAS RN 93697-74-6] (= Al-25);
rimsulfuron [CAS RN 122931-48-0] (= Al-26);
sulfometuron-methyl [CAS RN 74222-97-2] (= Al-27);
sulfosulfuron [CAS RN 141776-32-1] (= Al-28);
thifensulfuron-methyl [CAS RN 79277-27-3] (= Al-29);
triasulfuron [CAS RN 82097-50-5] (= Al-30);
tribenuron-methyl [CAS RN 101200-48-0] (= Al-31);
trifloxysulfuron [CAS RN 145099-21-4] (sodium) (= Al-32);
triflusulfuron-methyl [CAS RN 126535-15-7] (= Al-33);
tritosulfuron [CAS RN 142469-14-5] (= Al-34);
NC-330 [CAS RN 104770-29-8] (= Al-35);
NC-620 [CAS RN 868680-84-6] (= Al-36);
TH-547 [CAS RN 570415-88-2] (= Al-37);
monosulfuron-methyl [CAS RN 175076-90-1] (= Al-38);

2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide ( = Al-39);
a compound of the general formula (I)

where M⁺ denotes the respective salt of the compound (I), i.e.
its lithium salt ( = Al-40); its sodium salt ( = Al-41); its potassium salt ( = Al-42); its magnesium
salt ( = Al-43); its calcium ( = Al-44); its ammonium salt ( = Al-45); its methylammonium salt
( = Al-46); its dimethylammonium salt ( = Al-47); its tetr methylammonium salt ( = Al-48); its
ethylammonium salt ( = Al-49); its diethylammonium salt ( = Al-50); its tetaethylammonium
salt ( = Al-51); its propylammonium salt ( = Al-52); its tetr propylammonium salt ( = Al-53); its
isopropylammonium salt ( = Al-54); its diisopropylammonium salt ( = Al-55); its
butylammonium salt ( = Al-56); its tetrabutylammonium salt ( = Al-57); its (2-hydroxyeth-l-
yl)ammonium salt ( = Al-58); its bis-N,N-(2-hydroxyeth-l-yl)ammonium salt ( = Al-59); its tris-
N,N,N-(2-hydroxyeth-l-yl)ammonium salt ( = Al-60); its 1-phenylethlammonium salt ( = Al-
61); its 2-phenylethlammonium salt ( = Al-62); its trimethylsulfonium salt ( = Al-63); its
trimethyloxonium salt (= Al-64); its pyridinium salt (= Al-65); its 2-methylpyridinium salt (= Al-66); its 4-methylpyridinium salt (= Al-67); its 2,4-dimethylpyridinium salt (= Al-68); its 2,6-dimethylpyridinium salt (= Al-69); its pipendinium salt (= Al-70); its imidazolium salt (= Al-71); its mopholinium salt (= Al-72); its 1,5-diazabicyclo[4.3.0]non-7-enium salt (= Al-73); its 1,8-diazabicyclo[5.4.0]undec-7-enium salt (= Al-74);

or a compound of the formula (II) or salts thereof

\[
\begin{align*}
\text{O} & \quad \text{N} \\
\text{O} & \quad \text{SO}_2 \quad \text{O} \\
\text{N} & \quad \text{H} \\
\text{R}^2 & \quad \text{N} \quad \text{R}^3
\end{align*}
\]

(II)

with \( R^2 \) and \( R^3 \) having the meaning as defined in the below table

<table>
<thead>
<tr>
<th>Compound</th>
<th>( R^2 )</th>
<th>( R^3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1-75</td>
<td>OCH(_3)</td>
<td>OC(_3)H(_3)</td>
</tr>
<tr>
<td>A1-76</td>
<td>OCH(_3)</td>
<td>CH(_3)</td>
</tr>
<tr>
<td>A1-77</td>
<td>OCH(_3)</td>
<td>C(_2)H(_5)</td>
</tr>
<tr>
<td>A1-78</td>
<td>OCH(_3)</td>
<td>CF(_3)</td>
</tr>
<tr>
<td>A1-79</td>
<td>OCH(_3)</td>
<td>OCF(_2)H</td>
</tr>
<tr>
<td>A1-80</td>
<td>OCH(_3)</td>
<td>NHCH(_3)</td>
</tr>
<tr>
<td>A1-81</td>
<td>OCH(_3)</td>
<td>N(CH(_3))(_2)</td>
</tr>
<tr>
<td>A1-82</td>
<td>OCH(_3)</td>
<td>Cl</td>
</tr>
<tr>
<td>A1-83</td>
<td>OCH(_3)</td>
<td>OCH(_3)</td>
</tr>
<tr>
<td>A1-84</td>
<td>OC(_2)H(_5)</td>
<td>OC(_2)H(_3)</td>
</tr>
<tr>
<td>A1-85</td>
<td>OC(_2)H(_5)</td>
<td>CH(_3)</td>
</tr>
<tr>
<td>A1-86</td>
<td>OC(_2)H(_3)</td>
<td>C(_2)H(_5)</td>
</tr>
</tbody>
</table>

or the compound of formula (III) (= A1-87), i.e. the sodium salt of compound (A1-83)
or the compound of formula (IV) (=Al-88), i.e. the sodium salt of compound (Al-82)

\[
\begin{align*}
\text{(IV)}
\end{align*}
\]

the subgroup of the sulfonylaminocarbonyltriazolinones (subgroup (A2)), consisting of:
flucarbazone-sodium [CAS RN 181274-17-9] (= A2-1);
propoxycarbazone-sodium [CAS RN 181274-15-7] (= A2-2);
thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3);

the subgroup of the triazolopyrimidines (subgroup (A3)), consisting of:
cloransulam-methyl [147150-35-4] (= A3-1);
diclosulam [CAS RN 145701-21-9] (= A3-2);
florasulam [CAS RN 145701-23-1] (= A3-3);
flumetsulam [CAS RN 98967-40-9] (= A3-4);
metosulam [CAS RN 139528-85-1] (= A3-5);
penoxsulam [CAS RN 219714-96-2] (= A3-6);
pyroxsulam [CAS RN 422556-08-9] (= A3-7);

the subgroup of the sulfonanilides (subgroup (A4)), consisting of:
compounds or salts thereof from the group described by the general formula (I):

\[
\begin{align*}
\text{(V)}
\end{align*}
\]

in which
R\(^1\) is halogen, preferably fluorine or chlorine,
R² is hydrogen and R³ is hydroxyl or
R² and R³ together with the carbon atom to which they are attached are a carbonyl group C=O
and
R⁴ is hydrogen or methyl;

and more especially compounds of the below given chemical structure (A4-1) to (A4-8)

- the group of the imidazolinones (group (Bl)), consisting of:
  - imazamethabenzmethyl [CAS RN 81405-85-8] (= Bl-1);
  - imazamox [CAS RN 114311-32-9] (= Bl-2);
  - imazapic [CAS RN 104098-48-8] (= Bl-3);
imazapyr [CAS RN 81334-34-1] (= B1-4);
imazaquin [CAS RN 81335-37-7] (= B1-5);
imazethapyr [CAS RN 81335-77-5] (= Bl-6);
SYP-298 [CAS RN 557064-77-4] (= B1-7);
SYP-300 [CAS RN 374718-10-2] (= B1-8).

the group of the pyrimidinyl(thio)benzoates (group (C)), consisting of:
the subgroup of the pyrimidinloyxobenzoacids (subgroup (CI) ) consisting of:
  bispyribac-sodium [CAS RN 125401-92-5] (= C1-1);
  pyribenzoxim [CAS RN 168088-61-7] (= C1-2);
  pyriminobac-methyl [CAS RN 136191-64-5] (= C1-3);
  pyribambenz-isopropyl [CAS RN 420138-41-6] (= C1-4);
  pyribambenz-propyl [CAS RN 420138-40-5] (= C1-5);
the subgroup of the pyrimidinylthiobenzoacids (subgroup (C2)), consisting of:
  pyrifenylid [CAS RN 135186-78-6] (= C2-1);
  pyriothiobac-sodium [CAS RN 123343-16-8] (= C2-2).

[168] In this context, "tolerance" or "tolerant" means that the application of one or more ALS
inhibitor herbicide(s) belonging to any of the above defined groups (A), (B), (C) have reduced apparent
effect(s), as compared to effect(s) on wild type B. napus plants, concerning the physiological
functions/phytotoxicity when applied to the respective Brassica plant, such as B. napus plants according
to the invention, having mutations of its endogenous acetolactate synthase (ALS) genes, wherein the
ALS I Brassica, such as B. napus, gene encodes a first ALS Brassica, such as B. napus, polypeptide
containing serine instead of proline at a position corresponding to position 197 of SEQ ID NO: 10 and
wherein the ALS III Brassica, such as B. napus, gene encodes a second ALS III Brassica, such as B.
apus, polypeptide containing leucine instead of tryptophan at a position corresponding to position 574
of SEQ ID NO: 10 and whereas the application of the same amount of the respective ALS inhibitor
herbicide(s) on non-tolerant Brassica, such as B. napus, wild type plants leads to significant negative
effects concerning plant growth, its physiological functions or shows phytotoxic symptoms. Quality and
quantity of the observed effects may depend on the chemical composition of the respective ALS
inhibitor herbicide(s) applied, dose rate and timing of the application as well growth conditions/stage of
the treated plants.

[169] The "CAS RN" stated in square brackets after the names (common names) mentioned under
groups A to C corresponds to the "chemical abstract service registry number", a customary reference
number which allows the substances named to be classified unambiguously, since the "CAS RN"
distinguishes, inter alia, between isomers including stereoisomers.
ALS inhibitor herbicides which are preferably used for control of unwanted vegetation in *B. napus* growing areas which *B. napus* plants comprise mutations of its endogenous acetolactate synthase (ALS) genes, wherein the ALS I gene encodes an ALS I polypeptide containing serine instead of proline at a position 182 of said first ALS I polypeptide and wherein the ALS III gene encodes an ALS III polypeptide containing leucine instead of tryptophan at a position 559 of said ALS III polypeptide and thereby providing tolerance against the ALS inhibitor herbicide(s) according to this invention belonging to group (A) are:

amidosulfuron [CAS RN 120923-37-7] (= Al-1);
chlorimuron-ethyl [CAS RN 90982-32-4] (= Al-4);
chlorsulfuron [CAS RN 64902-72-3] (=Al-5);
ethametsulfuron-methyl [CAS RN 97780-06-8] (= Al-8);
ethoxysulfuron [CAS RN 126801-58-9] (= Al-9);
flupyrdsulfuron-methyl-sodium [CAS RN 144740-54-5] (= Al-12);
foramsulfuron [CAS RN 173159-57-4] (= Al-13);
iodosulfuron-methyl-sodium [CAS RN 144550-36-7] (= Al-16);
mesosulfuron-methyl [CAS RN 208465-21-8] (= Al-17);
metsulfuron-methyl [CAS RN 74223-64-6] (= Al-18);
monosulfuron [CAS RN 155860-63-2] (= Al-19);
nicosulfuron [CAS RN 111991-09-4] (= Al-20);
rimsulfuron [CAS RN 122931-48-0] (= Al-26);
sulfosulfuron [CAS RN 141776-32-1] (= Al-28);
thifensulfuron-methyl [CAS RN 79277-27-3] (= Al-29);
tribenuron-methyl [CAS RN 101200-48-0] (= Al-31);
triflusulfuron-methyl [CAS RN 126535-15-7] (= Al-33);
2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide (= Al-39);
2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide sodium salt (= Al-41);
(AI-83) or its sodium salt (=AI-87);
flucarbazone-sodium [CAS RN 181274-17-9] (= A2-1);
propoxycarbazone-sodium [CAS RN 181274-15-7] (= A2-2);
thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3);
florasulam [CAS RN 145701-23-1] (= A3-3);
metsulfuron [CAS RN 139528-85-1] (= A3-5);
pyroxsulam [CAS RN 422556-08-9] (= A3-7);
(A4-l); (A4-2) and (A4-3).
[17 1] ALS inhibitor herbicides which are more preferably used for control of unwanted vegetation in
B. napus growing areas which B. napus plants are described herein comprise mutations of its
endogenous acetylactate synthase (ALS) genes, wherein the ALS I gene encodes an ALS I polypeptide
containing serine instead of proline at a position 182 of said first ALS I polypeptide and wherein the
ALS III gene encodes an ALS III polypeptide containing leucine instead of tryptophan at a position 559
of said ALS III polypeptide and thereby providing tolerance against the ALS inhibitor herbicide(s)
according to this invention belonging to group (A) are:

<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>CAS Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>amidosulfuron</td>
<td>[CAS RN 120923-37-7] (= A1-1);</td>
</tr>
<tr>
<td>ethoxysulfuron</td>
<td>[CAS RN 126801-58-9] (= A1-9);</td>
</tr>
<tr>
<td>flupyr-sulfuron-methyl-sodium</td>
<td>[CAS RN 144470-54-5] (= A1-12);</td>
</tr>
<tr>
<td>foramsulfuron</td>
<td>[CAS RN 173159-57-4] (= A1-13);</td>
</tr>
<tr>
<td>iodosulfuron-methyl-sodium</td>
<td>[CAS RN 144550-36-7] (= A1-16);</td>
</tr>
<tr>
<td>mesosulfuron-methyl</td>
<td>[CAS RN 208465-21-8] (= A1-17);</td>
</tr>
<tr>
<td>metsulfuron-methyl</td>
<td>[CAS RN 74223-64-6] (= A1-18);</td>
</tr>
<tr>
<td>nicosulfuron</td>
<td>[CAS RN 111991-09-4] (= A1-20);</td>
</tr>
<tr>
<td>rimsulfuron</td>
<td>[CAS RN 122931-48-0] (= A1-26);</td>
</tr>
<tr>
<td>sulfosulfuron</td>
<td>[CAS RN 141776-32-1] (= A1-28);</td>
</tr>
<tr>
<td>thifensulfuron-methyl</td>
<td>[CAS RN 79277-27-3] (= A1-29);</td>
</tr>
<tr>
<td>tribenuron-methyl</td>
<td>[CAS RN 101200-48-0] (= A1-31);</td>
</tr>
<tr>
<td>the sodium salt of compound of formula (I)</td>
<td>(= A1-41);</td>
</tr>
<tr>
<td>compound of formula (III)</td>
<td>(= A1-87);</td>
</tr>
<tr>
<td>propoxycarbazone-sodium</td>
<td>[CAS RN 181224-15-7] (= A2-2);</td>
</tr>
<tr>
<td>thienocarbazone-methyl</td>
<td>[CAS RN 317815-83-1] (= A2-3);</td>
</tr>
<tr>
<td>florasulam</td>
<td>[CAS RN 145701-23-1] (= A3-3);</td>
</tr>
<tr>
<td>metosulfuron</td>
<td>[CAS RN 139528-85-1] (= A3-5);</td>
</tr>
<tr>
<td>pyroxasulam</td>
<td>[CAS RN 422556-08-9] (= A3-7).</td>
</tr>
</tbody>
</table>

[172] ALS inhibitor herbicides which are especially preferably used for control of unwanted
vegetation in B. napus growing areas which B. napus plants comprise mutations of its endogenous
acetylactate synthase (ALS) genes, wherein the ALS I gene encodes an ALS I polypeptide containing
serine instead of proline at a position 182 of said first ALS I polypeptide and wherein the ALS III gene
encodes an ALS III polypeptide containing leucine instead of tryptophan at a position 559 of said ALS
III polypeptide and thereby providing tolerance against the ALS inhibitor herbicide(s) according to this
invention belonging to group (A) are:
amidosulfuron [CAS RN 120923-37-7] (= Al-1);
foramsulfuron [CAS RN 173159-57-4] (= Al-13);
sodium salt of compound of formula (I) (= Al-41);
and
thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3).

