

(57) Abstract: The present invention refers to a plant or plant part comprising a polynucleotide encoding an herbicide tolerant IAA2 polypeptide having a deleted or disrupted degron tail region, the expression of said polynucleotide confers to the plant or plant part tolerance to synthetic auxin herbicides, such as 2,4-D. The present invention relates to methods and plants and that have a deleted or disrupted degron tail region of the IAA2 protein obtained by gene editing, transformation, mutagenesis, breeding, and the like.

**TITLE: HERBICIDE TOLERANT PLANTS AND PRODUCTION AND
DETECTION OF SAME**

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims priority to previously filed and co-pending applications USSN 62/816,773 filed March 11, 2019 and to USSN 62/963,630, filed January 21, 2020, the contents of each of which are incorporated herein by reference in their entirety.

SEQUENCE LISTING

10 The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 10, 2020, is named P13033WO00_SEQ_LISTING_ST25.txt and is 94,201 bytes in size.

15 **FIELD OF THE INVENTION**

 The present invention relates in general to compositions and methods for conferring plants with tolerance to herbicides. Particularly, the invention refers to plants that have a deleted or disrupted degron tail region of the IAA2 protein and having an increased tolerance to herbicides, more specifically to synthetic auxin herbicides.

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BACKGROUND

 Auxin, mainly as indoleacetic acid (IAA), is an important plant hormone essential for plant growth and development. It also plays a key role in the response of plants to changes in their environment. Auxins precipitate in a cascade of reactions, the best described of which is
25 the transport inhibitor response 1 (TIR1)/auxin-binding F-box (AFB) TIR1/AFB–auxin–Aux/IAA co-receptor system that is part of the Skp1-cullin-F-box protein (SCF) E3 ubiquitin ligase complex. Auxin interacts with both TIR1/AFB and the transcriptional repressor protein Aux/IAA. This leads to ubiquitination of the Aux/IAA protein, signaling the 26s proteasome to degrade the Aux/IAA protein resulting in de-repression of auxin response factors (ARFs)
30 and transcription of auxin-regulated genes.

 A number of synthetic auxins are used as commercial herbicides, with 2,4-D first being used in 1945. These herbicides belong to several chemical families and can have varying efficacy across plant species. The herbicides bind to the TIR1 receptor and the Aux/IAA repressor as auxin does; however, with herbicides the response leads to

hyperaccumulation of ethylene, abscisic acid (ABA), and the production of reactive oxygen species (ROS). The events cause stomatal closure, uncontrolled cell differentiation, and elongation visible through swelling, epinasty, leaf withering, and ultimately senescence.

The intensive use of synthetic auxin herbicides has resulted in the evolution of herbicide resistance in the target weeds. To date, 39 weed species have populations that have evolved resistance to one or more synthetic auxin herbicides. Two different mechanisms have been identified to synthetic auxin herbicides: enhanced herbicide metabolism and reduced translocation of the herbicide from the treated leaves.

Herbicide tolerant plants are useful in systems in which a plurality of such plants are planted, and can produce a crop, and either prior to planting, or after planting, an herbicide is applied that would otherwise kill or harm the plants but for their tolerance to the herbicide. Undesirable plants are killed or damaged, and the tolerant plants survive. There is a need to produce such plants.

SUMMARY

Applicants have surprisingly found that deletion or disruption of the degron tail region of an Indole-3-Acetic Acid Inducible (IAA) protein can confer tolerance to herbicides in plants. This novel mutation can be used to generate crops resistant to herbicides, including 2,4-D. Accordingly, the present invention provides for compositions and methods for producing plants that are resistant to herbicides.

The present invention provides plants having increased tolerance to herbicides when compared to a wild-type plant. In particular, the plants of the invention have increased resistance to synthetic auxin herbicides when compared to a wild-type plant. The herbicide-resistant plants of the invention comprise a polynucleotide that encodes an herbicide tolerant IAA protein, preferably IAA2. Such an herbicide tolerant IAA protein comprises a deletion or disruption of the degron tail region. The plants of the invention also include seeds and progeny plants that comprise a polynucleotide encoding an herbicide tolerant IAA protein of the invention.

Using the methods and materials of the present invention, herbicide tolerance may be conferred to a plant by, for example, introducing a heterologous nucleic acid of the present invention or by introducing a mutation or deletion of one or more amino acids of the degron tail region of an IAA protein through the use of a gene editing technique.

In another embodiment, the invention refers to methods for controlling undesired vegetation at a plant cultivation site. The methods comprise the steps of providing, at the site,

a plant according to the present invention having a deleted or disrupted degron tail region of an IAA protein which is tolerant to an herbicide and applying to the site an effective amount of the herbicide.

In yet another embodiment, the present invention provides methods for identifying
5 plant lines tolerant to the described herbicides and having a deleted or disrupted degron tail region of an IAA protein. The methods comprise the steps of obtaining a sample from the plant and assaying the plant for the absence or disruption of expression of a nucleic acid molecule encoding the degron tail or the deletion or disruption of the degron tail. The sequence information disclosed herein can be used in resistance diagnostics, including PCR
10 assays and/or protein antibody assays. In another embodiment, kits comprising a primer or probe that detects the presence or absence or disruption of a degron tail region of an IAA protein are provided.

The present invention provides a substantially purified or isolated nucleic acid or nucleic acid fragment encoding an herbicide tolerant IAA protein, complements thereof, and
15 functionally active fragments and variants thereof. The nucleic acid encoding an herbicide tolerant IAA includes those selected from the group consisting of: (a) the sequence shown in SEQ ID NO: 4; (b) a nucleotide sequence encoding the polypeptide shown in SEQ ID NO: 3; (c) complements of the sequences recited in (a) and (b); (d) functionally active fragments of the sequences recited in (a), (b), and (c); and (e) functionally active variants of the sequences
20 recited in (a), (b), (c), and (d). The nucleic acids encode an herbicide tolerant IAA protein comprising a deletion or disruption of the degron tail region as described herein.

While multiple embodiments are disclosed, still other embodiments of the present invention will become apparent to those skilled in the art from the following detailed description, which shows and describes illustrative embodiments of the invention.

25 Accordingly, the drawings and detailed description are to be regarded as illustrative in nature and not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the specification and are included to further
30 demonstrate certain embodiments or various aspects of the invention. In some instances, embodiments of the invention can be best understood by referring to the accompanying drawings in combination with the detailed description presented herein. The description and accompanying drawings may highlight a certain specific example, or a certain aspect of the

invention. However, one skilled in the art will understand that portions of the example or aspect may be used in combination with other examples or aspects of the invention.

Figure 1 shows the amino acid sequence of the *Sisymbrium orientale* IAA2 protein with the degron conserved core motif shown in bold (SEQ ID NO: 1).

Figures 2A - E show protein sequence alignments of herbicide sensitive and resistant *Sisymbrium orientale* IAA2 (SEQ ID NOs: 1 and 3, respectively) to Brassicaceae species with high similarity to IAA2 (SEQ ID NOs: 6-38). The conserved degron is noted as indicated with a box as is the degron tail.

Figure 3 shows a protein sequence alignment of the region comprising the IAA2 degron and degron tail including *Sisymbrium orientale* and 10 other species (SEQ ID NOs: 39-50). Full length sequences of the IAA proteins from the 10 species are shown in SEQ ID NOs: 51-60.

Figure 4 is a chart representing prediction of disordered protein regions based upon local amino acid sequence information and template proteins. Red is mIAA2 and blue is the wild-type template.

Figure 5 shows the transcripts that had differential expression between R and S RILs of both Port Broughton and P2 populations based on cutoff criteria of FDR <0.05 and fold change of > |2|.

Figure 6 shows the deletion of 27 nucleotides from the IAA2 gene results in a 9 amino acid deletion in the degron tail region of IAA2 (SEQ ID NOs: 1-4).

Figure 7 shows the deletion allele co-segregates with 2,4-D resistance in an F2 population and that 2,4-D treatment increases IAA2 expression in S but not in R.

Figure 8 shows that the wild-type Arabidopsis (panel A) and Arabidopsis transformed with IAA2 wild-type allele (panel B) are both sensitive to 2,4-D. Panel C shows Arabidopsis transformed with IAA2 Δ 27 that is resistant to 2,4-D treatment.

Figure 9 shows transformation of *Arabidopsis thaliana* with IAA2 wild-type allele and IAA2 Δ 27 allele from *Sisymbrium orientale* results in different responses of root growth to auxin and herbicides. Upper left panel: only IAA2 Δ 27 allele can grow on media containing 2,4-D. Right panels: IAA2 Δ 27 allele expressed in transgenic Arabidopsis confers resistance to IAA, 2,4-D, dicamba and clopyralid relative to wild-type Arabidopsis and wild-type IAA2 allele. MCPA and florpyrauxifen-benzyl were tested in root growth assays on *S. orientale* inbred lines containing IAA2 wild-type allele (SoIAA2) or deletion mutant allele (SoIAA2 Δ 27). The SoIAA2 Δ 27 mutant allele confers resistance to MCPA and florpyrauxifen-benzyl.

Figure 10 shows the surface plasmon resonance assay for IAA2 and IAA2Δ27 alleles from *Sisymbrium orientale*. The IAA2Δ27 peptide has a slower association constant (K_a) for the TIR1/auxin/IAA complex than the IAA2 wild-type allele for auxin (IAA), dicamba, and 2,4-D. The IAA2Δ27 peptide has a faster disassociation constant (K_d) for the

5 TIR1/auxin/IAA complex than the IAA2 wild-type allele for auxin (IAA), dicamba, and 2,4-D. Data from Richard Napier and Martin Kubes, University of Warwick, UK.

Figure 11 shows the predicted mechanistic effects of IAA2Δ27 deletion on association with the TIR1/synthetic auxin/IAA protein complex (top panel). The deletion is predicted to reduce the rate of association and increase the rate of dissociation, resulting in

10 less degradation of the IAA2 protein and plant survival. The loss of 9 amino acids reduces the distance of the domain III of IAA2 from domain II by 28 angstroms (middle panel), which is predicted to impair the ability of the IAA2 protein to interact with the TIR1/synthetic auxin complex (bottom panel).

15 DETAILED DESCRIPTION

Resistance to 2,4-D in *Sisymbrium orientale* was reported in South Australia in 2005. *S. orientale* is the sixth most costly weed of crops in southern Australia and is most problematic on alkaline soils. Resistance to 2,4-D has occurred in several populations of *S. orientale* in South Australia. This resistance is inherited as a single dominant allele and

20 results in significantly less 2,4-D translocating from the treated leaves to other parts of the plant compared to susceptible plants. A deletion of nine highly conserved amino acids after the degron of the auxin co-receptor IAA2 gene has been identified as a source of reduced translocation of 2,4-D in this species.

Indole-3-Acetic Acid proteins (IAA) are transcriptional repressors, the expression of which is stimulated by the phytohormone auxin. In the presence of a threshold concentration of auxin, the system triggers degradation of the protein. There are 29 gene family member that have been isolated in Arabidopsis and 55 from Brassica. *Brassica rapa* Aux/IAA family genes are discussed at length in Paul et al. (2016) “Genome-wide analysis and

25 characterization of Aux/IAA family genes in *Brassica rapa*” *PLoS One* 6;11(4).

Degradation signals or degrons are a region of a protein that is involved in regulation of the rate of degradation of protein. It is a minimal element within a protein that is sufficient for targeting the protein for degradation. Degrons may lead to a change in more or less ubiquitination and may be categorized as ubiquitin dependent or may be ubiquitin

30 independent and the presence of the degron is not necessary for polyubiquitination of the

protein. A degron may be identified by measuring the increase or decrease in amount of a protein when the region is present or absent.

Aux/IAA genes encode 25 to 35 kD proteins localized to the nucleus. The proteins have four conserved domains, domains I, II, III and IV. Domain II plays a role in destabilizing IAA proteins. The domain has the residues necessary for the receptor, auxin and co-receptor complex modulating the rate of Aux/IAA turnover and contain the degron that is necessary and sufficient for auxin-induced degradation. See Leyser et al. (2018) "Auxin Signaling" *Plant Physiol.* 176(1):465-479 and Zenser et al. (2001) "Auxin modulates the degradation rate of Aux/IAA proteins" *Proc. Natl. Acad. Sci USA* 98(20):11795-11800.

The degron has a conserved core GWPPV motif which is a binding site with TIR/AFB proteins and auxin. The auxin-responsive IAA2 protein of *Sisymbrium orientale* is shown in SEQ ID NO: 1:

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MAYEKVNELNLKDTELRLGLPGTEQVKEEQEVSCVRSNKRQFQIDNEENREEEESTPPTKTQ
IVGWPPVRSYRKNNNSVSYVKVSMGAPYLRKIDLKTYKNYPELLKALENMFKFTVGEYCER
EGYKGSEVVPTYEDKDGDWMLVGDVPWDMFSSCKRLRIMKGSDDSSL
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The degron conserved GWPPV motif is identified in bold in Figure 1.

The IAA2 protein of *Sisymbrium orientale* is encoded by the nucleotide sequence of SEQ ID NO: 2:

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ATGGCGTACGAGAAAGTCAATGAGCTTAACCTTAAGGACACAGAGCTTCGTCTTGGATTACCTG
GAACAGAGCAAGTTAAAGAAGAACAAGAGGTTTCTTGCGTTAGAAGCAACAAGCGTCAATTCCA
AATCGATAACGAGGAAAACCGTGAGGAAGAAGAATCTACACCTCCTACGAAAACCTCAAATCGTT
GGTTGGCCTCCGGTGAGATCTTACCGTAAGAACAACAACAGTGTGAGCTATGTGAAAGTGAGTA
TGGACGGAGCACCATACCTCCGCAAAATCGATCTCAAAACATACAAAACTATCCAGAGCTTCT
CAAGGCGTTAGAAAACATGTTCAAGTTCACGGTAGGTGAGTACTGTGAAAGAGAAGGATACAAA
GGATCTGAAGTCGTACCAACGTACGAGGATAAAGATGGAGACTGGATGTTGGTTGGTGATGTTT
CATGGGATATGTTCTCATCCTCTTGTAAGAGACTCAGAATCATGAAAGGATCTGATGACTCTTC
CTTATGA
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The disordered region following the degron is known as the "degron tail". The degron may be readily identified as described above. In any particular species of plant, the IAA region II degron may be identified and in an embodiment may be identified by alignment with the IAA2 *Sisymbrium orientale* sequence identified here. The disordered region does not have a fixed or ordered three-dimensional structure. Such degron tails may be partially or completely disordered. See, e.g., Uversky, Vladimir N. (2011) Intrinsically disordered

proteins from A to Z. <http://doi.org/10.1016/j.biocel.2011.04.001>. The degron tail described here has been found by the inventors to be highly disordered.

Bioinformatics can identify such disordered regions. For example, Liu et al. describe classification-based predictors of intrinsically disordered proteins (IDPs) and improves on these processes by describing a sequence labeling model based on conditional random fields. Their method uses IDP-FSP which uses three CRF-based predictors designed to predict long, short and generic disordered regions. See Liu et al. “Identification of Intrinsically Disordered Proteins and Regions by Length-Dependent Predictors Based on Conditional Random Fields” *Molecular Therapy Nucleic Acids* Vol. 17: 396-404. Various processes utilized involve machine learning techniques and computational predictors. Their method utilizes a predictor called IDP-FSP based on CRFs. IDP-FSP is a fusion of three CRF-based predictors—IDP-FSP-L, IDP-FSP-S, and IDP-FSP-G—that are specifically designed to predict long, short, and generic disordered regions, respectively.

By way of example, here is found that a deletion of the nine amino acid region of the degron tail of the IAA2 protein confers resistance to herbicides. The amino acid sequence deleted is shown in the protein alignment of IAA2 in Figure 2. As can be seen the deleted amino acids within this degron tail comprise YRKNNNSVS (SEQ ID NO. 5). Figure 3 shows alignment of BLASTP hits for the region comprising the IAA2 degron and degron tail to 10 species; all other species have sequence in between domain II and III (each highly conserved); the sequence is divergent, but no species have less sequence than IAA2 in the way that the deletion allele has the disordered amino acids missing.

The experimentation here checked how disordered the deletion region is (known as the ‘degron tail’ for its proximity to the degron in domain II) and found that this region, found in the wild-type IAA2, is highly disordered. Losing this ‘flexible intrinsically disordered’ region reduces the flexibility of the protein to change its conformation when binding to an herbicide such as 2,4-D. The disassociation rate is faster for this mutant. Therefore, deleting this region affects the binding kinetics with herbicides such as 2,4-D. Less of the protein will be degraded after herbicide application, or not as quickly, preventing in an example the rapid increase in gene expression of 2,4-D regulated genes normally caused by herbicide application.

