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# Herbicide tolerance protein, encoding gene thereof and use thereof


## Abstract

The present invention relates to an herbicide tolerance protein, an encoding gene thereof and use thereof, the herbicide tolerance protein comprising: a protein (a) having an amino acid sequence as shown in SEQ ID NO: 1, and having an alanine substitution at least at position 176 and/or having a valine substitution at position 178 of SEQ ID NO: 1; or (b) having an amino acid sequence as shown in SEQ ID NO: 3; or (c) having an amino acid sequence as shown in SEQ ID NO: 5; or (d) having an amino acid sequence as shown in SEQ ID NO: 7; or (e) being derived from (a) by means of the amino acid sequence of (a) undergoing substitution and/or deletion and/or by added one or several amino acids, and having the activity of thifensulfuron hydrolase. The herbicide tolerance protein of the present invention has a broad application prospects in plants.



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## References Cited

**U.S. PATENT DOCUMENTS**

| Patent No. | Issued Date | Patentee Name | U.S. Cl. | CPC |
|---|---|---|---|---|
| 2014/0325700 | 12/2013 | Li et al. | N/A | N/A |
| 2019/0029252 | 12/2018 | Xie et al. | N/A | N/A |
| 2019/0029257 | 12/2018 | Xie et al. | N/A | N/A |
| 2019/0106705 | 12/2018 | Xie et al. | N/A | N/A |
| 2019/0249187 | 12/2018 | He et al. | N/A | N/A |

**FOREIGN PATENT DOCUMENTS**

| Patent No. | Application Date | Country | CPC |
|---|---|---|---|
| 3608411 | 12/2019 | EP | N/A |

**OTHER PUBLICATIONS**

Hang et al, Appl. Environ. Microbiol. (2012) 78:1962-1968. cited by examiner
Cunningham et al, Science (1989) 244:1081-1085. cited by examiner
GenBank Accession No. JN617866, submitted Aug. 9, 2011, version of Feb. 28, 2012. cited by examiner
Arnold et al, Ed., Directed Evolution Library Creation, Methods and Protocols, Humana Press 2003. cited by examiner
Castle et al, Science (2004) 304:1151-1154. cited by examiner
Dillon et al, J. Biol. Chem. (2013) 39:27872-27880. cited by examiner
Lei et al, Environ. Res. (2023) 235:116570. cited by examiner
Hang, B. et al., SulE, a Sulfonylurea Herbicide De-Esterification Esterase from Hansschlegelia zhihuaiae S113, Applied and Environmental Microbiology, 78(6): 1962-1968, Mar. 2012. cited by applicant
English Translation of Office Action for Chinese Patent Application No. No. 201810124124.9, May 7, 2019. cited by applicant
English Translation Office Action for Chinese Patent Application No. No. 201810124124.9, Dec. 3,

2019. cited by applicant
English translation of Chinese Search Report for Chinese Application No. 201810124124.9, Mar. 29, 2019. cited by applicant
Brazilian Office Action for Brazilian Application No. BR112020015958, dated Mar. 1, 2024. cited by applicant
English Language Concise Explanation for Brazilian Office Action for Brazilian Application No. BR112020015958, dated Mar. 1, 2024. cited by applicant

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## Background/Summary

REFERENCE TO RELATED APPLICATIONS

(1) This application is a U.S. national stage of PCT/CN2018/124916, filed Dec. 28, 2018, which claims priority to Chinese application No. 201810124124.9, filed Feb. 7, 2018, the entire content of both of which is incorporated herein by reference.

SEQUENCE LISTING

(2) The instant application contains a Sequence Listing which has been submitted electronically as a file in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII format file, created on Dec. 1, 2020, is named 2020-12-1_Sequence_listing_BDBC0001PA txt and is 144,324 bytes in size.

TECHNICAL FIELD

(3) The present invention relates to a herbicide tolerant protein, a coding gene thereof and use thereof, and in particular to a sulfonylurea herbicide tolerant protein, a coding gene thereof and use thereof.

BACKGROUND

(4) Weeds may exhaust valuable nutrients required by crops and other plants of interest in the soil rapidly. Currently, there are many types of herbicides used to control weeds, among which a particularly popular herbicide is glyphosate. Crops resistant to glyphosate have been developed, such as maize, soybean, cotton, sugar beet, wheat and rice. Therefore, glyphosate can be sprayed onto the field where glyphosate resistant crops are planted, so as to control weeds without significant damage to the crops.

(5) Glyphosate has been widely used in the world for more than 20 years, resulting in an over-reliance on glyphosate and glyphosate tolerant crop technologies, as well as applying a high selection pressure to plants that are naturally more tolerant to glyphosate or have developed a glyphosate-resistant activity in wild weed species. It has been reported that a few weeds have developed resistance to glyphosate, including broad-leaved weeds and gramineous weeds, such as *Lolium rigidium*, *Lolium multiflorum*, *Eleusine indica Gaertn*, *Ambrosia artemisiifolia*, *Conyza canadensis*, *Conyza bonariensis* and *Plantago lanceolata*. Moreover, weeds that were not agricultural problems before the wide use of glyphosate tolerant crops have become prevalent gradually, and are difficult to control with glyphosate tolerant crops, wherein these weeds mainly appear together with (but not only with) difficult-to-control broad-leaved weeds, such as the *Amaranthus*, *Chenopodium*, *Taraxacum* and *Commelinaceae* species.

(6) In areas where glyphosate resistant weeds or difficult-to-control weed species are present, growers can compensate for the weakness of glyphosate by tank mixing or alternating with other herbicides that can control the missed weeds, such as sulfonylurea herbicides. Sulfonylurea

herbicides have become the third most popular herbicides after organophosphorus and acetamide herbicides, with global annual sales of more than $3 billion. The annual application area of sulfonylurea herbicides in our country has been more than 2 million hectares and still shows an expanding trend.

(7) With the emergence of glyphosate resistant weeds and the expanding application of sulfonylurea herbicides, there is a need for more genes capable of degrading sulfonylurea herbicides and for introducing the genes into plants of interest that are sensitive to sulfonylurea herbicides so as to increase the tolerance of the plants to sulfonylurea herbicides.

## SUMMARY OF THE INVENTION

(8) The objective of the present invention is to provide a herbicide tolerant protein, a coding gene thereof and use thereof, wherein the herbicide tolerant protein is capable of better degrading sulfonylurea herbicides and making the plants into which the herbicide tolerant protein-coding gene is introduced have higher tolerance to sulfonylurea herbicides.

(9) In order to achieve the above objective, the present invention provides a herbicide tolerant protein, comprising: (a) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 1, and at least having an alanine substitution at position 176 and/or a valine substitution at position 178 of SEQ ID NO: 1; or (b) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 19, and at least having an alanine substitution at position 140 and/or a valine substitution at position 142 of SEQ ID NO: 19; or (c) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 35, and at least having an alanine substitution at position 140 and/or a valine substitution at position 142 of SEQ ID NO: 35; or (d) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 51, and at least having an alanine substitution at position 131 and/or a valine substitution at position 133 of SEQ ID NO: 51; or (e) a protein which is derived from (a) to (d) by substituting and/or deleting and/or adding one or more amino acids in the amino acid sequences of (a) to (d), and has thifensulfuron hydrolase activity.

(10) Furthermore, said herbicide tolerant protein comprises: (f) an amino acid sequence of (a), wherein the amino acid sequence of (a) also has an arginine substitution at position 80 and/or an alanine substitution at position 81 and/or an arginine substitution at position 182 of SEQ ID NO: 1; or (g) an amino acid sequence of (b), wherein the amino acid sequence of (b) also has an arginine substitution at position 44 and/or an alanine substitution at position 45 and/or an arginine substitution at position 146 of SEQ ID NO: 19; or (h) an amino acid sequence of (c), wherein the amino acid sequence of (c) also has an arginine substitution at position 44 and/or an alanine substitution at position 45 and/or an arginine substitution at position 146 of SEQ ID NO: 35; or (i) an amino acid sequence of (d), wherein the amino acid sequence of (d) also has an arginine substitution at position 35 and/or an alanine substitution at position 36 and/or an arginine substitution at position 137 of SEQ ID NO: 51; or (j) a protein which is derived from (a) to (d) by substituting and/or deleting and/or adding one or more amino acids in the amino acid sequences of (f) to (i), and has thifensulfuron hydrolase activity.

(11) Furthermore, the herbicide tolerant protein comprises: (k) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 7, SEQ ID NO: 11 or SEQ ID NO: 15; or (l) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 23, SEQ ID NO: 27 or SEQ ID NO: 31; or (m) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 39, SEQ ID NO: 43 or SEQ ID NO: 47; or (n) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 55, SEQ ID NO: 59 or SEQ ID NO: 63.

(12) In order to achieve the above objective, the present invention further provides a herbicide tolerant gene, comprising: (o) a nucleotide sequence encoding the herbicide tolerant protein according to (a)-(n); or (p) a nucleotide sequence as shown in SEQ ID NO: 8, 9, 10, 12, 13, 14, 16, 17 or 18; or (q) a nucleotide sequence as shown in SEQ ID NO: 24, 25, 26, 28, 29, 30, 32, 33 or 34; or (r) a nucleotide sequence as shown in SEQ ID NO: 40, 41, 42, 44, 45, 46, 48, 49 or 50.

(13) In order to achieve the above objective, the present invention further provides an expression

cassette, wherein the expression cassette comprises the herbicide tolerant gene under the regulation of an effectively linked regulatory sequence.
(14) In order to achieve the above objective, the present invention further provides a recombinant vector containing the herbicide tolerant gene or the expression cassette.
(15) In order to achieve the above objective, the present invention also provides a method for producing a herbicide tolerant protein, comprising: obtaining a cell of a transgenic host organism containing the herbicide tolerant gene or the expression cassette; cultivating the cell of the transgenic host organism under conditions allowing production of a herbicide tolerant protein; and recovering the herbicide tolerant protein.
(16) Further, the transgenic host organism comprises plants, animals, bacteria, yeasts, baculoviruses, nematodes, or algae.
(17) In order to achieve the above objective, the present invention further provides a method for increasing herbicide tolerance ranges, comprising co-expressing the herbicide tolerant protein or the herbicide tolerant protein encoded by the expression cassette together with at least one second protein which is different from the herbicide tolerant protein or the herbicide tolerant protein encoded by the expression cassette in a plant.
(18) Furthermore, the second protein is 5-enolpyruvylshikimate-3-phosphate synthase, glyphosate oxidoreductase, glyphosate-N-acetyltransferase, glyphosate decarboxylase, glufosinate acetyltransferase, α-ketoglutarate-dependent dioxygenase, dicamba monooxygenase, 4-hydroxyphenylpyruvate dioxygenase, acetolactate synthase, cytochrome-like proteins and/or protoporphyrinogen oxidase.
(19) The expression of the herbicide tolerant protein of the present invention in a transgenic plant can be accompanied by the expression of one or more other herbicide (glyphosate or glufosinate) tolerant proteins. This co-expression of more than one herbicide tolerant protein in the same transgenic plant can be achieved by allowing the plant to comprise and express a desired gene through genetic engineering. In addition, a plant (the first parent) can express the herbicide tolerant protein of the present invention through genetic engineering manipulation, and a second plant (the second parent) can express other herbicide (glyphosate or glufosinate) tolerant proteins through genetic engineering manipulation. Progeny plants expressing all the genes introduced into the first parent and the second parent are obtained by hybridizing the first parent with the second parent.
(20) In order to achieve the above objective, the present invention also provides a method for selecting transformed plant cells, comprising: transforming a plurality of plant cells with the herbicide tolerant gene or the expression cassette, and cultivating the cells under a concentration of herbicide allowing the growth of the transformed cells expressing the herbicide tolerant gene or the expression cassette, while killing the untransformed cells or inhibiting the growth of the untransformed cells, wherein the herbicide is a sulfonylurea herbicide.
(21) In order to achieve the above objective, the present invention also provides a method for controlling weeds, comprising applying an effective dose of a sulfonylurea herbicide to a field for planting a target plant, the plant containing the herbicide tolerant gene or the expression cassette.
(22) In order to achieve the above objective, the present invention also provides a method for protecting a plant from damages caused by sulfonylurea herbicides, comprising introducing the herbicide tolerant gene, the expression cassette or the recombinant vector into a plant to make the resultant plant produce a sufficient amount of herbicide tolerant proteins for protecting the plant from damages caused by sulfonylurea herbicides.
(23) In order to achieve the above objective, the present invention also provides a method for controlling glyphosate resistant weeds in a field for a glyphosate tolerant plant, comprising applying an effective dose of a sulfonylurea herbicide to a field for planting a glyphosate tolerant plant, the glyphosate tolerant plant containing the herbicide tolerant gene or the expression cassette.
(24) In order to achieve the above objective, the present invention also provides a method for imparting sulfonylurea herbicide tolerance to a plant, comprising introducing the herbicide tolerant

gene, the expression cassette or the recombinant vector into the plant.
(25) In order to achieve the above objective, the present invention also provides a method for producing a sulfonylurea herbicide tolerant plant, comprising introducing the herbicide tolerant gene, the expression cassette or the recombinant vector into the genome of the plant.
(26) In order to achieve the above objective, the present invention also provides a method for cultivating a sulfonylurea herbicide tolerant plant, comprising: planting at least one plant propagule, whose genome contains the herbicide tolerant gene or the expression cassette; allowing the plant propagule to grow into a plant; applying an effective dose of a sulfonylurea herbicide to a plant growth environment comprising at least the plant, and harvesting the plant which has reduced plant damage and/or an increased plant yield compared to other plants which do not contain the herbicide tolerant gene or the expression cassette.
(27) Further, the plant is a monocotyledonous plant or a dicotyledonous plant.
(28) Preferably, the plant is maize, soybean, *Arabidopsis thaliana*, cotton, rape, rice, sorghum, wheat, barley, millet, sugar cane or oat.
(29) On the basis of the above-mentioned technical solution, the sulfonylurea herbicide is tribenuron-methyl, sulfometuron-methyl, halosulfuron-methyl, pyrazosulfuron-ethyl, thifensulfuron methyl, bensulfuron-methyl, metsulfuron-methyl, ethametsulfuron-methyl or chlorimuron-ethyl.
(30) In order to achieve the above objective, the present invention also provides a planting system for controlling weed growth, comprising a sulfonylurea herbicide and a plant growth environment in which at least one target plant exists, wherein the plant contains the herbicide tolerant gene or the expression cassette.
(31) In order to achieve the above objective, the present invention also provides a planting system for controlling glyphosate resistant weeds in a field of a glyphosate tolerant plant, comprising a sulfonylurea herbicide, a glyphosate herbicide and a field for planting at least one glyphosate tolerant plant, wherein the glyphosate tolerant plant contains the herbicide tolerant gene or the expression cassette.
(32) Further, the plant is a monocotyledonous plant or a dicotyledonous plant.
(33) Preferably, the plant is maize, soybean, *Arabidopsis thaliana*, cotton, rape, rice, sorghum, wheat, barley, millet, sugar cane or oat.
(34) On the basis of the above-mentioned technical solution, the sulfonylurea herbicide is tribenuron-methyl, sulfometuron-methyl, halosulfuron-methyl, pyrazosulfuron-ethyl, thifensulfuron methyl, bensulfuron-methyl, metsulfuron-methyl, ethametsulfuron-methyl or chlorimuron-ethyl.
(35) In order to achieve the above objective, the present invention also provides use of a herbicide tolerant protein for degrading sulfonylurea herbicides, wherein the herbicide tolerant protein comprises: (1) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 1, and at least having an alanine substitution at position 176 and/or a valine substitution at position 178 of SEQ ID NO: 1; or (2) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 19, and at least having an alanine substitution at position 140 and/or a valine substitution at position 142 of SEQ ID NO: 19; or (3) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 35, and at least having an alanine substitution at position 140 and/or a valine substitution at position 142 of SEQ ID NO: 35; or (4) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 51, and at least having an alanine substitution at position 131 and/or a valine substitution at position 133 of SEQ ID NO: 51; or (5) a protein which is derived from (1) to (4) by substituting and/or deleting and/or adding one or more amino acids in the amino acid sequences of (1) to (4), and has thifensulfuron hydrolase activity.
(36) Furthermore, said herbicide tolerant protein comprises: (6) an amino acid sequence of (1), wherein the amino acid sequence of (1) has an arginine substitution at position 80 and/or an alanine substitution at position 81 and/or an arginine substitution at position 182 of SEQ ID NO: 1; or (7)

an amino acid sequence of (2), wherein the amino acid sequence of (2) has an arginine substitution at position 44 and/or an alanine substitution at position 45 and/or an arginine substitution at position 146 of SEQ ID NO: 19; or (8) an amino acid sequence of (3), wherein the amino acid sequence of (3) has an arginine substitution at position 44 and/or an alanine substitution at position 45 and/or an arginine substitution at position 146 of SEQ ID NO: 35; or (9) an amino acid sequence of (4), wherein the amino acid sequence of (4) has an arginine substitution at position 35 and/or an alanine substitution at position 131 and/or a valine substitution at position 133 of SEQ ID NO: 51; or (10) a protein which is derived from (6) to (9) by substituting and/or deleting and/or adding one or more amino acids in the amino acid sequences of (6) to (9), and has thifensulfuron hydrolase activity.