[173] Another ALS inhibitor herbicide which is preferably used for control of unwanted vegetation in B. napus growing areas which B. napus plants comprise mutations of its endogenous acetolactate synthase (ALS) genes, wherein the ALS I gene encodes an ALS I polypeptide containing serine instead of proline at a position 182 of said first ALS I polypeptide and wherein the ALS III gene encodes an ALS III polypeptide containing leucine instead of tryptophan at a position 559 of said ALS III polypeptide and thereby providing tolerance against the ALS inhibitor herbicide(s) according to this invention belonging to group (B) is imazamox [CAS RN 114311-32-9] (= Bl-2).

[174] Another ALS inhibitor herbicide which is preferably used for control of unwanted vegetation in B. napus growing areas which B. napus plants comprise mutations of its endogenous acetolactate synthase (ALS) genes, wherein the ALS I gene encodes an ALS I polypeptide containing serine instead of proline at a position 182 of said first ALS I polypeptide and wherein the ALS III gene encodes an ALS III polypeptide containing leucine instead of tryptophan at a position 559 of said ALS III polypeptide and thereby providing tolerance against the ALS inhibitor herbicide(s) according to this invention belonging to group (C) is bispyribac-sodium [CAS RN 125401-92-5] (= CI-1).

[175] It is to be further understood that concerning all above defined ALS inhibitor herbicides and where not already specified by the respective CAS RN, all use forms, such as acids, and salts can be applied according to the invention.

[176] Additionally, the ALS inhibitor herbicide(s) to be used according to the invention may comprise further components, for example agrochemically active compounds of a different type of mode of action and/or the formulation auxiliaries and/or additives customary in crop protection, or may be used together with these.

[177] In a further embodiment, the herbicide combinations to be used according to the invention comprise effective amounts of the ALS inhibitor herbicide(s) belonging to groups (A), (B) and/or (C) and/or have synergistic actions. The synergistic actions can be observed, for example, when applying one or more ALS inhibitor herbicide(s) belonging to groups (A), (B), and/or (C) together, for example as a coformulation or as a tank mix; however, they can also be observed when the active compounds are applied at different times (splitting). It is also possible to apply the herbicides or the herbicide combinations in a plurality of portions (sequential application), for example pre-emergence applications.
followed by post-emergence applications or early post-emergence applications followed by medium or late post-emergence applications. Preference is given here to the joint or almost simultaneous application of the ALS-inhibitor herbicides belonging to groups (A), (B) and/or (C) of the combination in question.

[178] The synergistic effects permit a reduction of the application rates of the individual ALS inhibitor herbicides, a higher efficacy at the same application rate, the control of species which were as yet uncontrolled (gaps), control of species which are tolerant or resistant to individual ALS inhibitor herbicides or to a number of ALS inhibitor herbicides, an extension of the period of application and/or a reduction in the number of individual applications required and - as a result for the user - weed control systems which are more advantageous economically and ecologically.

[179] The herbicides to be used according to this invention are all acetolactate synthase (ALS) inhibitor herbicides and thus inhibit protein biosynthesis in plants.

[180] The application rate of the ALS inhibitor herbicides belonging to groups (A), (B) or (C) (as defined above) can vary within a wide range, for example between 0.001 g and 1500 g of ai/ha (ai/ha means here and below "active substance per hectare" = based on 100% pure active compound). Applied at application rates of from 0.001 g to 1500 g of ai/ha, the herbicides belonging to classes A, B and C according to this invention, preferably the compounds A1-1; A1-4; A1-9; A1-12; A1-13; A1-16; A1-17; A1-18; A1-20; A1-26; A1-28; A1-29; A1-31; A1-41; A1-87; A2-2; A3-3; A3-5; A3-7, control, when used by the pre- and post-emergence method, a relatively wide spectrum of harmful plants, for example of annual and perennial mono- or dicotyledonous weeds, and also of unwanted crop plants (together also defined as "unwanted vegetation").

[181] In many applications according to the invention, the application rates are generally lower, for example in the range of from 0.001 g to 1000 g of ai/ha, preferably from 0.1 g to 500 g of ai/ha, particularly preferably from 0.5 g to 250 g of ai/ha, and even more preferably 1.0 g to 200 g of ai/ha. In cases where the application of several ALS inhibitor herbicides is conducted, the quantity represents the total quantity of all of the applied ALS inhibitor herbicides.

[182] For example, the combinations according to the invention of ALS inhibitor herbicides (belonging to groups (A), (B) and/or (C)) allow the activity to be enhanced synergistically in a manner which, by far and in an unexpected manner, exceeds the activities which can be achieved using the individual ALS inhibitor herbicides (belonging to groups (A), (B) and/or (C)).

[183] For combinations of ALS inhibitor herbicides, the preferred conditions are illustrated below.
[184] Of particular interest according to present invention is the use of herbicidal compositions for control of unwanted vegetation in *B. napus* plants, preferably in mutated *B. napus* plants as described herein having a content of the following ALS inhibitor herbicides:

\[(Al-1) + (Al-9); (Al-1) + (Al-12); (Al-1) + (Al-13); (Al-1) + (Al-16); (Al-1) + (Al-17); (Al-1) + (Al-18); (Al-1) + (Al-20); (Al-1) + (Al-26); (Al-1) + (Al-28); (Al-1) + (Al-29); (Al-1) + (Al-31); (Al-1) + (Al-41); (Al-1) + (Al-87); (Al-1) + (A2-2); (Al-1) + (A2-3); (Al-1) + (A3-3); (Al-1) + (A3-5); (Al-1) + (A3-7); (Al-1) + (Bl-2); (Al-1) + (Cl-1); (Al-9) + (Al-12); (Al-9) + (Al-13); (Al-9) + (Al-16); (Al-9) + (Al-17); (Al-9) + (Al-18); (Al-9) + (Al-20); (Al-9) + (Al-26); (Al-9) + (Al-28); (Al-9) + (Al-29); (Al-9) + (Al-31); (Al-9) + (Al-41); (Al-9) + (Al-87); (Al-9) + (A2-2); (Al-9) + (A2-3); (Al-9) + (A3-3); (Al-9) + (A3-5); (Al-9) + (A3-7); (Al-9) + (Bl-2); (Al-9) + (Cl-1); (Al-12) + (Al-13); (Al-12) + (Al-16); (Al-12) + (Al-17); (Al-12) + (Al-18); (Al-12) + (Al-20); (Al-12) + (Al-26); (Al-12) + (Al-28); (Al-12) + (Al-29); (Al-12) + (Al-31); (Al-12) + (Al-41); (Al-12) + (Al-87); (Al-12) + (A2-2); (Al-12) + (A2-3); (Al-12) + (A3-3); (Al-12) + (A3-5); (Al-12) + (A3-7); (Al-12) + (Bl-2); (Al-12) + (Cl-1); (Al-13) + (Al-16); (Al-13) + (Al-17); (Al-13) + (Al-18); (Al-13) + (Al-20); (Al-13) + (Al-26); (Al-13) + (Al-28); (Al-13) + (A2-2); (Al-13) + (A2-3); (Al-13) + (A3-3); (Al-13) + (A3-5); (Al-13) + (A3-7); (Al-13) + (Bl-2); (Al-13) + (Cl-1); (Al-16) + (Al-17); (Al-16) + (Al-18); (Al-16) + (Al-20); (Al-16) + (Al-26); (Al-16) + (Al-28); (Al-16) + (Al-29); (Al-16) + (Al-41); (Al-16) + (Al-87); (Al-16) + (A2-2); (Al-16) + (A2-3); (Al-16) + (A3-3); (Al-16) + (A3-5); (Al-16) + (A3-7); (Al-16) + (Bl-2); (Al-16) + (Cl-1); (Al-17) + (Al-18); (Al-17) + (Al-20); (Al-17) + (Al-26); (Al-17) + (Al-28); (Al-17) + (Al-29); (Al-17) + (Al-31); (Al-17) + (Al-41); (Al-17) + (Al-87); (Al-17) + (A2-2); (Al-17) + (A2-3); (Al-17) + (A3-3); (Al-17) + (A3-5); (Al-17) + (A3-7); (Al-17) + (Bl-2); (Al-17) + (Cl-1); (Al-18) + (Al-20); (Al-18) + (Al-26); (Al-18) + (Al-28); (Al-18) + (Al-29); (Al-18) + (Al-31); (Al-18) + (Al-41); (Al-18) + (Al-87); (Al-18) + (A2-2); (Al-18) + (A2-3); (Al-18) + (A3-3); (Al-18) + (A3-5); (Al-18) + (A3-7); (Al-18) + (Bl-2); (Al-18) + (Cl-1);
(Al-20) + (Al-26); (Al-20) + (Al-28); (Al-20) + (Al-29); (Al-20) + (Al-31); (Al-20) + (Al-41);
(Al-20) + (Al-87); (Al-20) + (A2-2); (Al-20) + (A2-3); (Al-20) + (A3-3); (Al-20) + (A3-5);
(Al-20) + (A3-7); (Al-20) + (B1-2); (Al-20) + (Cl-1);

5
(Al-26) + (Al-28); (Al-26) + (Al-29); (Al-26) + (Al-31); (Al-26) + (Al-41); (Al-26) + (Al-87);
(Al-26) + (A2-2); (Al-26) + (A2-3); (Al-26) + (A3-3); (Al-26) + (A3-5); (Al-26) + (A3-7);
(Al-26) + (B1-2); (Al-26) + (Cl-1);

10
(Al-28) + (Al-29); (Al-28) + (Al-31); (Al-28) + (Al-41); (Al-28) + (Al-87); (Al-28) + (A2-2);
(Al-28) + (A2-3); (Al-28) + (A3-3); (Al-28) + (A3-5); (Al-28) + (A3-7); (Al-28) + (B1-2);
(Al-28) + (Cl-1);

15
(Al-29) + (Al-31); (Al-29) + (Al-41); (Al-29) + (Al-87); (Al-29) + (A2-2); (Al-29) + (A2-3);
(Al-29) + (A3-3); (Al-29) + (A3-5); (Al-29) + (A3-7); (Al-29) + (B1-2); (Al-29) + (Cl-1);

20
(Al-31) + (Al-41); (Al-31) + (Al-87); (Al-31) + (A2-2); (Al-31) + (A2-3); (Al-31) + (A3-3);
(Al-31) + (A3-5); (Al-31) + (A3-7); (Al-31) + (B1-2); (Al-31) + (Cl-1);

25
(Al-41) + (Al-87); (Al-41) + (A2-2); (Al-41) + (A2-3); (Al-41) + (A3-3); (Al-41) + (A3-5);
(Al-41) + (A3-7); (Al-41) + (B1-2); (Al-41) + (Cl-1);

30
(Al-87) + (A2-2); (Al-87) + (A2-3); (Al-87) + (A3-3); (Al-87) + (A3-5); (Al-87) + (A3-7);
(Al-87) + (B1-2); (Al-87) + (Cl-1);

35

(B1-2) + (Cl-1).
Additionally, the ALS inhibitor herbicides to be used according to the invention may comprise further components, for example agrochemically active compounds of a different type of mode of action and/or the formulation auxiliaries and/or additives customary in crop protection, or may be used together with these.

The ALS inhibitor herbicide(s) to be used according to the invention or combinations of various such ALS inhibitor herbicides may furthermore comprise various agrochemically active compounds, for example from the group of the safeners, fungicides, insecticides, or from the group of the formulation auxiliaries and additives customary in crop protection.

In a further embodiment, the invention relates to the use of effective amounts of ALS inhibitor herbicide(s) (i.e. members of the groups (A), (B) and/or (C)) and non-ALS inhibitor herbicides (i.e. herbicides showing a mode of action that is different to the inhibition of the ALS enzyme [acetohydroxyacid synthase; EC 2.2.1.6] (group D herbicides) in order obtain synergistic effect for the control of unwanted vegetation. Such synergistic actions can be observed, for example, when applying one or more ALS inhibitor herbicides (i.e. members of the groups (A), (B), and/or (C)) and one or more non ALS inhibitor herbicides (group D herbicides) together, for example as a coformulation or as a tank mix; however, they can also be observed when the active compounds are applied at different times (splitting). It is also possible to apply the ALS inhibitor herbicides and non ALS inhibitor herbicides in a plurality of portions (sequential application), for example pre-emergence applications followed by post-emergence applications or early post-emergence applications followed by medium or late post-emergence applications. Preference is given here to the joint or almost simultaneous application of the herbicides ((A), (B) and/or (C)) and (D) of the combination in question.