Figure 4 shows the disordered region in the degron tail of wild-type IAA2 (see the arrow). It was obtained using the server PrDOS predicting disordered regions by comparison with a template. (See, e.g., Ishida et al. (2007) “PrDOS: prediction of disordered protein

regions from amino acid sequence” *Nucleic Acids Res.* 35::W460-W464. The red area is the plot of mIAA2, note that the disordered degron tail is absent.

Experimentation included running BLASTP using IAA2 protein sequence against entire NCBI sequence database; 33 peptides with sequence identity $\geq 80\%$; mostly plants from within Brassicaceae family. See Ramos et al. (2001) “Rapid degradation of auxin/indoleacetic acid proteins requires conserved amino acids of domain II and is proteasome dependent” *Plant Cell* DOI: 10.2307/3871512. IAA2 and Figure 2 therein. This figure shows multiple sequence alignment of amino acids spanning conserved domain II, equivalent to amino acids 68 to 111 from IAA17, of 22 Aux/IAA proteins from Arabidopsis and two Aux/IAA proteins from pea. The shaded regions encompass amino acids outside of the conserved domain II, with the 13-amino acid consensus sequence noted at the bottom of the alignment. Invariant residues among all Aux/IAA proteins shown are underlined in the consensus sequence. Numbers below the 13-amino acid consensus sequences are reference points for mutants and variants tested.

The ‘degron tail’ is highly conserved among these sequences; a few variants have extra N (Asp) residues, but none are missing the 9 amino acids deleted in the mIAA2 sequence. The experiments also ran BLASTP using just degron and degron tail from IAA2 against 10 species in Ensembl Plants, ran alignment of these sequences; not necessarily the best IAA2 homologue from each species but the best hit based on the IAA2 degron and degron tail.

By way of example without limitation, deletion of the degron tail confers resistance to benzonitrile herbicides, cyclohexanedione herbicides, imidazolinone herbicides, phenoxy herbicides, sulfonylurea herbicides, triazine herbicides, 1-aminocyclopropane-1-carboxylic acid synthase-inhibiting herbicides, 4-hydroxyphenylpyruvate dioxygenase-inhibiting herbicides, acetolactate synthase-inhibiting herbicides, protoporphyrinogen oxidase-inhibiting herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), bromoxynil, dicamba, glufosinate, glyphosate, nicosulfuron, or quizalofop-p-ethyl. Preferably, the herbicide is a synthetic auxin herbicide. Synthetic auxin herbicides mimic the natural plant hormone and can inhibit cell division and growth. Synthetic auxin herbicides include phenoxy herbicides, benzoic acid herbicides, and carboxylic acid herbicides. In a preferred embodiment, deletion of the degron tail confers resistance to 2,4-D dichlorophenoxyacetic acid.

The inventors have determined a broad cross-resistance pattern of this mutation to other herbicides, in addition to 2,4-D as outlined below.

TABLE 1

Herbicide	Resistant	Susceptible	Testing in progress
Dicamba	X		
Clopyralid	X		
2,4-D	X		
Quinclorac		X	
Triclopyr		X	
Picloram			X
Fluroxypyr			X
Rinskor (florpyrauxifen-benzyl)	X		
MCPA	X		
Dichlorprop			X

Generally, the term “herbicide” is used herein to mean an active ingredient that kills, controls or otherwise adversely modifies the growth of plants. The preferred amount or concentration of the herbicide is an “effective amount” or “effective concentration.” By “effective amount” and “effective concentration” is intended an amount and concentration, respectively, that is sufficient to kill or inhibit the growth of a similar, wild-type, plant, plant tissue, plant cell, or host cell, but that said amount does not kill or inhibit as severely the growth of the herbicide-resistant plants, plant tissues, plant cells, and host cells of the present invention. Typically, the effective amount of an herbicide is an amount that is routinely used in agricultural production systems to kill weeds of interest. Such an amount is known to those of ordinary skill in the art. Herbicidal activity is exhibited by herbicides useful for the present invention when they are applied directly to the plant or to the locus of the plant at any stage of growth or before planting or emergence. The effect observed depends upon the plant species to be controlled, the stage of growth of the plant, the application parameters of dilution and spray drop size, the particle size of solid components, the environmental conditions at the time of use, the specific compound employed, the specific adjuvants and carriers employed, the soil type, and the like, as well as the amount of chemical applied. These and other factors can be adjusted as is known in the art to promote non-selective or selective herbicidal action. Generally, the herbicide treatments can be applied PPI (Pre Plant Incorporated), PPSA (Post plant surface applied), PRE- or POST-emergent. Postemergent treatment typically occurs to relatively immature undesirable vegetation to achieve the maximum control of weeds.

An embodiment provides for a plant having such a deleted or disrupted degron tail as described herein. The plant is resistance to one or more herbicides as described above. The plant may be any plant species, whether monocotyledonous or dicotyledonous, including but

not limited to corn (*Zea mays*), canola (*Brassica napus*, *Brassica rapa* ssp.), alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*, *Sorghum vulgare*), sunflower (*Helianthus annuus*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), oats (*Avena* spp.), barley (*Hordeum* spp.), vegetables, ornamentals, and conifers. Vegetables include tomatoes (*Solanum lycopersicum*), lettuce (e.g., *Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.) and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum. Conifers which may be employed in practicing the present invention include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). Preferably, the plant is a dicotyledonous plant.

The term plant or plant material or plant part is used broadly herein to include any plant at any stage of development, or to part of a plant, including a plant cutting, a plant cell, a plant cell culture, a plant organ, a plant seed, and a plantlet. A plant cell is the structural and physiological unit of the plant, comprising a protoplast and a cell wall. A plant cell can be in the form of an isolated single cell or aggregate of cells such as a friable callus, or a cultured cell, or can be part of a higher organized unit, for example, a plant tissue, plant organ, or plant. Thus, a plant cell can be a protoplast, a gamete producing cell, or a cell or collection of cells that can regenerate into a whole plant. As such, a seed, which comprises multiple plant cells and is capable of regenerating into a whole plant, is considered a plant

cell for purposes of this disclosure. A plant tissue or plant organ can be a seed, protoplast, callus, or any other groups of plant cells that is organized into a structural or functional unit. Particularly useful parts of a plant include harvestable parts and parts useful for propagation of progeny plants. A harvestable part of a plant can be any useful part of a plant, for
5 example, flowers, pollen, seedlings, tubers, leaves, stems, fruit, seeds, roots, and the like. A part of a plant useful for propagation includes, for example, seeds, fruits, cuttings, seedlings, tubers, rootstocks, and the like.

The plants here also include progeny which comprise said deleted or disrupted degron tail.

10 By an “herbicide-tolerant” or “herbicide-resistant” plant, it is intended that a plant that is tolerant or resistant to at least one herbicide at a level that would normally kill, or inhibit the growth of, a normal or wildtype plant. Levels of herbicide that normally inhibit growth of a non-tolerant plant are known and readily determined by those skilled in the art. Examples include the amounts recommended by manufacturers for application. The maximum rate is an
15 example of an amount of herbicide that would normally inhibit growth of a non-tolerant plant. For the present invention, the terms “herbicide-tolerant” and “herbicide-resistant” are used interchangeably and are intended to have an equivalent meaning and an equivalent scope. Similarly, the terms “herbicide-tolerance” and “herbicide-resistance” are used interchangeably and are intended to have an equivalent meaning and an equivalent scope.
20 Similarly, the terms “tolerant” and “resistant” are used interchangeably and are intended to have an equivalent meaning and an equivalent scope. As used herein, in regard to an herbicidal composition useful in various embodiments hereof, terms such as herbicides, and the like, refer to those agronomically acceptable herbicide active ingredients (A.I.) recognized in the art. Similarly, terms such as fungicide, nematocide, pesticide, and the like,
25 refer to other agronomically acceptable active ingredients recognized in the art.

Alterations to the ‘degron tail’ in one example alters the binding kinetics to 2,4-D and other synthetic auxin herbicides. Thus, introduction to a plant of the protein having the deleted degron tail, or otherwise causing any type of deletion or alteration via mutation of the region following the degron would confer herbicide resistance and in one example confers
30 resistance to synthetic auxin herbicide resistance. This can be accomplished using transgenic approaches (the mutation is dominant), gene editing, and crossing with a plant not having the deleted degron tail.

In another embodiment, the present invention provides a method for controlling undesired vegetation at a plant cultivation site. The term “control of undesired vegetation or

weeds” is to be understood as meaning the killing of weeds and/or otherwise retarding or inhibiting the normal growth of the weeds. Weeds, in the broadest sense, are understood as meaning all those plants which grow in locations where they are undesired. The weeds of the present invention include, for example, dicotyledonous and monocotyledonous weeds.

- 5 Dicotyledonous weeds include, but are not limited to, weeds of the genera: *Sinapis*, *Lepidium*, *Galium*, *Stellaria*, *Matricaria*, *Anthemis*, *Galinsoga*, *Chenopodium*, *Urtica*, *Senecio*, *Amaranthus*, *Portulaca*, *Xanthium*, *Convolvulus*, *Ipomoea*, *Polygonum*, *Sesbania*, *Ambrosia*, *Cirsium*, *Carduus*, *Sonchus*, *Solanum*, *Rorippa*, *Rotala*, *Lindernia*, *Lamium*, *Veronica*, *Abutilon*, *Emex*, *Datura*, *Viola*, *Galeopsis*, *Papaver*, *Centaurea*, *Trifolium*,
 10 *Ranunculus*, and *Taraxacum*. Monocotyledonous weeds include, but are not limited to, weeds of of the genera: *Echinochloa*, *Setaria*, *Panicum*, *Digitaria*, *Phleum*, *Poa*, *Festuca*, *Eleusine*, *Brachiaria*, *Lolium*, *Bromus*, *Avena*, *Cyperus*, *Sorghum*, *Agropyron*, *Cynodon*, *Monochoria*, *Fimbristylis*, *Sagittaria*, *Eleocharis*, *Scirpus*, *Paspalum*, *Ischaemum*, *Sphenoclea*, *Dactyloctenium*, *Agrostis*, *Alopecurus*, and *Apera*. In addition, the weeds of the present
 15 invention can include, for example, crop plants that are growing in an undesired location. For example, a volunteer maize plant that is in a field that predominantly comprises soybean plants can be considered a weed, if the maize plant is undesired in the field of soybean plants.

In yet another embodiment, the present invention provides a method for identifying plant lines tolerant to the described herbicides comprising supplying a sample from a plant,
 20 providing amplification primers for amplifying a region of a plant's genome corresponding to the nucleic acids encoding or the polypeptide of IAA2 with a deleted or disrupted degron tail present in said sample, applying said amplification primers to said nucleic acid sample such that amplification of a region that would comprise the degron tail, if present, and identifying plants tolerant to the herbicides based on the deletion or disruption of the degron tail in said
 25 sample and which confers herbicide tolerance. Such primers and probes are exemplified in the examples below. The methods here related to primers and probes with which to detect the presence or absence of the degron tail and/or its mutation. Such nucleic acids can have the sequence of at least 10, 12, 15, 20, 30, 40, 50 or more or less consecutive nucleotides of the region of IAA2 that would include the degron tail, such as SEQ ID NO: 2 from *Sisymbrium*
 30 *orientale*, by way of example. In an exemplary embodiment, the primers are selected from SEQ ID NOs: 61 and 62 or SEQ ID NOs: 63-65.

A kit may likewise be created that employs the use of such primers and/or probes. The presence of absence of the degron tail or its' mutation may be detected by sequencing, amplification and/or hybridization with a specific probe and primers as described here. A

sample containing a nucleic acid may be obtained from the plant or plant part or cell. The primers or probes may be labelled using any technique known to those skilled in the art. Examples include use of radioactive, fluorescent or enzymatic labelers. The nucleic acids may in embodiments be PCR amplified before detection. Methods are well known and in one example are described at Sambrook et al. Molecular Cloning – A Laboratory Manual Second Edition (Cold Spring Harbor Laboratory 1989).

Use of the absence or disruption of the degon tail as a marker for herbicide tolerance is available through the processes here. As used herein, the terms "marker" and "DNA marker" and "molecular marker" in reference to a "selectable marker" refers to a physiological or morphological trait that may be determined as a marker for its own selection or for selection of other traits closely linked to that marker. For example, such a marker could be a gene or trait that associates with herbicide tolerance including, but not limited to, simple sequence repeat (SSR), single nucleotide polymorphism (SNP), genetic insertions and/or deletions and the like.

The present processes provide a method of genotyping a plant comprising a polynucleotide of described here. Genotyping provides a means of distinguishing homologs of a chromosome pair and can be used to differentiate segregants in a plant population. Molecular marker methods can be used for phylogenetic studies, characterizing genetic relationships among crop varieties, identifying crosses or somatic hybrids, localizing chromosomal segments affecting monogenic traits, map based cloning, and the study of quantitative inheritance. See, e.g., Plant Molecular Biology: A Laboratory Manual, Chapter 7, Clark, Ed., Springer-Verlag, Berlin (1997). For molecular marker methods, see generally, The DNA Revolution by Andrew H. Paterson 1996 (Chapter 2) in: Genome Mapping in plants (Ed., Andrew H. Paterson) by Academic Press/R.G. Lands Company, Austin, Tex., pp. 7-21.

The particular method of genotyping may employ any number of molecular marker analytic techniques such as, but not limited to, restriction fragment length polymorphisms (RFLPs). RFLPs are the product of allelic differences between DNA restriction fragments resulting from nucleotide sequence variability. As is well known to those of skill in the art, RFLPs are typically detected by extraction of genomic DNA and digestion with a restriction enzyme. Generally, the resulting fragments are separated according to size and hybridized with a probe; single copy probes are preferred. Restriction fragments from homologous chromosomes are revealed. Differences in fragment size among alleles represent an RFLP. Thus, the present invention further provides a means to follow segregation of a gene or

nucleic acid of the present invention as well as chromosomal sequences genetically linked to these genes or nucleic acids using such techniques as RFLP analysis. Linked chromosomal sequences are within 50 centiMorgans (cM), often within 40 or 30 cM, preferably within 20 or 10 cM, more preferably within 5, 3, 2, or 1 cM of a gene of the present invention.

5 In the present methods, the nucleic acid probes employed for molecular marker mapping of plant nuclear genomes hybridize, under selective hybridization conditions, to a gene encoding a polynucleotide described here. In preferred embodiments, the probes are selected from polynucleotides of the present methods. Typically, these probes are cDNA probes or restriction enzyme treated genomic clones. The length of the probes is typically at
10 least 15 bases in length, more preferably at least 20, 25, 30, 35, 40, or 50 bases in length. Generally, however, the probes are less than about 1 kilobase in length. Preferably, the probes are single copy probes that hybridize to a unique locus in a haploid chromosome complement. Some exemplary restriction enzymes employed in RFLP mapping are EcoRI, EcoRV, and SstI. As used herein the term "restriction enzyme" includes reference to a
15 composition that recognizes and, alone or in conjunction with another composition, cleaves at a specific nucleotide sequence.

The method of detecting an RFLP comprises the steps of (a) digesting genomic DNA of a plant with a restriction enzyme; (b) hybridizing a nucleic acid probe, under selective hybridization conditions, to a sequence of a polynucleotide of the present invention of the
20 genomic DNA; (c) detecting therefrom a RFLP. Other methods of differentiating polymorphic (allelic) variants of polynucleotides of the present invention can be had by utilizing molecular marker techniques well known to those of skill in the art including such techniques as: 1) single stranded conformation analysis (SSCA); 2) denaturing gradient gel electrophoresis (DGGE); 3) RNase protection assays; 4) allele-specific oligonucleotides
25 (ASOs); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein; and 6) allele-specific PCR. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE); heteroduplex analysis (HA); and chemical mismatch cleavage (CMC). Thus, the present processes further provide a method of genotyping comprising the
30 steps of contacting, under stringent hybridization conditions, a sample suspected of comprising a polynucleotide of the present invention with a nucleic acid probe. Generally, the sample is a plant sample, preferably, a sample suspected of comprising a maize polynucleotide of the present invention (e.g., gene, mRNA). The nucleic acid probe selectively hybridizes, under stringent conditions, to a subsequence of a polynucleotide of the

present invention comprising a polymorphic marker. Selective hybridization of the nucleic acid probe to the polymorphic marker nucleic acid sequence yields a hybridization complex. Detection of the hybridization complex indicates the presence of that polymorphic marker in the sample. In preferred embodiments, the nucleic acid probe comprises a polynucleotide of the present invention.