(37) Furthermore, the herbicide tolerant protein comprises: (11) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 7, SEQ ID NO: 11 or SEQ ID NO: 15; or (12) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 23, SEQ ID NO: 27 or SEQ ID NO: 31; or (13) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 39, SEQ ID NO: 43 or SEQ ID NO: 47; or (14) a protein consisting of an amino acid sequence as shown in SEQ ID NO: 55, SEQ ID NO: 59 or SEQ ID NO: 63.

(38) Preferably, the sulfonylurea herbicide is tribenuron-methyl, sulfometuron methyl, halosulfuron-methyl, pyrazosulfuron-ethyl, thifensulfuron-methyl, bensulfuron-methyl, metsulfuron-methyl, ethametsulfuron-methyl or chlorimuron ethyl.

(39) The sulfometuron-methyl in the present invention refers to methyl 2-(4,6-dimethylpyrimidin-2-ylcarbamoylaminosulfonyl)benzoate as a white solid. Commonly used dosage forms are 10% sulfometuron-methyl wettable powder and 10% sulfometuron-methyl suspension (also known as dry suspension). Commercial formulations of sulfometuron-methyl include, but are not limited to, Oust and Sencaojing.

(40) The effective dose of sulfometuron-methyl according to the present invention is 9 to 120 g ai/ha, including 10-100 g ai/ha, 15-90 g ai/ha, 20-80 g ai/ha, 25-70 g ai/ha, 30-60 g ai/ha or 40-50 g ai/ha.

(41) The tribenuron-methyl in the present invention refers to methyl 2-[N-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)-N-methylcarbamoylaminosulfonyl]benzoate as a white solid. Commonly used dosage forms are 10% tribenuron-methyl wettable powder, and 75% tribenuron-methyl suspension (also known as dry suspension). Commercial formulations of tribenuron-methyl include, but are not limited to, GRANSTAR and kuoyejing.

(42) The effective dose of tribenuron-methyl according to the present invention is 9 to 144 g ai/ha, including 15-120 g ai/ha, 30-110 g ai/ha, 40-100 g ai/ha, 50-90 g ai/ha, 60-80 g ai/ha or 65-75 g ai/ha.

(43) The herbicide tolerant gene, the expression cassette or the recombinant vector according to the present invention is introduced into a plant. In order to introduce the exogenous DNA into plant cells in the present invention, the conventional transformation methods include, but are not limited to, the *Agrobacterium*-mediated transformation, microprojectile bombardment, the direct DNA uptake into the protoplast, electroporation or silicon whisker-mediated DNA introduction.

(44) The herbicide tolerant gene and the subsequent herbicide resistant crop according to the present invention provide an excellent choice for controlling glyphosate-resistant (or highly tolerant and successive) broad-leaved weed species in the crop. The sulfonylurea herbicides have a broad spectrum and are potent herbicides for broad-leaved weeds, and would provide excellent utility for planters if the stronger crop tolerance could be provided in both dicotyledons and monocotyledons alike. A transgenic dicotyledonous plant with a tolerance to sulfonylurea herbicide also has higher flexibilities in the timing and amount of application. Another use of the sulfonylurea herbicide resistant trait is that it can be used for preventing normally sensitive crops from damage caused by the drifting, volatilization, conversion (or other movement over a long distance), misuse, destruction, etc., of the sulfonylurea herbicides. The use of the herbicide tolerant

gene according to the present invention in a plant can provide protection against a broader spectrum of sulfonylurea herbicides, thereby improving the flexibility and spectra of weeds that can be controlled, and can provide protection against damage caused by the drifting of a full range of commercially available sulfonylurea herbicides or caused by other sulfonylurea herbicides over a long distance.

(45) It has now been identified that the herbicide tolerant gene according to the present invention has the characteristic of allowing the use of sulfonylurea herbicides in plants after being genetically modified for the expression in the plants, wherein the absence or lack of inherent tolerance in the plants does not allow the use of these herbicides. In addition, the herbicide tolerant gene of the present invention can provide protection against the sulfonylurea herbicides in plants where the natural tolerance is insufficient for selectivity. At present, the plants containing only the herbicide tolerant gene of the present invention can be treated sequentially or tank-mixed with one, two or a combination of several sulfonylurea herbicides. The application amount of each sulfonylurea herbicide for controlling a broad spectrum of dicotyledonous weeds ranges from 7.5 to 150 g ai/ha, more generally from 20 to 50 g ai/ha. Use of the herbicides of different chemical categories and having different modes and ranges of actions in the same field in combination (sequentially or tank-mixed) can provide control for most potential weeds that need to be controlled by the herbicides.

(46) Glyphosate is widely used, as it controls a very broad spectrum of broad-leaved and gramineous weed species. However, reusing glyphosate in glyphosate tolerant crops and non-crop applications has selected (and still will select) to make weeds evolve into naturally more tolerant species or glyphosate tolerant biotypes. Most herbicide tolerance management strategies suggest using an effective amount of tank-mixed herbicide partners as a means of delaying the emergence of tolerant weeds, wherein the herbicide partners provide control of the same species, but have different modes of action. Stacking the herbicide tolerant gene according to the present invention gene with a glyphosate tolerance trait (and/or another herbicide tolerance trait) can achieve control of glyphosate tolerant weed species (broad-leaved weed species controlled by one or more sulfonylurea herbicides) in glyphosate tolerant crops by allowing selective use of glyphosate and sulfonylurea herbicides in the same crop. The application of these herbicides can be performed by using simultaneously in a tank mixture containing two or more herbicides with different modes of action, or using a single herbicide composition alone in continuous use (e.g., before planting or before or after emergence) (with an interval time range used being from 2 hours to 3 months), or alternatively, can be performed by using a combination of any number of herbicides representative of each applicable compound category at any time (from any time within 7 months after planting a crop to the time when the crop is harvested (or the pre-harvest interval for a single herbicide, with the shortest being taken)).

(47) The flexibility in controlling broad-leaved weeds is very important, i.e., application time, single application amount of herbicide, and abilities to control the stubborn or resistant weeds. The application range of glyphosate stacked with a glyphosate tolerant gene/the herbicide tolerant gene of the present invention in crops can be from 200 to 1600 g ai/ha; and that of (one or more) sulfonylurea herbicides can be from 7.5 to 150 g ai/ha. The optimal combination of time for these applications depends on the specific conditions, species and environments.

(48) A herbicide preparation (e.g., an ester, acid or salt formula or soluble concentrate, emulsifying concentrate or soluble liquid) and a tank mix additive (e.g., an adjuvant or compatilizer) can significantly affect weed control of a given herbicide or a combination of one or more herbicides. Any chemical combination of any of the foregoing herbicides is within the scope of the present invention.

(49) It is well known for a person skilled in the art that the benefits of a combination of two or more modes of action in improving the controlled spectrum of weed and/or naturally more tolerant species or resistant weed species can also be extended to artificial (transgenic or non-transgenic)

production of herbicide tolerant chemicals in addition to glyphosate tolerant crops in crops. In fact, the traits encoding the following resistances can be stacked alone or in multiple combinations to provide the ability to effectively control or prevent weeds from developing tolerance to any of the above categories of herbicides: 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), glyphosate oxidoreductase (GOX), glyphosate-N-acetyltransferase (GAT), glyphosate decarboxylase, glufosinate acetyltransferase (PAT), α-ketoglutarate dependent dioxygenase (AAD), dicamba monooxygenase (DMO), 4-hydroxyphenylpyruvate dioxygenase (HPPD), acetolactate synthase (ALS), cytochrome-like proteins (P450) and/or protoporphyrinogen oxidase (Protox).

(50) In addition, the herbicide tolerant gene of the present invention alone or the herbicide tolerant gene of the present invention stacked with other characteristics of herbicide tolerant crops can be stacked with one or more other input traits (for example, insect tolerance, fungal tolerance or stress tolerance) or output traits (for example, increased yield, improved oil amount or increased fiber quality). Therefore, the present invention can be used to provide the abilities to flexibly and economically control any number of agricultural pests and complete agricultural solutions for improving qualities of crops.

(51) The herbicide tolerant gene of the present invention can degrade a sulfonylurea herbicide, and is an important basis of herbicide tolerant crops and the possibility for selecting marker features.

(52) Transgenic expression can be performed in the present invention, and almost all combinations of herbicides for broad-leaved weeds can be controlled. The herbicide tolerant gene of the present invention as an excellent trait of herbicide tolerant crops can be stacked with, for example, other traits of herbicide tolerant crops (for example, glyphosate tolerance, glufosinate tolerance, tolerance to other ALS inhibitor (for example, imidazolinones and triazolopyrimidinyl sulfonamides), bromoxynil tolerance, HPPD inhibitor tolerance, PPO inhibitor tolerance, and the like) and traits of insect tolerance (Cry1Ab, Cry1F, Vip3, other *Bacillus thuringiensis* proteins or insect tolerant proteins derived from non-bacillus bacterial species, etc.). In addition, the herbicide tolerant gene of the present invention can be used as a selective marker for the assistant selection of primary transformants of plants genetically modified with another gene or gene group.

(53) The traits of herbicide tolerant crops of the present invention can be used in a new combination with other traits (including but not limited to glyphosate tolerance) of herbicide tolerant crops. A new method for controlling the weed species can be produced by the combination of these traits due to newly obtained tolerance or inherent tolerance to a herbicide (for example, glyphosate). Therefore, apart from the traits of herbicide tolerant crops, the scope of the present invention includes the new method for controlling weeds with herbicides, wherein the tolerance to the herbicides can be produced by the enzyme in the transgenic crops.

(54) The present invention can be applied to various types of plants, and the dicotyledonous plant includes, but is not limited to, alfalfa, beans, cauliflowers, cabbages, carrots, celery, cotton, cucumbers, eggplants, lettuces, melon, peas, peppers, zucchinis, radishes, rape, spinach, soybeans, pumpkins, tomatoes, *Arabidopsis thaliana* or watermelons; preferably, the dicotyledonous plant refers to soybeans, *Arabidopsis thaliana*, tobacco, cotton or rape. The monocotyledonous plant includes, but is not limited to, maize, rice, sorghum, wheat, barley, rye, millet, sugar cane, oats or turfgrass; preferably, the monocotyledonous plant refers to maize, rice, sorghum, wheat, barley, millet, sugar cane or oats. The herbicide tolerant gene according to the present invention can be more positively used in gramineous crops with moderate tolerance, and thus the improved tolerance obtained by such traits can provide planters with the possibility of using these herbicides with a more effective application amount and a broader application time without crop damage risks.

(55) The planting system in the present invention refers to a combination of a plant and any herbicide tolerance thereof and/or an available herbicide treatment in different plant developmental stages, thus producing plants with high yields and/or reduced damage.

(56) In the present invention, the weeds refer to plants competing with the cultivated target plants in the plant growth environment.

(57) The term “control” and/or “prevention” in the present invention refers to at least a direct application (e.g., by spraying) of an effective dose of a sulfonylurea herbicide to the plant growth environment, so as to minimize weed development and/or stop weeds from growing. At the same time, the cultivated target plants should be morphologically normal and can be cultivated under conventional methods for product consumption and/or production; and preferably, compared to non-transgenic wild-type plants, the cultivated plants have reduced plant damage and/or an increased plant yield. The specific performances of the reduced plant damage include, but are not limited to, an improved stem resistance and/or an increased grain weight. The “control” and/or “prevention” effect of the herbicide tolerant protein of the present invention on weeds can exist independently, and will not be diminished and/or lost due to the presence of other substances that can “control” and/or “prevent” the weeds. Specifically, if any tissue of a transgenic plant (containing the herbicide tolerant gene of the present invention) has and/or produces the herbicide tolerant protein of the present invention and/or another substance that can control weeds simultaneously and/or separately, then the presence of the other substance will neither affect the “control” and/or “prevention” effect of the herbicide tolerant protein of the present invention on the weeds, nor result in that the “control” and/or “prevention” effect is achieved completely and/or partially by the other substance and has nothing to do with the herbicide tolerant protein of the present invention.

(58) The genome of a plant, plant tissue or plant cell in the present invention refers to any genetic material within the plant, plant tissue or plant cell, and includes cell nuclear, plastid and mitochondrial genome.

(59) The “plant propagule” in the present invention includes, but is not limited to, plant sexual propagules and plant vegetative propagules. The plant sexual propagules include, but are not limited to, plant seeds; and the plant vegetative propagules refer to vegetative organs or a specific tissue of a plant, which can generate a new plant under ex vivo conditions. The vegetative organs or the specific tissue include, but are not limited to, roots, stems and leaves; for example, plants with roots as the vegetative propagules include strawberries, sweet potatoes and the like; plants with stems as the vegetative propagules include sugar cane, potatoes (tubers) and the like; and plants with leaves as the vegetative propagules include aloe, begonias and the like.

(60) The “resistance” in the present invention is heritable, and allows a plant to grow and propagate in the case where an effective treatment by a general herbicide is performed on a given plant. As recognized by a person skilled in the art, even if a certain damage degree of a plant treated with a herbicide is apparent, the plant can still be considered “resistant”. The term “tolerance” in the present invention is more extensive than the term “resistance”, and includes “resistance” and an improved ability of a particular plant to resist various degrees of damage induced by a herbicide, and generally, damages to a wild-type plant with the same genotype can be caused at the same herbicide dose.