Suitable partner herbicides to be applied together with ALS inhibitor herbicides are, for example, the following herbicides which differ structurally from the herbicides belonging to the groups (A), (B), and (C) as defined above, preferably herbicidally active compounds whose action is based on inhibition of, for example, acetyl coenzyme A carboxylase, PS I, PS II, HPPDO, phytoene desaturase, protoporphyrinogen oxidase, glutamine synthetase, cellulose biosynthesis, 5-enolpyruvylshikimate 3-phosphate synthetase, as described, for example, in Weed Research 26, 441-445 (1986), or "The Pesticide Manual", 14th edition, The British Crop Protection Council, 2007, or 15th edition 2010, or in the corresponding "e-Pesticide Manual", Version 5 (2010), in each case published by the British Crop Protection Council, (hereinbelow in short also "PM"), and in the literature cited therein. Lists of common names are also available in "The Compendium of Pesticide Common Names" on the internet. Herbicides known from the literature (in brackets behind the common name hereinafter also classified by the indicators D1 to D426), which can be combined with ALS-inhibitor herbicides of groups (A), (B) and/or (C) and to be used according to present invention are, for example, the active compounds listed below: (note: the herbicides are referred to either by the "common name" in accordance with the
International Organization for Standardization (ISO) or by the chemical name, together where appropriate with a customary code number, and in each case include all use forms, such as acids, salts, esters and isomers, such as stereoisomers and optical isomers, in particular the commercial form or the commercial forms, unless the context indicates otherwise. The citation given is of one use form and in some cases of two or more use forms):

- acetochlor (D1),
- acibenzolar (D2),
- acibenzolar-S-methyl (D3),
- acifluorfen (D4),
- acifluorfen-sodium (D5),
- aclonifen (D6),
- alachlor (D7),
- alldichlor (D8),
- alloxynid (D9),
- alloxynid-sodium (D10),
- ametryn (D11),
- amicarbazone (D12),
- amidochlor (D13),
- aminocyclopyrachlor (D14),
- aminopyralid (D15),
- amitrole (D16),
- ammonium sulfamate (D17),
- ancydimol (D18),
- anilofos (D19),
- asulam (D20),
- atrazine (D21),
- azafenidin (D22),
- aziprotryn (D23),
- beflubutamid (D24),
- benazolin (D25),
- benazolin-ethyl (D26),
- bencarbazone (D27),
- benfluralin (D28),
- benfuresate (D29),
- bensulide (D30),
- bentazone (D31),
- benfendizone (D32),
- benzobicyclon (D33),
- benzfenap (D34),
- benzofoor (D35),
- benzyolprop (D36),
- bicyclopyrone (D37),
- bifenoxy (D38),
- bilanafos (D39),
- bilanafos-sodium (D40),
- bromacil (D41),
- bromobutide (D42),
- bromofenoxim (D43),
- bromoxynil (D44),
- bromuron (D45),
- bupafos (D46),
- busoxinone (D47),
- butachlor (D48),
- butafenacil (D49),
- butamifos (D50),
- butenachlor (D51),
- butralin (D52),
- butroxydim (D53),
- butylate (D54),
- cafenstrole (D55),
- carbetamide (D56),
- carfentrazone (D57),
- carfentrazone-ethyl (D58),
- chlormethoxyfen (D59),
- chloramben (D60),
- chlorazifop (D61),
- chlorazifop-butyl (D62),
- chlorbromuron (D63),
- chlorbufam (D64),
- chlorfenac (D65),
- chlorfenac-sodium (D66),
- chlorfenprop (D67),
- chlorflurenol (D68),
- chlorflurenol-methyl (D69),
- chloridazon (D70),
- chlorimequat-chloride (D71),
- clomnitrofen (D72),
- chloropthalim (D73),
- chlorothal-dimethyl (D74),
- chlorotoluron (D75),
- cinidon (D76),
- cinidon-ethyl (D77),
- cinnmethlin (D78),
- clathidim (D79),
- clodinafop (D80),
- clodinafop-propargyl (D81),
- clofencet (D82),
- clomazone (D83),
- clomeprop (D84),
- cloproph (D85),
- clopyralid (D86),
- clorsulam (D87),
- clorsulam-methyl (D88),
- cumyluron (D89),
- cyanamide (D90),
- cyanazine (D91),
- cyclanilide (D92),
- cycloate (D93),
- cycloxydim (D94),
- cycluron (D95),
- cyhalofop (D96),
- cyhalofop-butyl (D97),
- cyperquat (D98),
- cyprazine (D99),
- cyprazole (D100),
- 2,4-D (D101),
- 2,4-DB (D102),
- daimuron/dymron (D103),
- dalapon (D104),
- daminozide (D105),
- dazomet (D106),
- d-necanol (D-107),
- desmedipham (D108),
- desmetryn (D109),
- detosyl-pyrazolate (D110),
- diallate (D111),
- dicamba (D112),
- dichlofensil (D12),
- dichloprop (D114),
- dichlorprop (D115),
- diclofop (D116),
- diclofop-methyl (D117),
- diclofop-P-methyl (D118),
- diethatyl (D119),
- diethatyl-ethyl (D120),
- difenoxuron (D121),
- difenzoquat (D122),
- difluflenican (D123),
- difluflenzopyr (D124),
- difluflenzopyr-sodium (D125),
- dimefuron (D126),
- dikegulac-sodium (D127),
- dimefuron (D128),
- dipropertin (D140),
- diquat (D141),
- diquat-dibromide (D142),
- dithio.pyr (D143),
- diuron
(= D144), DNOC (= D145), eglinazine-ethyl (= D146), endothal (= D147), EPTC (= D148), esprocarb (= D149), ethalfluralin (= D150), ethephon (= D151), ethidimuron (= D152), ethoziun (= D153), ethofumesate (= D154), ethoxyfen (= D155), ethoxyfen-ethyl (= D156), etobenzanid (= D157), F-5331 (= 2-Chlor-4-fluor-5-[4-(3-fluorpropyl)-4,5-dihydro-5-oxo-1H-tetrazol-1-yl]-phenyl)-ethansulfonamid)

(= D158), F-7967 (= 3-[7-Chlor-5-fluor-2-(trifluoroethyl)-1H-benzimidazol-4-yl]-1-methyl-6-(trifluoromethyl)pyrimidin-2,4-(IH,3H)-dion) (= D159), fenoprop (= D160), fenoxaprop (= D161), fenoxaprop-P (= D162), fenoxaprop-ethyl (= D163), fenoxaprop-P-ethyl (= D164), fenoxasulfone (= D165), fentrazamide (= D166), fenuron (= D167), fiamprop (= D168), flampop-M-isopropyl (= D169), flampop-M-methyl (= D170), fluazifop (= D171), fluazifop-P (= D172), fluazifop-butyl (= D173), fluazifop-P-butyl (= D174), fluazolinate (= D175), flufenacet (thiafluamide) (= D177), flufenpyr (= D178), flufenpyr-ethyl (= D179), flumetrin (= D180), flumiclorac (= D181), flumiclorac-pentyl (= D182), flumioxazin (= D183), flumipropyn (= D184), flumuron (= D185), fluorodifen (= D186), fluoroglycofen (= D187), fluoroglycofen-ethyl (= D188), fluopyram (= D189), flupropacil (= D190), flupropionate (= D191), flurenol (= D192), flurenol-butyl (= D193), fluridone (= D194), flurochloridone (= D195), fluroxypyr (= D196), fluroxypyr-mercaptanil (= D197), flurprimidol (= D198), flurtamone (= D199), fluthiacet (= D200), fluthiacet-methyl (= D201), fluthiamide (= D202), fomesafen (= 203), forchlorfenuron (= D204), fosamine (= D205), furyloxyfen (= D206), gibberellic acid (= D207), glufosinate (= D208), glufosinate-ammonium (= D209), glufosinate-P (= D210), glufosinate-P-ammonium (= D211), glufosinate-P-sodium (= D212), glyphosate (= D213), glyphosate-isopropylammonium (= D214), H-9201 (=0-(2,4-Dimethyl-6-nitrophenyl)-0-ethyl-isopropylphosphorotriothioate) (= D215), halosafen (= D216), haloxyfop (= D217), haloxyfop-P (= D218), haloxyfop-ethoxyethyl (= D219), haloxyfop-P-ethoxyethyl (= D220), haloxyfop-methyl (= D221), haloxyfop-P-methyl (= D222), hexazinone (= D223), HW-02 (= 1-(Dimethoxyphosphoryl)-ethyl(2,4-dichlorophenoxy)acetate) (= D224), inabenfide (= D225), indanofan (= D226), indaziflam (= D227), indol-3-acetic acid (IAA) (= D228), 4-indol-3-ylbutyric acid (IBA) (= D229), ioxynil (= D230), ipfencarbazone (= D231), isocarbammid (= D232), isopropalin (= D233), isoproturon (= D234), isouron (= D235), isoxaben (= D236), isoxachlortole (= D237), isoxaflutole (= D238), isoxapril (= D239), KUH-043 (= 3-[(5-(Difluoromethyl)-1-methyl-3-(trifluoroethyl)-1H-pyrazol-4-yl)methyl]sulfonyl)-5,5-dimethyl-4,5-dihydro-1,2-oxazol) (= D240), karbutilate (= D241), ketospiradox (= D242), lactofen (= D243), lenacil (= D244), linuron (= D245), maleic hydrazide (= D246), MCPA (= D247), MCPB (= D248), MCPB-methyl, ethyl and -sodium (= D249), mecoprop (= D250), mecoprop-sodium (= D251), mecoprop-butyl (= D252), mecoprop-P-butyl (= D253), mecoprop-P-dimethylammonium (= D254), mecoprop-P-2-ethylhexyl (= D255), mecoprop-P-potassium (= D256), mefenacet (= D257), mefludide (= D258), mepikat-chloride (= D259), mesotrione (= D260), methabenzthiazuron (= D261), metam (= D262), metamifop (= D263), metanitron (= D264), metazachlor (= D265), metazole (= D266), methiopyrsulfuron (= D267), methiozolin (= D268), methoxyphenone (= D269), methylidymron (= D270), 1-methylcyclopropen (= D271), methylisothiocyanate (= D272), metobenzuron (= D273), metobromuron (= D274), metolachlor (= D275), S-metolachlor (= D-276), metoxuron (= D277),
metribuzin (= D278), molinate (= D279), monalide (= D280), monocarbamide (= D281), monocarbamide-dihydrogensulfate (= D282), monolinuron (= D283), monosulfuron-ester (= D284), monuron (= D285), MT-128 (= 6-Chlor-N-[(2E)-3-chloroprop-2-en-1-yl]-5-methyl-N-pentenylpyridazin-3-amine) (= D286), MT-5950 (=N-[3-Chlor-4-(1-methylethyl)-phenyl]-2-methylpentanamide) (= D287), NGGC-01 1 (= D288), naproanlide (= D289), napropamide (= D290), naptalam (= D291), NC-310 (= 4-(2,4-Dichlorobenzoyl)-1-methyl-5-benzoxypyrazole) (= D292), neburon (= D293), nipyralofen (= D294), nitralin (= D295), nitrofen (= D296), nitrophenolat-sodium (isomer mixture) (= D297), nitrofluorfen (= D298), nonanoic acid (= D299), norflurazon (= D300), orbecarb (= D301), oryzalin (= D302), oxadiargyl (= D303), oxadiazon (= D304), oxaziclomefone (= D305), oxyfluorfen (= D306), paclobutrazol (= D307), paraquat (= D308), paraquat-dichloride (= D309), pelargonic acid (nonanoic acid) (= D310), pendimethalin (= D311), pendralin (= D312), pentanochlor (= D313), pentoxazone (= D314), perludone (= D315), pethoxamid (= D316), phenisopham (= D317), phenmedipham (= D318), phenmedipham-ethyl (= D319), picloram (= D321), picolinifen (= D322), pinoxaden (= D323), piperophos (= D324), pirifenop (= D325), pirifenop-butyl (= D326), pretilachlor (= D327), probenazole (= D328), proflualoz (= D329), procyzine (= D330), prodimine (= D331), prifuraline (= D332), profoxydim (= D333), prohexadione (= D334), prohexadione-calcium (= D335), prohydrojasnone (= D336), prometon (= D337), prometryn (= D338), propachlor (= D339), propanil (= D340), propaquizafop (= D341), propazone (= D342), prophen (= D343), propisochlor (= D344), propyzamide (= D345), prosulfalin (= D346), prosulfocarb (= D347), prynychlor (= D348), pyraclonil (= D349), pyraflufen (= D350), pyraflufen-ethyl (= D351), pyrasulfotole (= D352), pyrazolinate (pyrazolate) (= D353), pyrazoxyfen (= D354), pyribenzox = D355), pyributicarb (= D356), pyridafol (= D357), pyridate (= D358), pyriminobac (= D359), pyrimsulfan (= D360), pyroxasulfone (= D361), quinclorac (= D362), quinmerac (= D363), quinoclamine (= D364), quinalofop (= D365), quinalofop-ethyl (= D366), quinalofop-P (= D367), quinalofop-P-ethyl (= D368), quinalofop-P-tetryl (= D369), saflufenacil (= D370), secbumeton (= D371), sethoxydim (= D372), siduron (= D373), simazine (= D374), simetry (D375), SN-106279 (= Methyl-(2R)-2-[(7-chlor-4-(trifluoromethyl)phenoxy]-naphthyl)oxy)-propanoate) (= D376), sulcotrione (= D377), sulfallate (CDEC) (= D378), sulfentrazone (= D379), sulfosate (glyosphate-trismsium) (= D380), SYN-523 (= D381), SYP-249 (= 1-Ethoxy-3-methyl-1-oxobut-3-en-2-y1-5-[2-chlor-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate) (= D382), tebutam (= D383), tebufthiuron (= D384), tecnazene (= D385), tefuryltronie (= D386), tembotrione (= D387), tepraloxydim (= D388), terbacin (= D389), terbacarb (= D390), terbuchlor (= D391), terbumeton (= D392), terbutylazine (= D393), terbutryn (= D394), thenylchlor (= D395), thiamfluamide (= D396), thiazafuron (= D397), thiazopyr (= D398), thidiazim (= D399), thidiazuron (= D400), thiobencarb (= D401), тioсarbazil (= D402), topramezone (= D403), tralkoxydim (= D404), triallate (= D405), triazolam (= D406), triazofenamid (= D407), trichloracetic acid (TCA) (= D408), triclopyr (= D409), tridiphane (= D410), trietazine (= D411), trifluralin (= D412), trimeturon (= D413), trinexapac (= D414), trinexapac-ethyl (= D415), tsitodef (= D416), uniconazole (= D417), uniconazole-P (= D418), vernolate (= D419),
ZJ-0862 (= 3,4-Dichlor-N-{2-[(4,6-dimethoxypyrimidin-2-yl)oxy]benzyl}aniline) (= D420), the below
compounds defined by their chemical structure, respectively:

![Chemical structures](image)

and propachlor (D 427).