Other methods employed include use of KASP™, that is, Kompetitive Allele Specific PCR. It is based on competitive allele-specific PCR and allows scoring of single nucleotide polymorphisms (SNPs), as well as deletions and insertions at specific loci. Two allele specific forward primers are used having the target SNP at the 3' end and a common reverse primer is used for both. The primers have a unique "tail" sequence (reporter nucleotide sequence) compatible with a different fluorescent reporter (reporter molecule). The primers are contacted with the sample along with a mix which includes a universal Fluorescence Resonant Energy Transfer (FRET) cassette and Taq polymerase. During rounds of PCR cycling, the tail sequences allow the FRET cassette to bind to the DNA and emit fluorescence. See, e.g. Yan et al. "Introduction of high throughput and cost effective SNP genotyping platforms in soybean" *Plant Genetics, Genomic and Biotechnology* 2(1): 90 – 94 (2014); Semagn et al. "Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): overview of the technology and its application in crop improvement" *Molecular Breeding* 33(1): 1 – 14 (2013). In the present process, emission of one fluorescent signal (reporter molecule) or the other indicates the plant is one of the two species, where presence of both signals indicates a hybrid. Examples here show use of 6- carboxyfluorescein (FAM); and 6 - carboxy - 2',4,4',5',7,7' – hexachlorofluorescein (HEX) fluorophores, however any convenient means of producing a measurable signal may be used. Examples without intending to be limiting include tetrachlorofluorescein (TET); cyan florescent protein, yellow fluorescent protein, luciferase, SyBR Green I; ViC; CAL Fluor Gold 540, ROX Texas Red; CAL Fluor Red 610; CY5; Quasar 670; Quasar 705; and Fret.

In sum, a first primer is produced recognizing a first target nucleotide sequence in the genome of a first species, a second primer is produced recognizing a second target nucleotide sequence of a second species and the third common reverse primer universal to all genotypes allows for amplification. A "tail" reporter sequence is provided with the primer. The expression cassette comprises sequences complementary to the reporter sequence. With rounds of PCR, the cassette is no longer quenched and a measurable signal is produced.

When referring to the nucleic acid molecules and polypeptides of IAA and the degron and degron tail is intended to include those variants of the sequences retaining the properties so defined.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm.

Optimal alignment of sequences for comparison can use any means to analyze sequence identity (homology) known in the art, e.g., by the progressive alignment method of termed "PILEUP" (Morrison, (1997) *Mol. Biol. Evol.* 14:428-441, as an example of the use of PILEUP); by the local homology algorithm of Smith & Waterman (*Adv. Appl. Math.* 2: 482 (1981)); by the homology alignment algorithm of Needleman & Wunsch (*J. Mol. Biol.* 48:443-453 (1970)); by the search for similarity method of Pearson (*Proc. Natl. Acad. Sci. USA* 85: 2444 (1988)); by computerized implementations of these algorithms (e.g., GAP, BEST FIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.); ClustalW (CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, Calif., described by, e.g., Higgins(1988), *Gene* 73: 237-244; Corpet (1988), *Nucleic Acids Res.* 16:10881-10890; Huang, *Computer Applications in the Biosciences* 8:155-165 (1992); and Pearson (1994), *Methods in Mol. Biol.* 24:307-331); Pfam (Sonnhammer (1998), *Nucleic Acids Res.* 26:322-325); TreeAlign (Hein (1994), *Methods Mol. Biol.* 25:349-364); MEG-ALIGN, and SAM sequence alignment computer programs; or, by manual visual inspection.

Another example of algorithm that is suitable for determining sequence similarity is the BLAST algorithm, which is described in Altschul et al, (1990) *J. Mol. Biol.* 215: 403-410. The BLAST programs (Basic Local Alignment Search Tool) of Altschul, S. F., et al., searches under default parameters for identity to sequences contained in the BLAST "GENEMBL" database. A sequence can be analyzed for identity to all publicly available DNA sequences contained in the GENEMBL database using the BLASTN algorithm under the default parameters.

Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information, www.ncbi.nlm.nih.gov/; see also Zhang (1997), *Genome Res.* 7:649-656 for the "PowerBLAST" variation. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the

neighborhood word score threshold (Altschul et al (1990), *J. Mol. Biol.* 215: 403-410). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a wordlength (W) of 11, the BLOSUM62 scoring matrix (see Henikoff (1992), *Proc. Natl. Acad. Sci. USA* 89:10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The term BLAST refers to the BLAST algorithm which performs a statistical analysis of the similarity between two sequences; see, e.g., Karlin (1993), *Proc. Natl. Acad. Sci. USA* 90:5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

In an embodiment, GAP (Global Alignment Program) can be used. GAP uses the algorithm of Needleman and Wunsch (*J. Mol. Biol.* 48:443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. Default gap creation penalty values and gap extension penalty values in the commonly used Version 10 of the Wisconsin Package® (Accelrys, Inc., San Diego, CA) for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. A general purpose scoring system is the BLOSUM62 matrix (Henikoff and Henikoff (1993), *Proteins* 17: 49-61), which is currently the default choice for BLAST programs. BLOSUM62 uses a combination of three matrices to cover all contingencies. Altschul, *J. Mol. Biol.* 36: 290-300 (1993), herein incorporated by reference in its entirety and is the scoring matrix used in Version 10 of the Wisconsin Package®

(Accelrys, Inc., San Diego, CA) (see Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window.

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Identity to the sequence of described would mean a polynucleotide or polypeptide sequence having at least 65% sequence identity, more preferably at least 70% sequence identity, more preferably at least 75% sequence identity, more preferably at least 80% identity, more preferably at least 85% 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity.

As used herein, the terms "nucleic acid" or "polynucleotide" refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. As such, the terms include RNA and DNA, which can be a gene or a portion thereof, a cDNA, a synthetic polydeoxyribonucleic acid sequence, or the like, and can be single-stranded or double-stranded, as well as a DNA/RNA hybrid. Furthermore, the terms are used herein to include naturally-occurring nucleic acid molecules, which can be isolated from a cell, as well as synthetic molecules, which can be prepared, for example, by methods of chemical synthesis or by enzymatic methods such as by the polymerase chain reaction (PCR). Unless specifically limited, the terms encompass nucleic acids containing known analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. A nucleic acid often comprises standard nucleotides typically found in naturally occurring DNA or RNA (which can include modifications such as methylated nucleobases), joined by phosphodiester bonds. In some embodiments a nucleic acid may comprise one or more non-standard nucleotides, which may be naturally occurring or non-naturally occurring (i.e.,

artificial; not found in nature) in various embodiments and/or may contain a modified sugar or modified backbone linkage. Nucleic acid modifications (e.g., base, sugar, and/or backbone modifications), non-standard nucleotides or nucleosides, etc., such as those known in the art as being useful in the context of RNA interference (RNAi), aptamer, CRISPR technology, polypeptide production, reprogramming, or antisense-based molecules for research or therapeutic purposes may be incorporated in various embodiments. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. Such modifications may, for example, increase stability (e.g., by reducing sensitivity to cleavage by nucleases), decrease clearance in vivo, increase cell uptake, or confer other properties that improve the translation, potency, efficacy, specificity, or otherwise render the nucleic acid more suitable for an intended use. Various non-limiting examples of nucleic acid modifications are described in, e.g., Deleavey G F, et al., Chemical modification of siRNA. Curr. Protoc. Nucleic Acid Chem. 2009; 39:16.3.1-16.3.22; Crooke, S T (ed.) Antisense drug technology: principles, strategies, and applications, Boca Raton: CRC Press, 2008; Kurreck, J. (ed.) Therapeutic oligonucleotides, RSC biomolecular sciences. Cambridge: Royal Society of Chemistry, 2008; U.S. Pat. Nos. 4,469,863; 5,536,821; 5,541,306; 5,637,683; 5,637,684; 5,700,922; 5,717,083; 5,719,262; 5,739,308; 5,773,601; 5,886,165; 5,929, 226; 5,977,296; 6,140,482; 6,455,308 and/or in PCT application publications WO 00/56746 and WO 01/14398. Different modifications may be used in the two strands of a double-stranded nucleic acid. A nucleic acid may be modified uniformly or on only a portion thereof and/or may contain multiple different modifications. Where the length of a nucleic acid or nucleic acid region is given in terms of a number of nucleotides (nt) it should be understood that the number refers to the number of nucleotides in a single-stranded nucleic acid or in each strand of a double-stranded nucleic acid unless otherwise indicated. An "oligonucleotide" is a relatively short nucleic acid, typically between about 5 and about 100 nt long. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al. (1991) *Nucleic Acid Res.* 19:5081; Ohtsuka et al. (1985) *J. Biol. Chem.* 260:2605-2608; Rossolini et al. (1994) *Mol. Cell. Probes* 8:91-98). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

In certain embodiments, the nucleic acids include at least one base substitution, insertion, or deletion so that they do not recite a naturally occurring nucleic acid sequence.

Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g. degenerate codon substitutions)

5 and complementary sequences as well as the sequence explicitly indicated. The term conservatively modified variants applies to both amino acid and nucleic acid sequences.

With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of

10 functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are silent variations and represent one species of conservatively modified variation. Every nucleic acid
15 sequence herein that encodes a polypeptide also, by reference to the genetic code, describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine; and UGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which
20 encodes a polypeptide is implicit in each described polypeptide sequence and is within the scope of the products and processes described.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded
25 sequence is a "conservatively modified variant" referred to herein as a "variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. See, for example, Davis et al., "Basic Methods in Molecular Biology" Appleton & Lange, Norwalk, Conn. (1994).

30 The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, e.g., see, e.g.,

Creighton, *Proteins: Structures and Molecular Properties* (WH Freeman & Co.; 2nd edition (December 1993)).

The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. In general, a polypeptide may contain only standard amino acids or may comprise one or more non-standard amino acids (which may be naturally occurring or non-naturally occurring amino acids) and/or amino acid analogs in various embodiments. A "standard amino acid" is any of the 20 L-amino acids that are commonly utilized in the synthesis of proteins and are encoded by the genetic code. A "non-standard amino acid" is an amino acid that is not commonly utilized in the synthesis of proteins. Non-standard amino acids include naturally occurring amino acids (other than the 20 standard amino acids) and non-naturally occurring amino acids. An amino acid, e.g., one or more of the amino acids in a polypeptide, may be modified, for example, by addition, e.g., covalent linkage, of a moiety such as an alkyl group, an alkanoyl group, a carbohydrate group, a phosphate group, a lipid, a polysaccharide, a halogen, a linker for conjugation, a protecting group, a small molecule (such as a fluorophore), etc.

The terms "residue" or "amino acid residue" or "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide, or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

In certain embodiments, a polypeptide disclosed herein may be a chimeric protein. A "chimeric protein" or "fusion protein" is a molecule in which different portions of the protein are derived from different origins such that the entire molecule is not naturally occurring. A chimeric protein may contain amino acid sequences from the same species or different species as long as they are not arranged together in the same way that they exist in a natural state. Examples of a chimeric protein include sequences disclosed herein that contain one, two or more amino acids attached to the C-terminal or N-terminal end that are not identical to any naturally occurring protein, such as in the case of adding an amino acid containing an amine side chain group, e.g., lysine, an amino acid containing a carboxylic acid side chain group such as aspartic acid or glutamic acid, a polyhistidine tag, e.g. typically four or more histidine amino acids.

In certain embodiments, a polypeptide disclosed herein may further comprise a label. A "label" refers to a detectable compound or composition that is conjugated directly or indirectly to another molecule, such as a protein, to facilitate detection of that molecule.

Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes. A label includes the incorporation of a radiolabeled amino acid or the covalent attachment of biotinyl moieties to a polypeptide that can be detected by marked avidin (for example, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Various methods of labeling polypeptides are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionucleotides (such as ^{35}S or ^{131}I) fluorescent labels (such as fluorescein isothiocyanate (FITC), rhodamine, lanthanide phosphors), enzymatic labels (such as horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase), chemiluminescent markers, biotinyl groups, or predetermined polypeptide epitopes recognized by a secondary reporter (such as a leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

When referring to hybridization techniques, all or part of a known nucleotide sequence can be used as a probe that selectively hybridizes to other corresponding nucleotide sequences present in a population of cloned genomic DNA fragments or cDNA fragments (i.e., genomic or cDNA libraries) from a chosen organism. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P , or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the DNA sequences. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed (Sambrook *et al.*, 2001).

For example, the sequence disclosed herein, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding sequences. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among the sequences to be screened and are preferably at least about 10 nucleotides in length, and most preferably at least about 20 nucleotides in length. Such sequences may alternatively be used to amplify corresponding sequences from a chosen plant by PCR. This technique may be used to isolate sequences from a desired plant or as a diagnostic assay to determine the presence of sequences in a plant. Hybridization techniques include hybridization screening of DNA libraries plated as either plaques or colonies (Sambrook *et al.*, 2001).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under

which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 50°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 0.1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, *Anal. Biochem.*, 138:267-284 (1984): $T_m = 81.5^\circ\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a

defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, Part I, Chapter 2 (Elsevier, New York); and Ausubel et al., eds. (1995) Current Protocols in Molecular Biology, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual (3rd ed., Cold Spring Harbor Laboratory Press, Plainview, N.Y.) and Haymes et al. (1985) In: Nucleic Acid Hybridization, a Practical Approach, IRL Press, Washington, D.C.

In general, sequences that correspond to the nucleotide sequences described and hybridize to the nucleotide sequence disclosed herein will be at least 50% homologous, 70% homologous, and even 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% homologous or more with the disclosed sequence. That is, the sequence similarity between probe and target may range, sharing at least about 50%, about 70%, and even about 85% or more sequence similarity.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity" and (d) "percentage of sequence identity."

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length promoter sequence, or the complete promoter sequence.

(b) As used herein, "comparison window" makes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the

reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to accurately reflect the similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

As discussed, the degron tail region of the IAA2 protein may be deleted or may be disrupted. When referring to disruption is meant that expression or activity of the region is reduced or prevented such that when the plant, plant part or cell is exposed to herbicide, in one embodiment the herbicide 2,4-D, the plant is tolerant to the herbicide.

The present invention provides, *inter alia*, isolated nucleic acids of DNA, RNA, homologs, paralogs and orthologs and/or chimeras thereof, comprising an herbicide tolerant IAA gene polynucleotide. This includes naturally occurring as well as synthetic variants and homologs of the sequences. Sequences homologous, i.e., that share significant sequence identity or similarity, to those provided herein derived from other plants of choice, are also an aspect of the invention.

Homologous sequences as described above can comprise orthologous or paralogous sequences. Several different methods are known by those of skill in the art for identifying and defining these functionally homologous sequences. Three general methods for defining orthologs and paralogs are described; an ortholog, paralog or homolog may be identified by one or more of the methods described below.

Orthologs and paralogs are evolutionarily related genes that have similar sequence and similar functions. Orthologs are structurally related genes in different species that are derived by a speciation event. Paralogs are structurally related genes within a single species that are derived by a duplication event.

Within a single plant species, gene duplication may result in two copies of a particular gene, giving rise to two or more genes with similar sequence and often similar function known as paralogs. A paralog is therefore a similar gene formed by duplication within the same species. Paralogs typically cluster together or in the same clade (a group of similar genes) when a gene family phylogeny is analyzed using programs such as CLUSTAL (Thompson et al. (1994) *Nucleic Acids Res.* 22: 4673-4680; Higgins et al. (1996) *Methods Enzymol.* 266: 383-402). Groups of similar genes can also be identified with pair-wise BLAST analysis (Feng and Doolittle (1987) *J. Mol. Evol.* 25: 351-360).

For example, a clade of very similar MADS domain transcription factors from *Arabidopsis* all share a common function in flowering time (Ratcliffe et al. (2001) *Plant Physiol.* 126: 122-132), and a group of very similar AP2 domain transcription factors from *Arabidopsis* are involved in tolerance of plants to freezing (Gilmour et al. (1998) *Plant J.* 16: 433-442). Analysis of groups of similar genes with similar function that fall within one clade can yield sub-sequences that are particular to the clade. These sub-sequences, known as consensus sequences, can not only be used to define the sequences within each clade, but define the functions of these genes; genes within a clade may contain paralogous sequences, or orthologous sequences that share the same function (see also, for example, Mount (2001), in *Bioinformatics: Sequence and Genome Analysis* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., page 543.)

Speciation, the production of new species from a parental species, can also give rise to two or more genes with similar sequence and similar function. These genes, termed orthologs, often have an identical function within their host plants and are often interchangeable between species without losing function. Because plants have common ancestors, many genes in any plant species will have a corresponding orthologous gene in another plant species. Once a phylogenetic tree for a gene family of one species has been constructed using a program such as CLUSTAL (Thompson et al. (1994) *Nucleic Acids Res.* 22: 4673-4680; Higgins et al. (1996) *supra*) potential orthologous sequences can be placed into the phylogenetic tree and their relationship to genes from the species of interest can be determined. Orthologous sequences can also be identified by a reciprocal BLAST strategy. Once an orthologous sequence has been identified, the function of the ortholog can be deduced from the identified function of the reference sequence.