(61) The polynucleotide and/or nucleotide in the present invention form a complete “gene”, which encodes a protein or a polypeptide in a desired host cell. A person skilled in the art will readily appreciate that the polynucleotide and/or nucleotide in the present invention can be placed under the control of a regulatory sequence in a host of interest.

(62) As is well known to a person skilled in the art, DNA is typically present in a double-stranded form. In this arrangement, one strand is complementary to the other, and vice versa. Additional complementary strand of DNA is produced as DNA is replicated in a plant. As such, the present invention includes the use of the polynucleotides as exemplified in the sequence listing and complementary strands thereof. The “coding strand” commonly used in the art refers to a strand bound to an antisense strand. In order to express a protein in vivo, one strand of DNA is typically transcribed to one complementary strand of mRNA, which acts as a template for translating the protein. Actually, mRNA is transcribed from the “antisense” strand of DNA. The “sense” or “coding” strand has a series of codons (a codon is composed of three nucleotides, and a specific

amino acid can be produced by reading three codons at a time), which can be read as an open reading frame (ORF) to form a protein or peptide of interest. The present invention also includes RNA having an equivalent function to the exemplary DNA.

(63) The nucleic acid molecule or a fragment thereof in the present invention hybridizes with the herbicide tolerant gene of the present invention under stringent conditions. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of the herbicide tolerant gene of the present invention. A nucleic acid molecule or a fragment thereof is capable of specifically hybridizing with other nucleic acid molecules under certain circumstances. In the present invention, if two nucleic acid molecules can form an anti-parallel double stranded nucleic acid structure, then it can be considered that these two nucleic acid molecules can be specifically hybridized with each other. If two nucleic acid molecules exhibit a complete complementarity, then one nucleic acid molecule of the two is said to be the “complement” of the other nucleic acid molecule. In the present invention, when each nucleotide of a nucleic acid molecule is complementary to the corresponding nucleotide of another nucleic acid molecule, then these two nucleic acid molecules are said to exhibit a “complete complementarity”. If two nucleic acid molecules can be hybridized with each other with a sufficient stability such that they are annealed and bound to each other at least under conventional “low stringency” conditions, then these two nucleic acid molecules are said to be “minimally complementary”. Similarly, if two nucleic acid molecules can be hybridized with each other with a sufficient stability such that they are annealed and bound to each other under conventional “high stringency” conditions, then these two nucleic acid molecules are said to be “complementary”. Deviation from a complete complementarity is permissible, as long as this deviation does not completely prevent two molecules from forming a double-stranded structure. In order to enable a nucleic acid molecule to act as a primer or probe, it is only necessary to ensure that the nucleic acid molecule has a sufficient complementarity in its sequence to allow a stable double-stranded structure to be formed in case of the particular solvent and salt concentration used.

(64) In the present invention, a substantially homologous sequence is a nucleic acid molecule, wherein the nucleic acid molecule can be specifically hybridized with the complementary strand of a matched nucleic acid molecule under high stringency conditions. Suitable stringent conditions that promote DNA hybridization are well known to a person skilled in the art; for example, the suitable stringent conditions can be achieved by treating with 6.0× sodium chloride/sodium citrate (SSC) under conditions of approximately 45, and then washing with 2.0×SSC under conditions of 50. For example, the salt concentration in the washing step can be selected from the low stringency condition of about 2.0×SSC and 50° C. to the high stringency condition of about 0.2×SSC and 50° C. In addition, the temperature condition in the washing step can rise from the low stringency condition of room temperature (about 22° C.) to the high stringency condition of about 65° C. The temperature condition and the salt concentration can both vary, and it is also possible that one of the two remains unchanged, while the other variable varies. Preferably, the stringent conditions in the present invention can be achieved by specifically hybridizing a sequence with the herbicide tolerant gene in the present invention in a 6×SSC, 0.5% SDS solution at 65° C., and then washing the membrane once with 2×SSC, 0.1% SDS and once with 1×SSC, 0.1% SDS.

(65) Consequently, sequences which have the herbicide tolerant activity and are hybridized with the herbicide tolerant gene of the present invention under stringent conditions are included in the present invention. These sequences are at least approximately 40%-50% homologous, or approximately 60%, 65% or 70% homologous, or even at least approximately 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more to the sequence of the present invention.

(66) The present invention provides a functional protein. In the present invention, the “functional activity” (or “activity”) means that the protein/enzyme used in the present invention (alone or in combination with other proteins) has the ability to degrade a sulfonylurea herbicide or diminish the

activity of a sulfonylurea herbicide. A plant producing the herbicide tolerant protein of the present invention preferably produces an “effective amount” of the protein, so that when the plant is treated with a herbicide, the protein expression level is sufficient to impart to the plant a complete or partial resistance or tolerance to the sulfonylurea herbicide (unless otherwise specified, in a general amount). The herbicide can be used in an amount which would usually kill a target plant or in a normal field amount and concentration. Preferably, the plant cell and plant of the present invention are protected from growth inhibition or damage caused by treatment with the herbicide. The transformed plant and plant cell of the present invention preferably has tolerance or resistance to sulfonylurea herbicides; that is, the transformed plant and plant cell can grow in the presence of an effective amount of sulfonylurea herbicides.

(67) The gene and protein in the present invention not only comprise a specific exemplary sequence, but also comprise a portion and/or a fragment (including an internal deletion and/or terminal deletion compared to the full-length protein), a variant, a mutant, a substitute (a protein having substituted amino acids), a chimera and a fusion protein, which retain the herbicide tolerance activity characteristic of the specific exemplary protein. The “variant” or “variation” refers to a nucleotide sequence that encodes the same protein or encodes an equivalent protein having a herbicide resistance activity. The “equivalent protein” refers to a protein having the same or substantially the same bioactivity of herbicide tolerance as the claimed protein.

(68) The “fragment” or “truncation” of a DNA molecule or protein sequence in the present invention refers to a portion of the original DNA or protein sequence (nucleotides or amino acids) or an artificially modified form thereof (e.g., a sequence suitable for plant expression), wherein the length of the foregoing sequences may vary, but the length is sufficient to ensure that the (encoded) protein is a herbicide tolerant protein.

(69) Because of the degeneracy of the genetic codon, a variety of different DNA sequences may encode the same amino acid sequence. It is within the skill of a person skilled in the art to produce these alternative DNA sequences encoding the same or substantially the same protein. These different DNA sequences are included in the scope of the present invention. The aforementioned “substantially the same” sequence refers to a sequence with an amino acid substitution, deletion, addition or insertion that does not substantively affect the herbicide tolerance activity, and includes a fragment retaining the herbicide tolerance activity.

(70) The substitution, deletion or addition of an amino acid sequence in the present invention is a conventional technique in the art. Preferably, this amino acid change is a small characteristic change, that is, a conservative amino acid substitution that does not significantly affect the folding and/or activity of a protein; a small deletion, typically a deletion of about 1-30 amino acids; a small amino or carboxyl terminal extension, e.g., a methionine residue extending at the amino terminus; or a small linker peptide, e.g., about 20-25 residues in length.

(71) Examples of conservative substitutions are substitutions occurring within the following amino acid groups: basic amino acids (e.g., arginine, lysine and histidine), acidic amino acids (e.g., glutamic acid and aspartic acid), polar amino acids (e.g., glutamine and asparagine), hydrophobic amino acids (e.g., leucine, isoleucine and valine), aromatic amino acids (e.g., phenylalanine, tryptophan and tyrosine) and small molecule amino acids (e.g., glycine, alanine, serine, threonine and methionine). Those amino acid substitutions that generally do not alter the specific activity are well known in the art, and have been described by, for example, N. Neurath and R. L. Hill in *Protein*, published by Academic Press in New York in 1979. The most common substitutions are Ala/Ser, Val/Ile, Asp/Glu, Thu/Ser, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu and Asp/Gly, as well as the reverse substitutions thereof.

(72) As will be apparent to a person skilled in the art, this substitution can occur outside the region that is important for molecular functions, and still produces an active polypeptide. Amino acid residues that are essential for the activity of the polypeptide of the present invention and are thus chosen not to be substituted can be identified according to methods known in the art, such as site-

directed mutagenesis or alanine-scanning mutagenesis (see e.g., Cunningham and Wells, 1989, Science 244: 1081-1085). The latter technique is to introduce a mutation at each positively charged residue in a molecule and detect the herbicide resistance activity of the resulting mutant molecule to determine the amino acid residues that are important for the molecular activity. Substrate-enzyme interaction sites can also be determined by analyzing the three-dimensional structure thereof, wherein this three-dimensional structure can be determined by nuclear magnetic resonance analysis, crystallography, photoaffinity labeling and other techniques (see e.g., de Vos et al., 1992, Science 255: 306-312; Smith et al., 1992, J. Mol. Biol. 224: 899-904; and Wlodaver et al., 1992, FEBS Letters 309: 59-64).

(73) The regulatory sequence in the present invention includes, but is not limited to, a promoter, a transit peptide, a terminator, an enhancer, a leader sequence, an intron and other regulatory sequences operably linked to the herbicide tolerant gene of the present invention.

(74) The promoter is a plant expressible promoter. The “plant expressible promoter” refers to a promoter that ensures the expression of the coding sequence linked thereto in a plant cell. The plant expressible promoter can be a constitutive promoter. Examples of the promoters directing the constitutive expression in plants include, but are not limited to, a 35S promoter derived from a cauliflower mosaic virus, maize Ubi promoters, rice GOS2 gene promoters, and the like. Alternatively, the plant expressible promoter can be a tissue specific promoter; i.e. the promoter directs the expression of a coding sequence in several tissues, such as green tissues, at a level higher than in other tissues of the plant (which can be measured through conventional RNA trials), such as a PEP carboxylase promoter. Alternatively, the plant expressible promoter can be a wound-inducible promoter. The wound-inducible promoter or a promoter directing a wound-induced expression pattern means that when a plant suffers from a wound caused by a mechanical factor or the gnawing of insects, the expression of the coding sequence under the regulation of the promoter is significantly improved compared to normal growth conditions. Examples of the wound-inducible promoters include, but are not limited to, promoters of potato and tomato protease inhibitor genes (pin I and pin II) and a maize protease inhibitor gene (MPI).

(75) The transit peptide (also known as secretion signal sequence or targeting sequence) directs a transgenic product to a specific organelle or cell compartment. For a receptor protein, the transit peptide may be heterologous, for example, targeting the chloroplast using a sequence encoding the chloroplast transit peptide, or targeting the endoplasmic reticulum using a ‘KDEL’ retention sequence, or targeting the vacuole using CTPP of a barley phytolectin gene.

(76) The leader sequence includes, but is not limited to, a small RNA virus leader sequence, such as an EMCV leader sequence (a 5′ non-coding region of encephalomyocarditis virus); a potato virus Y group leader sequence, such as a MDMV (Maize Dwarf Mosaic Virus) leader sequence; human immunoglobulin heavy chain binding protein (BiP); an untranslated leader sequence of the coat protein mRNA of alfalfa mosaic virus (AMV RNA4); and a tobacco mosaic virus (TMV) leader sequence.

(77) The enhancer includes, but is not limited to, a cauliflower mosaic virus (CaMV) enhancer, figwort mosaic virus (FMV) enhancer, carnation etched ring virus (CERV) enhancer, cassava vein mosaic virus (CsVMV) enhancer, mirabilis mosaic virus (MMV) enhancer, cestrum yellow leaf curling virus (CmYLCV) enhancer, cotton leaf curl Multan virus (CLCuMV) enhancer, commelina yellow mottle virus (CoYMV) enhancer and peanut chlorotic streak virus (PCLSV) enhancer.

(78) For use in a monocotyledonous plant, the intron includes, but is not limited to, a maize hsp70 intron, maize ubiquitin intron, Adh intron 1, sucrose synthase intron or rice Actl intron. For use in a dicotyledonous plant, the intron includes, but is not limited to, a CAT-1 intron, pKANNIBAL intron, PIV2 intron and “super ubiquitin” intron.

(79) The terminator can be a suitable polyadenylation signal sequence that functions in a plant, including, but not limited to, a polyadenylation signal sequence derived from the *Agrobacterium tumefaciens* nopaline synthetase (NOS) gene, a polyadenylation signal sequence derived from the

protease inhibitor II (pinII) gene, a polyadenylation signal sequence derived from the pea ssRUBISCO E9 gene and a polyadenylation signal sequence derived from the α-tubulin gene.
(80) The “effective linking” in the present invention indicates the binding of nucleic acid sequences, wherein the binding enables a sequence to provide a function required for the sequence linked thereto. The “effective linking” in the present invention can be achieved by linking a promoter to a sequence of interest, so that the transcription of the sequence of interest is controlled and regulated by the promoter. When a sequence of interest encodes a protein and the expression of the protein is desired, “effective linking” means that a promoter is linked to the sequence in such a manner that the resulting transcript is efficiently translated. If the linking of a promoter to a coding sequence is a transcript fusion and expression of the encoded protein is intended to be achieved, such linking is created that the first translation initiation codon in the resulting transcript is the initiation codon in the coding sequence. Alternatively, if the linking of a promoter to a coding sequence is a translation fusion and expression of the encoded protein is intended to be achieved, such a linking is created that the first translation initiation codon contained in the 5′ untranslated sequence is linked to the promoter in such a manner that the relationship of the resulting translation product with the translation open reading frame encoding the desired protein is in-frame. Nucleic acid sequences that can be “effectively linked” include, but are not limited to: sequences providing gene expression functions (i.e., gene expression elements, such as promoters, 5′ untranslated regions, introns, protein coding regions, 3′ untranslated regions, polyadenylation sites and/or transcription terminators), sequences providing DNA transfer and/or integration functions (i.e., T-DNA boundary sequences, site-specific recombinase recognition sites and integrase recognition sites), sequences providing selective functions (i.e., antibiotic resistance markers and biosynthesis genes), sequences providing marker scoring functions, sequences assisting in sequence manipulation in vitro or in vivo (i.e., polylinker sequences and site-specific recombination sequences) and sequences providing replication functions (i.e., bacterial origins of replication, autonomously replicating sequences and centromeric sequences).
(81) The present invention may impart a new herbicide resistance trait to a plant, and no adverse effects on phenotypes (including yields) are observed. The plant in the present invention can tolerate, e.g., 2×, 3×, 4× or 8× the general application level of at least one herbicide tested. The improvement of these levels of tolerance is within the scope of the present invention. For example, foreseeable optimization and further development can be performed on various techniques known in the art, to increase the expression of a given gene.
(82) The herbicide tolerant protein of the present invention can be a protein consisting of an amino acid sequence shown in SEQ ID NO: 1 and at least having an alanine substitution at position 176 and/or a valine substitution at position 178 of SEQ ID NO: 1, with an example shown in SEQ ID NO: 7, SEQ ID NO: 11 or SEQ ID NO: 15 in the sequence listing. The herbicide tolerant gene of the present invention can be a coding gene of the above-mentioned herbicide tolerant protein, with examples shown in SEQ ID NOs: 8-10, SEQ ID NOs: 12-14 and SEQ ID NOs: 16-18 in the sequence listing.
(83) The herbicide tolerant protein of the present invention can be a protein consisting of an amino acid sequence shown in SEQ ID NO: 19 and at least having an alanine substitution at position 140 and/or a valine substitution at position 142 of SEQ ID NO: 19, with an example shown in SEQ ID NO: 23, SEQ ID NO: 27 or SEQ ID NO: 31 in the sequence listing. The herbicide tolerant gene of the present invention can be a coding gene of the above-mentioned herbicide tolerant protein, with examples shown in SEQ ID NOs: 24-26, SEQ ID NOs: 28-30 and SEQ ID NOs: 32-34 in the sequence listing.
(84) The herbicide tolerant protein of the present invention can be a protein consisting of an amino acid sequence shown in SEQ ID NO: 35 and at least having an alanine substitution at position 140 and/or a valine substitution at position 142 of SEQ ID NO: 35, with an example shown in SEQ ID NO: 39, SEQ ID NO: 43 or SEQ ID NO: 47 in the sequence listing. The herbicide tolerant gene of