[189] Preferably, further herbicides which differ structurally and via their mode of action from the
ALS inhibitor herbicides belonging to the groups (A), (B), and (C) as defined above and to be applied
to the present invention for control of unwanted vegetation in ALS inhibitor herbicide tolerant
*B. napus* plants, preferably in mutated *B. napus* plants as described herein. In connection with ALS
inhibitor herbicides belonging to the groups (A), (B), and (C) are those selected from the group
consisting of acetochlor (= D1), carbetamide (= D56), fenoxaprop-P-ethyl (= D164), fluazifop-P-butyl
(= D174), haloxyfop-P-methyl (= D222), metolachlor (= D275), dimethenamid (= D132), napropamide
(= D290), pethoxamid (= D317), propaquizafop (= D341), propisochlor (= D344), propyzamide (=
D345), quinmerac (= D363), propachlor (D 427), clomazone (= D83), clopyralid (= D86), dimethachlor
(= D130), metazachlor (= D265), picloram (= D321), and quizalofop-P-ethyl (= D368).

[190] Even more preferably, further herbicides which differ from the ALS inhibitor herbicides
belonging to the groups (A), (B), and (C) as defined above and to be applied according to the invention
in connection with ALS inhibitor herbicides belonging to the groups (A), (B), and (C) are those selected
from the group consisting of clomazone (= D83), clopyralid (= D86), dimethachlor (= D130),
metazachlor (= D265), picloram (= D321), and quizalofop-P-ethyl (= D368).

[191] Mixtures containing ALS inhibitor herbicides and non ALS inhibitor herbicides, compositions
comprising mixtures of one or more ALS inhibitor herbicide(s) (compounds belonging to one or more of
groups (A), (B) and (C)) and non ALS inhibitor herbicide(s) (group (D) members; as defined above)
that are of very particular interest in order to be used according to present invention for control of
unwanted vegetation are:
(Al-1) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);
(Al-9) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-12) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-13) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-16) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-17) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-18) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-20) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-26) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-28) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-29) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-31) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-41) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A1-87) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A2-2) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A2-3) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A3-3) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A3-5) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);

(A3-7) + (D83); (Al-1) + (D86); (Al-1) + (D130); (Al-1) + (D265); (Al-1) + (D321); (Al-1) + (D368);
The application of ALS inhibitor herbicides also act efficiently on perennial weeds which produce shoots from rhizomes, root stocks and other perennial organs and which are difficult to control. Here, the substances can be applied, for example, by the pre-sowing method, the pre-emergence method or the post-emergence method, for example jointly or separately. Preference is given, for example, to application by the post-emergence method, in particular to the emerged harmful plants.

Specific examples may be mentioned of some representatives of the monocotyledonous and dicotyledonous weed flora which can be controlled by the ALS inhibitor herbicides, without the enumeration being restricted to certain species.

Examples of weed species on which the application according to present invention act efficiently are, from amongst the monocotyledonous weed species, *Avena* spp., *Alopecurus* spp., *Apera* spp., *Brachiaria* spp., *Bromus* spp., *Digitaria* spp., *Lolium* spp., *Echinochloa* spp., *Panicum* spp., *Phalaris* spp., *Poa* spp., *Setaria* spp., volunteer cereals (*Triticum sp.*, *Hordeum sp.*) and also *Cyperus* species from the annual group, and, among the perennial species, *Agropyron*, *Cynodon*, *Imperata* and *Sorghum* and also perennial *Cyperus* species.


Another embodiment provides a *Brassica*, such as *B. napus*, plant as described herein to which one or more ALS inhibitor herbicide(s) alone or in combination with one or more herbicide(s) that do(es) not belong to the class of ALS inhibitor herbicides are applied for control of unwanted vegetation in *Brassica*, such as *B. napus*, plant comprising an ALS I polypeptide containing serine instead of
proline at a position of said ALS I a \textit{Brassica}, such as \textit{B. napus}, polypeptide corresponding to position 197 of SEQ ID NO: 10 and an ALS III \textit{Brassica}, such as \textit{B. napus}, polypeptide containing leucine instead of tryptophan at a position of said ALS III polypeptide corresponding to position 574 of SEQ ID NO: 10.

[197] In another embodiment, a \textit{Brassica}, such as \textit{B. napus}, plant is provided as described herein to which one or more ALS inhibitor herbicide(s) alone or in combination with one or more herbicide(s) that do(es) not belong to the class of ALS inhibitor herbicides are applied for control of unwanted vegetation in \textit{Brassica}, such as \textit{B. napus}, plant comprising mutations of its endogenous acetolactate synthase (ALS) \textit{Brassica}, such as \textit{B. napus}, genes, wherein the ALS I \textit{Brassica}, such as \textit{B. napus}, gene encodes an ALS I \textit{Brassica}, such as \textit{B. napus}, polypeptide containing serine instead of proline at a position corresponding to position 197 of SEQ ID NO: 10 and wherein the ALS III \textit{Brassica}, such as \textit{B. napus}, gene encodes an ALS III \textit{Brassica}, such as \textit{B. napus}, polypeptide containing leucine instead of tryptophan at a position corresponding to position 574 of SEQ ID NO: 10.

[198] In yet another embodiment, a \textit{Brassica}, such as \textit{B. napus}, plant as described herein is homozygous regarding the mutation of an ALS I gene and an ALS II gene, respectively, as described herein.

[199] In one embodiment, the present invention relates to the use of one or more ALS inhibitor herbicide(s) alone or in combination with one or more non ALS inhibitor herbicide(s) for weed control in \textit{B. napus} growing areas which \textit{B. napus} comprise an endogenous ALS I gene, wherein the ALS I gene comprises a codon encoding Ser instead of Pro at a position corresponding to position 544-546 of the nucleotide sequence of the \textit{B. napus} ALS I gene shown in SEQ ID NO: 1, and an endogenous ALS III gene, wherein the ALS III gene comprises Leu instead of Trp at a position corresponding to position 1666-1668 of the nucleotide sequence of the \textit{B. napus} ALS III gene shown in SEQ ID NO: 3, which plants are heterozygous or homozygous, preferably homozygous concerning the mutation in codon 544-546 of the endogenous ALS I gene and the mutation in codon 1666-1668 of the endogenous ALS III gene.

[200] Owing to their herbicidal and plant growth-regulatory properties, ALS inhibitor herbicides belonging to one or more of the groups (A), (B), and (C) either alone or in combination with non ALS inhibitor herbicides can be employed for controlling harmful plants in known \textit{Brassica}, such as \textit{B. napus}, plants but also in tolerant or genetically modified crop plants that do already exist or need still to be developed. In general, the transgenic plants are distinguished by specific advantageous properties, in addition to tolerances to the ALS inhibitor herbicides according to the invention, for example, by tolerances to non ALS inhibitor herbicides, resistances to plant diseases or the causative organisms of plant diseases such as certain insects or microorganisms, such as fungi, bacteria or viruses. Other specific characteristics relate, for example, to the harvested material with regard to quantity, quality,
storability, composition and specific constituents. Thus, transgenic plants are known whose oil content is increased, or whose oil quality is altered, or those where the harvested material has a different fatty acid composition.

[201] Conventional methods of generating novel plants which have modified properties in comparison to plants occurring to date consist, for example, in traditional breeding methods and the generation of mutants. Alternatively, novel plants with altered properties can be generated with the aid of recombinant methods (see, for example, EP-A-0221044, EP-A-0131624). For example, the following have been described in several cases:

- the modification, by recombinant technology, of crop plants with the aim of modifying the starch synthesized in the plants (for example WO 92/1 1376, WO 92/14827, WO 91/19806),
- transgenic crop plants which exhibit tolerance to non ALS inhibitor herbicides,
- transgenic crop plants with the capability of producing Bacillus thuringiensis toxins (Bt toxins), which make the plants resistant to certain pests (EP-A-0142924, EP-A-0193259),
- transgenic crop plants with a modified fatty acid composition (WO 91/13972).

[202] The plants according to the invention may additionally contain an endogenous or a transgene, which confers herbicide resistance, such as the bar or pat gene, which confer resistance to glufosinate ammonium (Liberty or Basta) [EP 0 242 236 and EP 0 242 246 incorporated by reference]; or any modified EPSPS gene, such as the 2mEPSPS gene from maize [EP0 508 909 and EP 0 507 698 incorporated by reference], or glyphosate acetyltransferase, or glyphosate oxidoreductase, which confer resistance to glyphosate (RoundupReady), or bromoxynitril nitrilase to confer bromoxynitril tolerance.

Further, the plants according to the invention may additionally contain an endogenous or a transgene which confers increased oil content or improved oil composition, such as a 12:0 ACP thioesterase increase to obtain high laureate; which confers increased digestibility, such as 3-phytase; which confers pollination control, such as such as barnase under control of an anther-specific promoter to obtain male sterility, or barstar under control of an anther-specific promoter to confer restoration of male sterility, or such as the Ogura cytoplasmic male sterility and nuclear restorer of fertility.


[204] To carry out such recombinant manipulations, nucleic acid molecules which allow mutagenesis or sequence changes by recombination of DNA sequences can be introduced into plasmids. For example, the abovementioned standard methods allow base exchanges to be carried out, subsequences to be removed, or natural or synthetic sequences to be added. To connect the DNA fragments to each other,
adapters or linkers may be added to the fragments.

[205] For example, the generation of plant cells with a reduced activity of a gene product can be achieved by expressing at least one corresponding antisense RNA, a sense RNA for achieving a cosuppression effect or by expressing at least one suitably constructed ribozyme which specifically cleaves transcripts of the abovementioned gene product.

[206] To this end, it is possible to use DNA molecules which encompass the entire coding sequence of a gene product inclusive of any flanking sequences which may be present, and also DNA molecules which only encompass portions of the coding sequence, it being necessary for these portions to be long enough to have an antisense effect in the cells. The use of DNA sequences which have a high degree of homology to the coding sequences of a gene product, but are not completely identical to them, is also possible.

[207] When expressing nucleic acid molecules in plants, the protein synthesized can be localized in any desired compartment of the plant cell. However, to achieve localization in a particular compartment, it is possible, for example, to link the coding region with DNA sequences which ensure localization in a particular compartment. Such sequences are known to those skilled in the art (see, for example, Braun et al., EMBO J. 11 (1992), 3219-3227; Wolter et al., Proc. Natl. Acad. Sci. USA 85 (1988), 846-850; Sonnewald et al., Plant J. 1 (1991), 95-106).

[208] The transgenic plant cells can be regenerated by known techniques to give rise to entire plants. Thus, transgenic Brassica, such as B. napus, plants can be obtained whose properties are altered by overexpression, suppression or inhibition of homologous (= natural) genes or gene sequences or the expression of heterologous (= foreign) genes or gene sequences.

[209] The present invention furthermore provides a method for controlling unwanted plants in B. napus growing areas of B. napus plants according to the invention as described herein which comprises applying one or more ALS inhibitor herbicides belonging to groups (A), (B) and/or (C) to the plants (for example harmful plants, such as monocotyledonous or dicotyledonous weeds or unwanted crop plants), the seed (seeds or vegetative propagation organs, such as tubers or shoot parts) or to the area in which the plants grow (for example the area under cultivation), for example together or separately.

[210] The present invention furthermore provides a method for controlling unwanted plants in B. napus growing areas of B. napus plants according to the invention as described herein which comprises applying one or more ALS inhibitor herbicide(s) belonging to groups (A), (B) and/or (C) alone or in combination with non ALS inhibitor herbicides belonging to class (D) compound according to the invention to the plants (for example harmful plants, such as monocotyledonous or dicotyledonous weeds or unwanted crop plants), the seed (seeds or vegetative propagation organs, such as tubers or shoot parts)
or to the area in which the plants grow (for example the area under cultivation), for example together or separately. One or more non ALS inhibitor herbicides may be applied in combination with one or more ALS inhibitor herbicide(s) before, after or simultaneously with the ALS inhibitor herbicide(s) to the plants, the seed or the area in which the plants grow (for example the area under cultivation).

[21] "Unwanted plants" or "unwanted vegetation" are to be understood as meaning all plants which grow in locations where they are unwanted. This can, for example, be harmful plants (for example monocotyledonous or dicotyledonous species or other unwanted crop plants (volunteers)) such as *Geranium dissectum*, *Centaurea cyanus*, *Sinapis arvensis* and/or *Alopecurus myosuroides*.


[213] In yet another embodiment, an unwanted plant is at least one monocotyledonous plant selected from the group consisting of *Agropyron repens*, *Alopecurus myosuroides*, *Apera spica-venti*, *Avena sp.*, Bromus sp., Cyperus sp., Digitaria sp., Echinochloa sp., *Hoxdeum marinum*, *Lolium multiflorum*, *Panicum dichotomiflorum*, *Phalaris canariensis*, *Poa sp.*, *Setaria sp.*, *Sorghum halepense*, *Leptochloa filiformis*. In another embodiment, an unwanted plant is at least one plant selected from the group consisting of *Agropyron repens*, *Alopecurus myosuroides*, *Apera spica-venti*, *Avena sp.* and *Poa sp.*
In yet another embodiment, an unwanted plant is at least one monocotyledonous plant selected from the group consisting of *Beta vulgaris*, *Helianthus annuus*, *Solarium tuberosum*, *Triticum vulgare*, *Hordeum vulgare*, *Secale cereale*, *Avena sativa*. In another embodiment, an unwanted plant is *Triticum vulgare* and *Hordeum vulgare*.

The herbicide combinations to be used according to the invention can be prepared by known processes, for example as mixed formulations of the individual components, if appropriate with further active compounds, additives and/or customary formulation auxiliaries, which combinations are then applied in a customary manner diluted with water, or as tank mixes by joint dilution of the components, formulated separately or formulated partially separately, with water. Also possible is the split application of the separately formulated or partially separately formulated individual components.