Orthologous genes from different organisms have highly conserved functions, and very often essentially identical functions (Lee et al. (2002) *Genome Res.* 12: 493-502; Remm et al. (2001) *J. Mol. Biol.* 314: 1041-1052). Paralogous genes, which have diverged through gene duplication, may retain similar functions of the encoded proteins. In such cases, paralogs can be used interchangeably with respect to certain embodiments of the instant invention (for example, transgenic expression of a coding sequence).

Exemplary orthologous sequences of *Sisymbrium orientale* IAA2 from *Helianthus annuus*, *Vitis vinifera*, *Glycine max*, *Coffea canephora*, *Gossypium raimondii*, *Phaseolus vulgaris*, *Beta vulgaris*, *Capsicum annuum*, *Solanum lycopersicum*, and *Solanum tuberosum* are shown in SEQ ID NOs: 51-60. Additional sequences of orthologs as well as IAA paralogs

are known to those of skill in the art and are readily available through sources such as GENBANK[®] or Ensembl Plants.

As used herein, the term "mutant" or "functional mutant" when made in reference to a gene or to a gene product refers, respectively, to a gene or to a gene product which displays
5 modifications in sequence and/or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. Thus, the terms "modified" and "mutant" when used in reference to a nucleotide sequence refer to a nucleic acid sequence that differs by one or more nucleotides from another such that a disrupted or deleted degtron tail region in the IAA protein is produced.

As used herein, "genetically modified plant" includes reference to a plant or ancestor thereof, to which has been introduced a heterologous polynucleotide. In some instances, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. In some instances, such as gene editing the heterologous polynucleotide engineers a chromosomal change that is passed to successive
15 generations while the polynucleotide itself is not. The heterologous polynucleotide may be introduced alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by subsequent sexual crosses or asexual propagation from the
20 initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

The term introduced in the context of inserting a nucleic acid into a cell, includes transfection or transformation or transduction and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

When referring to introduction of a nucleotide sequence into a plant is meant to include transformation into the cell, as well as crossing a plant having the sequence with another plant, so that the second plant contains the heterologous sequence, as in conventional plant breeding techniques. Such breeding techniques are well known to one skilled in the art. For a discussion of plant breeding techniques, see Poehlman (1995) Breeding Field Crops. AVI

Publication Co., Westport Conn, 4th Edit. Backcrossing methods may be used to introduce a gene into the plants. This technique has been used for decades to introduce traits into a plant. An example of a description of this and other plant breeding methodologies that are well known can be found in references such as Poehlman, supra, and Plant Breeding Methodology, edit. Neal Jensen, John Wiley & Sons, Inc. (1988). In a typical backcross protocol, the original variety of interest (recurrent parent) is crossed to a second variety (nonrecurrent parent) that carries the single gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent are recovered in the converted plant, in addition to the single transferred gene from the nonrecurrent parent.

To achieve this one could, for example, perform the following steps:

- (a) plant seeds of the first (starting line) and second (donor plant line that comprises a gene of the invention) parent plants;
- (b) grow the seeds of the first and second parent plants into plants that bear flowers;
- (c) pollinate a flower from the first parent plant with pollen from the second parent plant; and
- (d) harvest seeds produced on the parent plant bearing the fertilized flower.

Backcrossing is herein defined as the process including the steps of:

- (a) crossing a plant of a first genotype containing a desired gene, DNA sequence or element to a plant of a second genotype lacking the desired gene, DNA sequence or element;
- (b) selecting one or more progeny plant containing the desired gene, DNA sequence or element;
- (c) crossing the progeny plant to a plant of the second genotype; and
- (d) repeating steps (b) and (c) for the purpose of transferring a desired DNA sequence from a plant of a first genotype to a plant of a second genotype.

The terms "introgress" and "introgressing" and "introgression" refer to conventional (i.e. classic) pollination breeding techniques to incorporate foreign genetic material into a line of breeding stock. For example, the present invention provides for plants introgressed with a modified IAA2 protein having a disrupted or deleted degron tail region for herbicide tolerance by crossing two plant generations.

Transformation of a plant to produce expression of a polypeptide having a disrupted or deleted degron tail are processes well known to one of skill in the art.

A construct is a package of genetic material inserted into the genome of a cell via various techniques. The term nucleic acid construct refers to a coding sequence or sequences operably linked to appropriate regulatory sequences and inserted into a vector for transforming a cell. Such a nucleic acid construct may contain a coding sequence for a gene product of interest, along with a marker gene and/or a reporter gene.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from its original form by deliberate human intervention.

As used herein, the term vector refers broadly to any plasmid or virus encoding an exogenous nucleic acid. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into virions or cells, such as, for example, polylysine compounds and the like. The vector may be a viral vector that is suitable as a delivery vehicle for delivery of the nucleic acid, or mutant thereof, to a cell, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, Proc. Natl. Acad. Sci. U.S.A. 94:12744-12746). Examples of viral vectors include, but are not limited to, a recombinant vaccinia virus, a recombinant adenovirus, a recombinant retrovirus, a recombinant adeno-associated virus, a recombinant avian pox virus, and the like (Cranage et al., 1986, EMBO J. 5:3057-3063; US Patent No. 5,591,439). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

As used herein, a nucleotide segment is referred to as operably linked when it is placed into a functional relationship with another DNA segment. For example, DNA for a signal sequence is operably linked to DNA encoding a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it stimulates the transcription of the sequence. Operably linked elements may be contiguous or non-contiguous. When used to refer to the

joining of two protein coding regions, by operably linked it is intended that the coding regions are in the same reading frame. Alternatively, the additional gene(s) can be provided on multiple expression cassettes. Such an expression cassette is provided with a plurality of restriction sites and/or recombination sites for insertion of the polynucleotide to be under the transcriptional regulation of the regulatory regions. The expression cassette can include one or more enhancers in addition to the promoter. By enhancer is intended a cis-acting sequence that increases the utilization of a promoter. Such enhancers can be native to a gene or from a heterologous gene. Further, it is recognized that some promoters can contain one or more enhancers or enhancer-like elements. An example of one such enhancer is the 35S enhancer, which can be a single enhancer, or duplicated. See for example, McPherson et al, US Patent 5,322,938.

As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses, and bacteria which comprise genes expressed in plant cells such *Agrobacterium* or *Rhizobium*. Examples are promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, seeds, fibres, xylem vessels, tracheids, or sclerenchyma. Such promoters are referred to as "tissue- preferred." A "cell type" specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An "inducible" or "regulatable" promoter is a promoter which is under environmental control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions or the presence of light. Another type of promoter is a developmentally regulated promoter, for example, a promoter that drives expression during pollen development. Tissue preferred, cell type specific, developmentally regulated, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter, which is active under most environmental conditions.

The method of transformation/transfection is not critical to the instant invention; various methods of transformation or transfection are currently available. As newer methods are available to transform crops or other host cells they may be directly applied. Accordingly, a wide variety of methods have been developed to insert a DNA sequence into the genome of a host cell to obtain the transcription or transcript and translation of the

sequence to effect phenotypic changes in the organism. Thus, any method which provides for efficient transformation/transfection may be employed.

Methods for introducing expression vectors into plant tissue available to one skilled in the art are varied and will depend on the plant selected. Procedures for transforming a wide variety of plant species are well known and described throughout the literature. (See, for example, Miki and McHugh (2004) *Biotechnol.* 107, 193-232; Klein et al. (1992) *Biotechnology* (N Y) 10, 286-291; and Weising et al. (1988) *Annu. Rev. Genet.* 22, 421-477). For example, the DNA construct may be introduced into the genomic DNA of the plant cell using techniques such as microprojectile-mediated delivery (Klein et al. 1992, *supra*), electroporation (Fromm et al., 1985 *Proc. Natl. Acad. Sci. USA* 82, 5824-5828), polyethylene glycol (PEG) precipitation (Mathur and Koncz, 1998 *Methods Mol. Biol.* 82, 267-276), direct gene transfer (WO 85/01856 and EP-A-275 069), *in vitro* protoplast transformation (U.S. Pat. No. 4,684,611), and microinjection of plant cell protoplasts or embryogenic callus (Crossway, A. (1985) *Mol. Gen. Genet.* 202, 179-185). *Agrobacterium* transformation methods of Ishida et al. (1996) and also described in U.S. Pat. No. 5,591,616 are yet another option. Co-cultivation of plant tissue with *Agrobacterium tumefaciens* is a variation, where the DNA constructs are placed into a binary vector system (Ishida et al., 1996 *Nat. Biotechnol.* 14, 745-750). The virulence functions of the *Agrobacterium tumefaciens* host will direct the insertion of the construct into the plant cell DNA when the cell is infected by the bacteria. See, for example, Fraley et al. (1983) *Proc. Natl. Acad. Sci. USA*, 80, 4803-4807. *Agrobacterium* is primarily used in dicots, but monocots including maize can be transformed by *Agrobacterium*. See, for example, U.S. Pat. No. 5,550,318. In one of many variations on the method, *Agrobacterium* infection of corn can be used with heat shocking of immature embryos (Wilson et al. U.S. Pat. No. 6,420,630) or with antibiotic selection of Type II callus (Wilson et al., U.S. Pat. No. 6,919,494).

Rice transformation is described by Hiei et al. (1994) *Plant J.* 6, 271-282 and Lee et al. (1991) *Proc. Nat. Acad. Sci. USA* 88, 6389-6393. Standard methods for transformation of canola are described by Moloney et al. (1989) *Plant Cell Reports* 8, 238-242. Corn transformation is described by Fromm et al. (1990) *Biotechnology* (N Y) 8, 833-839 and Gordon-Kamm et al. (1990) *supra*. Wheat can be transformed by techniques similar to those used for transforming corn or rice. Sorghum transformation is described by Casas et al. (Casas et al. (1993) Transgenic sorghum plants via microprojectile bombardment. *Proc. Natl. Acad. Sci. USA* 90, 11212-11216) and barley transformation is described by Wan and Lemaux (Wan and Lemaux (1994) Generation of large numbers of independently

transformed fertile barley plants. *Plant Physiol.* 104, 37-48). Soybean transformation is described in a number of publications, including U.S. Pat. No. 5,015,580.

The degron tail may be deleted or disrupted through a variety of gene editing techniques available to one of skill in the art. Any method that can prevent or disrupt expression or activity of the degron tail may be employed. Examples are provided below and are not intended to be limiting.

Methods which provide for targeting of the molecule of interest (MOI) to the target site of the target gene may be utilized in the method as well as means to delete or mutate one or more bases or residues. The following is provided by way of example rather than

limitation. A guide nucleic acid molecule is one that directs the nuclease to the specific cut site in the genome, whether via use of a binding domain, recognition domains, guide RNAs or other mechanisms. The guide nucleic acid molecule is introduced into the cell under conditions appropriate for operation of the guide nucleic acid molecule in directing cleavage to the target locus. A person of skill in the art will have available a number of methods that

may be used, the most common utilizing a nuclease to cleave the target region of the gene, along with sequences which will recognize sequences at the target locus and direct cleavage to the locus. Any nuclease that can cleave the phosphodiester bond of a polynucleotide chain may be used in the methods described here. By way of example without limitation, available systems include those utilizing site specific nucleases (SSN) such as ZFNs (Zinc finger

nuclease), (Whyte, et al. Cell Biology Symposium: Zinc finger nucleases to create custom-designed modifications. *J Anim Sci* 90, 1111-1117 (2012)); TALENs (Transcription activator-like effector nucleases) (see, Carlson, D.F. et al. Efficient TALEN-mediated gene knockout in animals. *Proc Natl Acad Sci U S A* 109, 17382-17387 (2012); Tan, W. et al.

Efficient nonmeiotic allele introgression in livestock using custom endonucleases. *Proc Natl Acad Sci U S A* 110, 16526-16531 (2013); and the CRISPR (Clustered regularly interspaced short palindromic repeats) -associated (Cas) nuclease system (Hai, T., Teng, F., Guo, R., Li, W. & Zhou, Q. One-step generation of knockout pigs by zygote injection of CRISPR/Cas system. *Cell Res* 24, 372-375 (2014)) that have permitted editing of animal genomes. The use

of recombinases such as FLP/FRT as described in US Patent No. 6,720,475, or CRE/LOX as described in US Patent No. 5,658,772, can be utilized to integrate a polynucleotide sequence into a specific chromosomal site. Meganucleases have been used for targeting donor polynucleotides into a specific chromosomal location as described in Puchta *et al.*, *PNAS USA* 93 (1996) pp. 5055-5060. ZFNs work with proteins or domains of proteins binding to a binding domain having a stabilized structure as a result of use a zinc ion. TALENs utilize

domains with repeats of amino acids which can specifically recognize a base pair in a DNA sequence. For a discussion of both systems see Voytas et al. US Patent No. 8,697,853, incorporated herein by reference in its entirety. These systems utilize enzymes prepared for each target sequence.

5 A still further example of potential uses provides for introduction into a plant or cell of interfering nucleic acid molecules. For example, double-stranded RNA molecules (dsRNA) may be employed. In this process, in summary, RNA which is double stranded, in part, or completely, is produced based upon the sequence of the target nucleic acid molecule. Specifics of the means of producing the dsRNA may vary as one skilled in the art
10 appreciates, and include, by way of example without intending to be limiting, the approach of Graham et al., US Patent No. 6,573,099 where two copies of a sequence corresponding to a target sequence is used, or that of Fire et al., US Patent 6,326,193 (both incorporated herein by reference), where the first strand is an RNA sequence corresponding to the target nucleic acid, and the second is one which is complementary to the target sequence, each of which are
15 incorporated herein by reference in their entirety. These strands hybridize with each other to form the inhibiting dsRNA. The strand which corresponds to the target nucleic acid molecule can correspond to all or a portion thereof, as long as a dsRNA is formed. Where a strand is used which is the complement (antisense) of the target nucleic acid is used, it can be complementary to all or a portion of the target nucleic acid molecule, so long as the dsRNA
20 formed interferes with the target nucleic acid molecule. The dsRNA triggers a response in which the RNase III Dicer enzyme process dsRNA into small interfering RNAs (siRNA) of approximately 21 - 23 nucleotides, which are formed into a RNA-induced silencing complex RISC which destroys homologous mRNAs. (See, Hammond, S. M., et al., *Nature* (2000) 404:293-296). Generally, sequences of up to 10 nucleotides 20 nucleotides, 30 nucleotides,
25 40 nucleotides, 50 nucleotides, 100 nucleotides, 200 nucleotides, 300, 500, 550, 500, 550, or greater and any amount in-between may be used.

Any method of deleting or disrupting degon tail expression of activity may be used in the present methods, and a vast variety of such methods are known. A few examples of such methods are provided by way of example below, ranging from physical or chemical mutagen
30 applications, to gene editing which has provided many accurate and precise techniques, cosuppression, antisense silencing, RNA interference, or any of many others available.

Methods are also provided to reduce or eliminate the activity of a degon tail by transforming a plant cell with an expression cassette that expresses a polynucleotide that inhibits the expression of the degon tail. The polynucleotide may inhibit the expression of

the degron tail directly, by preventing transcription or translation of the degron tail synthase messenger RNA, or indirectly, by encoding a polypeptide that inhibits the transcription or translation of an degron tail nucleic acid molecule encoding an degron tail polypeptide.

Methods for inhibiting or eliminating the expression of a gene in a plant are well known in the art, and any such method may be used in the present invention to inhibit the expression of the degron tail polypeptide. Many methods may be used to reduce or eliminate the activity of a degron tail polypeptide. In addition, more than one method may be used to reduce the activity of a single degron tail polypeptide.

In some embodiments of the present invention, a plant is transformed with an expression cassette that is capable of expressing a polynucleotide that inhibits the expression of a degron tail polypeptide of the invention. The term "expression" as used herein refers to the biosynthesis of a gene product, including the transcription and/or translation of said gene product. For example, for the purposes of the present invention, an expression cassette capable of expressing a polynucleotide that inhibits the expression of at least one degron tail polypeptide is an expression cassette capable of producing an RNA molecule that inhibits the transcription and/or translation of at least one degron tail synthase polypeptide of the invention. The "expression" or "production" of a protein or polypeptide from a DNA molecule refers to the transcription and translation of the coding sequence to produce the protein or polypeptide, while the "expression" or "production" of a protein or polypeptide from an RNA molecule refers to the translation of the RNA coding sequence to produce the protein or polypeptide.