the present invention can be a coding gene of the above-mentioned herbicide tolerant protein, with examples shown in SEQ ID NOs: 40-42, SEQ ID NOs: 44-46 and SEQ ID NOs: 48-50 in the sequence listing.
(85) The herbicide tolerant protein of the present invention can be a protein consisting of an amino acid sequence shown in SEQ ID NO: 51 and at least having an alanine substitution at position 131 and/or a valine substitution at position 133 of SEQ ID NO: 51, with an example shown in SEQ ID NO: 55, SEQ ID NO: 59 or SEQ ID NO: 63 in the sequence listing. The herbicide tolerant gene of the present invention can be a coding gene of the above-mentioned herbicide tolerant protein, with examples shown in SEQ ID NOs: 56-58, SEQ ID NOs: 60-62 and SEQ ID NOs: 64-66 in the sequence listing.
(86) The herbicide tolerant gene of the present invention can be used in plants; and can contain, apart from a coding region of the herbicide tolerant gene of the present invention, other elements such as a coding region encoding a transit peptide, and a coding region encoding a selective marker protein or a protein imparting insect resistance.
(87) The herbicide tolerant protein of the present invention has tolerance to most of the sulfonylurea herbicides. The plant in the present invention contains an exogenous DNA in its genome, wherein the exogenous DNA comprises the herbicide tolerant gene of the present invention, and the plant is protected from the threat of a sulfonylurea herbicide by expressing an effective amount of the protein. The effective amount refers to a dose causing no or minor damage. At the same time, the plant should be morphologically normal and can be cultivated under conventional methods for product consumption and/or production.
(88) The expression level of the herbicide tolerant protein in a plant material can be detected by a variety of methods described in the art, for example, by quantifying the mRNA encoding the herbicide tolerant protein produced in a tissue by employing specific primers, or specifically detecting the amount of the produced herbicide tolerant protein directly.
(89) The present invention provides a herbicide tolerant protein, a coding gene thereof and a use thereof, having the following advantages: 1. The herbicide tolerant protein of the present invention has a strong tolerance to sulfonylurea herbicides and can tolerate eight-fold field concentration of tribenuron-methyl. 2. The herbicide tolerant protein of the present invention has a broad prospect of application in plants.
(90) The technical solution of the present invention is further described in details through the figures and examples below.

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## Description

DESCRIPTION OF THE FIGURES
(1) FIG. **1** is a construction flow chart of a recombinant cloning vector DBN01-T containing an ALT02M1-01 nucleotide sequence for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention;
(2) FIG. **2** is a construction flow chart of a recombinant expression vector DBN100825 containing an ALT02M1-01 nucleotide sequence for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention;
(3) FIG. **3** is a schematic structural diagram of a control recombinant expression vector DBN100828N for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention;
(4) FIG. **4** is a diagram showing the tolerance of a transgenic soybean T.sub.1 plant to benzenesulfonic acid for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention; A: ALT02-01 transgenic soybean plant; B: ALT02M1-01 transgenic soybean plant; C: ALT02M2-01 transgenic soybean plant; D: ALT02M3-01 transgenic soybean plant; E:

control soybean plant; F: wild-type soybean plant;
(5) FIG. **5** is a construction flow chart of a recombinant cloning vector DBN02-T containing an ALT02M1-02 nucleotide sequence for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention;
(6) FIG. **6** is a construction flow chart of a recombinant expression vector DBN100833 containing an ALT02M1-02 nucleotide sequence for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention;
(7) FIG. **7** is a schematic structural diagram of a control recombinant expression vector DBN100830N for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention; and
(8) FIG. **8** is a diagram showing the tolerance of a transgenic maize T.sub.1 plant to benzenesulfonic acid for the herbicide tolerant protein, the coding gene thereof and a use thereof in the present invention; A: ALT02-02 transgenic maize plant; B: ALT02M1-02 transgenic maize plant; C: ALT02M2-02 transgenic maize plant; D: ALT02M3-02 transgenic maize plant; E: control maize plant; F: wild-type maize plant.
PARTICULAR EMBODIMENTS
(9) The technical solutions of the herbicide tolerant protein, the coding gene thereof and use thereof in the present invention are further described through specific examples below.
Example 1. Mutation and Screening of ALT Gene
(10) 1. Synthesis of ALT Gene
(11) The nucleotide sequence (1197 nucleotides) of the ALT01 gene as shown in SEQ ID NO: 2 in the sequence listing was synthesized, which encodes the ALT01 protein (398 amino acids) as shown in SEQ ID NO: 1 in the sequence listing. The nucleotide sequence (SEQ ID NO: 2) of the synthetic ALT01 gene was ligated with a SpeI restriction enzyme site at the 5′ end and a KasI restriction enzyme site at the 3′ end. The ALT01-01 nucleotide sequence as shown in SEQ ID NO: 3 in the sequence listing encoding the amino acid sequence corresponding to ALT01 was obtained based on soybean codon usage bias, and the ALT01-02 nucleotide sequence as shown in SEQ ID NO: 4 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT01 was obtained based on the maize codon usage bias.
(12) 2. Construction of a Mutant Library of ALT01 Gene
(13) The above synthetic ALT01 gene was amplified by PCR, and then was cloned into the vector pGEM-T according to the operational procedure in the instructions of product pGEM-T vector (Promega, Madison, USA, CAT: A3600) of Promega Corporation. Then, the above ligated product was introduced into *Escherichia coli* DH5α as a template to carry out error-prone PCR using primer 1 and primer 2, so that the ALT01 gene was mutated due to random base mismatch. The primers and error-prone PCR reaction system were as follows:
(14) TABLE-US-00001 primer 1: ATGGAAACCGATAAAAAAACCG, as shown in SEQ ID NO: 5 in the sequence listing; primer 2: TCAGCTTTCGTTCTGATCTAAG, as shown in SEQ ID NO: 6 in the sequence listing;
Error-Prone PCR Reaction System (Total Volume: 50 μL):
(15) TABLE-US-00002 2 × StarMut Random System 25 μL Plasmid DNA template 1 μL Primer 1 1 μL Primer 2 1 μL StarMut Enhancer 0-20 μL Water (ddH.sub.2O) added to 50 μL
(16) The plasmid DNA template having a concentration of 1-10 ng/μL, the primer 1 having a concentration of 10 μM, and the primer 2 having a concentration of 10 μM, were stored at 4° C. in an amber tube.
(17) Error-Prone PCR Reaction Conditions:
(18) TABLE-US-00003 Step temperature time 11 95° C. 2 min 12 94° C. 30 s 13 55° C. 1 min 14 72° C. 1.5 min 15 back to step 12, 30 cycles 16 72° C. 10 min
(19) The above error-prone PCR product was transformed into tribenuron-methyl-sensitive

*Escherichia coli* DH10B ilvG.sup.+ by heat shock at 42° C. to construct a random mutant library of ALT01 gene.

(20) 3. Screening of a Mutant Library of ALT01 Gene

(21) The transformed product in the above mutant library was inoculated onto a screening medium (glucose 5 g/L, ampicillin 100 mg/L, valine 200 mg/L, leucine 200 mg/L, (NH.sub.4).sub.2SO.sub.4 2 g/L, MgSO.sub.4.Math.7H.sub.2O 200 mg/L, CaCl.sub.2.Math.2H.sub.2O 10 mg/L, FeSO.sub.4.Math.7H.sub.2O 1 mg/L, Na.sub.2HPO.sub.4.Math.12H.sub.2O 1.5 g/L and KH.sub.2PO.sub.4 1.5 g/L) containing tribenuron-methyl at a concentration of 200 mg/L, and was cultured at a temperature of 37° C. for 24 h.

(22) In view of the ability of a resistance gene to transform tribenuron-methyl to benzenesulfonic acid which is non-toxic to bacteria, the above mutant library was subjected to high-throughput screening using the principle, and *Escherichia coli* DH10B ilvG.sup.+, which is still able to grow on the screening medium containing tribenuron-methyl at a concentration of 200 mg/L, was isolated to obtain a resistance gene.

(23) 4. Acquisition of Mutant Resistance Genes

(24) The sequencing results showed acquisition of three mutant resistance genes of ALT01, which were named ALT01M1, ALT01M2 and ALT01M3 genes respectively. The nucleotide sequence of ALT01M1 was mutated at position 527 from G to C, resulting in mutation from glycine to alanine at position 176 of the amino acid sequence of ALT01M1; the nucleotide sequence of ALT01M2 was mutated at positions 532 and 533 from TC to GT, resulting in mutation from serine to valine at position 178 of the amino acid sequence of ALT01M2; the nucleotide sequence of ALT01M3 was mutated at positions 239 to 242 from CATA to GAGC, and at positions 527 to 544 from GAAACTCCAGTAAAGAAG to CAAACGTCAGTAAAGAAA, resulting in mutation from proline and tyrosine to arginine and alanine at positions 80 to 81 and mutation from glycine, serine and glycine to alanine, valine and arginine at positions 176, 178 and 182 of the amino acid sequence of ALT01M3.

(25) The amino acid sequence of the herbicide tolerant protein ALT01M1 is shown in SEQ ID NO: 7 in the sequence listing, and the ALT01M1 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT01M1 is shown in SEQ ID NO: 8 in the sequence listing; the ALT01M1-01 nucleotide sequence as shown in SEQ ID NO: 9 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT01M1 was obtained based on the soybean codon usage bias; the ALT01M1-02 nucleotide sequence as shown in SEQ ID NO: 10 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT01M1 was obtained based on the maize codon usage bias.

(26) The amino acid sequence of the herbicide tolerant protein ALT01M2 is shown in SEQ ID NO: 11 in the sequence listing, and the ALT01M2 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT01M2 is shown in SEQ ID NO: 12 in the sequence listing; the ALT01M2-01 nucleotide sequence as shown in SEQ ID NO: 13 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT01M2 was obtained based on the soybean codon usage bias; the ALT01M2-02 nucleotide sequence as shown in SEQ ID NO: 14 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT01M2 was obtained based on the maize codon usage bias.

(27) The amino acid sequence of the herbicide tolerant protein ALT01M3 is shown in SEQ ID NO: 15 in the sequence listing, and the ALT01M3 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT01M3 is shown in SEQ ID NO: 16 in the sequence listing; the ALT01M3-01 nucleotide sequence as shown in SEQ ID NO: 17 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT01M3 was obtained based on the soybean codon usage bias; the ALT01M3-02 nucleotide sequence as shown in SEQ ID NO: 18 in the sequence listing encoding the amino acid sequence corresponding to the

herbicide tolerant protein ALT01M3 was obtained based on the maize codon usage bias.
(28) Synthesis of the Following Nucleotide Sequences:
(29) The amino acid sequence (369 amino acids) of ALT02 is shown in SEQ ID NO: 19 in the sequence listing, and the ALT02 nucleotide sequence (1110 nucleotides) which encodes the amino acid sequence of ALT02 is shown in SEQ ID NO: 20 in the sequence listing; the ALT02-01 nucleotide sequence as shown in SEQ ID NO: 21 in the sequence listing encoding the amino acid sequence corresponding to the ALT02 was obtained based on the soybean codon usage bias; the ALT02-02 nucleotide sequence as shown in SEQ ID NO: 22 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02 was obtained based on the maize codon usage bias.
(30) The herbicide tolerant protein ALT02M1 includes a mutation from glycine to alanine at position 140 of the amino acid sequence of the ALT02. The amino acid sequence of ALT02M1 is shown in SEQ ID NO: 23 in the sequence listing, and the ALT02M1 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT02M1 is shown in SEQ ID NO: 24 in the sequence listing; the ALT02M1-01 nucleotide sequence as shown in SEQ ID NO: 25 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02M1 was obtained based on the soybean codon usage bias; the ALT02M1-02 nucleotide sequence as shown in SEQ ID NO: 26 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02M1 was obtained based on the maize codon usage bias.
(31) The herbicide tolerant protein ALT02M2 includes a mutation from serine to valine at position 142 of the amino acid sequence of the ALT02. The amino acid sequence of the ALT02M2 is shown in SEQ ID NO: 27 in the sequence listing, and the ALT02M2 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT02M2 is shown in SEQ ID NO: 28; the ALT02M2-01 nucleotide sequence as shown in SEQ ID NO: 29 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02M2 was obtained based on the soybean codon usage bias; the ALT02M2-02 nucleotide sequence as shown in SEQ ID NO: 30 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02M2 was obtained based on the maize codon usage bias.
(32) The herbicide tolerant protein ALT02M3 includes mutations from proline and tyrosine to arginine and alanine at positions 44 to 45 and mutations from glycine, serine and glycine to alanine, valine and arginine at positions 140, 142 and 146 of the amino acid sequence of the ALT02. The amino acid sequence of the ALT02M3 is shown in SEQ ID NO: 31 in the sequence listing, and the ALT02M3 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT02M3 is shown in SEQ ID NO: 32 in the sequence listing; the ALT02M3-01 nucleotide sequence as shown in SEQ ID NO: 33 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02M3 was obtained based on the soybean codon usage bias; the ALT02M3-02 nucleotide sequence as shown in SEQ ID NO: 34 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT02M3 was obtained based on the maize codon usage bias.
(33) The amino acid sequence (362 amino acids) of ALT03 is shown in SEQ ID NO: 35 in the sequence listing, and the ALT03 nucleotide sequence (1089 nucleotides) which encodes the amino acid sequence of the ALT03 is shown in SEQ ID NO: 36 in the sequence listing; the ALT03-01 nucleotide sequence as shown in SEQ ID NO: 37 in the sequence listing encoding the amino acid sequence corresponding to the ALT03 was obtained based on the soybean codon usage bias; the ALT03-02 nucleotide sequence as shown in SEQ ID NO: 38 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03 was obtained based on the maize codon usage bias.
(34) The herbicide tolerant protein ALT03M1 includes a mutation from glycine to alanine at position 140 of the amino acid sequence of the ALT03. The amino acid sequence of the ALT03M1

is shown in SEQ ID NO: 39 in the sequence listing, and the ALT03M1 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT03M1 is shown in SEQ ID NO: 40 in the sequence listing; the ALT03M1-01 nucleotide sequence as shown in SEQ ID NO: 41 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03M1 was obtained based on the soybean codon usage bias; the ALT03M1-02 nucleotide sequence as shown in SEQ ID NO: 42 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03M1 was obtained based on the maize codon usage bias.