It is also possible to apply ALS inhibitor herbicides or the combination comprising ALS inhibitor herbicide(s) and non ALS inhibitor herbicide(s) in a plurality of portions (sequential application) using, for example, pre-emergence applications followed by post-emergence applications or using early post-emergence applications followed by medium or late post-emergence applications. Preference is given here to the joint or almost simultaneous application of the active compounds of the combination in question.

The herbicides belonging to any of the above defined groups (A), (B), (C) and (D) and to be applied according to present invention can be converted jointly or separately into customary formulations, such as solutions, emulsions suspensions, powders, foams, pastes, granules, aerosols, natural and synthetic materials impregnated with active compound and microencapsulations in polymeric materials. The formulations may comprise the customary auxiliaries and additives.

These formulations are produced in a known manner, for example by mixing the active compounds with extenders, that is liquid solvents, pressurized liquefied gases and/or solid carriers, if appropriate with the use of surfactants, that is emulsifiers and/or dispersants, and/or foam formers.

If the extender used is water, it is also possible to use, for example, organic solvents as auxiliary solvents. Suitable liquid solvents are essentially: aromatics, such as xylene, toluene, alkynaphthalenes, chlorinated aromatics or chlorinated aliphatic hydrocarbons, such as chlorobenzenes, chloroethylenes, or methylene chloride, aliphatic hydrocarbons, such as cyclohexane or paraffins, for example mineral oil fractions, mineral and vegetable oils, alcohols, such as butanol or glycol, and ethers and esters thereof, ketones, such as acetone, methyl ethyl ketone, methyl isobutyl ketone or cyclohexanone, strongly polar solvents, such as dimethylformamide or dimethyl sulfoxide, and also water.
[220] Suitable solid carriers are: for example ammonium salts and ground natural minerals, such as kaolins, clays, talc, chalk, quartz, attapulgite, montmorillonite or diatomaceous earth, and ground synthetic minerals, such as finely divided silica, alumina and silicates; suitable solid carriers for granules are: for example crushed and fractionated natural rocks, such as calcite, marble, pumice, sepiolite and dolomite, and also synthetic granules of inorganic and organic meals, and granules of organic material, such as sawdust, coconut shells, corn cobs and tobacco stalks; suitable emulsifiers and/or foam formers are: for example nonionic and anionic emulsifiers, such as polyoxyethylene fatty acid esters, polyoxyethylene fatty alcohol ethers, for example alkylaryl polyglycol ethers, alkylsulfonates, alkyl sulfates, arylsulfonates and also protein hydrolysates; suitable dispersants are: for example lignosulfite waste liquors and methylcellulose.

[221] Tackifiers such as carboxymethylcellulose and natural and synthetic polymers in the form of powders, granules or latices, such as gum arabic, polyvinyl alcohol and polyvinyl acetate, and also natural phospholipids, such as cephalins and lecithins and synthetic phospholipids, can be used in the formulations. Other possible additives are mineral and vegetable oils.

[222] The herbicidal action of the herbicide combinations to be used according to the invention can be improved, for example, by surfactants, preferably by wetting agents from the group of the fatty alcohol polyglycol ethers. The fatty alcohol polyglycol ethers preferably comprise 10 - 18 carbon atoms in the fatty alcohol radical and 2 - 20 ethylene oxide units in the polyglycol ether moiety. The fatty alcohol polyglycol ethers may be present in nonionic form, or ionic form, for example in the form of fatty alcohol polyglycol ether sulfates, which may be used, for example, as alkali metal salts (for example sodium salts and potassium salts) or ammonium salts, or even as alkaline earth metal salts, such as magnesium salts, such as C_{12}/C_{14}-fatty alcohol diglycol ether sulfate sodium (Genapol® LRO, Clariant GmbH); see, for example, EP-A-0476555, EP-A-0048436, EP-A-0336151 or US-A-4,400,196 and also Proc. EWRS Symp. "Factors Affecting Herbicidal Activity and Selectivity", 227 - 232 (1988). Nonionic fatty alcohol polyglycol ethers are, for example, (Cio-Cis)-, preferably (Cio-Ci4)-fatty alcohol polyglycol ethers (for example isotridecyl alcohol polyglycol ethers) which comprise, for example, 2 - 20, preferably 3 - 15, ethylene oxide units, for example those from the Genapol® X-series, such as Genapol® X-030, Genapol® X-060, Genapol® X-080 or Genapol® X-150 (all from Clariant GmbH).

[223] The present invention further comprises the combination of ALS inhibitor herbicides belonging to any of the groups (A), (B), and (C) according to present invention with the wetting agents mentioned above from the group of the fatty alcohol polyglycol ethers which preferably contain 10 - 18 carbon atoms in the fatty alcohol radical and 2 - 20 ethylene oxide units in the polyglycol ether moiety and which may be present in nonionic or ionic form (for example as fatty alcohol polyglycol ether sulfates). Preference is given to Cn/Cw-fatty alcohol diglycol ether sulfate sodium (Genapol® LRO, Clariant
GmbH) and isotridecyl alcohol polyglycol ether having 3 - 15 ethylene oxide units, for example from the Genapol® X-series, such as Genapol® X-030, Genapol® X-060, Genapol® X-080 and Genapol® X-150 (all from Clariant GmbH). Furthermore, it is known that fatty alcohol polyglycol ethers, such as nonionic or ionic fatty alcohol polyglycol ethers (for example fatty alcohol polyglycol ether sulfates) are also suitable for use as penetrants and activity enhancers for a number of other herbicides (see, for example, EP-A-0502014).

[224] Furthermore, it is known that fatty alcohol polyglycol ethers, such as nonionic or ionic fatty alcohol polyglycol ethers (for example fatty alcohol polyglycol ether sulfates) are also suitable for use as penetrants and activity enhancers for a number of other herbicides (see, for example, EP-A-0502014).

[225] The herbicidal action of the herbicide combinations according to the invention can also be enhanced by using vegetable oils. The term vegetable oils is to be understood as meaning oils of oleaginous plant species, such as soybean oil, rapeseed oil, corn oil, sunflower oil, cottonseed oil, linseed oil, coconut oil, palm oil, thistle oil or castor oil, in particular rapeseed oil, and also their transesterification products, for example alkyl esters, such as rapeseed oil methyl ester or rapeseed oil ethyl ester.

[226] The vegetable oils are preferably esters of G0-C22-, preferably G2-C20-, fatty acids. The G0-C22-fatty acid esters are, for example, esters of unsaturated or saturated Go-C22-fatty acids, in particular those having an even number of carbon atoms, for example erucic acid, lauric acid, palmitic acid and in particular C18-fatty acids, such as stearic acid, oleic acid, linoleic acid or linolenic acid.

[227] Examples of Go-C22-fatty acid esters are esters obtained by reacting glycerol or glycol with the Cio-C22-fatty acids contained, for example, in oils of oleaginous plant species, or Ci-C2o-alkyl-Cio-C22-fatty acid esters which can be obtained, for example, by transesterification of the aforementioned glycerol- or glycol-Cio-C22-fatty acid esters with Ci-C2o-alcohols (for example methanol, ethanol, propanol or butanol). The transesterification can be carried out by known methods as described, for example, in RSmpp Chemie Lexikon, 9th edition, Volume 2, page 1343, Thieme Verlag Stuttgart.

[228] Preferred Ci-C2o-alkyl-Cio-C22-fatty acid esters are methyl esters, ethyl esters, propyl esters, butyl esters, 2-ethylhexyl esters and dodecyl esters. Preferred glycol- and glycerol-Go-C22-fatty acid esters are the uniform or mixed glycol esters and glycerol esters of Go-C22-fatty acids, in particular fatty acids having an even number of carbon atoms, for example erucic acid, lauric acid, palmitic acid and, in particular, C18-fatty acids, such as stearic acid, oleic acid, linoleic acid or linolenic acid.

[229] In the herbicidal compositions to be used according to the invention, the vegetable oils can be present, for example, in the form of commercially available oil-containing formulation additives, in particular those based on rapeseed oil, such as Hasten® (Victorian Chemical Company, Australia,
hereinbelow referred to as Hasten, main ingredient: rapeseed oil ester), Actirob®B (Novance, France, hereinbelow referred to as ActirobB, main ingredient: rapeseed oil methyl ester), Rako-Binol® (Bayer AG, Germany, hereinbelow referred to as Rako-Binol, main ingredient: rapeseed oil), Renol® (Stefes, Germany, hereinbelow referred to as Renol, vegetable oil ingredient: rapeseed oil methyl ester) or Stefes Mero® (Stefes, Germany, hereinbelow referred to as Mero, main ingredient: rapeseed oil methyl ester).

[230]  In a further embodiment, herbicidal combinations to be used according to present invention can be formulated with the vegetable oils mentioned above, such as rapeseed oil, preferably in the form of commercially available oil-containing formulation additives, in particular those based on rapeseed oil, such as Hasten® (Victorian Chemical Company, Australia, hereinbelow referred to as Hasten, main ingredient: rapeseed oil ethyl ester), Actirob®B (Novance, France, hereinbelow referred to as ActirobB, main ingredient: rapeseed oil methyl ester), Rako-Binol® (Bayer AG, Germany, hereinbelow referred to as Rako-Binol, main ingredient: rapeseed oil), Renol® (Stefes, Germany, hereinbelow referred to as Renol, vegetable oil ingredient: rapeseed oil methyl ester) or Stefes Mero® (Stefes, Germany, hereinbelow referred to as Mero, main ingredient: rapeseed oil methyl ester).

[231]  It is possible to use colorants, such as inorganic pigments, for example iron oxide, titanium oxide, Prussian Blue, and organic dyes, such as alizarin dyes, azo dyes and metal phthalocyanine dyes, and trace nutrients such as salts of iron, manganese, boron, copper, cobalt, molybdenum and zinc.

[232]  The formulations to be used according to present invention generally comprise from 0.1 to 95% by weight of active compounds, preferably from 0.5 to 90% by weight.

[233]  As such or in their formulations, the ALS inhibitor herbicides belonging to any of the above defined groups (A), (B), and (C) can also be used as a mixture with other agrochemically active compounds, such as known non ALS inhibitor herbicides, for controlling unwanted vegetation, for example for controlling weeds or for controlling unwanted crop plants, finished formulations or tank mixes, for example, being possible.

[234]  The use of a mixture of ALS inhibitor herbicides belonging to any of the above defined groups (A), (B), and (C) with other known active compounds, such as fungicides, insecticides, acaricides, nematicides, safeners, bird repellants, plant nutrients and soil structure improvers is likewise possible.

[235]  The ALS inhibitor herbicides belonging to any of the above defined groups (A), (B), (C) can be used as such, in the form of their formulations or in the use forms prepared therefrom by further dilution, such as ready-to-use solutions, suspensions, emulsions, powders, pastes and granules. Application is carried out in a customary manner, for example by watering, spraying, atomizing, broadcasting.
According to the invention, one or more of the ALS inhibitor herbicides belonging to any of the above defined groups (A), (B), and (C) can be applied either alone or in combination with one or more non ALS inhibitor herbicides belonging to group (DO) to the plants (for example harmful plants, such as monocotyledonous or dicotyledonous weeds or unwanted crop plants), the seed (for example grains, seeds or vegetative propagation organs, such as tubers or shoot parts with buds) or the area under cultivation (for example the soil), preferably to the green plants and parts of plants and, if appropriate, additionally the soil. One possible use is the joint application of the active compounds in the form of tank mixes, where the optimally formulated concentrated formulations of the individual active compounds are, together, mixed in a tank with water, and the spray liquor obtained is applied.

**Agronomically exploitable**

The skilled person will understand that it is generally preferred that the *B. napus* plants of the present invention and parts thereof are agronomically exploitable.

"Agronomically exploitable" means that the *B. napus* plants and parts thereof are useful for agronomical purposes. For example, the *B. napus* plants should serve for the purpose of being useful for rape seed oil production for, e.g., bio fuel or bar oil for chainsaws, animal feed or honey production, for oil, meal, grain, starch, flour, protein, fiber, industrial chemical, pharmaceutical or neutraceutical production. The term "agronomically exploitable" when used herein also includes that the *B. napus* plants of the present invention are less sensitive against an ALS-inhibitor herbicide, such as 5 times, or 10 times, or 50 times, or 100 times, or 500 times, or 1000 times, or 2000 times less sensitive as compared to wild type plants. The ALS inhibitor herbicide is one or more described herein, preferably it is foramsulfuron either alone or in combination with one or more further ALS-inhibitor herbicide(s) either from the sub-class of the sulfonyurea herbicides or any other sub-class of the ALS-inhibitor herbicides, most preferably it is foramsulfuron in combination with a further sulfonylurea herbicide and/or an ALS-inhibitor of the sulfonylaminocarbonyltriazolinone herbicide sub-class.

Another aspect of the present invention is the use of the *B. napus* plant described herein and/or the harvestable parts or propagation material described herein for the manufacture/breeding of *B. napus* plants. Methods for the manufacture/breeding of *B. napus* plants are described herein elsewhere. Such manufacture/breeding methods may be used to generate *B. napus* plants of the present invention further comprising novel plant traits such as stress-resistance, like but not limited to drought, heat, cold, or salt stress and the like.

In a still further aspect, the present invention envisages the use of the herbicide tolerant *B. napus* plant described herein and/or harvestable parts or propagation material derived thereof in a screening method for the selection of ALS inhibitor herbicides.
A better understanding of the present invention and of its many advantages will be had from the following examples, offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

SEQUENCES

5  A. thaliana sequences  SEQ ID Nos: 9 (nucleotide AY042819) and 10 (protein AAK68759), and
wild type B. napus sequences  SEQ ID Nos: 1 (ALS1 nucleotide Z1 1524) and 3 (ALS3 nucleotide
Z1 1526) were taken from the ncbi-genebank (see world wide web: http://www.ncbi.nlm.nih.gov/genbank/).  SEQ ID Nos: 2 and 4 are the protein sequences encoded by
SEQ ID Nos: 1 and 3, respectively.