Examples of polynucleotides that inhibit the expression of an degron tail polypeptide include sense suppression/cosuppression, where an expression cassette is designed to express an RNA molecule corresponding to all or part of a messenger RNA encoding an degron tail polypeptide in the "sense" orientation and over expression of the RNA molecule can result in reduced expression of the native gene. With antisense suppression the expression cassette is designed to express an RNA molecule complementary to all or part of a messenger RNA encoding the degron tail polypeptide and over expression of the antisense RNA molecule can result in reduced expression of the native gene. In double-Stranded RNA interference, a sense RNA molecule like that described above for cosuppression and an antisense RNA molecule that is fully or partially complementary to the sense RNA molecule are expressed in the same cell, resulting in inhibition of the expression of the corresponding endogenous messenger RNA. Hairpin RNA interference and intron-containing hairpin RNA interference utilizes an expression cassette is designed to express an RNA molecule that hybridizes with

itself to form a hairpin structure that comprises a single-stranded loop region and a base-paired stem. Small Interfering RNA or Micro RNA employs an expression cassette is designed to express an RNA molecule that is modeled on an endogenous miRNA gene.

In one embodiment, the polynucleotide encodes a zinc finger protein that binds to a gene encoding an degron tail polypeptide, resulting in reduced expression of the gene, Methods of selecting sites for targeting by zinc finger proteins have been described, for example, in U.S. Patent Nos. 6,453,242, and methods for using zinc finger proteins to inhibit the expression of genes in plants are described, for example, in U.S. Patent Publication Nos. 2003/0037355; each of which is herein incorporated by reference.

In some embodiments, the polynucleotide encodes an antibody that binds to at least one degron tail and reduces the activity of the degron tail polypeptide. The expression of antibodies in plant cells and the inhibition of molecular pathways by expression and binding of antibodies to proteins in plant cells are well known in the art. See, for example, Conrad and Sonnewald, (2003) *Nature Biotech.* 21:35-36.

In some embodiments, the activity of a degron tail polypeptide is reduced or eliminated by disrupting the gene encoding the degron tail polypeptide. The gene encoding the degron tail polypeptide may be disrupted by any method known in the art. For example, in one embodiment, the gene is disrupted by transposon tagging. In another embodiment, the gene is disrupted by mutagenizing plants using random or targeted mutagenesis, and selecting for plants that have reduced degron tail activity.

Unless otherwise specifically explained, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this disclosure belongs. It is to be understood that all terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting in any manner or scope. For example, as used in this specification and the appended claims, the singular forms "a," "an" and "the" can include plural referents unless the content clearly indicates otherwise. Further, all units, prefixes, and symbols may be denoted in its SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

The following is provided by way of exemplification and is not intended to limit the scope of the invention. All references cited herein are herein by reference.

EXAMPLES

5 *Example 1: Candidate gene identification through RNAseq*

Plant material

A population of 2,4-D-resistant *S. orientale* was collected from a wheat field in Port Broughton (PB-R1), a second 2,4-D resistant population was collected 9.2 km away (PB-R2), while the susceptible population originated from Roseworthy (S), South Australia, Australia,
10 about 120 km away from the resistant populations. PB-R1 plants were reported to be 22-fold more resistant to 2,4-D and 20-fold more resistant to MCPA compared to S plants.

Two biparental crosses were made to study inheritance of 2,4-D resistance, using PB-R1 as the male parent for Cross A and PB-R2 as the male parent for Cross B, and Roseworthy S as the female parent for both crosses. Crosses were performed by hand
15 emasculation of S and hand pollination with pollen from the R parent. The F1 progeny were self-pollinated to produce F2 progeny, and F2 individuals were self-pollinated to produce F3 progeny. The F3 progeny were sprayed with 150 g a.i. ha⁻¹ 2,4-D (2,4-D amine, Shredder, Winfield) using an overhead track sprayer (DeVries Manufacturing) equipped with a flat-fan nozzle tip (TEEJET[®] 8002EVS, Spraying System) calibrated to deliver 187 L ha⁻¹ of spray
20 solution at 172 kPa to visually determine 2,4-D resistance segregation, evaluated 21 DAT. Homozygous resistant and homozygous susceptible F3 lines were inbred via single-seed descent to create recombinant inbred lines (RILs) to the F4 generation (Cross A). The F3 generation was used for Cross B.

RNA-Sequencing

25 The transcriptome of six F4 recombinant inbred lines (RILs) from a cross between 2,4-D resistant PB-1 and susceptible S (Cross A), and six F3 RILs from a cross between 2,4-D resistant PB-2 and susceptible S (Cross B) was sequenced. From Cross A, three RILs were homozygous 2,4-D-resistant and three RILs were homozygous 2,4-D-susceptible. From Cross B, two RILs were homozygous 2,4-D-resistant and four RILs were homozygous 2,4-D-
30 susceptible. The samples used for RNA-Sequencing were first germinated on agar plates, transplanted to 3×3cm pots filled with potting soil, and grown in a growth chamber at 25/20 °C, 16/8 h light/dark photoperiod. At the four-leaf stage, the youngest expanded leaf was collected. Samples were frozen immediately in liquid nitrogen and total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen). Sample quality was assessed by RIN score

(Agilent TapeStation) as well as by gel electrophoresis. DNase treatment, library preparation, and sequencing were conducted at the Roy J. Carver Biotechnology Center at the University of Illinois. Libraries were prepared using the TruSeq Stranded mRNA Sample Prep Kit (Illumina). Sequencing was performed on a HiSeq 4000 (Illumina) using bar-coded adapters in two lanes of the Illumina flow-cell for 101 cycles, yielding 1.6 billion paired-end reads. Individual library yields ranged from 52 to 75 million paired-end reads. Fastq files were generated and demultiplexed with bcl2fastq v2.17.1.13 conversion software (Illumina) and adaptors were trimmed from the 3'-end reads.

A de novo reference transcriptome was obtained by separately assembling Illumina reads from a Cross A 2,4-D-resistant RIL (R206) and a susceptible RIL (S242) with Trinity. The assembled contigs were filtered for minimum contig length of 500 bp. The two assemblies were compared using CDHit v4.6.6 with a 95% confidence interval to eliminate redundant contigs and retain all contigs unique to either the R or S assembly. This resulted in a 72-Mb transcriptome with 37,223 contigs. Putative annotations were assigned using Trinotate and the TAIR10 protein database. Read alignments to the *de novo* reference transcriptome were conducted with Bowtie2 using the default "end-to-end" mode and the "sensitive" option. The minimum allowed fragment length was set to 100 and the maximum to 800 bp.

Differential expression and SNP analysis

Raw read counts were extracted using SAMtools. Counts-per-million (CPM) and gene expression differences were calculated with the package 'edgeR' using the statistical software R v3.3 and an expression threshold of ≥ 1 CPM in at least two samples. After normalization of the RNA composition the data was analyzed using the 'classic approach'. Expression differences were compared between 2,4-D resistant and susceptible RILs within Cross A and B, respectively, as well as between all 2,4-D-resistant and -susceptible RILs. Differentially expressed transcripts were then filtered for a fold-change of $\geq |2|$ and a false discovery rate (FDR) adjusted p-value ≤ 0.05 . Single nucleotide polymorphism (SNP) calling was performed using SAMtools (v. 1.3.1) with the default options and the "mpileup" command. The command "bcftools" was used to retain only SNPs that had a quality score higher than 10 and read depth higher than 10. An additional filtering step was for SNPs that were heterozygous or homozygous in at least three individuals of all R and all S RILs. Contigs with annotations in the TIR1/AFB family and the Aux/IAA gene family were manually inspected for sequence variants between R and S RILs using IGV to view BAM file read alignments.

Only three transcripts had differential expression between R and S RILs of both Port Broughton and P2 populations based on cutoff criteria of FDR <0.05 and fold change of > |2| (Figure 5). These included IAA2 (3.3 fold lower in R than S, FDR<0.0001, homology to AT3G23030), PRP39-2 (10 fold lower in R than S, FDR<0.0001, homology to AT5G46400), and ABCB13 (6 fold higher in R than S, FDR <0.01, homology to AT1G27940). Notably, PRP39-2 and ABCB13 both had at least one RIL replicate inconsistent with the general pattern, while IAA2 had lower read counts in all R RILs compared to all S RILs. No SNPs were identified that were shared among all R RILs and different from all S RILs from the two populations. However, inspection of the read alignments to IAA2 identified a small gap in read coverage for all R RILs, whereas all S RILs had continuous read coverage at this position.

Example 2: Candidate gene validation

IAA2 gene sequencing

To further investigate and validate the results found for IAA2 through RNAseq, a region of the *IAA2* gene was sequenced. A 239 bp region of the *IAA2* gene was amplified (forward primer 5'-AACTCAAATCGTTGGTTGGC-3' (SEQ ID NO: 61) and reverse primer 5'-CTTTATCCTCGTACGTTGGTACG-3' (SEQ ID NO: 62)) and sequenced for 3 R and 3 S RILs and the R allele was found to contain a 27 bp deletion (*IAA2*_{Δ27}) (Figure 6).

The deletion results in a functional, in-frame *IAA2* protein, but missing amino acids 73 to 81, removing most of the degron tail region located between the degron and the PB1 domain (Figure 6). No S alleles contained a deletion in *IAA2*, suggesting the deletion was correlated with resistance. The deletion also explains the observed lower expression value in R RILs, rather than a reduced transcriptional rate.

Deletion and resistance correlation in other resistant populations

The presence of *IAA2*_{Δ27} in other 2,4-D resistant populations of *S. orientale* was also investigated. Seeds of *S. orientale* populations were germinated by sowing directly onto the soil surface of seedling trays containing standard potting mix. Once emerged, seedlings were transplanted into 9.5 × 8.5 × 9.5 cm punnet pots and grown outdoors at the Waite Campus, University of Adelaide, Australia. At the 4-leaf stage, plants were treated with a single rate of 250 g. a. ha⁻¹ 2,4-D (2,4-D amine 650, FMC) to screen for resistance. Herbicide was applied using a laboratory moving boom pesticide applicator and applied at an equivalent of 109 L ha⁻¹ of water at a pressure of 250 kPa and a speed of 1 m s⁻¹ using Tee-Jet 001 nozzles

(Tee-Jet 8001E; Spraying Systems Co., Wheaton, IL). Leaf curl was assessed 48 h after treatment, with any plants showing signs of leaf curl considered susceptible and tissue harvested, while the remaining plants were considered resistant and allowed to grow for a further 21 days to confirm resistance and tissue subsequently harvested. Eight populations from Southern Australia, including parent populations PB-1R and PB-2R were screened for resistance to 2,4-D, with four identified as resistant.

The IAA2 gene was sequenced to determine the presence or absence of IAA2_{Δ27} in a number of individual plants from each populations. Genomic DNA was extracted using the Isolate II Plant DNA kit (Bioline, Alexandria, New South Wales, Australia) according to the manufacturer's instructions. The concentration of nucleic acids was determined spectrophotometrically on a nanodrop ND-1000 (Thermo Scientific, Wilmington, DE, USA) at 260 nm. For polymerase chain reaction (PCR) amplification, ~100 ng of gDNA was added to a standard 25 μL PCR reaction mix containing 1× MyFi reaction buffer (containing 0.2 mM of dNTPs and 0.6 mM of MgCl₂), 0.4 μM of each gene-specific primer (as above) and 1 μL of MyFi DNA polymerase (Bioline). Amplification was carried out in an automated DNA thermal cycler (GeneTouch, Bioer Technology, Binjiang, Hangzhou, China) with PCR conditions as follows: 1 min denaturing at 95°C; 35 cycles of 15 s denaturation at 95°C, 30 s annealing at 58°C, 15 s elongation at 72°C and a final extension for 7 min at 72°C. PCR products were prepared with 1× Ficoll loading dye [15% (w/v) Ficoll 4000, 0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF] and visualised on SYBR Safe (Life Technologies, Mulgrave, Victoria, Australia) stained agarose gels. Samples were electrophoresed in 1× TAE buffer (40 mM of Trizma base, 1 mM of Na₂EDTA, pH to 8 with glacial acetic acid) at 100 V and photographed under UV light ($\lambda = 302$ nm). DNA fragment sizes were estimated by comparing their mobility with bands of known sizes of a low-molecular-weight marker (EasyLadder & HyperLadder; Bioline).

Amplified fragments were cloned using the Topo TA cloning kit (Life Technologies) according to the manufacturer's instructions to facilitate sequencing. Colony PCR was performed to determine positive clones carrying the fragment, using the same PCR protocol as used for amplification, but replacing the template DNA with a single clone colony, and increasing the initial denaturing step to 10 min to aid cell lysis. Before addition into the PCR reaction, colonies were streaked onto standard LB/kan plates and plasmid DNA of positive clones isolated from the regrown streaked colonies (Isolate II Plasmid Mini Kit, Bioline). Plasmids were sequenced using the standard M13 vector primers with sequencing conducted

by the Australian Genome Research Facility (AGRF). Sequence data were analyzed using ContigExpress from the Vector-NTI Suite 6 programs (Life Technologies).

Only wild type IAA2 was present in individuals from four susceptible populations P15, P31, P49 and P50 (Table 2). All individuals from the two parent resistant populations, PB-1R and PB-2R, contained IAA2 Δ 27. Individuals from resistant population P17 were found to contain either IAA2 Δ 27 alone, or both IAA2 Δ 27 and the wild type IAA2 with no deletion IAA2_{WT}, suggesting a segregating population. Population P28, although resistant, did not contain IAA2 Δ 27.

TABLE 2

Population	No. of individuals	Banding pattern		
		IAA2 _{WT}	IAA2 Δ 27	IAA2 _{WT} /IAA2 Δ 27
P2 (R)	20		20	
P13 (R)	20		20	
P15 (S)	15	15		
P17 (R)	20		14	6
P28 (R)	20	20		
P31 (S)	5	5		
P49 (S)	5	5		
P50 (S)	5	5		

Segregation analysis

A KASP genotyping assay was developed using a forward primer specific to the R allele appended with a linker sequence for the HEX fluorophore (gene specific sequence *italicized*) (5'-GAAGGTCGGAGTCAACGGATTCTCCGGTGAGATCTTATGTG-3')(SEQ ID NO:63) and a forward primer specific to the S allele appended with a linker sequence for the FAM fluorophore (5'-GAAGGTGACCAAGTTCATGCTCGTAAGAACAACAACAGTGTGAGC-3')(SEQ ID NO: 64), together with a universal reverse primer (5'-ATTTTGCGGAGGTATGGTGC-3') (SEQ ID NO: 65). The analysis was performed by mixing a primer master mix containing the 3 primers and a KASP master mix. 4 μ l of the master mix was mixed to 4 μ l of plant DNA at 20 nM μ L⁻¹. FAM and HEX fluorescence was obtained and corrected by removing background fluorescence obtained in a non-template control.

A progeny test segregation analysis was performed on 219 F3 plants derived from self-pollination of a heterozygous F2 individual from cross B, identified via progeny test due to segregation of 2,4-D resistance in the F3 progeny. Plants were sprayed with 200 gai ha⁻¹ 2,4-D at the 5 true leaf stage. Damage percentage was evaluated at 28 days after treatment.

Leaf tissue was harvested and genomic DNA was extracted by CTAB. A KASP assay was performed with primers and methods as described above. To further test the co-segregation between the resistant phenotype and IAA2 Δ 27, nine each of R and S RILs from cross A and three each of R and S RILs from cross B were genotyped using the KASP assay. Two
 5 individuals were genotyped from each RIL.

The association between IAA2 Δ 27 and the resistant phenotype was confirmed by a segregation analysis, where all sensitive plants exhibited 70% visual injury and were homozygous for IAA2_{WT}; heterozygous plants showed an average of 24% injury and homozygous resistant 12%. This significant difference between heterozygous and
 10 homozygous R is probably due to semi-dominance for the resistant gene. The relative expression (RE) of IAA2 also confirmed the results of the RNAseq, where untreated seedlings showed 3-fold higher expression in S (RE = 0.21) compared to R (RE = 0.07). Auxin treatment significantly increased the expression of IAA2 in R (2.5-fold for 2,4-D and 9.5-fold for IAA) and S (4-fold for 2,4-D and 7-fold for IAA) seedlings compared to
 15 untreated plants and DMSO control. Treated susceptible plants (2,4-D RE = 0.55; IAA RE = 1) showed significant increased expression compared to resistant (2,4-D RE = 0.16; IAA RE = 0.62) (Figure 7).