(35) The herbicide tolerant protein ALT03M2 includes a mutation from serine to valine at position 142 of the amino acid sequence of the ALT03. The amino acid sequence of the ALT03M2 is shown in SEQ ID NO: 43 in the sequence listing, and the ALT03M2 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT03M2 is shown in SEQ ID NO: 44 in the sequence listing; the ALT03M2-01 nucleotide sequence as shown in SEQ ID NO: 45 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03M2 was obtained based on the soybean codon usage bias; the ALT03M2-02 nucleotide sequence as shown in SEQ ID NO: 46 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03M2 was obtained based on the maize codon usage bias.

(36) The herbicide tolerant protein ALT03M3 includes mutations from proline and tyrosine to arginine and alanine at positions 44 to 45 and mutations from glycine, serine and glycine to alanine, valine and arginine at positions 140, 142 and 146 of the amino acid sequence of the ALT03. The amino acid sequence of the ALT03M3 is shown in SEQ ID NO: 47 in the sequence listing, and the ALT03M3 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT03M3 is shown in SEQ ID NO: 48 in the sequence listing; the ALT03M3-01 nucleotide sequence as shown in SEQ ID NO: 49 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03M3 was obtained based on the soybean codon usage bias; the ALT03M3-02 nucleotide sequence as shown in SEQ ID NO: 50 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT03M3 was obtained based on the maize codon usage bias.

(37) The amino acid sequence (350 amino acids) of ALT04 is shown in SEQ ID NO: 51 in the sequence listing, and the ALT04 nucleotide sequence (1053 nucleotides) which encodes the amino acid sequence of the ALT04 is shown in SEQ ID NO: 52 in the sequence listing; the ALT04-01 nucleotide sequence as shown in SEQ ID NO: 53 in the sequence listing encoding the amino acid sequence corresponding to the ALT04 was obtained based on the soybean codon usage bias; the ALT04-02 nucleotide sequence as shown in SEQ ID NO: 54 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04 was obtained based on the maize codon usage bias.

(38) The herbicide tolerant protein ALT04M1 includes a mutation from glycine to alanine at position 131 of the amino acid sequence of the ALT04. The amino acid sequence of the ALT04M1 is shown in SEQ ID NO: 55 in the sequence listing, and the ALT04M1 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT04M1 is shown in SEQ ID NO: 56 in the sequence listing; the ALT04M1-01 nucleotide sequence as shown in SEQ ID NO: 57 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04M1 was obtained based on the soybean codon usage bias; the ALT04M1-02 nucleotide sequence as shown in SEQ ID NO: 58 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04M1 was obtained based on the maize codon usage bias.

(39) The herbicide tolerant protein ALT04M2 includes a mutation from serine to valine at position 133 of the amino acid sequence of the ALT04. The amino acid sequence of the ALT04M2 is shown in SEQ ID NO: 59 in the sequence listing, and the ALT04M2 nucleotide sequence which encodes

the amino acid sequence of the herbicide tolerant protein ALT04M2 is shown in SEQ ID NO: 60 in the sequence listing; the ALT04M2-01 nucleotide sequence as shown in SEQ ID NO: 61 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04M2 was obtained based on the soybean codon usage bias; the ALT04M2-02 nucleotide sequence as shown in SEQ ID NO: 62 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04M2 was obtained based on the maize codon usage bias.

(40) The herbicide tolerant protein ALT04M3 includes mutations from proline and tyrosine to arginine and alanine at positions 35 to 36 and mutations from glycine, serine and glycine to alanine, valine and arginine at positions 131, 133 and 137 of the amino acid sequence of the ALT04. The amino acid sequence of the ALT04M3 is shown in SEQ ID NO: 63 in the sequence listing, and the ALT04M3 nucleotide sequence which encodes the amino acid sequence of the herbicide tolerant protein ALT04M3 is shown in SEQ ID NO: 64 in the sequence listing; the ALT04M3-01 nucleotide sequence as shown in SEQ ID NO: 65 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04M3 was obtained based on the soybean codon usage bias; the ALT04M3-02 nucleotide sequence as shown in SEQ ID NO: 66 in the sequence listing encoding the amino acid sequence corresponding to the herbicide tolerant protein ALT04M3 was obtained based on the maize codon usage bias.

Example 2. Expression and Purification of Protein

(41) 1. PCR Amplification of Genes

(42) A pair of primers were designed:

(43) TABLE-US-00004 primer 3: TGCAGACATATGGAAACCGATAAAAAAAC (the portion underlined being Nde I restriction enzyme site), as shown in SEQ ID NO: 67 in the sequence listing; primer 4: CCCAAGCTTCTAGCTTTCGTTCTGATCTAAGCCGTGC (the portion underlined being Hind III restriction enzyme site), as shown in SEQ ID NO: 68 in the sequence listing;

(44) The ALT01M1 gene (terminal containing Nde I and Hind III restriction enzyme sites) was amplified using the following PCR amplification system:

(45) TABLE-US-00005 Taq DNA polymerase (5 U/μL) 0.5 μL 5 × PrimeSTARBuffer (Mg.sup.2+ Plus) 25 μL dNTP mixture (each 2.5 mM) 5 μL Template DNA (M1 gene) 10 ng Primer 3 (25 μM) 1 μL Primer 4 (25 μM) 1 μL Water (ddH.sub.2O) added to 50 μL

(46) PCR reaction conditions: denaturation at 98° C. for 1 min; then entering the following cycle: denaturation at 98° C. for 15 s, annealing at 55° C. for 15 s, extension at 72° C. for 1 min, totally including 29 cycles; finally extension at 72° C. for 10 min, and cooling to room temperature.

(47) According to the above PCR amplification method, the ALT01M2 nucleotide sequence, the ALT01M3 nucleotide sequence, the ALT01 nucleotide sequence, the ALT03M1 nucleotide sequence, the ALT03M2 nucleotide sequence, the ALT03M3 nucleotide sequence, ALT03 nucleotide sequence, ALT04M1 nucleotide sequence, ALT04M2 nucleotide sequence, ALT04M3 nucleotide sequence and ALT04 nucleotide sequence, which contain the Nde I and Hind III restriction enzyme sites at terminals, were amplified. ALT02M1 nucleotide sequence, ALT02M2 nucleotide sequence, ALT02M3 nucleotide sequence, and ALT02 nucleotide sequence (terminals of which contain Nde I and Hind III restriction enzyme sites, respectively) were synthesized.

(48) 2. Construction of a Bacterial Expression Vector and Acquisition of Recombinant Microorganisms

(49) The above PCR amplification product (the ALT01M1 nucleotide sequence, the ALT01M2 nucleotide sequence, the ALT01M3 nucleotide sequence, the ALT01 nucleotide sequence, the ALT02M1 nucleotide sequence, the ALT02M2 nucleotide sequence, the ALT02M3 nucleotide sequence, the ALT02 nucleotide sequence, the ALT03M1 nucleotide sequence, the ALT03M2 nucleotide sequence, the ALT03M3 nucleotide sequence, the ALT03 nucleotide sequence, the

ALT04M1 nucleotide sequence, the ALT04M2 nucleotide sequence, the ALT04M3 nucleotide sequence and the ALT04 nucleotide sequence, which contain the Nde I and Hind III restriction enzyme sites at terminals) and a bacterial expression vector pET-30a (+) were digested respectively with restriction enzymes Nde I and Hind III, the excised gene fragments mentioned above were enzymatically linked respectively with the bacterial expression vector pET-30a (+) after enzyme digestion, and the enzymatically linked products were transformed respectively to the expression host strain BL21 (DE3) to obtain the recombinant microorganisms BL21 (ALT01M1), BL21 (ALT01M2), BL21 (ALT01M3), BL21 (ALT01), BL21 (ALT02M1), BL21 (ALT02M2), BL21 (ALT02M3), BL21 (ALT02), BL21 (ALT03M1), BL21 (ALT03M2), BL21 (ALT03M3), BL21 (ALT03), BL21 (ALT04M1), BL21 (ALT04M2), BL21 (ALT04M3), and BL21 (ALT04).

(50) 3. Expression and Purification of Herbicide Tolerant Protein in *Escherichia coli*

(51) The recombinant microorganisms BL21 (ALT01M1), BL21 (ALT01M2), BL21 (ALT01M3), BL21 (ALT01), BL21 (ALT02M1), BL21 (ALT02M2), BL21 (ALT02M3), BL21 (ALT02), BL21 (ALT03M1), BL21 (ALT03M2), BL21 (ALT03M3), BL21 (ALT03), BL21 (ALT04M1), BL21 (ALT04M2), BL21 (ALT04M3), and BL21 (ALT04) were cultured in 100 mL of LB medium (10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl and 100 mg/L of ampicillin, adjusted to pH 7.5 with NaOH) to a concentration of OD.sub.600nm=0.6-0.8, and induced with isopropyl thiogalactoside (IPTG) added at a concentration of 0.4 mM at a temperature of 16° C. for 20 hours. Bacterial cells were collected by centrifugation and resuspended in 20 ml of Tris-HCl buffer (100 mM, pH 8.0), followed by performing ultrasonication (X0-900D ultrasonic processor ultrasonic processor, 30% intensity) for 10 min, then centrifuging, collecting the supernatant, purifying the acquired herbicide tolerant proteins mentioned above with nickel ion affinity chromatography column, and detecting the purification result using SDS-PAGE protein electrophoresis with the band size being consistent with theoretically predicted band size.

Example 3. Determination of Enzymatic Activity of Herbicide Tolerant Protein

(52) Enzymatic reaction system (1 mL) contains 0.2 μg of reactive enzyme (the herbicide tolerant proteins ALT01M1, ALT01M2, ALT01M3, ALT01, ALT02M1, ALT02M2, ALT02M3, ALT02, ALT03M1, ALT03M2, ALT03M3, ALT03, ALT04M1, ALT04M2, ALT04M3 and ALT04 obtained from the above purification), 0.2 mM of thifensulfuron-methyl (metsulfuron-methyl, chlorimuron-ethyl, bensulfuron-methyl, sulfometuron-methyl or tribenuron-methyl), and a buffer system of phosphate buffer at a concentration of 50 mM (pH 7.0), which were reacted in a water bath at a temperature of 30° C. for 20 min. Each reaction was timed beginning with the addition of reactive enzyme, and was terminated with 1 mL of dichloromethane. The organic phase after delamination was dehydrated with anhydrous sodium sulfate.

(53) The above dehydrated reaction solution was blown dry with nitrogen and filtered by adding 1 mL of methanol, and 20 μL of the filtrate was subjected to liquid chromatography-mass spectrometry (LC-MS). High performance liquid chromatography (HPLC) conditions were as follows: mobile phase being methanol:water (80:20, V/V), Zorbax XDB-C18 chromatographic column (3.5 μm, 2.1×50 mm, Agilent, USA), column temperature being room temperature, UV detector, with a detection wavelength of 255 nm, a sample injection volume of 20 μL, and a flow rate of 0.25 mL/min. The primary ion mass spectrometry conditions were as follows: ion detection mode being multi-reactive ion detection; ion polarity being negative ion; ionization mode being electrospray ionization; a capillary voltage of 4000 volts; a dry gas temperature of 330° C., a flow rate of 10 L/min, an atomizing gas pressure of 35 psi, a collision voltage of 135 volts; and a mass scan range of 300-500 m/z. The secondary ion mass spectrometry conditions were as follows: a collision voltage of 90 volts; a mass scanning range of 30-400 m/z, and other conditions being the same as those of the primary ion mass spectrometry. It was identified by LC-MS that the metabolite of thifensulfuron-methyl was thiophene sulfonic acid, and the metabolite of metsulfuron-methyl, chlorimuron-ethyl, bensulfuron-methyl, sulfometuron-methyl or tribenuron-methyl was its corresponding sulfonic acid. The amount of the generated thiophene sulfonic acid (metabolite) was

detected using high performance liquid chromatography (HPLC). An enzyme activity unit was defined as the amount of enzyme required for catalyzing the degradation of thifensulfuron-methyl (metsulfuron-methyl, chlorimuron-ethyl, bensulfuron-methyl, sulfometuron-methyl or tribenuron-methyl) at pH 7.0, at a temperature of 30° C. within 1 min to decrease 1 μmol of thifensulfuron-methyl (metsulfuron-methyl, chlorimuron-ethyl, bensulfuron-methyl, sulfometuron-methyl or tribenuron-methyl), which is expressed as U. Experimental results were shown in Table 1.

(54) TABLE-US-00006 TABLE 1 Experimental results of degradation of sulfonylurea herbicides by herbicide tolerant proteins specific enzyme activity tribenuron- bensulfuron- thifensulfuron- metsulfuron- chlorimuron- sulfometuron- (μmol/min/mg) methyl methyl methyl methyl ethyl methyl ALT01 1.8 1.7 27.4 2.0 2.7 1.9 ALT01M1 3.1 3.9 89.9 2.2 10.4 5.4 ALT01M2 10.8 2.4 106.0 1.2 9.0 3.5 ALT01M3 3.3 0.68 17.8 4.2 38.4 1.1 ALT02 1.9 1.8 28.8 2.1 2.8 2.0 ALT02M1 3.3 4.1 94.4 2.3 10.9 5.7 ALT02M2 11.3 2.5 111.3 1.3 9.5 3.7 ALT02M3 3.5 0.7 18.7 4.4 40.3 1.2 ALT03 1.7 1.6 26.0 1.9 2.6 1.8 ALT03M1 2.9 3.7 85.4 2.1 9.9 5.1 ALT03M2 10.3 2.3 100.7 1.1 8.6 3.3 ALT03M3 3.1 0.6 16.9 4.0 36.5 1.0 ALT04 1.6 1.5 24.7 1.8 2.4 1.7 ALT04M1 2.8 3.5 80.9 2.0 9.4 4.9 ALT04M2 9.7 2.2 95.4 1.1 8.1 3.2 ALT04M3 3.0 0.6 16.0 3.8 34.6 1.0

(55) The above experimental results indicate that compared with the herbicide tolerant protein ALT01, the purified herbicide tolerant protein ALT01M1 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 1.7, 2.3 and 3.3-fold of those of ALT01 respectively; the purified herbicide tolerant protein ALT01M2 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 6.0, 1.4 and 3.9-fold of those of ALT01 respectively; the purified herbicide tolerant protein ALT01M3 degrades tribenuron-methyl, metsulfuron-methyl and chlorimuron-ethyl at efficiencies that are 1.9, 2.1 and 14.2-fold of those of ALT01 respectively.

(56) Compared with the herbicide tolerant protein ALT02, the purified herbicide tolerant protein ALT02M1 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 1.7, 2.3 and 3.3-fold of those of ALT02 respectively; the purified herbicide tolerant protein ALT02M2 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 5.9, 1.4 and 3.9-fold of those of ALT02 respectively; the purified herbicide tolerant protein ALT02M3 degrades tribenuron-methyl, metsulfuron-methyl and chlorimuron-ethyl at efficiencies that are 1.8, 2.1 and 14.2-fold of those of ALT02 respectively.