10  | Nucleic acid sequence encoding B. napus wild type ALS 1 gb Z1 1524.
      | SEQ ID No.2; B. napus ALS 1 amino acid sequence derived from SEQ ID No. 1.
      | SEQ ID No.3: Nucleic acid sequence encoding B. napus wild type ALS 11 gb Z1 1526.
      | SEQ ID No.4: B. napus ALS III amino acid sequence derived from SEQ ID No. 3.
      | SEQ ID No.5: Nucleic acid sequence encoding B. napus ALS 1 protein comprising an P197S
      | mutation.
      | SEQ ID No.6: B. napus P197S ALS 1 amino acid sequence derived from SEQ ID No. 5 (position
      | 182 of SEQ ID NO. 6 corresponds to position 197 of SEQ ID NO: 10).
      | SEQ ID No.7: Nucleic acid sequence encoding B. napus ALS III protein comprising an W574L
      | mutation.
      | SEQ ID No.8: B. napus W574L ALS 1 amino acid sequence derived from SEQ ID No.7 (position
      | 556 of SEQ ID NO: 8 corresponds to position 574 of SEQ ID NO: 10).
      | SEQ ID No.9: Nucleic acid sequence encoding A. thaliana ALS gene.
      | SEQ ID No.10: A. thaliana amino acid sequence derived from SEQ ID No.9.

EXAMPLES

Example 1 - Generation and isolation of mutant Brassica AHAS alleles

[243]  Brassica napus lines with the HETO108 mutation, i.e. comprising a C to T substitution at
position 544 of ALS 1, resulting in a Proline to Serine amino acid substitution at position 182 of the
encoded protein, were generated and identified as described in WO 201 1/076345. The nucleotide
sequence of HETO108 is given in SEQ ID No. 5, and the protein encoded by HETO108 is given in SEQ
ID No. 6.
Brassica napus lines with the HET0121 mutation, i.e. comprising a G to T substitution at position 1667 of ALS III, resulting in a Tryptophan to Leucine amino acid substitution at position 556 of the encoded protein, were generated as follows.

Microspore isolation and embryo induction

Unopened oilseed rape seed flower buds of sizes +/- 3 mm have been isolated from donor Brassica napus plants. The donor plants were grown till flowering in controlled environment in the greenhouse. The buds were surface sterilized 20 min in 5% NaOCl bleach and rinsed three times with sterile water. Microspores were released from buds by mechanical squeezing in a mortar. The slurry was poured through a fine mesh with minimum pore size of 45 µm and washed through with liquid B5 medium (Gamborg et al. 1968). The filtrate was centrifuged for 3 min at 1,500 rpm two times, spores being re-suspended in fresh B5 medium each time. The isolated microspores were finally suspended in liquid Lichter's medium (Lichter 1982) and plated at a concentration of 60,000-100,000 spores/mL. The microspores were cultured at 32°C in dark for 3 days and then transferred to the culture room at 25°C in low light intensity for embryo induction. The embryos were grown 2-3 weeks to reach morphological maturity (approximately 5 mm long).

Selection of mutant HETQ121

The microspore derived embryos were transferred to agar (0.8%) solidified B5 medium comprising 2 x 10⁻⁷ M of the ALS inhibitor herbicide foramsulfuron (CAS RN 173159-57-4). Six weeks later the surviving embryos were transferred onto fresh agar medium of the same composition and then sub-cultured in 2-4 intervals. The cultures were kept at 25°C under dim light at 12h/12h light/dark cycle.

Preliminary, microspore derived embryos were transferred to non-selective medium to check the viability of the embryos obtained from the isolated microspores. The in vitro cultured microspores divided and developed into embryos able to grow into normal haploid plantlets.

Following the selection on 2 x 10⁻³ M foramsulfuron, one embryo was able to grow in presence of the ALS inhibitor and to regenerate to plant. The presence of a single point mutation in the tryptophan 556 codon (corresponding to the tryptophane 574 codon in A. thaliana), i.e. a G to T substitution at position 1667 of the coding sequence of ALS III gene, resulting in a Tryptophan to Leucine amino acid substitution at position 556 of the encoded protein, was confirmed by sequencing analysis (SEQ ID No. 7 for coding sequence, SEQ ID No. 8 for encoded protein). The same mutation was found in canola by Hattori et al. (1995, Mol Gen Genet 246:419). Prior transfer to the greenhouse, the haploid plants of the mutant HET0121 were treated with colchicine (0.1%, 6h) for chromosome doubling and later seed production. After colchicine treatment, the HET0121 plants were transferred into sterile plant containers filled with wet, sterilized perlite, watered with half strength MS inorganic ingredients (Murashige, T. & Skoog, F. 1962) and cultured for one week till transplantation in soil.
Example 2 - Combination of HETO108 and HET0121 alleles

[249] Brassica plants comprising HETO108 have been backcrossed 5 times with an elite Brassica line (BC1 to 5). After each backcrossing step, plants comprising the HETO108 mutation have been identified as described in WO 2011/076345. BC5 plants have been selfed, and progeny homozygous for the HETO108 mutation have been identified using the methods as described in WO 2011/076345.

[250] Brassica plants comprising HET0121 were grown to maturity and self-pollinated, and offspring homozygous for the HET0121 mutation were selected.

[251] The Brassica plants homozygous for HETO108 were crossed with the Brassica plants homozygous for HET0121. F1 offspring, heterozygous for HETO108 and heterozygous for HET0121 were selfed twice to obtain the following genotypes:

<table>
<thead>
<tr>
<th>ALS I</th>
<th>ALS III</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>+/-</td>
<td>HETO121/+</td>
</tr>
<tr>
<td>+/-</td>
<td>HETO121/HETO121</td>
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<tr>
<td>HETO108/+</td>
<td>+/-</td>
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<td>HETO108/+</td>
<td>HETO121/+</td>
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<td>HETO108/+</td>
<td>HETO121/HETO121</td>
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<td>HETO108/HETO108</td>
<td>+/-</td>
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<tr>
<td>HETO108/HETO108</td>
<td>HETO121/+</td>
</tr>
<tr>
<td>HETO108/HETO108</td>
<td>HETO121/HETO121</td>
</tr>
</tbody>
</table>

wherein + indicates the wild-type allele for ALS I and ALS III.

[252] Seeds heterozygous for both HETO108 and HET0121 have been deposited at the NCIMB Limited (Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, Scotland, AB21 9YA, UK) on December 15, 2011, under accession number NCIMB 41912.

Example 3 - Measurement of herbicide tolerance of Brassica plants comprising mutant AHAS alleles

[253] The correlation between the presence of mutant AHAS alleles in a Brassica plant grown in the greenhouse and tolerance to thienoicarbazone-methyl and foramsulfuron was determined as follows. Treatment post-emergence at the 1-2 leaf stage was carried out with a dose of 5 g a.i./ha of thienoicarbazone-methyl and 8.75 g a.i./ha of foramsulfuron. The plants were evaluated for phenotype (height, side branching and leaf morphology) on scale of 5 to 1, where; type 5 = normal (corresponding to wildtype unsprayed phenotype); type 4 = normal height, some side branching, normal leaves; type 3 =
intermediate height, intermediate side branching, normal leaves; type 2 = short, severe side branching ("bushy"), some leave malformations; type 1 = short, severe side branching ("bushy"), severe leave malformations. Phytotoxicity (PPTOX) was determined and evaluated on a scale of 1 to 9, where 1 = completely yellowing, 5 = 50% of plant is yellow and 9 = no yellowing. For assessment of vigor scores, plants were evaluated on a scale of 1 to 9, where 1 = very poor (+/- dead), 5 = average, 9 = vigorous.

Table 1a: Vigor scores (10 and 26 days after spraying), phytotoxicity (PPTOX) (10 days after spraying and phenotype (pheno) (26 days after spraying) scores upon spay testing post-emergence (post) of homozygous and heterozygous plants. + = wild-type allele.

<table>
<thead>
<tr>
<th>Allele combination</th>
<th>Vigor 10 days</th>
<th>PPTOX 10 days</th>
<th>Pheno 26 days</th>
<th>Vigor 26 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHASI + / +</td>
<td>1</td>
<td>1</td>
<td>dead 1</td>
<td>1</td>
</tr>
<tr>
<td>+ / +</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>HETO108 / +</td>
<td>2</td>
<td>1</td>
<td>dead 1</td>
<td>1</td>
</tr>
<tr>
<td>HETO108 / HETO108</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>HETO108 / HETO108</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Mixture of lines untreated</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>9</td>
</tr>
</tbody>
</table>
[255] Table 1b: Vigor scores (5, 8, 13 and 20 days after spraying), phytotoxicity (PPTOX) (5 days after spraying) and phenotype (pheno) (20 days after spraying) scores upon spray test in spray-cabinet post-emergence of S2 generations of homozygous genotypes and wild-type segregants. WT: homozygous for wild-type AHAS I or AHAS III; HETO108: homozygous for HETOI allele in AHAS I; HETO121: homozygous for HETO121 allele in AHAS III. HT: Herbicide treatment: + relates to treated; 0 relates to untreated plants. The control is an elite *Brassica* line homozygous for wild-type AHAS alleles. The experiments were repeated three times (columns 1, 2 and 3).

<table>
<thead>
<tr>
<th>Allele combination</th>
<th>HT</th>
<th>Vigor 5 days</th>
<th>PPTOX 5 days</th>
<th>Vigor 8 days</th>
<th>Vigor 13 days</th>
<th>Vigor 20 days</th>
<th>Pheno 20 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>HETO108</td>
<td>+</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>HETO121</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>HETO108</td>
<td>+</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>HETO108</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>WT</td>
<td>+</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>HETO121</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>HETO108</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HETO108</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The control is an elite *Brassica* line homozygous for wild-type AHAS alleles. The experiments were repeated three times (columns 1, 2 and 3).
Although spraying caused some developmental changes of the plants comprising HETO 108 and HETO 121 (not shown), Table 1 clearly shows that these plants have an increased tolerance to the combination of thiencarbazone-methyl and foramsulfuron. Further, plants comprising HETO108 and HETO121 show a better herbicide tolerance as compared to the plants comprising a wild-type AHAS I allele and HETO121, and as compared to the plants comprising HETO108 and a wild-type AHAS III allele. A further phenotypic analysis of the plants also revealed that indeed the HETO 108 mutation in AHAS I adds to the tolerance provided by the HETO 121 mutation in AHAS III (not shown). Further, it can be seen that in the absence of herbicide spraying, the vigor of the plants with the HETO 108 and/or the HETO121 is similar to that of the wild-types.

In conclusion, the data in Table 1 show that the presence of the P197S mutation in AHAS I, or, to a higher extent, of the W574L mutation in AHAS III increases tolerance of Brassica plants to a combination of the herbicides thiencarbazone-methyl and foramsulfuron in the greenhouse, and that the combination of these two mutations increases the tolerance even further.
Example 4 - Measurement of herbicide tolerance of *Brassica* plants comprising mutant AHAS alleles in the field

[259] Seeds of spring oilseed rape homozygous for HETO108 and HETO121 were sown in a field according to typical practical agricultural methods. The registered spring oilseed rape variety ABILITY served as a comparison. All plants were cultivated up to BBCH stage 14 (four true leaves of the oilseed rape plants). Afterwards, the herbicides mentioned in table 2 have been applied to the oilseed rape plants by using specific spray equipment for small plot applications. The water amount used was 200L/ha. 18 days after application the visible phytotoxicity on the oilseed rape plant was assessed according to a scale from 0% to 100%: 0% = no phytotoxic effects, comparable to untreated 100% = complete control, all plants killed. The assessments led to the results shown in table 2. The use of all herbicides in the test led to a clearly better tolerance of the spring oilseed rape lines homozygous for HETO108 and HETO121 as compared to the standard variety ABILITY.

[260] Table 2: Phytotoxicity in the oilseed rape variety ABILITY and oilseed rape homozygous for HETO108-HETO121 upon herbicide spraying in the field. % phytotox: percentage phytotoxicity; AI = active ingredieng; gai/ha = gram active ingredient / hectare.

<table>
<thead>
<tr>
<th>Variety</th>
<th>ABILITY</th>
<th>HETO108-HETO121</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rating Data Typ</strong></td>
<td><strong>Active ingredient</strong></td>
<td><strong>AI dose rate (gai/ha)</strong></td>
</tr>
<tr>
<td><strong>Product</strong></td>
<td><strong>Active ingredient</strong></td>
<td></td>
</tr>
<tr>
<td>AE F130360 00 WG50 A1</td>
<td>Foramsulfuron</td>
<td>50</td>
</tr>
<tr>
<td>BYH18636</td>
<td>Thiencarbazone</td>
<td>30</td>
</tr>
<tr>
<td>SP102000025743</td>
<td>Foramsulfuron + Thiencarbazone</td>
<td>25+15</td>
</tr>
<tr>
<td>SP102000025743</td>
<td>Foramsulfuron + Thiencarbazone</td>
<td>50+30</td>
</tr>
<tr>
<td>AE F115008 00 WG10 A2</td>
<td>Iodosulfuron</td>
<td>7</td>
</tr>
<tr>
<td>KATANA</td>
<td>Flazasulfuron</td>
<td>50</td>
</tr>
<tr>
<td>MONITOR</td>
<td>Sulfoximuron</td>
<td>10</td>
</tr>
<tr>
<td>PRIMUS</td>
<td>Floraam</td>
<td>10</td>
</tr>
<tr>
<td>SIMPLICITY</td>
<td>Pyroxim</td>
<td>50</td>
</tr>
<tr>
<td>AE F130060 00 WG75 A2</td>
<td>Mesosulfuron - methyl</td>
<td>15</td>
</tr>
<tr>
<td>AE1887196+EXS</td>
<td>Ethoxysulfuron</td>
<td>180</td>
</tr>
<tr>
<td>HOESTAR</td>
<td>Amidosulfuron</td>
<td>30</td>
</tr>
</tbody>
</table>
Table 3. For the determination of $K_m$- and $V_{max}$-values, ALS activity was determined by variation of the pyruvate concentrations. Assays were initiated by adding enzyme and terminated after 75 min incubation at 30°C by the addition of 40 µl 0.5 M H2SO4. After 60 min at room temperature, 80 µl of a solution of 1.4% a-naphtol and 0.14% creatine in 0.7 M NaOH was added and after an additional 45 min incubation at room temperature, the absorbance was determined at 540 nm. pI50-values for inhibition of ALS were determined as described by Ray (1984) (supra), using the XLFit Excel add-in version 4.3.1 curve fitting program of ID business Solutions Limited. The pI50 values measured for the different mutant AHAS proteins and calculated for the mixtures of two different AHAS enzymes are shown in Table 3. For the determination of $K_m$- and $V_{max}$-values, ALS activity was determined by variation of the pyruvate concentrations.