Example 3: Arabidopsis transformation

Arabidopsis transformation was performed based on established protocols with
 20 modifications. IAA2 alleles were amplified by PCR from cDNA generated from RNA extracts of homozygous R and S F5 lines (cross A). RNA was extracted using Direct-zol RNA Miniprep Plus Kit (Zymo). cDNA synthesis was performed using oligo(dT)₂₀ primers from SuperScript™ II Reverse Transcriptase (Invitrogen). Wild-type and mutant alleles were
 25 amplified by PCR using PrimeSTAR HS Premix (Takara), using primers linked with restriction enzyme sites for cloning. Purified PCR amplicons were ligated into the pGEM-T Easy Vector (Promega), transformed into *E. coli*, sequenced by Sanger sequencing, and digested using the restriction sites AscI and BamHI (New England BioLabs). The digested product was then ligated into the binary vector pFGC5941 and cloned into *Agrobacterium*
 30 *tumefaciens* (strain GV3101). For plant transformation, *Agrobacterium* 400 mL liquid cultures were incubated at 28 C overnight and after centrifugation, resuspended in a 300 mL transformation solution (5% sucrose and 0.02% Silwet L-77). Each allele of IAA2 was transformed into *Arabidopsis thaliana* (Col-0) by floral dip and grown to seed production.

For transformant selection, 40 mg of transformed seeds for each IAA2 allele were cleaned with bleach solution (12.5%) and plated at half MS selective media with Cefotaxime (200 mg/L) and glufosinate-ammonium (7.5 mg/L). After two weeks, positive transformants were transferred to a second selective plate with half MS, Cefotaxime (100 mg/L) and glufosinate-ammonium (5 mg/L). After selection, potential transformants were transferred to soil and grown until seed production. For T2 selection, 36 seeds were cleaned and grown on half MS and glufosinate-ammonium (5 mg/L) media. T3 homozygous plants containing just one copy of the T-DNA vector were selected and IAA2 expression was analyzed using the same primers from mustard expression assay (see below), *AtCyclophilin* and *AtActin2* were used as normalization genes. Three expressing homozygous lines from each version of the IAA2 WT and $\Delta 27$ with just one copy of the T-DNA inserted vector were selected and used for the root growth experiments.

Primers used for transformation:

1AA2-FW-AscI:

5'-TTGGCGCGCC ATGGCGTACGAGAAAGTCAATGAGCTTA-3' (SEQ ID NO:66)

1AA2-RW-BamHI:

5'-CGGGATCCTCATAAGGAAGAGTCATCAGATCCTTTCATGATTC-3' (SEQ ID NO:67)

The wild type *Arabidopsis* and *Arabidopsis* transformed with the wild type *Sisymbrium orientale* IAA2 were susceptible when treated with 200 g a.i. ha⁻¹ 2,4-D (Figure 8). *Arabidopsis* transformed with *Sisymbrium orientale* IAA2 $\Delta 27$ with the 27 base pair deletion, was resistant to 2,4-D. This confirms that the deletion within the degtron region of *Sisymbrium orientale* IAA2 was sufficient to provide resistance to 2,4-D.

Root assays and gene expression analysis of AUX/IAA2 after auxin treatment

Resistance was further confirmed using transformed *Arabidopsis* using a root growth assay. In this assay, germinated seedlings were placed on agar plates containing IAA, 2,4-D, or dicamba and new root growth measured after 7 days. *Arabidopsis* transformed with the null vector, not containing *S. orientale* IAA2 were susceptible to inhibition by 2,4-D, dicamba, and to a lesser extent higher concentrations of IAA (Figure 9). *Arabidopsis* transformed with the wild type *S. orientale* IAA2 had similar responses to 2,4-D and IAA compared with the null vector. However, these plants were slightly more tolerant to dicamba (Figure 9). *Arabidopsis* transformed with the mutant *S. orientale* IAA2 $\Delta 27$ showed higher tolerance in the root growth assay to 2,4-D, dicamba, clopyralid, and IAA compared with

either the null vector or wild type gene. From this assay, it is clear that the IAA2 Δ 27 can provide resistance to dicamba as well as 2,4-D. *S. orientale* RILs homozygous for the IAA2 Δ 27 showed higher tolerance to MCPA and fluorpyrauxifen-benzyl (Figure 9).

All root assays were performed on 120 mm square petri dishes with half MS and 1% phytoagar. Natural and synthetic auxins were bought from Caisson Labs (2,4-D, dicamba, and IAA) Stock solutions were made in DMSO at 1 mM and 0.001 mM and finally dissolved into 100 ml depending on the final concentration of the treatment. For the auxin treatments 2,4-D and dicamba were 0.01, 0.05, 0.1, 0.5, 1, 10 and 100 μ M; for IAA 0.1, 0.5, 1, 10, 100, and 1000 μ M; DMSO at 1 μ L mL⁻¹ was used for the control. For the mustard root assay, ten seedlings (3 days after germination) of F5 RILs of each phenotype were transferred to an auxin treated or control plate, the root tip of each plant was marked and root growth was evaluated seven days after treatment. For Arabidopsis, sterilized seeds were planted directly on the treated petri dish and incubated at 4 °C for seed scarification and moved to the growth chamber after 3 days. T3 homozygous plants of 35::SoIAA₂WT, 35:: SoIAA2 Δ 27, the T-DNA insertion line on *AtIAA₂* and the empty T-DNA from PFGC5941 plasmid as control were used in this study. Twelve plants per construct were used in total and roots were measured three weeks after the plates were moved to the growth conditions. For all root assays, seeds were gas sterilized for 6 hours using bleach and hydrochloric acid. The plants were grown under controlled conditions of 180 μ Mol m⁻² light intensity, relative humidity of 60%, 16 hours of day and day/night temperatures of 22/18 °C.

To study IAA2 expression under auxin treatments sterilized seeds from F5 RILs for each phenotype were planted in half MS and grown until expanded cotyledon stage (size was about 2 cm from root to cotyledon tips). Four plants of each phenotype were then dipped in different half MS liquid media containing DMSO as control, dicamba, IAA, or 2,4-D for 2 hr. After exposure plants were collected into 2 ml centrifuge tubes and frozen in liquid nitrogen. RNA was extracted using Direct-zol RNA Microprep from Zymo research, DNAase treatment was done using DNaseI from Invitrogen – ThermoFisher and cDNA was generated using iScript[™] according to each manufacture protocol. Q-PCR (CFX Connect TM Real-Time PCR Detection System thermal cycler, BioRad, Hercules, CA) was used to determine IAA2 expression. The qPCR reaction contained 12.5 μ l of SYBR Green (PerfeCTa SYBR Green FastMix), 1 μ l each of forward and reverse primers (5 μ M), and 1 μ L of cDNA for a final reaction volume of 25 μ l. Q-PCR parameters were 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 30 s and 60°C for 1 min. Relative expression quantification was

calculated by $\text{Expression} = 2^{\Delta C_t}$, where $\Delta C_t = C_t(\text{average of Actin2 and Cyclophilin}) - C_t(\text{IAA2})$. Each biological sample was run in two technical replicates.

Primers used for expression measurements:

SoIAA2_{WT}:

5 FW 5'-GAACAACAACAGTGTGAGCTATG-3' (SEQ ID NO:68)

RV 5'-GCCTTGAGAAGCTCTGGATAG-3' (SEQ ID NO:69)

SoIAA2 Δ 27:

FW 5'-CTCCGGTGAGATCTTATGTG-3' (SEQ ID NO:70)

RV 5'-CTCCGGTGAGATCTTATGTG-3' (SEQ ID NO:71)

10 *SoCyclophilin*

FW 5'-CATGTGCCAAGGAGGAGATT-3' (SEQ ID NO:72)

RV 5'-GTGTGCTTCCTCTCGAAGTT-3' (SEQ ID NO:73)

SoActin2

FW 5'-GTGGAACCACTATGTTCTCTGG-3' (SEQ ID NO:74)

15 RV 5'-GGAGGTGCAACGACCTTAAT-3' (SEQ ID NO:75)

Example 4: Surface Plasmon Resonance (SPR) affinity binding analysis

The mechanism whereby the deletion in IAA2 interferes with auxinic herbicide action was explored using surface plasmon resonance (SPR) assay. In short, this assay measures the binding of auxin and IAA to the receptor protein TIR1 and their unbinding. The assay showed that the presence of the mutant IAA2 Δ 27 resulted in slower association of the herbicide/IAA/TIR1 complex and more rapid dissociation of the complex (Figure 10). This was true in the presence of IAA, 2,4-D and dicamba. This shows that the mechanism of action of all three is impaired by the deletion.

25 Surface plasmon resonance (SPR) experiments were done according to published protocols. TIR1/AFB5 was expressed in insect cell culture using a recombinant baculovirus. The construct contained sequences for three affinity tags, namely 6 His, maltose-binding protein (MBP) and FLAG. Initial purification using the His tag was followed by clean-up using FLAG chromatography, the purified protein was used for SPR assays by passing it over a streptavidin chip loaded with biotinylated IAA2/IAA7 degron peptides.

The SPR buffer was Hepes-buffered saline with 10 mM Hepes, 3 mM EDTA, 150 mM NaCl and 0.05% Tween 20. Compounds to be tested were premixed with the protein to a final 50 μ M concentration. Binding experiments were run at a flow rate of 30 μ l min⁻¹ using 2.0 min of injection time and 4.0 min of dissociation time. Data from a control channel

(mIAA7) and from a buffer+DMSO-only run were subtracted from each sensogram following the standard double reference subtraction protocol.

Example 5: Mechanistic model of *S. orientale* IAA2_{Δ27}

5 A mechanistic model, which explains how the deletion within the degron would interfere with the action of auxinic herbicides is presented in Figure 11. The IAA protein and TIR1 protein form an auxin receptor complex where the auxin, or auxinic herbicide, is bound to the TIR1 protein just under the IAA protein. The complex is recognized by SCF^{TIR1} complexes that ubiquitinate lysine residues on the IAA protein leading to degradation of the IAA protein and subsequent signal transduction. The deletion in the degron region reduces the length of the degron tail between Domains II and III by approximately 28 Å. This impairs the ability of the IAA protein to interact with the TIR1 protein, because it is no longer long enough to associate with sites on the TIR1 protein and keep the herbicide at its binding site. The result is that the IAA/herbicide/TIR1 complex dissociates faster and the IAA protein is ubiquitinated more slowly. Failure to ubiquitinate the IAA protein results in preventing the rapid increase in gene expression of 2,4-D regulated genes that is normal in the presence of 2,4-D. This model suggests that the deletion in the IAA2 gene would likely result in varying levels of resistance to any herbicide that binds to the IAA2/TIR1 complex. Our current data shows this includes 2,4-D, MCPA, and dicamba.

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What is claimed is:

1. A method of producing a plant having tolerance to an herbicide, the method comprising producing a plant having a deleted or disrupted degron tail region of the IAA2 protein of said plant and having tolerance to said herbicide.
- 5 2. The method of claim 1, wherein said degron tail region comprises the region that aligns with amino acid residues 72 to 80 of SEQ ID NO: 1.
3. The method of claim 1, wherein said degron tail region comprises SEQ ID NO: 5.
4. The method of claim 1, wherein the plant comprises a nucleic acid having at least 80% sequence identity SEQ ID NO: 4, or a nucleic acid encoding a polypeptide
- 10 having at least 80% sequence identity to SEQ ID NO: 3.
5. The method of claim 1, wherein said herbicide is a synthetic auxin herbicide.
6. The method of claim 5, wherein said herbicide is selected from 2,4-dichlorophenoxyacetic acid, dicamba, dichlorprop, clopyralid, fluroxypyr, floryrauxifen-benzyl, and 2-methyl-4-chlorophenoxyacetic acid.
- 15 7. The method of claim 6, wherein said herbicide is 2,4-dichlorophenoxyacetic acid.
8. The method of claim 1, wherein said plant is a member of the Brassicaceae family.
9. The method of any of claims 1 – 8, wherein said plant is produced by introducing a heterologous nucleic acid encoding an IAA2 polypeptide comprising a deleted or disrupted degron tail region into said plant.
- 20 10. The method of any of claims 1 – 8, wherein said plant is produced by introducing a mutation into said degron tail region of the endogenous IAA2 protein in said plant.
11. The method of any of claims 1 – 8, wherein said plant is produced by crossing a plant comprising said degron tail region with a plant not comprising said degron tail region to produce a progeny plant comprising said deleted or disrupted degron tail region.
- 25 12. The method of claim 10, wherein said mutation of said degron tail region is introduced by gene editing.
13. The method of claim 12, wherein said gene editing is selected from a CRISPR/Cas process, a TALEN process, or a zinc finger nuclease process.
14. A plant or plant part or cell of a plant or progeny of a plant produced by any of the methods of claims 1 – 13, wherein said plant, plant part, cell or progeny comprises
- 30 said deleted or disrupted degron tail region.
15. Seed of a plant of claim 14.
16. An herbicide tolerant plant comprising a deleted or disrupted degron tail region of an IAA2 protein of said plant.

17. The herbicide tolerant plant of claim 16, wherein said degon tail region comprises the region that aligns with amino acid residues 72 to 80 of SEQ ID NO: 1.
18. The herbicide tolerant plant of claim 16, wherein said degon tail region comprises SEQ ID NO: 5.
- 5 19. The herbicide tolerant plant of claim 16, wherein the plant comprises a nucleic acid molecule having at least 80% sequence identity SEQ ID NO: 4, or a nucleic acid molecule encoding a polypeptide having at least 80% sequence identity to SEQ ID NO: 3.
- 10 20. The herbicide tolerant plant of claim 16, wherein said plant has tolerance to a synthetic auxin herbicide.
21. The herbicide tolerant plant of claim 20, wherein said herbicide is selected from 2,4-dichlorophenoxyacetic acid, dicamba, dichlorprop, clopyralid, fluroxypyr, floryrauxifen-benzyl, and 2-methyl-4-chlorophenoxyacetic acid.
- 15 22. The herbicide tolerant plant of claim 21, wherein said herbicide is 2,4-dichlorophenoxyacetic acid.
23. The herbicide tolerant plant of claim 16, wherein said plant is a member of the Brassicaceae family.
24. The herbicide tolerant plant of any of claims 16 – 23, wherein the plant comprises a heterologous nucleic acid encoding the IAA2 protein having a deleted or disrupted
20 degon tail region.
25. The herbicide tolerant plant of any of claims 16 – 23, wherein the plant comprises a mutation of said degon tail region in an endogenous IAA2 protein of said plant.
26. A method for controlling undesired vegetation at a plant cultivation site, the method comprising the steps of:
- 25 a. providing, at said site, the plant of any of claims 16 – 25 which is resistant or tolerant to an herbicide; and
- b. applying to said site an effective amount of said herbicide.
27. The method of claim 26, wherein the plant having the deleted or disrupted degon tail region of the IAA2 protein has no or reduced injury after applying said herbicide.
- 30 28. The method of claim 26, wherein said herbicide is a synthetic auxin herbicide.
29. The method of claim 28, wherein said herbicide is selected from 2,4-dichlorophenoxyacetic acid, dicamba, dichlorprop, clopyralid, fluroxypyr, floryrauxifen-benzyl, and 2-methyl-4-chlorophenoxyacetic acid.
30. The method of claim 29, wherein said herbicide is 2,4-dichlorophenoxyacetic acid.

31. A method for identifying a plant comprising a deleted or disrupted degron tail region of IAA2, said method comprising:
- a. obtaining a sample from said plant; and
 - b. assaying said plant for the absence or disruption of expression of a nucleic acid molecule encoding said degron tail or the deletion or disruption of said degron tail.
32. The method of claim 31, wherein said nucleic acid molecule comprises SEQ ID NO: 2 or 4 and/or said degron tail region comprises SEQ ID NO: 5.
33. The method of claim 31, wherein said assaying comprises hybridizing said nucleic acid sample with at least one probe or primer comprising at least 10 consecutive nucleotides of SEQ ID NO: 2 or 4, or with a complement of said probe or primer.
34. The method of claim 33, wherein said primer or probe is selected from SEQ ID NOs: 61-65.
35. A kit comprising a primer or probe that detects the presence or absence or disruption of a degron tail region of IAA2.
36. The kit of claim 35, wherein said primer or probe comprises at least 10 consecutive nucleotides of SEQ ID NO: 2 or 4, or with a complement of said probe or primer.
37. The kit of claim 35, wherein said primer or probe is selected from SEQ ID NOs: 61-65.
38. A substantially purified or isolated nucleic acid or nucleic acid fragment encoding an herbicide tolerant IAA2 protein, or complementary to a sequence encoding an herbicide tolerant IAA2 protein, said nucleic acid or nucleic acid fragment including a nucleotide sequence selected from the group consisting of:
- (a) the sequence shown in SEQ ID NO: 4;
 - (b) a nucleotide sequence encoding the polypeptide shown in SEQ ID NO: 3;
 - (c) complements of the sequences recited in (a) and (b);
 - (d) functionally active fragments of the sequences recited in (a), (b), and (c); and
 - (e) functionally active variants of the sequences recited in (a), (b), (c), and (d).
39. The nucleic acid of claim 38, wherein said functionally active variants have at least 80% sequence identity to the sequence recited in (a), (b), or (c), and wherein said functionally active fragments have a size of at least 100 nucleotides.
40. A genetic construct comprising a nucleic acid or nucleic acid fragment of claim 38
41. The genetic construct of claim 40, further comprising an operably linked heterologous regulatory sequence.