(57) Compared with the herbicide tolerant protein ALT03, the purified herbicide tolerant protein ALT03M1 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 1.5, 2.1 and 3.0-fold of those of ALT03 respectively; the purified herbicide tolerant protein ALT03M2 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 5.4, 1.3 and 3.5-fold of those of ALT03 respectively; the purified herbicide tolerant protein ALT03M3 degrades tribenuron-methyl, metsulfuron-methyl and chlorimuron-ethyl at efficiencies that are 1.6, 1.9 and 13.0-fold of those of ALT03 respectively.

(58) Compared with the herbicide tolerant protein ALT04, the purified herbicide tolerant protein ALT04M1 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 1.5, 1.9 and 2.8-fold of those of ALT04 respectively; the purified herbicide tolerant protein ALT04M2 degrades tribenuron-methyl, bensulfuron-methyl and thifensulfuron-methyl at efficiencies that are 5.1, 1.2 and 3.3-fold of those of ALT04 respectively; the purified herbicide tolerant protein ALT03M3 degrades tribenuron-methyl, metsulfuron-methyl and chlorimuron-ethyl at efficiencies that are 1.6, 1.8 and 12.4-fold of those of ALT04 respectively.

(59) It thus can be seen that, in the amino acid sequence of the herbicide tolerant protein ALT01, mutation at position 176 from glycine to alanine and/or mutation at position 178 position from serine to valine both can enhance the ability of mutant genes (such as the ALT01M1, ALT01M2 or ALT01M3 gene) to degrade sulfonylurea herbicides, especially tribenuron-methyl. In the amino acid sequence of the herbicide tolerant protein ALT02 (or ALT03), mutation at position 140 from glycine to alanine and/or mutation at position 142 from serine to valine both can enhance the ability

of mutant genes (such as the ALT02M1, ALT02M2, ALT02M3, ALT03M1, ALT03M2 or ALT03M3 gene) to degrade sulfonylurea herbicides, especially tribenuron-methyl. In the amino acid sequence of the herbicide tolerant protein ALT04, mutation at position 131 from glycine to alanine and/or mutation at position 133 from serine to valine both can enhance the ability of mutant genes (such as the ALT04M1, ALT04M2 or ALT04M3 gene) to degrade sulfonylurea herbicides, especially tribenuron-methyl.

Example 4. Construction of Recombinant Expression Vectors for Soybean

(60) 1. Construction of Recombinant Cloning Vectors Containing ALT02M1-01 Nucleotide Sequence for Soybean

(61) The ALT02M1-01 nucleotide sequence was ligated into cloning vector pGEM-T (Promega, Madison, USA, CAT: A3600) according to the operational procedure in the instructions of product pGEM-T vector of Promega Corporation, thereby obtaining a recombinant cloning vector DBN01-T, the construction process of which was as shown in FIG. **1** (wherein, Amp represents the ampicillin resistance gene; fl represents the origin of replication of phage fl; LacZ is LacZ initiation codon; SP6 is SP6 RNA polymerase promoter; T7 is T7 RNA polymerase promoter; ALT02M1-01 is the ALT02M1-01 nucleotide sequence (SEQ ID NO: 25); and MCS is a multiple cloning site).

(62) Then, *Escherichia coli* T1 competent cells (Transgen, Beijing, China, CAT: CD501) were transformed with the recombinant cloning vector DBN01-T using the heat shock method under the following heat shock conditions: maintaining 50 μL of *Escherichia coli T*1 competent cells and 10 μL of plasmid DNA (recombinant cloning vector DBN01-T) in water bath at 42° C. for 30 seconds; shake culturing at 37° C. for 1 hour (using a shaker at a rotation speed of 100 rpm for shaking); and growing on an LB plate (10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl, and 15 g/L of agar, with a pH adjusted to 7.5 with NaOH) of ampicillin (100 mg/L) having its surface coated with IPTG (isopropylthio-β-D-galactoside) and X-gal (5-bromo-4-chloro-3-indole-β-D-galactoside) overnight. White colonies were picked out and cultured in an LB liquid culture medium (10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl, and 100 mg/L of ampicillin, with a pH adjusted to 7.5 with NaOH) at a temperature of 37° C. overnight. The plasmids in the cells were extracted through an alkaline method: centrifuging the bacteria solution at a rotation speed of 12000 rpm for 1 min, removing the supernatant, and suspending the precipitated thalli with 100 μL of ice pre-cooled solution I (25 mM Tris-HCl, 10 mM EDTA (ethylenediaminetetraacetic acid), and 50 mM glucose, with a pH of 8.0); adding 200 μL of newly formulated solution II (0.2M NaOH, 1% SDS (sodium dodecyl sulfate)), inverting the tube 4 times, and mixing and placing on ice for 3-5 min; adding 150 μL of ice-cold solution III (3 M potassium acetate, 5 M acetic acid), mixing uniformly immediately and placing on ice for 5-10 min; centrifuging under the conditions of a temperature of 4° C. and a rotation speed of 12000 rpm for 5 min, adding 2-fold volumes of anhydrous ethanol to the supernatant and placing at room temperature for 5 min after mixing uniformly; centrifuging under the conditions of a temperature of 4° C. and a rotation speed of 12000 rpm for 5 min, discarding the supernatant, and air drying the precipitate after washing with ethanol at a concentration of 70% (V/V); adding 30 μL of TE (10 mM Tris-HCl, and 1 mM EDTA, with a pH of 8.0) containing RNase (20 μg/mL) to dissolve the precipitate; water bathing at a temperature of 37° C. for 30 min to digest the RNA; and storing at a temperature of −20° C. for use.

(63) After identifying the extracted plasmid by SpeI and KasI digestion, positive clones were verified by sequencing. The results showed that the inserted ALT02M1-01 nucleotide sequence in the recombinant cloning vector DBN01-T was the nucleotide sequence as shown in SEQ ID NO: 25 in the sequence listing, that is, the ALT02M1-01 nucleotide sequence was inserted correctly.

(64) 2. Construction of Recombinant Expression Vectors Containing ALT02M1-01 Nucleotide Sequence for Soybean

(65) The recombinant cloning vector DBN01-T and an expression vector DBNBC-01 (vector backbone: pCAMBIA2301 (which can be provided by the CAMBIA institution)) were both digested with restriction enzymes SpeI and KasI respectively; the excised ALT02M1-01 nucleotide

sequence fragment was inserted between the SpeI and KasI sites in the expression vector DBNBC-01; and it is well known to a person skilled in the art to construct a vector using conventional enzyme digestion methods, wherein a recombinant expression vector DBN100825 was constructed, the construction process of which was as shown in FIG. **2** (Spec: the spectinomycin gene; RB: the right boundary; prAtUbi10: the *Arabidopsis thaliana* Ubiquitin 10 gene promoter (SEQ ID NO: 69); ALT02M1-01: the ALT02M1-01 nucleotide sequence (SEQ ID NO: 25); tNos: the terminator of a nopaline synthase gene (SEQ ID NO:70); prBrCBP: the rape eukaryotic elongation factor gene 1α (Tsfl) promoter (SEQ ID NO: 71); spAtCTP2: the *Arabidopsis thaliana* chloroplast transit peptide (SEQ ID NO: 72); EPSPS: the 5-enolpyruvylshikimate-3-phosphate synthase gene (SEQ ID NO: 73); tPsE9: the pea RbcS gene terminator (SEQ ID NO: 74); LB: the left boundary).

(66) *Escherichia coli* T1 competent cells were transformed with the recombinant expression vector DBN100825 by a heat shock method under the following heat shock conditions: maintaining 50 μL of *Escherichia coli* T1 competent cells and 10 μL of plasmid DNA (recombinant expression vector DBN100825) in water bath at 42° C. for 30 seconds; shake culturing at 37° C. for 1 hour (using a shaker at a rotation speed of 100 rpm for shaking); then culturing under the condition of a temperature of 37° C. on an LB solid plate containing 50 mg/L of spectinomycin (10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl, and 15 g/L of agar, with a pH adjusted to 7.5 with NaOH) for 12 hours, picking white colonies, and culturing under the condition of a temperature of 37° C. overnight in an LB liquid culture medium (10 g/L of tryptone, 5 g/L of yeast extract, 10 g/L of NaCl, and 50 mg/L of spectinomycin, with a pH adjusted to 7.5 with NaOH). The plasmids in the cells were extracted through the alkaline method. The extracted plasmid was identified after digesting with restriction enzymes SpeI and KasI, and positive clones were identified by sequencing. The results showed that the nucleotide sequence between the SpeI and KasI sites in the recombinant expression vector DBN100825 was the nucleotide sequence as shown in SEQ ID NO: 25 in the sequence listing, i.e., the ALT02M1-01 nucleotide sequence.

(67) The recombinant expression vector DBN100826 containing ALT02M2-01 nucleotide sequence, the recombinant expression vector DBN100827 containing ALT02M3-01 nucleotide sequence, and the recombinant expression vector DBN100828 containing ALT02-01 nucleotide sequence were constructed according to the method for constructing the recombinant expression vector DBN100825 containing ALT02M1-01 nucleotide sequence as described above. Positive clones were verified by sequencing, with the results showing that ALT02M2-01 nucleotide sequence, ALT02M3-01 nucleotide sequence and ALT02-01 nucleotide sequence inserted into the recombinant expression vectors DBN100825, DBN100826, DBN100827 and DBN100828 were the nucleotide sequences as shown in SEQ ID NO: 29, SEQ ID NO: 33 and SEQ ID NO: 21 in the sequence listing respectively, namely ALT02M2-01 nucleotide sequence, ALT02M3-01 nucleotide sequence and ALT02-01 nucleotide sequence were inserted correctly.

(68) According to the method for constructing the recombinant expression vector DBN100825 containing ALT02M1-01 nucleotide sequence as described above, a control recombinant expression vector DBN100828N was constructed, the structure of which is as shown in FIG. **3** (vector backbone: pCAMBIA2301 (which can be provided by the CAMBIA institution); Spec: the spectinomycin gene; RB: the right boundary; prBrCBP: the rape eukaryotic elongation factor gene 1α (Tsfl) promoter (SEQ ID NO: 71); spAtCTP2: the *Arabidopsis thaliana* chloroplast transit peptide (SEQ ID NO: 72); EPSPS: the 5-enolpyruvylshikimate 3-phosphate synthase gene (SEQ ID NO: 73); tPsE9: the pea RbcS gene terminator (SEQ ID NO: 74); LB: the left boundary). Positive clones were verified by sequencing, with the results showing that the control recombinant expression vector DBN100828N was correctly constructed.

(69) 3. Transformation of *Agrobacterium* with the Recombinant Expression Vectors

(70) *Agrobacterium* LBA4404 (Invitrogen, Chicago, USA, CAT: 18313-015) was transformed with the recombinant expression vectors DBN100825, DBN100826, DBN100827, DBN100828, and DBN100828N which had been constructed correctly using a liquid nitrogen method, under the

following transformation conditions: placing 100 μL of *Agrobacterium* LBA4404 and 3 μL of plasmid DNA (recombinant expression vector) in liquid nitrogen for 10 minutes, and warm water bathing at 37° C. for 10 minutes; inoculating the transformed *Agrobacterium* LBA4404 into an LB tube, culturing under the conditions of a temperature of 28° C. and a rotation speed of 200 rpm for 2 hours, spreading on an LB plate containing 50 mg/L of rifampicin and 50 mg/L of spectinomycin until positive single clones were grown, picking out single clones for culturing and extracting the plasmids thereof, and performing enzyme digestion verification using restriction enzymes. The results showed that the structures of the recombinant expression vectors DBN100825, DBN100826, DBN100827, DBN100828, and DBN100828N were completely correct.

Example 5. Acquisition and Verification of Transgenic Soybean Plants

(71) 1. Acquisition of Transgenic Soybean Plants

(72) According to the *Agrobacterium* infection method conventionally used, the cotyledonary node tissue of sterilely cultured soybean variety Zhonghuang13 was co-cultured with the *Agrobacterium* in part 3 of Example 4, so as to introduce the T-DNA (including the *Arabidopsis thaliana* Ubiquitin10 gene promoter sequence, the ALT02M1-01 nucleotide sequence, the ALT02M2-01 nucleotide sequence, the ALT02M3-01 nucleotide sequence, the ALT02-01 nucleotide sequence, the tNos terminator, the rape eukaryotic elongation factor gene 1α promoter, the *Arabidopsis thaliana* chloroplast transit peptide, a 5-enolpyruvylshikimate-3 phosphate synthase gene, and the pea RbcS gene terminator) in the recombinant expression vectors DBN100825, DBN100826, DBN100827, DBN100828, and DBN100828N constructed in Part 2 of Example 4 into the soybean chromosome sets, thereby obtaining soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, soybean plants into which the ALT02M2-01 nucleotide sequence was introduced, soybean plants into which the ALT02M3-01 nucleotide sequence was introduced, and soybean plants into which the ALT02-01 nucleotide sequence was introduced; meanwhile, control soybean plants into which T-DNA in a control recombinant expression vector DBN100828N was introduced and wild-type soybean plants were used as the control.

(73) As regards the *Agrobacterium*-mediated soybean transformation, briefly, mature soybean seeds were germinated in a soybean germination culture medium (3.1 g/L of B5 salt, B5 vitamin, 20 g/L of sucrose, and 8 g/L of agar, with a pH of 5.6), and the seeds were inoculated on a germination culture medium and cultured under the conditions of a temperature of 25±1° C.; and a photoperiod (light/dark) of 16 h/8 h. After 4-6 days of germination, soybean sterile seedlings swelling at bright green cotyledonary nodes were taken, hypocotyledonary axes were cut off 3-4 mm below the cotyledonary nodes, the cotyledons were cut longitudinally, and apical buds, lateral buds and seminal roots were removed. A wound was made at a cotyledonary node using the knife back of a scalpel, and the wounded cotyledonary node tissues were contacted with an *Agrobacterium* suspension, wherein the *Agrobacterium* can transfer the ALT02M1-01 nucleotide sequence (ALT02M2-01 nucleotide sequence, ALT02M3-01 nucleotide sequence or ALT02-01 nucleotide sequence) to the wounded cotyledonary node tissues (step 1: the infection step). In this step, the cotyledonary node tissues were preferably immersed in the *Agrobacterium* suspension (OD.sub.660=0.5-0.8, an infection culture medium (2.15 g/L of MS salt, B5 vitamin, 20 g/L of sucrose, 10 g/L of glucose, 40 mg/L of acetosyringone (AS), 4 g/L of 2-morpholine ethanesulfonic acid (MES), and 2 mg/L of zeatin (ZT), with a pH of 5.3)) to initiate the inoculation. The cotyledonary node tissues were co-cultured with *Agrobacterium* for a period of time (3 days) (step 2: the co-culturing step). Preferably, the cotyledonary node tissues were cultured in a solid culture medium (4.3 g/L of MS salt, B5 vitamin, 20 g/L of sucrose, 10 g/L of glucose, 4 g/L of MES, 2 mg/L of ZT, and 8 g/L of agar, with a pH of 5.6) after the infection step. After this co-culturing stage, there can be an optional “recovery” step. In the “recovery” step, there may be at least one antibiotic (cephalosporin) known to inhibit the growth of *Agrobacterium* in a recovery culture medium (3.1 g/L of B5 salt, B5 vitamin, 1 g/L of MES, 30 g/L of sucrose, 2 mg/L of ZT, 8 g/L of agar, 150 mg/L of cephalosporin, 100 mg/L of glutamic acid, and 100 mg/L of aspartic acid, with a

pH of 5.6), without the addition of a selective agent for a plant transformant (step 3: the recovery step). Preferably, tissue blocks regenerated from the cotyledonary nodes were cultured in a solid culture medium with an antibiotic, but without a selective agent, to eliminate *Agrobacterium* and provide a recovery stage for the infected cells. Subsequently, the tissue blocks regenerated from the cotyledonary nodes were cultured in a culture medium containing a selective agent (glyphosate), and growing transformed calli were selected (step 4: the selection step). Preferably, the tissue blocks regenerated from the cotyledonary nodes were cultured in a screening solid culture medium (3.1 g/L of B5 salt, B5 vitamin, 1 g/L of MES, 30 g/L of sucrose, 1 mg/L of 6-benzyladenine (6-BAP), 8 g/L of agar, 150 mg/L of cephalosporin, 100 mg/L of glutamic acid, 100 mg/L of aspartic acid, and 0.25 mol/L of N-(phosphonomethyl)glycine, with a pH of 5.6) containing a selective agent, thus resulting in selective growth of the transformed cells. Then, plants were regenerated from the transformed cells (step 5: the regeneration step). Preferably, the tissue blocks regenerated from the cotyledonary nodes grown in a culture medium containing a selective agent were cultured in solid culture media (a B5 differentiation culture medium and B5 rooting culture medium) to regenerate plants.