Example 5 - In vitro ALS inhibitor sensitivity and kinetic parameters of proteins encoded by different AHAS mutants

[261] The coding sequences of the Arabidopsis thaliana wild-type and P197S-, W574L-, and S653N-mutant ALS genes were cloned into Novagen pET-32a(+) vectors and the vectors transformed into Escherichia coli AD494 according to the instructions of the manufacturer. Bacteria were grown at 37°C in LB-medium containing 100 µg/ml carbenicillin and 25 µg/ml canamycin, induced with 1 mM isopropyl-β-D-thiogalactopyranoside at an OD600 of 0.6, cultivated for 16 hours at 18°C and harvested by centrifugation. Bacterial pellets were resuspended in 100 mM sodium phosphate buffer pH 7.0 containing 0.1 mM thiamine-pyrophosphate, 1 mM MgCl₂, and 1 µM FAD at a concentration of 1 gram wet weight per 25 ml of buffer and disrupted by sonification. The crude protein extract obtained after centrifugation was used for ALS activity measurements.

[262] ALS assays were carried out in 96-well microtiter plates using a modification of the procedure described by Ray (1984), Plant Physiol 75:827-831. The reaction mixture contained 20 mM potassium phosphate buffer pH 7.0, 20 mM sodium pyruvate, 0.45 mM thiamine-pyrophosphate, 0.45 mM MgCl₂, 9 µM FAD, ALS enzyme and various concentrations of ALS inhibitors in a final volume of 90 µl. Assays were initiated by adding enzyme and terminated after 75 min incubation at 30°C by the addition of 40 µl 0.5 M H2SO4. After 60 min at room temperature, 80 µl of a solution of 1.4% a-naphtol and 0.14% creatine in 0.7 M NaOH was added and after an additional 45 min incubation at room temperature, the absorbance was determined at 540 nm. pI50-values for inhibition of ALS were determined as described by Ray (1984) (supra), using the XLFit Excel add-in version 4.3.1 curve fitting program of ID business Solutions Limited. The pI50 values measured for the different mutant AHAS proteins and calculated for the mixtures of two different AHAS enzymes are shown in Table 3. For the determination of $K_m$- and $V_{max}$-values, ALS activity was determined by variation of the pyruvate concentrations.
concentration in the assay mixture from 0 - 120 mM. Reaction velocities were tiffed to the Michalis-Menten equation with the XLFit curve fitting program. The $k_m$ and $v_{max}$ values for the different mutant AHAS proteins are shown in Table 4.

[263] To characterize the response of a mixture of two different ALS enzymes to a given ALS inhibitor the following assumptions were made:

a. inhibition is competitive and reversible

b. in the absence of inhibitor, equal activities of the ALS (mutant) enzymes 1 and 2 are present

[264] Using experimentally determined pI50-values for various ALS-inhibiting herbicides, enzyme activities at different inhibitor concentrations were calculated (see Theory) for each ALS enzyme. Total ALS activity at any given inhibitor concentration is the sum of the activity of (mutant) enzyme 1 and (mutant) enzyme 2. If the experimentally determined pI50-value was <4, pI50 = 3.0 was used for the calculations. If the calculated pI50-value was 3.0, the pI50 was redefined as <4.

[265] Theory

For competitive inhibition

$$v = \frac{VS}{k_m \left(1 + \frac{I}{k_i}\right) + S}$$

and

$$I_{50} = k_i \left(1 + \frac{S}{k_m}\right)$$

It can be shown that $v$ at any given inhibitor concentration is given by

$$v = v_{I=0} \frac{I_{50}}{1 + I_{50}}$$

[266] From Table 3, it is clear that, for most of the herbicides, the resistance factors for the enzymes comprising the P197S and W574L mutations and combinations thereof are higher than for S653N alone and S653N in combination with W574L. Further, as can be seen in Table 4, the $k_m$ of the enzymes with the different mutations are somewhat higher than for the wild-type enzyme. The $v_{max}$ of the enzymes with the P197S or the W574L mutation is higher than that of wild-type, whereas the $v_{max}$ of the enzyme with the S653N mutation is lower than that of the wild-type enzyme.
Table 3. ALS inhibitor sensitivity - pI50 values and resistance factor for different AHAS mutants. 1: Sulfonylureas, 2: pyrimidinylbenzoates, 3: Triazolopyrimidines, 4: Sulfonylaminocarbonyltriazolinones, 5: Imidozolinones.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>pI50</th>
<th>Resistance Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>P197S &amp; W574L</td>
</tr>
<tr>
<td>Amidosulfuron</td>
<td>6.7</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Ethoxysulfuron</td>
<td>7.8</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Flazasulfuron</td>
<td>9.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Flupyrsulfuron-methyl</td>
<td>8.7</td>
<td>7.0</td>
</tr>
<tr>
<td>Foramsulfuron</td>
<td>8.2</td>
<td>7.0</td>
</tr>
<tr>
<td>Jodosulfuron-methysodium</td>
<td>8.6</td>
<td>6.4</td>
</tr>
<tr>
<td>Mesosulfuron-methyl</td>
<td>8.9</td>
<td>7.0</td>
</tr>
<tr>
<td>Metsulfuron-methyl</td>
<td>8.0</td>
<td>6.3</td>
</tr>
<tr>
<td>Nicosulfuron</td>
<td>6.9</td>
<td>6.0</td>
</tr>
<tr>
<td>Rimsulfuron</td>
<td>7.5</td>
<td>6.4</td>
</tr>
<tr>
<td>Sulfosulfuron</td>
<td>7.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Thifensulfuron-Methyl</td>
<td>7.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Bispyribac-sodium</td>
<td>7.7</td>
<td>7.1</td>
</tr>
<tr>
<td>Florasulam</td>
<td>7.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Metosulam</td>
<td>8.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Pyroxsulam</td>
<td>8.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Imazamox</td>
<td>5.4</td>
<td>5.3</td>
</tr>
<tr>
<td>Propoxycarbazone</td>
<td>7.7</td>
<td>5.5</td>
</tr>
<tr>
<td>Thiencarbazone-methyl</td>
<td>7.9</td>
<td>4.8</td>
</tr>
</tbody>
</table>

measured, measured, measured, measured, calculated, calculated, measured, measured, measured, calculated, calculated.
Table 4. Kinetic parameters of enzymatic activity of different AHAS mutants

<table>
<thead>
<tr>
<th>ALS</th>
<th>$K_m$ (mM)</th>
<th>$V_{max}$ (AE/min mg)$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>2.1 ± 0.1</td>
<td>42.7 ± 1.3</td>
</tr>
<tr>
<td>P197S</td>
<td>5.5 ± 0.8</td>
<td>50.0 ± 6.1</td>
</tr>
<tr>
<td>W574L</td>
<td>6.9 ± 0.2</td>
<td>54.4 ± 2.8</td>
</tr>
<tr>
<td>S653N</td>
<td>5.8 ± 0.4</td>
<td>32.0 ± 1.4</td>
</tr>
</tbody>
</table>

Example 6 - ALS enzyme activity in leaves of plants with different mutant AHAS alleles

The inhibitory effect of ALS inhibiting herbicides on ALS enzymes encoded by the different mutant AHAS alleles was tested in leaf material essentially as described by Shimizu et al. 2008, Plant Physiol. 147:1976. 50 mg leaf material from *Brassica* lines homozygous for the HETO108 mutation, from *Brassica* lines homozygous for the HETO121 mutation, *Brassica* lines homozygous for both the HETO108 and the HETO121 mutations, and wild-type *Brassica* lines not comprising the HETO108 and the HETO121 mutation was incubated with different concentrations Foramsulfuron (FS) and Thien carbazole-methyl (TCM), and the ALS enzyme activity was tested. The result of the ALS enzyme activity is shown in Figure 3. For both FS (A) and TCM (B), the ALS activity in wild-type leaves (row IV) is inhibited at lower concentrations of the herbicides than in leaves comprising the mutant AHAS alleles (rows I-III). In the mutant lines, the herbicide tolerance is higher for TCM than for FS, whereas, especially for FS, the tolerance in lines with the P197S mutation (row II) is lower than the tolerance in lines with the W574L mutation (row III) and with both the P197S and W574L mutation (row I). These results are in line with the resistance factors as determined in Example 5, and the spray data of Example 4.

Example 7 - Detection and/or transfer of mutant AHAS alleles into (elite) *Brassica* lines

The mutant AHAS genes are transferred into (elite) *Brassica* breeding lines by the following method: A plant containing a mutant AHAS gene (donor plant), is crossed with an (elite) *Brassica* line (elite parent / recurrent parent) or variety lacking the mutant AHAS gene. The following introgression scheme is used (the mutant AHAS allele is abbreviated to *ahas* while the wild type is depicted as AHAS):

Initial cross:  
ahas / ahas (donor plant)   X   AHAS / AHAS (elite parent)  
F1 plant:  
AHAS / ahas

BC1 cross:  
AHAS / ahas   X   AHAS / AHAS (recurrent parent)  
BC1 plants:  50% AHAS / ahas and 50% AHAS / AHAS

The 50%, ahas / AHAS are selected by direct sequencing or using molecular markers (e.g.
AFLP, PCR, Invader™, TaqMan® and the like) for the mutant AHAS allele (ahas).

BC2 cross:   AHAS / AHAS (BC1 plant)  X  AHAS / AHAS (recurrent parent)

BC2 plants:  50% AHAS / ahas and 50% AHAS / AHAS

The 50% AHAS / AHAS are selected by direct sequencing or using molecular markers for the mutant AHAS allele (ahas).

Backcrossing is repeated until BC3 to BC6

BC3-6 plants:  50% AHAS / ahas and 50% AHAS / ahas

The 50% AHAS / ahas are selected using molecular markers for the mutant AHAS allele (ahas). To reduce the number of backcrossings (e.g. until BC3 instead of BC6), molecular markers can be used specific for the genetic background of the elite parent.

BC3-6 SI cross:  AHAS / ahas  X  AHAS / ahas

BC3-6 SI plants:  25% AHAS / AHAS and 50% AHAS / ahas and 25% ahas / ahas

Plants containing ahas are selected using molecular markers for the mutant AHAS allele (AHAS). Individual BC3-6 SI or BC3-6 S2 plants that are homozygous for the mutant AHAS allele (ahas / ahas) are selected using molecular markers for the mutant and the wild-type AHAS alleles. These plants are then used for seed production.

[271] To select for plants comprising a point mutation in an AHAS allele, direct sequencing by standard sequencing techniques known in the art, such as those described in Example 1, can be used.
CLAIMS:

1. An ALS inhibitor herbicide tolerant *Brassica napus* plant or parts thereof comprising an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

2. The *B. napus* plant or parts thereof according to claim 1, wherein said ALS I polypeptide is at least 90% identical to SEQ ID NO: 6 and wherein said ALS III polypeptide is at least 90% identical to SEQ ID NO: 8.

3. The *B. napus* plant or parts thereof according to claim 2, wherein said ALS I polypeptide is identical to SEQ ID NO: 6 and wherein said ALS III polypeptide is identical to SEQ ID NO: 8.

4. The *B. napus* plant or parts thereof according to claim 3, wherein said ALS I polypeptide is encoded by the nucleotide sequence corresponding to SEQ ID NO: 5, and said ALS III protein is encoded by the nucleotide sequence corresponding to SEQ ID NO: 7.

5. The *B. napus* plant or parts thereof according to claim 4 which is obtainable from seeds deposited at NCIMB under accession number NCIMB 41912.

6. The *B. napus* plant or parts thereof according to any one of claims 1 to 5, which are tolerant to one or more ALS-inhibitor herbicides belonging to the group consisting of sulfonylurea herbicides, sulfonylaminocarbonyltriazolinone herbicides, imidazolinone herbicides, triazolopyrimidine herbicides, and pyrimidinyl(thio)benzoate herbicides.

7. The *B. napus* plant or parts thereof according to any one of claims 1 to 6, characterized in that both ALS I alleles encode an ALS I polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine, and that both ALS III alleles encode an ALS III polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

8. Parts of the *B. napus* plant according to any one of claims 1 to 7, wherein the parts are organs, tissues, cells or seeds.
9. Food, feed, or an industrial product obtainable from a plant according to any one of claims 1 to 7.

10. The food, feed or industrial product according to claim 9, wherein
   a) the food or feed is oil, meal, grain, starch, flour or protein; or
   b) the industrial product is biofuel, fiber, industrial chemicals, a pharmaceutical or a nutraceutical.

11. Progeny of a B. napus plant according to any one of claims 1 to 7 obtained by further breeding with
    said plant, wherein said progeny contains an ALS I polypeptide comprising at a position
    corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline
    the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to
    position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino
    acid leucine.

12. A method of producing a hybrid seed, comprising crossing a parent B. napus plant according to any
    one of claims 1 to 7 with a second parent Brassica plant and harvesting a resultant hybrid seed.

13. A hybrid plant produced from crossing a parent B. napus plant according to any one of claims 1 to 7
    with a second parent Brassica plant and harvesting a resultant hybrid seed and growing said seed,
    wherein said hybrid plant having at least an ALS I polypeptide comprising at a position
    corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline
    the amino acid serine, and an ALS III polypeptide comprising at a position corresponding to
    position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino
    acid leucine.

14. A method of producing food, feed, or an industrial product comprising
    a) obtaining the plant or a part thereof, of any one of claims 1 to 8, 11, and 13; and
    b) preparing the food, feed or industrial product from the plant or part thereof.

15. The method of claim 14 wherein
    a) the food or feed is oil, meal, grain, starch, flour or protein; or
    b) the industrial product is biofuel, fiber, industrial chemicals, a pharmaceutical or a nutraceutical.

16. Use of one or more ALS inhibitor herbicide(s) for controlling unwanted vegetation in Brassica
    growing area, such as B. napus growing area, which Brassica plants, such as B. napus plants,
    comprise an altered ALS I Brassica, such as B. napus, polypeptide comprising at a position
    corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline
    the amino acid serine; and an altered ALS III Brassica, such as B. napus, polypeptide comprising at
    a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino
acid tryptophan the amino acid leucine.