- 42. The genetic construct of claim 40, wherein said construct is a vector.
- 43. A plant, plant part, plant cell, or plant seed comprising the genetic construct of any of claims 40-42.

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MAYEKVNELNLKDTELRLGLPGTEQVKEEQEVSCVRSNKRQFQIDNEENREEEES
TPPTKTQIV**GWPPV**RSYRKNNNSVSYVKVSMDGAPYLRKIDLKTYKNYPELLKAL
ENMFKFTVGEYCEREGYKGSEVVPTYEDKDGDWMLVGDVPWDMFSSSCKRLRIMK
GSDDSSL

FIG.1

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KFK39590.1	-----	0
VVA98621.1	-----	0
XP_002865542.1	-----	0
ADL70486.1	-----	0
ADC29363.1	-----	0
NP_188943.1	-----	0
NP_001326722.1	MGGLKLRVPLLPSPCLFLAFHASNLSSEFNPEDAP--LYISLPFLQNSSTSPRIT	58
ADL70491.1	-----	0
AAB97164.1	-----	0
XP_006298679.1	-----	0
XP_010466600.1	-----	0
XP_018486538.1	-----	0
XP_018486510.1	-----	0
XP_018486509.1	-----	0
VDD45187.1	MGHSHVCVLPSPCLILAFHAPDNFYLSSFKPNFYASSPTFIYLSPISSKSLNQTHQOE	60
XP_013638497.1	-----	0
XP_022562911.1	MGHSHVCVLPSPCLILAFHAPDNFYLSSFKPNFYASSPTFIYLSPISSKSLNQTHQOE	60
CDY10692.1	-----	0
VDC71733.1	MGHSHVCVLPSPCLILAFHAPDNFYLSSFKPNFYASSPTFIYLSPISSKSLNQTHQOE	60
XP_009145242.1	-----	0
XP_022575253.1	MGHSHVCVLPSPCLILAFHAPDNFYLSSFKPNFYASSPTFIYLSPISSKSLNQTHQOE	60
RID71991.1	-----	0
XP_013625564.1	-----	0
VDC94217.1	MDGQSVYVFPSPCLILASHAPDNFYLSSFYPNFYASSPTFIYLSPISSSKTLTNKKNH	60
XP_022572324.1	-----	0
XP_018434635.1	-----MLFTSLSL-----FLTLT-----SMH-LPPVYLSLKLPINQ---T-H	36
XP_013636218.1	-----	0
XP_013751214.1	-----	0
XP_013643498.1	MGHSHVCVFPSPCLFLAFHAPDNLSSEFNPNFYASSPSCIYLSFKPINQ---T-H	55
CDY40837.1	-----	0
XP_013751366.1	-----	0
XP_009108345.1	-----	0
XP_006406101.1	-----	0
IAA2_full_protein_S	-----	0
IAA2_deletion_protein_R	-----	0

FIG. 2A

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KFK39590.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
VVA98621.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
XP_002865542.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
ADL70486.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
ADC29363.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
NP_188943.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
NP_001326722.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 113
ADL70491.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
AAB97164.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
XP_006298679.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
XP_010466600.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
XP_018486538.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
XP_018486510.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
XP_018486509.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 26
VDD45187.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_013638497.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 108
XP_022562911.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
CDY10692.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
VDC71733.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 108
XP_009145242.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_022575253.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 106
RID71991.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_013625564.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
VDC94217.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 116
XP_022572324.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_018434635.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 89
XP_013636218.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_013751214.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_013643498.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 109
CDY40837.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_013751366.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_009108345.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
XP_006406101.1 -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
IAA2_full_protein_S -----MAY-EKVNEINIKDTELRLGLPGTEEE 25
IAA2_deletion_protein_R -----MAY-EKVNEINIKDTELRLGLPGTEEE 25

```

FIG. 2B

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	Conserved "degron"	Deletion of "degron tail"
KFK39590.1	VKEQEVSVCVRNKRQFDEET---RDEESMPPTKTQIVGWPPVRSYRKN	74
VVA98621.1	VKEKEVSCVRNKRQFDEET---LNEESMPPTKTQIVGWPPVRSYRKN	75
XP_0028685542.1	IKEEQEVSVCVRNKRQFET---REHEESTPTTKTQIVGWPPVRSYRKN	74
ADL70486.1	IKEEQEVSVCVRNKRQFET---RDEESIPPTKTQIVGWPPVRSYRKN	74
ADC29363.1	IKEEQEVSVCVRNKRQFET---RDEESTPTTKTQIVGWPPVRSYRKN	74
NP_188943.1	IKEEQEVSVCVRNKRQFET---RDEESTPTTKTQIVGWPPVRSYRKN	74
NP_001326722.1	IKEEQEVSVCVRNKRQFET---RDEESTPTTKTQIVGWPPVRSYRKN	161
ADL70491.1	IKEEQEVSVCVRNKRQFET---RDEESTPTTKTQIVGWPPVRSYRKN	74
AAB97164.1	IKEEQEVSVCVRNKRQFET---RDEESTPTTKTQIVGWPPVRSYRKN	74
XP_006298679.1	IKEEQEVSVCVRNKRQFET---LDEESTPTTKTQIVGWPPVRSYRKN	73
XP_010466600.1	IKEEQEVSVCVRNKRQFET---RDEESTPTTKTQIVGWPPVRSYRKN	74
XP_018486538.1	VKEEQDVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_018486510.1	VKEEQDVSCVRNKRQFQSDNE---EESTTEFTKTQIVGWPPVRSYRKN	73
XP_018486509.1	VKEEQDVSCVRNKRQFQSDNE---EESTTPPTKTQIVGWPPVRSYRKN	81
VDD45187.1	VKEEQDVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	154
XP_013638497.1	VKEEQDVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_022562911.1	VKEEQDVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	152
CDY10692.1	VKEEQDVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
VDC71733.1	VKEKEVSCVRNKRQFQSDNE---EES-TEFTKTQIVGWPPVRSYRKN	154
XP_009145242.1	VKEKEVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_022573253.1	VKEKEVSCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	152
RID71991.1	AKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_013625564.1	AKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
VDC94217.1	AKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	162
XP_022572324.1	AKEEQEVSVCVRNKRQFQSDNE---EES-TEFTKTQIVGWPPVRSYRKN	71
XP_018434635.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	135
XP_013636218.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_013751214.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_013643498.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	155
CDY40837.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_013751366.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_009108345.1	DKEEQEVSVCVRNKRQFQSDNE---EES-TPPTKTQIVGWPPVRSYRKN	71
XP_006406101.1	NKKEQEVSVCVRNKRQFHECTCEQEEHEESTPTTKTQIVGWPPVRSYRKN	76
IAA2_full_protein_S	VKEQEVSVCVRNKRQFQDNEENREHEESTPTTKTQIVGWPPVRSYRKN	76
IAA2_deletion_protein_R	VKEQEVSVCVRNKRQFQDNEENREHEESTPTTKTQIVGWPPVRSYRKN	71

FIG. 2C

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Deletion of "degron tail"

```

KFK39590.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFPTTIGEYSEREGYK-GSG 129
VVA98621.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYQELLKALENMFPTTIGEYNREGEYKRGSG 132
XF_002885542.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVTIGEYCRREGYK-GSG 129
ADL70486.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
ADC29363.1  ---TSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
NF_188943.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
NF_001326722.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 216
ADL70491.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
AAB97164.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
XF_006298679.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 128
XF_010466600.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 216
XF_018486538.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
XF_018486510.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
XF_018486509.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 128
VDD45187.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 129
XF_013638497.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_022562911.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 130
CDY10692.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 140
VDC71733.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 210
XF_009145242.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 127
XF_022575253.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 208
R1D71991.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_013625564.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
VDC94217.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 217
XF_022572324.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_018434635.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 190
XF_013636218.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_013751214.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_013643498.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 210
CDY40837.1  ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_013751366.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_009108245.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 126
XF_006406101.1 ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 131
IAA2_full_protein_S ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 131
IAA2_deletion_protein_R ---NSVSVKVSMDGAPYLKIDLTQYKNYPPELLKALENMFVIMIGEYCRREGYK-GSG 122
***** *****; ****; ** :***; **; **

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FIG. 2D

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KFK39590.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
VVA98621.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 177
XF_002865542.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
ADL70486.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
ADC29363.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
NP_188943.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
NF_001326722.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 261
ADL70491.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
AAB97164.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_006298679.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_010466600.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_018486538.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_018486510.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_018486509.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
VDD45187.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_013638497.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_022562911.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
CDY10692.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
VDC71733.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_009145242.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_022573253.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
RID71991.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_013625564.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
VDC94217.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_022572324.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_018434635.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_013636218.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_013751214.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_013643498.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
CDY40837.1      EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_013751366.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_009108345.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
XF_006406101.1  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 174
IAA2_full_protein_S  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 172
IAA2_deletion_protein_R  EVPTYEDKDGWMLVGDVPWDMFSSCKRLRIMKGSAPALDSSL- 163
:.;*****

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FIG. 2E

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```

sunflower_HannXRQ_Chr10g0314921
grape_VIT_18s0001g08090
soybean_GLYMA_20G210400
coffea_GSCC_T00041837001
cotton_B456_005G04600
IAA2_full_protein_S
IAA2_deletion_protein_R
bean_PHAVB_007G109700g
sugarbeet_BVRB_5g120300
pepper_T459_18490
tomato_Solyc06g084070.3
potato_PSSC0003DMG400020139

-----GKGGKNNMEVAVGWPPVCSYRKRTII-----K
NETTENHTGTGNRSSAPATKAQVGVWPPPIKSFRRNTLATSKTEVD-----CKAGPCA
--NV--SKETLLDKPAKQVGVWPPVPSYRKNNMAVQKVSTEDVAEKTTSSTANPG
261
213
CEKK-PQVSAANGHAAAPAAKAQVGVWPPPIRSFRNNSMAINPFIDEDAD---AKLTGC
217
93
CGVN-GCKSDQDETAPPPKQIVGVWPPPIRSYRKNNIOTKKNE-----SEGGG
79
-----NEENREEESTPTKTIQVGVWPPVRS-----Y
71
-----NEENREEESTPTKTIQVGVWPPVRS-----
96
KG-----SNAPKTVVTEAPPAKAKIVGWPPPIRSYRKNSLQ-----NEGAG
102
STNN-SSVSEARREDCPEPSKTIQVGVWPPPIRSYRKNNLOEKTE-----TQASG
64
-----SMMINNDDEPAPKAQVGVWPPVPSYRKNVLQ-----A
60
-----SH--RRNNDPPQKAQVGVWPPVPSYRKNILE-----A
61
-----SH-----INDEPPKQVIGWPPVPSYRKNILE-----A
:****:*