(74) The resistant tissue blocks obtained from screening were transferred onto the B5 differentiation culture medium (3.1 g/L of B5 salt, B5 vitamin, 1 g/L of MES, 30 g/L of sucrose, 1 mg/L of ZT, 8 g/L of agar, 150 mg/L of cephalosporin, 50 mg/L of glutamic acid, 50 mg/L of aspartic acid, 1 mg/L of gibberellin, 1 mg/L of auxin, and 0.25 mol/L of N-(phosphonomethyl)glycine, with a pH of 5.6), and cultured at 25° C. for differentiation. The differentiated seedlings were transferred onto the B5 rooting culture medium (3.1 g/L of B5 salt, B5 vitamin, 1 g/L of MES, 30 g/L of sucrose, 8 g/L of agar, 150 mg/L of cephalosporin, and 1 mg/L of indole-3-butyric acid (IBA)), cultured in the rooting culture medium until reaching a height of about 10 cm at 25° C., and transferred to a greenhouse for culturing until fruiting. In the greenhouse, the plants were cultured at 26° C. for 16 hours, and then cultured at 20° C. for 8 hours every day.

(75) 2. Verification of the Transgenic Soybean Plants Using TaqMan

(76) About 100 mg of leaves from the soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M2-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M3-01 nucleotide sequence was introduced, the soybean plants into which the ALT02-01 nucleotide sequence was introduced and control soybean plants respectively were taken as samples, and the genomic DNA thereof was extracted with a DNeasy Plant Maxi Kit of Qiagen, and copy numbers of an EPSPS gene were detected by the Taqman probe fluorescence quantitative PCR method so as to determine the copy numbers of the genes of interest. At the same time, wild-type soybean plants were used as controls, and detected and analyzed according to the above-mentioned method. Triple repeats were set for the experiments, and were averaged.

(77) The specific method for detecting the copy number of the EPSPS gene was as follows: Step 21. 100 mg of leaves from the soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, soybean plants into which the ALT02M2-01 nucleotide sequence was introduced, soybean plants into which the ALT02M3-01 nucleotide sequence was introduced and soybean plants into which the ALT02-01 nucleotide sequence was introduced, control soybean plants and wild-type soybean plants respectively were taken, and ground into a homogenate using liquid nitrogen in a mortar respectively, and triple repeats were taken for each sample; Step 22. The genomic DNA of the above-mentioned samples was extracted using a DNeasy Plant Mini Kit of Qiagen (for the particular method, refer to the product instructions thereof); Step 23. The concentrations of the genomic DNA of the above-mentioned samples were detected using NanoDrop 2000 (Thermo Scientific); Step 24. The concentrations of the genomic DNA of the above-mentioned samples were adjusted to a consistent concentration value which ranges from 80 to 100 ng/μL; Step 25. The copy numbers of the samples were identified using the Taqman probe

fluorescence quantitative PCR method, wherein samples for which the copy numbers had been identified and known were taken as standards, the samples of the wild-type soybean plants were taken as the control, and triple repeats were taken for each sample, and were averaged; the sequences of fluorescence quantitative PCR primers and a probe were as follows:

(78) The following primers and probe were used to detect the EPSPS gene sequence:

(79) TABLE-US-00007 primer 5: CTGGAAGGCGAGGACGTCATCAATA as shown in SEQ ID NO: 75 in the sequence listing; primer 6: TGGCGGCATTGCCGAAATCGAG as shown in SEQ ID NO: 76 in the sequence listing; probe 1: ATGCAGGCGATGGGCGCCCGCATCCGTA as shown in SEQ ID NO: 77 in the sequence listing;

PCR Reaction System:

(80) TABLE-US-00008 JumpStart ™ Taq ReadyMix ™ (Sigma) 10 μL 50 × primer/probe mixture 1 μL genomic DNA 3 μL water (ddH.sub.2O) 6 μL

(81) The 50×primer/probe mixture comprises 45 μL of each primer at a concentration of 1 mM, 50 μL of the probe at a concentration of 100 μM, and 860 μL of 1×TE buffer, and was stored at 4° C. in an amber tube.

(82) PCR Reaction Conditions:

(83) TABLE-US-00009 Step temperature time 31 95° C. 5 min 32 95° C. 30 s 33 60° C. 1 min 34 back to step 32, repeated 40 times

(84) Data was analyzed using software SDS2.3 (Applied Biosystems).

(85) It was further demonstrated, by analyzing the experimental results of the copy number of the EPSPS gene, that the ALT02M1-01 nucleotide sequence, the ALT02M2-01 nucleotide sequence, the ALT02M3-01 nucleotide sequence and the ALT02-01 nucleotide sequence had all been integrated into the chromosome set of the detected soybean plants, and all of the soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, soybean plants into which the ALT02M2-01 nucleotide sequence was introduced, soybean plants into which the ALT02M3-01 nucleotide sequence was introduced and soybean plants into which the ALT02-01 nucleotide sequence was introduced and control soybean plants resulted in single-copy transgenic soybean plants.

Example 6. Detection of Herbicide Tolerance Effects of the Transgenic Soybean Plants

(86) The effect of herbicide tolerance to tribenuron-methyl was detected on the soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M2-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M3-01 nucleotide sequence was introduced, the soybean plants into which the ALT02-01 nucleotide sequence was introduced, control soybean plants and wild-type soybean plants (at seedling stage V3-V4), respectively.

(87) The soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M2-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M3-01 nucleotide sequence was introduced, the soybean plants into which the ALT02-01 nucleotide sequence was introduced, control soybean plants and wild-type soybean plants were taken and sprayed with tribenuron-methyl (144 g ai/ha, eight-fold field concentration) or a blank solvent (water), respectively. The degree of damage caused by the herbicide was measured for each plant according to the leaf curl degree and the growth point damage degree 3 days after spraying (3 DAT), 7 days after spraying (7 DAT), 14 days after spraying (14 DAT) and 21 days after spraying (21 DAT): the case where the leaves are flat as untreated plants and the growth points are intact is defined as having a damage degree of 0%; the case where veins are locally browned, new leaves are malformed and plant growth is slow is defined as having a damage degree of 50%; and the case where veins are purple, until the whole plant is dead and the growth points are browned and dry is defined as having a damage degree of 100%. The soybean plants into which the ALT02M1-01 nucleotide sequence was introduced were

of three strains in total (S1, S2 and S3), the soybean plants into which the ALT02M2-01 nucleotide sequence was introduced were of three strains in total (S4, S5 and S6), the soybean plants into which the ALT02M3-01 nucleotide sequence was introduced were of three strains in total (S7, S8 and S9), the soybean plants into which the ALT02-01 nucleotide sequence was introduced were of three strains in total (S10, S11 and S12), the control soybean plants were of two strains in total (S13 and S14), and the wild-type soybean plants were of one strain in total (CK1); and 10-15 plants were selected from each strain and tested. The results were as shown in Table 2 and FIG. **4**.

(88) TABLE-US-00010 TABLE 2 Experimental results of the herbicide tolerance of transgenic soybean T.sub.1 plants Average Average Average Average Soybean damage % damage % damage % damage % Treatment genotypes 3DAT 7DAT 14DAT 21DAT Blank S1 0 0 0 0 solvent S2 0 0 0 0 (water) S3 0 0 0 0 S4 0 0 0 0 S5 0 0 0 0 S6 0 0 0 0 S7 0 0 0 0 S8 0 0 0 0 S9 0 0 0 0 S10 0 0 0 0 S11 0 0 0 0 S12 0 0 0 0 S13 0 0 0 0 S14 0 0 0 0 CK1 0 0 0 0 144 g ai/ha S1 15 8 0 0 tribenuron- S2 16 9 0 0 methyl S3 10 3 0 0 (8x Tri.) S4 0 0 0 0 S5 0 0 0 0 S6 0 0 0 0 S7 12 3 0 0 S8 11 2 0 0 S9 10 1 0 0 S10 25 15 5 0 S11 24 14 3 0 S12 30 17 4 0 S13 63 91 100 100 S14 58 95 100 100 CK1 76 87 100 100

(89) For soybeans, eight-fold field concentration of tribenuron-methyl is an effective dose for high pressure treatment. The results in Table 2 and FIG. **4** showed that the herbicide tolerant proteins ALT02M1-01, ALT02M2-01, ALT02M3-01 and ALT02-01 all can impart transgenic soybean plants with the tolerance to benzenesulfonic acid; compared with the soybean plants into which the ALT02-01 nucleotide sequence was introduced, all of the soybean plants into which the ALT02M1-01 nucleotide sequence was introduced, the soybean plants into which the ALT02M2-01 nucleotide sequence was introduced and the soybean plants into which the ALT02M3-01 nucleotide sequence was introduced had a significantly increased tolerance to benzenesulfonic acid; while the control soybean plants and the wild-type soybean plants had no tolerance to benzenesulfonic acid.

Example 7. Construction of Recombinant Expression Vectors for Maize

(90) 1. Construction of Recombinant Cloning Vectors Containing ALT02M1-02 Nucleotide Sequence for Maize

(91) The ALT02M1-02 nucleotide sequence was ligated into cloning vector pGEM-T (Promega, Madison, USA, CAT: A3600) according to the operational procedure in the instructions of product pGEM-T vector of Promega Corporation, thereby obtaining a recombinant cloning vector DBN02-T, the construction process of which is as shown in FIG. **5** (wherein, Amp represents the ampicillin resistance gene; fl represents the origin of replication of phage fl; LacZ is LacZ initiation codon; SP6 is SP6 RNA polymerase promoter; T7 is T7 RNA polymerase promoter; ALT02M1-02 is the ALT02M1-02 nucleotide sequence (SEQ ID NO: 26); and MCS is a multiple cloning site).

(92) According to the method in Part 1 of Example 4, *Escherichia coli* T.sub.1 competent cells were transformed with the recombinant cloning vector DBN01-T using the heat shock method, and the plasmids in the cells were extracted through the alkaline method. The extracted plasmid was identified after digesting with restriction enzymes SpeI and KasI, and positive clones were identified by sequencing. The results showed that the nucleotide sequence between the SpeI and KasI sites in the recombinant cloning vector DBN02-T was the nucleotide sequence as shown in SEQ ID NO: 26 in the sequence listing, i.e., the ALT02M1-02 nucleotide sequence.

(93) 2. Construction of Recombinant Expression Vectors Containing ALT02M1-02 Nucleotide Sequence for Maize

(94) The recombinant cloning vector DBN02-T and an expression vector DBNBC-02 (vector backbone: pCAMBIA2301 (which can be provided by the CAMBIA institution)) were both digested with restriction enzymes SpeI and KasI; the excised ALT02M1-02 nucleotide sequence fragment was inserted between the SpeI and KasI sites in the expression vector DBNBC-02; and it is well known to a person skilled in the art to construct a vector using conventional enzyme digestion methods, wherein a recombinant expression vector DBN100833 was constructed, the construction process of which was as shown in FIG. **6** (Spec: the spectinomycin gene; RB: the right

boundary; prUbi: the maize Ubiquitin 1 gene promoter (SEQ ID NO: 78); ALT02M1-02: the ALT02M1-02 nucleotide sequence (SEQ ID NO:26); tNos: the terminator of a nopaline synthase gene (SEQ ID NO:70); PMI: the phosphomannose isomerase gene (SEQ ID NO: 79); LB: the left boundary).

(95) According to the method in Part 2 of Example 4, *Escherichia coli* T1 competent cells were transformed with the recombinant expression vector DBN100833 using the heat shock method, and the plasmids in the cells were extracted through the alkaline method. The extracted plasmid was identified after digesting with restriction enzymes SpeI and KasI, and positive clones were identified by sequencing. The results showed that the nucleotide sequence between the SpeI and KasI sites in the recombinant expression vector DBN100833 was the nucleotide sequence as shown in SEQ ID NO: 26 in the sequence listing, i.e., the ALT02M1-02 nucleotide sequence.

(96) The recombinant expression vector DBN100832 containing ALT02M2-02 nucleotide sequence, the recombinant expression vector DBN100831 containing ALT02M3-02 nucleotide sequence, and the recombinant expression vector DBN100830 containing ALT02-02 nucleotide sequence were constructed according to the method for constructing the recombinant expression vector DBN100833 containing ALT02M1-02 nucleotide sequence as described above. Positive clones were verified by sequencing, with the results showing that ALT02M2-02 nucleotide sequence, ALT02M3-02 nucleotide sequence and ALT02-02 nucleotide sequence inserted into the DBN100832, DBN100831 and DBN100830 were the nucleotide sequences as shown in SEQ ID NO: 30, SEQ ID NO: 34 and SEQ ID NO: 22 in the sequence listing respectively, namely ALT02M2-02 nucleotide sequence, ALT02M3-02 nucleotide sequence and ALT02-02 nucleotide sequence were inserted correctly.

(97) According to the method for constructing the recombinant expression vector DBN100833 containing ALT02M1-02 nucleotide sequence as described above, a control recombinant expression vector DBN100830N was constructed, the structure of which is as shown in FIG. **7** (vector backbone: pCAMBIA2301 (which can be provided by the CAMBIA institution); Spec: the spectinomycin gene; RB: the right boundary; prUbi: the maize Ubiquitin 1 gene promoter (SEQ ID NO: 78); PMI: the phosphomannose isomerase gene (SEQ ID NO: 79); tNos: the terminator of a nopaline synthase gene (SEQ ID NO:70); LB: the left boundary). Positive clones were verified by sequencing, with the results showing that the control recombinant expression vector DBN100830N was correctly constructed.