17. Use of one or more ALS inhibitor herbicide(s) according to claim 16, wherein the ALS inhibitor herbicide(s) belong(s) to:

the group of the (sulfon)amides (group (A)) consisting of:

- amidosulfuron [CAS RN 120923-37-7] (= Al-1);
- azimsulfuron [CAS RN 120162-55-2] (= Al-2);
- bensulfuron-methyl [CAS RN 83055-99-6] (= A1-3);
- chlorimuron-ethyl [CAS RN 90982-32-4] (= Al-4);
- chlorosulfuron [CAS RN 64902-72-3] (= Al-5);
- cinosulfuron [CAS RN 94593-91-6] (= Al-6);
- cyclosulfamuron [CAS RN 136849-15-5] (= Al-7);
- ethametsulfuron-methyl [CAS RN 97780-06-8] (= Al-8);
- ethosulfuron [CAS RN 126801-58-9] (= Al-9);
- flazasulfuron [CAS RN 104040-78-0] (= Al-10);
- flucetosulfuron [CAS RN 412928-75-7] (= AM 1);
- flupyr-sulfuron-methyl-sodium [CAS RN 144740-54-5] (= Al-12);
- foramsulfuron [CAS RN 173159-57-4] (= Al-13);
- halosulfuron-methyl [CAS RN 100784-20-1] (= Al-14);
- imazosulfuron [CAS RN 122548-33-8] (= Al-15);
- iodosulfuron-methyl-sodium [CAS RN 144550-36-7] (= AM 6);
- mesosulfuron-methyl [CAS RN 208465-21-8] (= AI-7);
- metsulfuron-methyl [CAS RN 74223-64-6] (= AM 8);
- monosulfuTon [CAS RN 155860-63-2] (= Al-19);
- nicosulfuron [CAS RN 111991-09-4] (= Al-20);
- orthosulfamuron [CAS RN 213464-77-8] (= AI-21);
- oxasulfuron [CAS RN 14465-1-06-9] (= AI-22);
- primisulfuron-methyl [CAS RN 86209-51-0] (= AI-23);
- prosulfuron [CAS RN 94125-34-5] (= AI-24);
- pyrazosulfuron-ethyl [CAS RN 93697-74-6] (= Al-25);
- rimsulfuron [CAS RN 12293-1-48-0] (= Al-26);
- sulfometuron-methyl [CAS RN 74222-97-2] (= AI-27);
- sulfosulfuron [CAS RN 141776-32-1] (= Al-28);
- thifensulfuron-methyl [CAS RN 79277-27-3] (= AI-29);
- triasulfuron [CAS RN 82097-50-5] (= Al-30);
- tribenuron-methyl [CAS RN 101200-48-0] (= AI-31);
- trifloxysulfuron [CAS RN 145099-21-4] (sodium) (= Al-32);
triflusulfuron-methyl [CAS RN 126535-15-7] (= Al-33);
tritosulfuron [CAS RN 142469-14-5] (= Al-34);
NC-330 [CAS RN 104770-29-8] (= Al-35);
NC-620 [CAS RN 868680-84-6] (= Al-36);
TH-547 [CAS RN 570415-88-2] (= Al-37);
monosulfuron-methyl [CAS RN 175076-90-1] (= Al-38);
2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide (= Al-39);
where M⁺ denotes the respective salt of the compound (I), i.e. its lithium salt (= Al-40); its sodium salt (= Al-41); its potassium salt (= Al-42); its magnesium salt (= Al-43); its calcium (= Al-44); its ammonium salt (= Al-45); its methylammonium salt (= Al-46); its dimethylammonium salt (= Al-47); its tetramethylammonium salt (= Al-48); its ethylammonium salt (= Al-49); its diethylammonium salt (= Al-50); its tetraethylammonium salt (= Al-51); its propylammonium salt (= Al-52); its tripropylammonium salt (= Al-53); its isopropylammonium salt (= Al-54); its diisopropylammonium salt (= Al-55); its butylammonium salt (= Al-56); its tetrabutylammonium salt (= Al-57); its (2-hydroxyethyl-1-y1)ammonium salt (= Al-58); its bis-N,N-(2-hydroxyethyl-1-y1)ammonium salt (= Al-59); its tris-N,N,N-(2-hydroxyethyl-1-y1)ammonium salt (= Al-60); its 1-phenylethylammonium salt (= Al-61); its 2-phenylethylammonium salt (= Al-62); its trimethylsulfonium salt (= Al-63); its trimethyloxonium salt (= Al-64); its pyridinium salt (= Al-65); its 2-methylpyridinium salt (= Al-66); its 4-methylpyridinium salt (= Al-67); its 2,4-dimethylpyridinium salt (= Al-68); its 2,6-dimethylpyridinium salt (= Al-69); its piperidinium salt (= Al-70); its imidazolium salt (= Al-71); its morpholinium salt (= Al-72); its 1,5-diazabicyclo[4.3.0]non-7-enum salt (= Al-73); its 1,8-diazabicyclo[5.4.0]undec-7-enium salt (= Al-74);
or a compound of the formula (II) or salts thereof
with $R^2$, and $R^3$ having the meaning as defined in the below table

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or the compound of formula (III) (= Al-87), i.e. the sodium salt of compound (Al-83)

or the compound of formula (IV) (=Al-88), i.e. the sodium salt of compound (Al-82)
the subgroup of the sulfonylamino carbonyltriazolinones (subgroup \((A2)\), consisting of: flucarbazone-sodium [CAS RN 181274-17-9] (= A2-1); propoxycarbazone-sodium [CAS RN 181274-15-7] (= A2-2); thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3);

the subgroup of the triazolopyrimidines (subgroup \((A2)\), consisting of: cloransulam-methyl [147150-35-4] (= A3-1); diclosulam [CAS RN 145701-21-9] (= A3-2); florasulam [CAS RN 145701-23-1] (= A3-3); flumetsulam [CAS RN 98967-40-9] (= A3-4); metosulam [CAS RN 139528-85-1] (= A3-5); penoxsulam [CAS RN 219714-96-2] (= A3-6); pyroxsulam [CAS RN 422556-08-9] (= A3-7);

the subgroup of the sulfonanilides (subgroup \((A4)\), consisting of: compounds or salts thereof from the group described by the general formula \((I)\):

\[
\begin{align*}
\text{in which} & \\
R^1 & \text{is halogen, preferably fluorine or chlorine}, \\
R^2 & \text{is hydrogen and } R^3 \text{ is hydroxyl or } \\
R^2 \text{ and } R^3 \text{ together with the carbon atom to which they are attached are a carbonyl group } C=0 \text{ and } \\
R^4 & \text{ is hydrogen or methyl;}
\end{align*}
\]
and more especially compounds of the below given chemical structure (A4-1) to (A4-8)

the group of the imidazolinones (group (B)), consisting of:
imazamethabenzmethyl [CAS RN 81405-85-8] (= Bl-1);
imazamox [CAS RN 114311-32-9] (= Bl-2);
imazapic [CAS RN 104098-48-8] (= Bl-3);
imazapyr [CAS RN 81334-34-1] (= Bl-4);
imazaquin [CAS RN 81335-37-7] (= Bl-5);
imazethapyr [CAS RN 81335-77-5] (= Bl-6);
SYP-298 [CAS RN 557064-77-4] (= Bl-7); and
SYP-300 [CAS RN 374718-10-2] (= Bl-8).

the group of the pyrimidinyl(thio)benzoates (group (C)), consisting of:
the subgroup of the pyrimidinyloxybenzoeacids (subgroup (CI)) consisting of:
- bispyribac-sodium [CAS RN 125401-92-5] (= C1-1);
- pyribenzoxim [CAS RN 168088-61-7] (= C1-2);
- pyriminobac-mefhyl [CAS RN 136191-64-5] (= C1-3);
- pyribambenz-isopropyl [CAS RN 420138-41-6] (= C1-4); and
- pyribambenz-propyl [CAS RN 420138-40-5] (= C1-5);

the subgroup of the pyrimidinylthiobenzoeacids (subgroup (C2)), consisting of:
- pyriftalid [CAS RN 135186-78-6] (= C2-1); and
- pyrithiobac-sodium [CAS RN 123343-16-8] (= C2-2).

18. Use of one or more ALS inhibitor herbicide(s) according to claim 16 or 17, wherein the ALS inhibitor herbicide(s) belong(s) to the group consisting of:
- amidosulfuron [CAS RN 120923-37-7] (= AM);
- chlorimuron-ethyl [CAS RN 90982-32-4] (= Al-4);
- chlorimuron [CAS RN 64902-72-3] (= Al-5);
- ethametsulfuron-methyl [CAS RN 97780-06-8] (= Al-8);
- ethoxysulfuron [CAS RN 126801-58-9] (= Al-9);
- flupyrdsulfuron-methyl-sodium [CAS RN 144740-54-5] (= Al-12);
- foramsulfuron [CAS RN 173159-57-4] (= Al-13);
- iodosulfuron-methyl-sodium [CAS RN 144550-36-7] (= AM 6);
- mesosulfuron-methyl [CAS RN 208465-21-8] (= AM 7);
- metsulfuron-methyl [CAS RN 74223-64-6] (= AM 8);
- monosulfuron [CAS RN 155860-63-2] (= Al-19);
- nicosulfuron [CAS RN 111991-09-4] (= Al-20);
- rimsulfuron [CAS RN 122931-48-0] (= Al-26);
- sulfosulfuron [CAS RN 141776-32-1] (= Al-28);
- thifensulfuron-methyl [CAS RN 79277-27-3] (= Al-29);
- tribenuron-methyl [CAS RN 101200-48-0] (= Al-31);
- triflusulfuron-methyl [CAS RN 126535-15-7] (= Al-33);
- 2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide (= Al-39);
- 2-iodo-N-[(4-methoxy-6-methyl-1,3,5-triazinyl)carbamoyl]benzene-sulfonamide sodium salt (= Al-41); or its sodium salt (= Al-87);
- flucarbazone-sodium [CAS RN 181274-17-9] (= A2-1);
- propoxycarbazone-sodium [CAS RN 181274-15-7] (= A2-2);
- thiencarbazone-methyl [CAS RN 317815-83-1] (= A2-3);
florasulam [CAS RN 145701-23-1] (= A3-3);  
metosulam [CAS RN 139528-85-1] (= A3-5);  
pyroxsulam [CAS RN 422556-08-9] (= A3-7)  
(A4-1);  
(A4-2);  
(A4-3);  
imazamox [CAS RN 1143 11-32-9] (= B1-2); and  
bispyribac-sodium [CAS RN 125401-92-5] (= C1-1).

19. Use of one or more ALS inhibitor herbicide(s) according to claim 16 or 17, wherein the ALS 
inhibitor herbicide(s) belong(s) to the group consisting of:

amidosulfuron [CAS RN 120923-37-7] (= A1-1);  
foramsulfuron [CAS RN 173 159-57-4] (= A1-13);  
sodium salt of compound of formula (I) (= A1-41);  
compound of formula (III) (=A1-41);  
thiencarbazone-methyl [CAS RN 3178 15-83-1] (= A2-3);  
imazamox [CAS RN 1143 11-32-9] (= B1-2); and  
bispyribac-sodium [CAS RN 125401-92-5] (= C1-1).

20. Use of one or more ALS inhibitor herbicide(s) according to any one of claims 16 to 19, wherein the 
Brassica plants are B. napus plants comprising an ALS I B. napus polypeptide comprising at a 
position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino 
acid proline the amino acid serine, and wherein an ALS III B. napus polypeptide comprising at a 
position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino 
acid tryptophan the amino acid leucine.

21. Use of one or more ALS inhibitor herbicide(s) according to any one of claims 16 to 20, in 
combination with non-ALS inhibitor herbicides (i.e. herbicides showing a mode of action that is 
different to the inhibition of the ALS enzyme [acetohydroxyacid synthase; EC 2.2.1.6] (group D 
herbicides), and wherein the non ALS inhibitor herbicide(s) is/are selected form the group 
consisting of:

acetochlor (= D1), carbetamide (= D56), fenoxaprop-P-ethyl (= D164), fluazifop-P-butyl (= D174), 
haloxyfop-P-methyl (= D222), metolachlor (= D275), dimethenamin (= D132), napropamide (= 
D290), pethoxamid (= D317), propaquizafop (= D341), propisochlor (= D344), propyzamide (= 
D345), quinmerac (= D363), propachlor (D 427), clomazone (= D83), clopyralid (= D86), 
dimethachlor (= D130), metazachlor (= D265), picloram (= D321), and quizalofop-P-ethyl (= 
D368).
22. Use of one or more ALS inhibitor herbicide(s) according to claim 21, and wherein the non ALS inhibitor herbicide(s) is/are selected from the group consisting of:
clomazone (D83), clopyralid (D86), dimethachlor (D130), metazachlor (D265), picloram (D321), and quizalofop-P-ethyl (D368).

23. Method for controlling unwanted vegetation in *B. napus* plant growing areas by applying one or more ALS inhibitor herbicide(s) alone or in combination with one or more herbicide(s) that do(es) not belong to the class of ALS inhibitor herbicides for weed control in *B. napus* growing areas which *B. napus* plants comprise an altered ALS I *Brassica*, such as *B. napus*, polypeptide comprising at a position corresponding to position 182 of SEQ ID NO: 2 instead of the naturally encoded amino acid proline the amino acid serine; and an altered ALS III *Brassica*, such as *B. napus*, polypeptide comprising at a position corresponding to position 556 of SEQ ID NO: 4 instead of the naturally encoded amino acid tryptophan the amino acid leucine.

24. Method according to claim 23 for controlling unwanted vegetation, and wherein the ALS inhibitor herbicide(s) are taken from the groups as defined in claim 17.

25. Method according to claim 24, and wherein the ALS inhibitor herbicide(s) are taken from the groups as defined in claim 18.

26. Method according to claim 24 or 25, and wherein the non ALS inhibitor herbicide(s) are taken from the group as defined in claim 21.

27. Method according to claim 24 or 25, and wherein the non ALS inhibitor herbicide(s) are taken from the group as defined in claim 22.
SEQ ID NO 9  1 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**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C12N15/82 A01H5/10 C12N9/88

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

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

C12N A01H

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

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

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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[X] Further documents are listed in the continuation of Box C.  
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Date of the actual completion of the international search: 22 April 2013  
Date of mailing of the international search report: 03/05/2013

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Authorized officer: Kani a., Thomas
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