(98) 3. Transformation of *Agrobacterium* with the Recombinant Expression Vectors for Maize

(99) *Agrobacterium* LBA4404 (Invitrogen, Chicago, USA, CAT: 18313-015) was transformed with the recombinant expression vectors DBN100833, DBN100832, DBN100831, DBN100830, and DBN100830N which had been constructed correctly using a liquid nitrogen method, under the following transformation conditions: placing 100 μL of *Agrobacterium* LBA4404, and 3 μL of plasmid DNA (recombinant expression vector) in liquid nitrogen for 10 minutes, and warm water bathing at 37° C. for 10 minutes; inoculating the transformed *Agrobacterium* LBA4404 into an LB tube, culturing under the conditions of a temperature of 28° C. and a rotation speed of 200 rpm for 2 hours, spreading on an LB plate containing 50 mg/L of rifampicin and 50 mg/L of spectinomycin until positive single clones were grown, picking out single clones for culturing and extracting the plasmids thereof, and performing enzyme digestion verification using restriction enzymes. The results showed that the structures of the recombinant expression vectors DBN100833, DBN100832, DBN100831, DBN100830, and DBN100830N were completely correct.

Example 8. Acquisition and Verification of Transgenic Maize Plants

(100) 1. Acquisition of Transgenic Maize Plants

(101) According to the conventionally used *Agrobacterium* infection method, young embryos of sterilely cultured maize variety Zong31 (Z31) were co-cultured with the *Agrobacterium* in Part 3 of Example 7, so as to introduce T-DNA (including the maize Ubiquitin1 gene promoter sequence, ALT02M1-02 nucleotide sequence, ALT02M2-02 nucleotide sequence, ALT02M3-02 nucleotide

sequence and ALT02-02 nucleotide sequence, the PMI gene and the tNos terminator sequence) in the recombinant expression vectors DBN100833, DBN100832, DBN100831, DBN100830, and DBN100830N constructed in Part 2 of Example 7 into the maize chromosome set, thereby obtaining maize plants into which ALT02M1-02 nucleotide sequence was introduced, maize plants into which ALT02M2-02 nucleotide sequence was introduced, maize plants into which ALT02M3-02 nucleotide sequence was introduced and maize plants into which ALT02-02 nucleotide sequence was introduced; meanwhile, the control maize plants into which T-DNA in the control recombinant expression vector DBN100830N was introduced and wild type maize plants were used as the control.

(102) As regards the *Agrobacterium*-mediated maize transformation, briefly, immature young embryos were separated from maize, and contacted with an *Agrobacterium* suspension, wherein the *Agrobacterium* can transfer the ALT02M1-02 nucleotide sequence (ALT02M2-02 nucleotide sequence, ALT02M3-02 nucleotide sequence or ALT02-02 nucleotide sequence) to at least one cell of one of the young embryos (step 1: the infection step). In this step, the young embryos were preferably immersed in an *Agrobacterium* suspension (OD.sub.660=0.4-0.6, an infection culture medium (4.3 g/L of MS salt, MS vitamin, 300 mg/L of casein, 68.5 g/L of sucrose, 36 g/L of glucose, 40 mg/L of acetosyringone (AS), and 1 mg/L of 2,4-dichlorphenoxyacetic acid (2,4-D), with a pH of 5.3)) to initiate the inoculation. The young embryos were co-cultured with *Agrobacterium* for a period of time (3 days) (step 2: the co-culturing step). Preferably, the young embryos were cultured in a solid culture medium (4.3 g/L of MS salt, MS vitamin, 300 mg/L of casein, 20 g/L of sucrose, 10 g/L of glucose, 100 mg/L of acetosyringone (AS), 1 mg/L of 2,4-dichlorphenoxyacetic acid (2,4-D), and 8 g/L of agar, with a pH of 5.8) after the infection step. After this co-culturing stage, there can be an optional “recovery” step. In the “recovery” step, there may be at least one antibiotic (cephalosporin) known to inhibit the growth of *Agrobacterium* in a recovery culture medium (4.3 g/L of MS salt, MS vitamin, 300 mg/L of casein, 30 g/L of sucrose, 1 mg/L of 2,4-D, and 3 g/L of phytagel, with a pH of 5.8), without the addition of a selective agent for a plant transformant (step 3: the recovery step). Preferably, the young embryos were cultured in a solid culture medium with an antibiotic, but without a selective agent, in order to eliminate *Agrobacterium* and provide a recovery stage for the infected cells. Subsequently, the inoculated young embryos were cultured in a culture medium containing a selective agent (mannose), and growing transformed calli were selected (step 4: the selection step). Preferably, the young embryos were cultured in a screening solid culture medium (4.3 g/L of MS salt, MS vitamin, 300 mg/L of casein, 30 g/L of sucrose, 12.5 g/L of mannose, 1 mg/L of 2,4-D, and 3 g/L of phytagel, with a pH of 5.8) with a selective agent, resulting in the selective growth of transformed cells. Then, plants were regenerated from the calli (step 5: the regeneration step). Preferably, the calli grown in a culture medium containing a selective agent were cultured in solid culture media (an MS differentiation culture medium and MS rooting culture medium) to regenerate plants.

(103) Resistant calli obtained from screening were transferred onto the MS differentiation culture medium (4.3 g/L of MS salt, MS vitamin, 300 mg/L of casein, 30 g/L of sucrose, 2 mg/L of 6-benzyladenine, 5 g/L of mannose, and 3 g/L of phytagel, with a pH of 5.8), and cultured at 25° C. for differentiation. The differentiated seedlings were transferred onto the MS rooting culture medium (2.15 g/L of MS salt, MS vitamin, 300 mg/L of casein, 30 g/L of sucrose, 1 mg/L of indole-3-acetic acid, and 3 g/L of phytagel, with a pH of 5.8), cultured at 25° C. to a height of about 10 cm, and transferred to a greenhouse for culturing until fruiting. In the greenhouse, the plants were cultured at 28° C. for 16 hours, and then cultured at 20° C. for 8 hours every day.

(104) 2. Verification of the Transgenic Maize Plants Using TaqMan

(105) The maize plant into which the ALT02M1-02 nucleotide sequence was introduced, the maize plant into which the ALT02M2-02 was introduced, the maize plant into which the ALT02M3-02 was introduced, the maize plant into which the ALT02-02 was introduced and the control maize plant were detected and analyzed according to the method for verifying transgenic soybean plants

with TaqMan as described in part 2 of Example 5. The copy number of the PMI gene was detected by the Taqman probe fluorescence quantitative PCR method so as to determine the copy number of the target gene. Meanwhile, wild-type maize plants were used as the control, and detected and analyzed according to the above-mentioned method. Triple repeats were set for the experiments, and were averaged.

(106) The following primers and probe were used to detect the PMI gene sequence:

(107) TABLE-US-00011 primer 7: GCTGTAAGAGCTTACTGAAAAAATTAACA as shown in SEQ ID NO: 80 in the sequence listing; primer 8: CGATCTGCAGGTCGACGG as shown in SEQ ID NO: 81 in the sequence listing; probe 2: TCTCTTGCTAAGCTGGGAGCTCGATCC as shown as SEQ ID NO: 82
in the sequence listing.

(108) It was further demonstrated, by analyzing the experimental results of the copy number of PMI gene, that the ALT02M1-02 nucleotide sequence, the ALT02M2-02 nucleotide sequence, the ALT02M3-02 nucleotide sequence and the ALT02-02 nucleotide sequence had all been integrated into the chromosome set of the detected maize plants, and all of the maize plants into which the ALT02M1-02 nucleotide sequence was introduced, the maize plants into which the ALT02M2-02 nucleotide sequence was introduced, the maize plants into which the ALT02M3-02 nucleotide sequence was introduced, the maize plants into which the ALT02-02 nucleotide sequence was introduced and control maize plants resulted in single-copy transgenic maize plants.

Example 9. Detection of Herbicide Tolerance Effects of the Transgenic Maize Plants

(109) The effect of herbicide tolerance to tribenuron-methyl was detected on the maize plants into which the ALT02M1-02 nucleotide sequence was introduced, maize plants into which the ALT02M2-02 nucleotide sequence was introduced, maize plants into which the ALT02M3-02 nucleotide sequence was introduced, maize plants into which the ALT02-02 nucleotide sequence was introduced, control maize plants and wild-type maize plants (at V3-V4 stages) respectively.

(110) The maize plants into which the ALT02M1-02 nucleotide sequence was introduced, the maize plants into which the ALT02M2-02 nucleotide sequence was introduced, the maize plants into which the ALT02M3-02 nucleotide sequence was introduced, the maize plants into which the ALT02-02 nucleotide sequence was introduced, control maize plants and wild-type maize plants were taken and sprayed with tribenuron-methyl (144 g ai/ha, eight-fold field concentration) or a blank solvent (water), respectively. The degree of damage caused by the herbicide was measured for each plant according to the plant growth status 3 days after spraying (3 DAT), 7 days after spraying (7 DAT), 14 days after spraying (14 DAT) and 21 days after spraying (21 DAT): a growth status equivalent to that of the untreated plants is defined as having a damage degree of 0%; the case where leaves are locally chlorotic and yellow but the normal plant growth is substantially not affected is defined as having a damage degree of 50%; and the case where the whole plant is purple and dying is defined as having a damage degree of 100%. The maize plants into which the ALT02M1-02 nucleotide sequence was introduced were of three strains in total (S15, S16 and S17), the maize plants into which the ALT02M2-02 nucleotide sequence was introduced were of three strains in total (S18, S19 and S20), the maize plants into which the ALT02M3-02 nucleotide sequence was introduced were of three strains in total (S21, S22 and S23), the maize plants into which the ALT02-02 nucleotide sequence was introduced were of three strains in total (S24, S25 and S26), the control maize plants were of two strains in total (S27 and S28), and the wild-type maize plants were of one strain in total (CK2); and 10-15 plants were selected from each strain and tested. The results were as shown in Table 3 and FIG. **8**.

(111) TABLE-US-00012 TABLE 3 Experimental results of the herbicide tolerance of transgenic maize T.sub.1 plants Average Average Average Average Maize damage % damage % damage % damage % Treatment genotypes 3DAT 7DAT 14DAT 21DAT Blank S15 0 0 0 0 solvent S16 0 0 0 0 (water) S17 0 0 0 0 S18 0 0 0 0 S19 0 0 0 0 S20 0 0 0 0 S21 0 0 0 0 S22 0 0 0 0 S23 0 0 0 0 S24 0 0

0 0 S25 0 0 0 0 S26 0 0 0 0 S27 0 0 0 0 S28 0 0 0 0 CK2 0 0 0 0 144 g ai/ha S15 5 0 0 0 tribenuron-S16 6 0 0 0 methyl S17 3 0 0 0 (8x Tri.) S18 0 0 0 0 S19 0 0 0 0 S20 0 0 0 0 S21 3 0 0 0 S22 2 0 0 0 S23 0 0 0 0 S24 14 5 0 0 S25 15 4 0 0 S26 20 7 0 0 S27 61 82 100 100 S28 53 78 100 100 CK2 46 86 100 100

(112) For the maize, eight-fold field concentration of tribenuron-methyl is an effective dose for high pressure treatment. The results in Table 3 and FIG. **8** showed that the herbicide tolerant proteins ALT02M1-02, ALT02M2-02, ALT02M3-02 and ALT02-02 all can impart transgenic maize plants with the tolerance to benzenesulfonic acid; compared with the maize plants into which the ALT02-02 nucleotide sequence was introduced, all of the maize plants into which the ALT02M1-02 nucleotide sequence was introduced, the maize plants into which the ALT02M2-02 nucleotide sequence was introduced and the maize plants into which the ALT02M3-02 nucleotide sequence was introduced had a significantly increased tolerance to benzenesulfonic acid; while the control maize plants and the wild-type maize plants had no tolerance to benzenesulfonic acid.

(113) In conclusion, the herbicide tolerant protein ALT01 of the present invention can exhibit a higher tolerance to sulfonylurea herbicides, particularly tribenuron-methyl when its amino acid sequence is mutated at position 176 from glycine to alanine and/or at position 178 from serine to valine (such as the herbicide tolerant proteins ALT01M1, ALT01M2 or ALT01M3); the herbicide tolerant protein ALT02 (or ALT03) can exhibit a higher tolerance to sulfonylurea herbicides, particularly tribenuron-methyl when its amino acid sequence is mutated at position 140 from glycine to alanine and/or at position 142 from serine to valine (such as the herbicide tolerant proteins ALT02M1, ALT02M2, ALT02M3, ALT03M1, ALT03M2 or ALT03M3); the herbicide tolerant protein ALT04 can exhibit a higher tolerance to sulfonylurea herbicides, particularly tribenuron-methyl when its amino acid sequence is mutated at position 131 from glycine to alanine and/or at position 133 from serine to valine (such as the herbicide tolerant proteins ALT04M1, ALT04M2 or ALT04M3). Moreover, the coding genes of the above-mentioned herbicide tolerant proteins are particularly suitable for expression in plants due to the use of the preferred codons of plants. The soybean and maize plants into which the above-mentioned herbicide tolerant proteins are introduced have a strong tolerance to sulfonylurea herbicides, and can tolerate tribenuron-methyl of an eight-fold field concentration particularly. Therefore, the above-mentioned herbicide tolerant proteins have a broad application prospect in plants.

(114) Finally, it should be stated that the above examples are merely used for illustrating, rather than limiting, the technical solution of the present invention; and although the present invention has been described in detail with reference to the preferred examples, a person skilled in the art should understand that modifications or equivalent substitutions may be made to the technical solution of the present invention without departing from the spirit and scope of the technical solution of the present invention.

## Claims

1. An herbicide tolerance protein, comprising: the amino acid sequence as shown in SEQ ID NO: 27.

2. The herbicide tolerance protein according to claim 1, wherein the herbicide tolerance protein consists of the amino acid sequence as shown in SEQ ID NO: 27.

3. An herbicide tolerance protein, comprising the amino acid sequence as shown in SEQ ID NO: 31.

4. The herbicide tolerance protein according to claim 3, wherein the herbicide tolerance protein consists of the amino acid sequence as shown in SEQ ID NO: 31.

5. A method of making an herbicide tolerant plant, comprising: co-expressing the herbicide tolerance protein of claim 2 in said plant together with at least one additional protein which is different from the herbicide tolerance protein of claim 2.

6. A method of making an herbicide tolerant plant, comprising: co-expressing the herbicide tolerance protein of claim 3 in said plant together with at least one additional protein which is different from the herbicide tolerance protein of claim 3.
7. A method of making an herbicide tolerant plant, comprising: co-expressing the herbicide tolerance protein of claim 4 in said plant together with at least one additional protein which is different from the herbicide tolerance protein of claim 4.
8. The method according to claim 5, wherein the additional protein is 5-enolpyruvylshikimate-3-phosphate synthase, glyphosate oxidoreductase, glyphosate-N-acetyltransferase, glyphosate decarboxylase, glufosinate acetyltransferase, α-ketoglutarate-dependent dioxygenase, dicamba monooxygenase, 4-hydroxyphenylpyruvate dioxygenase, acetolactate synthase, cytochrome-like proteins or protoporphyrinogen oxidase.
9. A method of making an herbicide tolerant plant, comprising: co-expressing the herbicide tolerance protein of claim 1 in said plant together with at least one additional protein which is different from the herbicide tolerance protein of claim 1.