MYVKVSMDCAPFLRKIDINGKNGYSDVFVMALEKIFG-----
109
LFVKVSMDCAPYLRKVDLNYSAQYQELSSALEKMFSCFTIQYVSHGAPGREMLSEKIK
321
AFVKVSMDCAPYLRKVDLTMYRSYKELSDALAKMFSSFTMGNYGAQGM--IDFMESKIM
271
LYVKVSMDCAPYLRKVLDIKIYSSYKDLSSALEKMFSCFTTIGQCGSHGVHIRDGNSESLM
277
134
LYVKVSMDCAPYLRKIDLNKIVSGYPRLLQAIENMFKFTIVL-----
134
SYVKVSMDCAPYLRKIDLTNNYPELLKALENMFNFTVGEYCER-----
124
--YVKVSMDCAPYLRKIDLTNNYPELLKALENMFNFTVGEYCER-----
115
LYVKVSMDCAPYLRKIDLVYGGYTGILLIAETMEKLTIGEYSER-----
141
MYVKVSMDCAPYLRKIDLNKIQSYAELLKLESMFRCIGKYSER-----
147
SYVKVSMDCAPYLRKIDLNKIVKSYPPQLLKALENMFKCSIGVYSER-----
109
SYVKVSMDCAPYLRKIDLTNYKSYPPQLLKALENMFKCSIDVYSER-----
105
SYVKVSMDCAPYLRKIDLNKIVKSYPPQLLKALENMFKCSIE---T-----
102
:*****:****: : a : : : :

```

FIG. 3

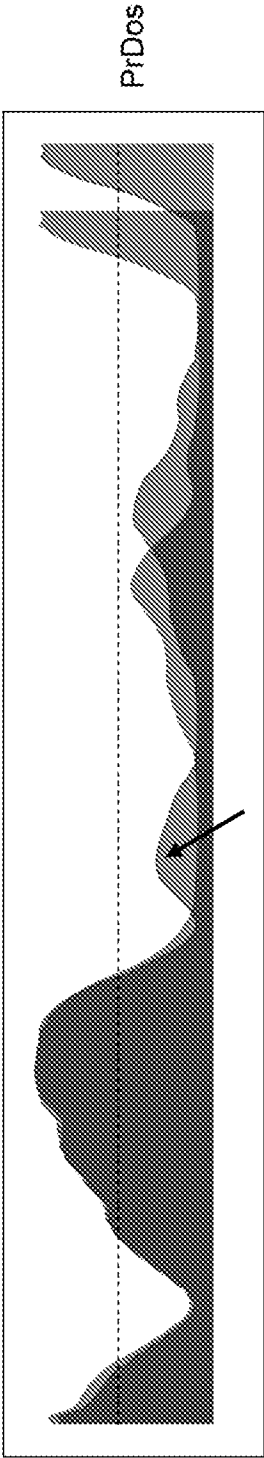


FIG. 4

	Fold change	Gene	Function
↓	0.1	PRP39-2	Tetratricopeptide repeat (TPR)-like superfamily protein
↓	0.3	AUX/IAA2	Auxin-responsive protein
↓	0.5	SNARE-like protein	Involved in vacuolar sorting and storage
↑	5.7	Glycoprotein ABCB13	Cellular auxin efflux-transporter

FIG. 5

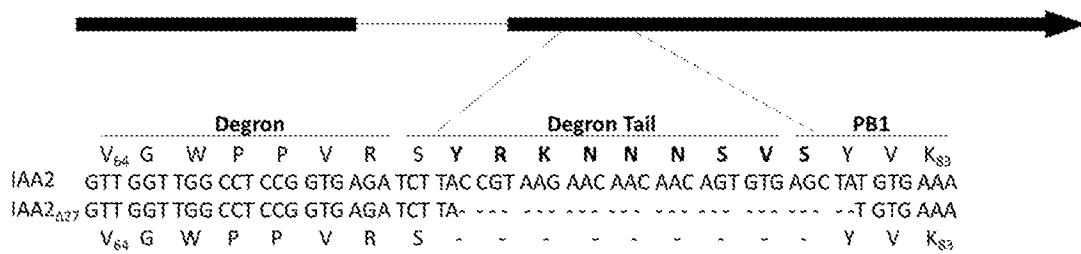


FIG. 6

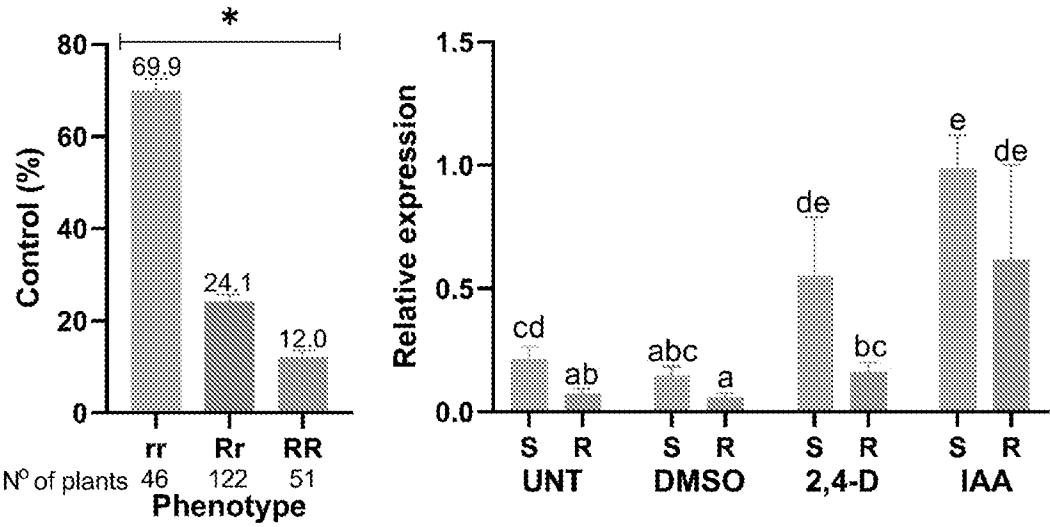


FIG. 7

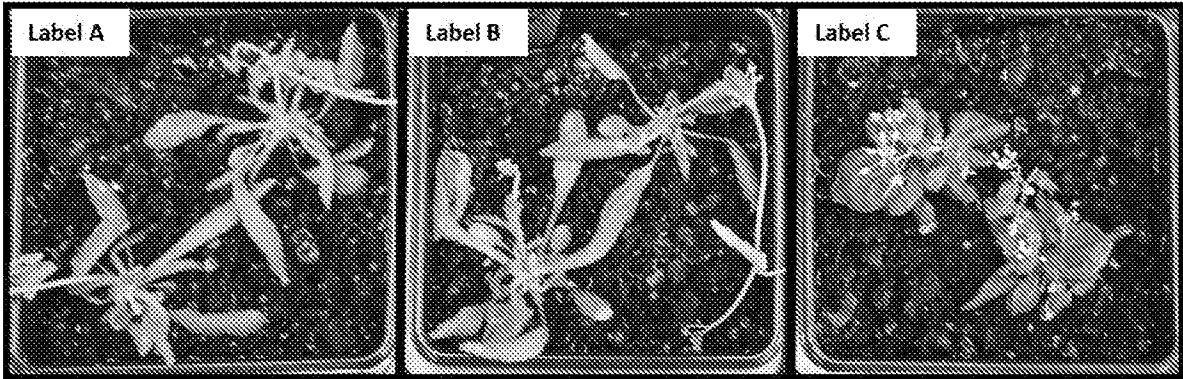


FIG. 8

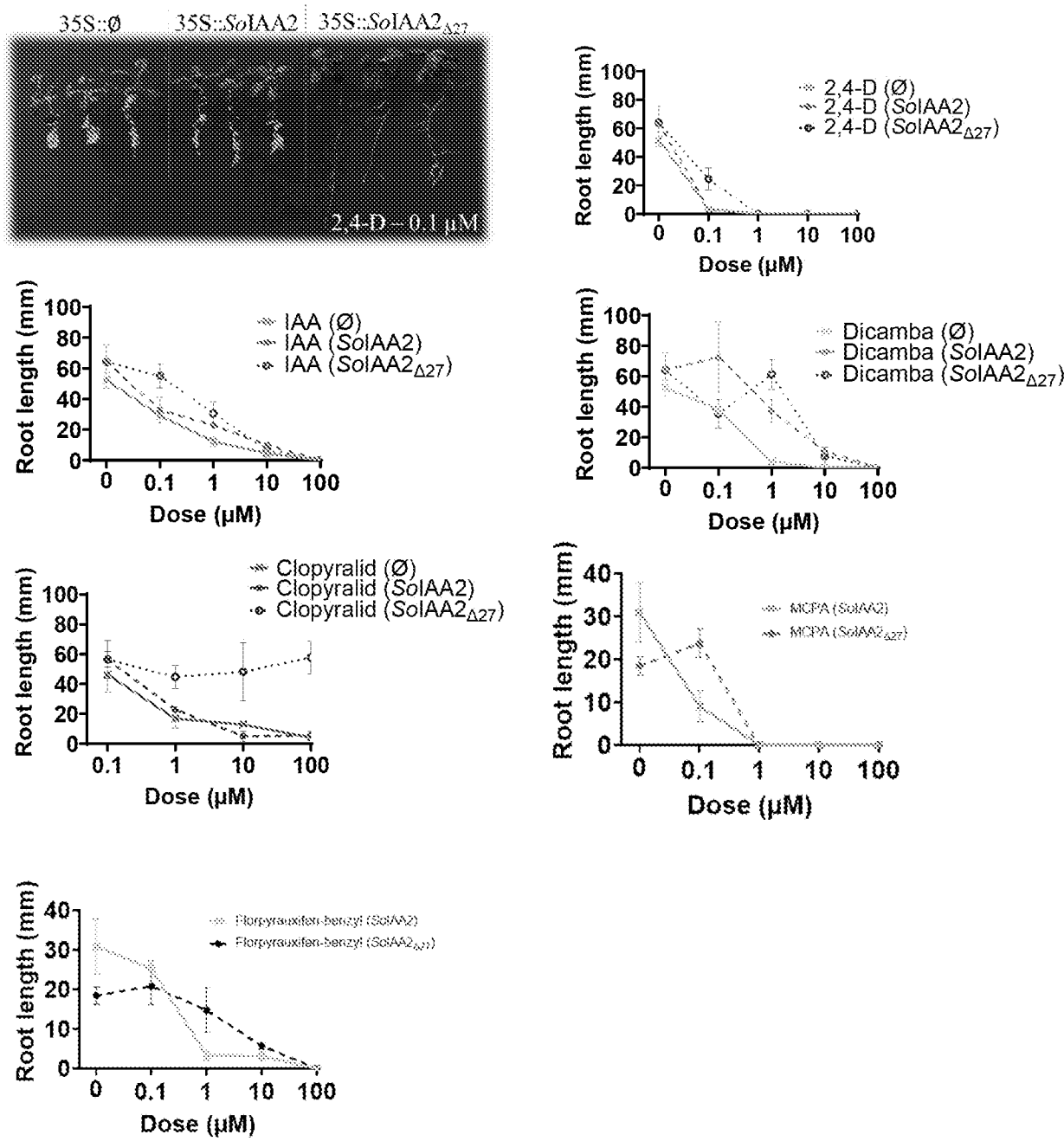


FIG. 9

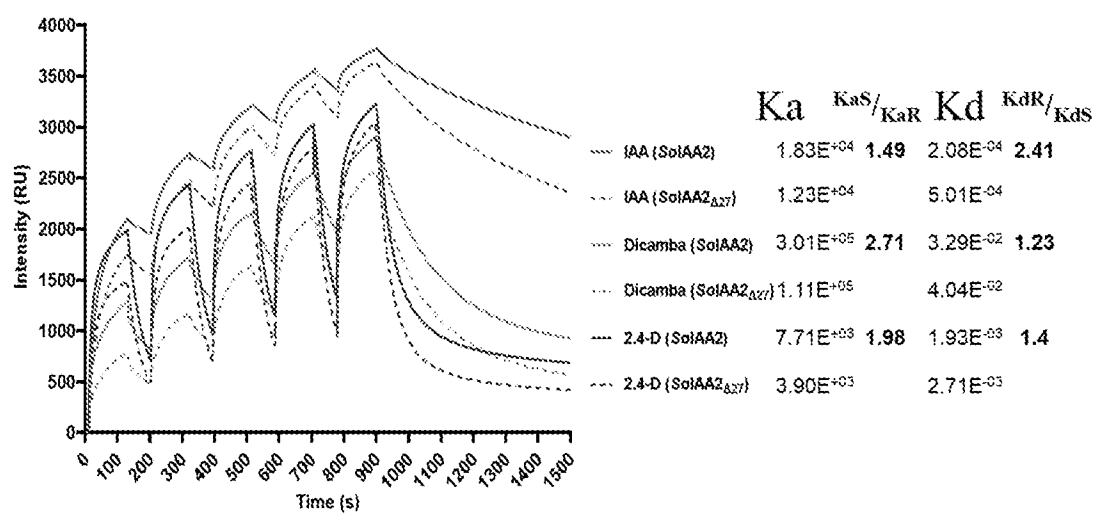


FIG. 10

13/13

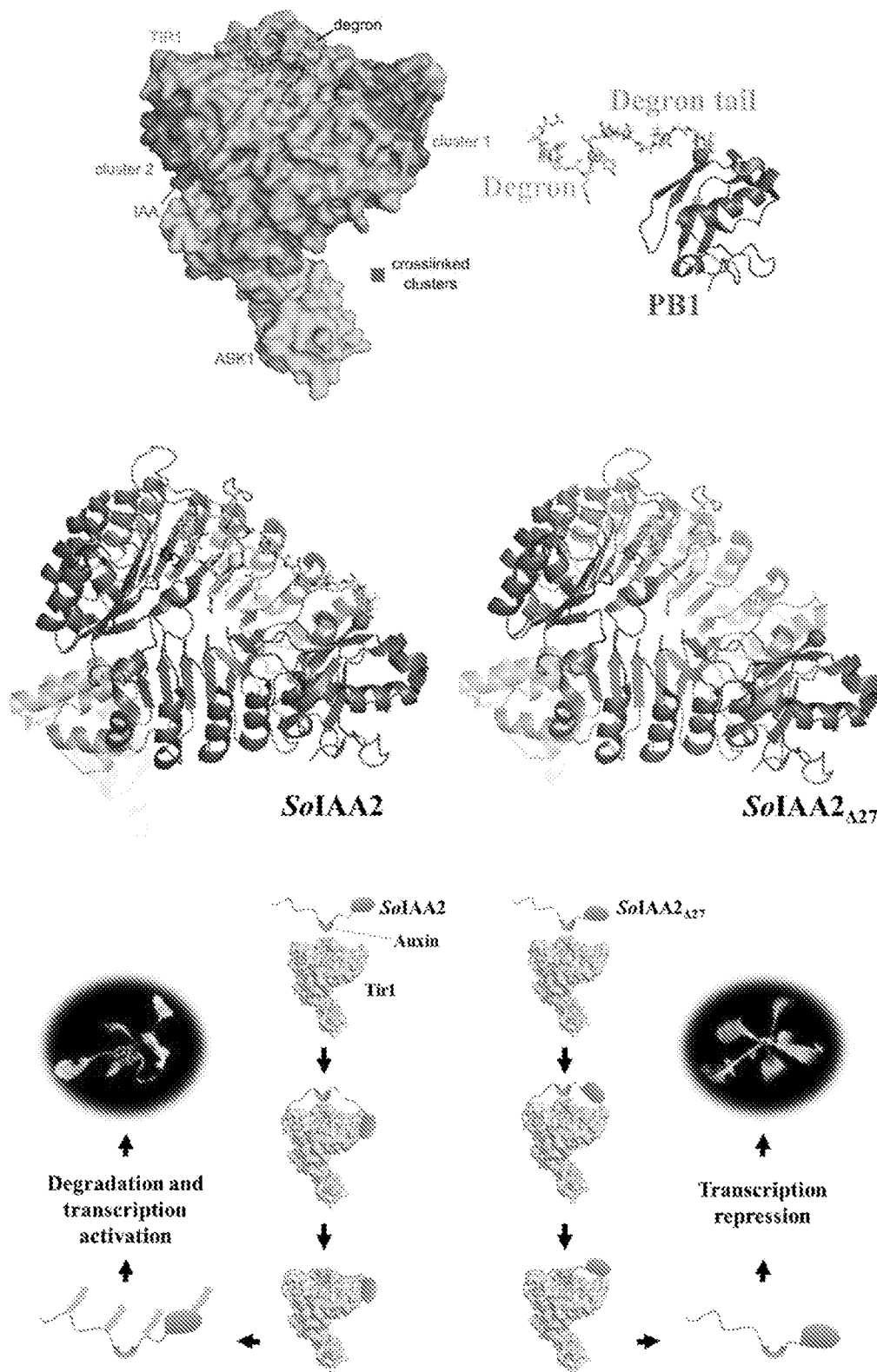


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/22118

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A01H 1/04, C07H 21/04, A01H 1/00, C12N 15/01, C12Q 1/6895 (2020.01)

CPC - C12N 15/8275, C07K 14/415, C12N 15/8241, C12N 15/8247, C12N 15/8273, C12N 15/8274, C12Q 1/6895, C12Q 2600/13, C12N 15/8261, C12N 2310/20, C12N 15/8213, Y02A 40/146

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LECLERE et al., Cross-resistance to dicamba, 2,4-D, and fluroxypyr in Kochia scoparia is endowed by a mutation in an AUX/IAA gene. PNAS, 12 March 2018, Vol. 115, No. 13, E2911-E2920. Abstract; pE2911, col 1, para 1; E2913, col 1, para 2; E2915, col 2, para 1-3	1, 5-8, (9-11)/(1, 5-8)
Y		2-4, (9-11)/(2-4), (12, 13)/(1-8)
Y	US 2009/0144847 A1 (SHAIKH et al.) 4 June 2009 (04.06.2009) Abstract; Claim 3; SEQ ID NO 2404; para [0008]	2, 4, (9-13)/(2, 4)
Y	US 2004/0216190 A1 (KOVALIC) 28 October 2004 (28.10.2004) Abstract; Claim 2; Claim 3; SEQ ID NO:6914	3, (9-13)/3
Y	US 2015/0082478 A1 (E I DU PONT DE NEMOURS AND COMPANY et al.) 19 March 2015 (19.03.2015) Abstract; para [0234]	(12, 13)/(1-8)

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 July 2020

Date of mailing of the international search report

07 AUG 2020

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/22118

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 14-15, 26-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

---Please see continuation in first extra sheet -----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US 20/22118

Continuation of Box No. III. Observations where unity of invention is lacking.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-13, directed to a method of producing a plant having tolerance to a herbicide.

Group II, claims 16-25, directed to a herbicide tolerant plant comprising a deleted or disrupted degron tail region of an IAA2 protein of said plant.

Group III, claims 31-34, directed to a method for identifying a plant comprising a deleted or disrupted degron tail region of IAA2.

Group IV, claims 35-37, directed to a kit for detecting presence or absence or disruption of a degron tail region of IAA2.

Group V, claims 38-43, directed to a substantially purified or isolated nucleic acid or nucleic acid fragment encoding a herbicide tolerant IAA2 protein.

The inventions listed as Groups I-V do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features:

Group I has the special technical feature of a method of producing a plant having tolerance to a herbicide, that is not required by Groups II-V.

Group II has the special technical feature of a herbicide tolerant plant comprising a deleted or disrupted degron tail region of an IAA2 protein, that is not required by Groups I and III-V.

Group III has the special technical feature of method for identifying a plant comprising a deleted or disrupted degron tail region of IAA2, that is not required by Groups I-II and IV-V.

Group IV has the special technical feature of a kit for detecting presence or absence or disruption of a degron tail region of IAA2, that is not required by Groups I-III and V.

Group V has the special technical feature of a substantially purified or isolated nucleic acid or nucleic acid fragment encoding a herbicide tolerant IAA2 protein, that is not required by Groups I-IV.

Common technical features:

Groups I-V share the common technical feature of a plant comprising a deleted or disrupted degron tail region of IAA2. However, this shared technical feature does not represent a contribution over prior art, because this shared technical feature is anticipated by the article entitled "Cross-resistance to dicamba, 2,4-D, and fluroxypyr in *Kochia scoparia* is endowed by a mutation in an AUX/IAA gene" by LeClere et al., (PNAS, 27 March 2018, Vol. 115, No. 13, E2911-E2920) (hereinafter 'LeClere').

LeClere teaches a herbicide resistant plant comprising a mutation in the degron tail region of an IAA gene (Abstract - 'We have further characterized the auxin responses of one previously reported dicamba-resistant biotype isolated from western Nebraska and found that it is additionally cross-resistant to other auxin herbicides, including 2,4-dichlorophenoxyacetic acid (2,4-D) and fluroxypyr. We have utilized transcriptome sequencing and comparison to identify a 2-nt base change in this biotype, which results in a glycine to asparagine amino acid change within a highly conserved region of an AUX/indole-3-acetic acid (IAA) protein, KsIAA16. Through yeast two-hybrid analysis, characterization of F2 segregation, and heterologous expression and characterization of the gene in *Arabidopsis thaliana*, we show that that the single dominant KsIAA16R resistance allele is the causal basis for dicamba resistance in this population'; E2913, col 1, para 2 - 'The identified difference is a GG to AA nucleotide change, which results in a glycine (G) to asparagine (N) amino acid substitution within the highly conserved degron region (GWPPV/I) of a homolog of IAA16 (Fig. 2 A and C). This degron region has been shown to be indispensable for auxin binding and interaction between the TIR/F-box proteins and AUX/IAA proteins (17, 21)'). Since LeClere teaches mutation of a single amino acid in a highly conserved region of auxin degron disrupts its function, resulting in herbicide resistance, it would have been obvious to one of ordinary skill in the art that disruption or deletion of this auxin protein region could also be expected to produce a similar outcome because auxin binding to TIR/F-box would be disrupted. Further, since LeClere also teaches additional IAA genes including IAA2 from a phylogenetic analysis, it would have been obvious to one of ordinary skill in the art to screen additional IAA proteins such as IAA2 to determine the effect of deletion or disruption of auxin degron tail region on herbicide resistance since different genera may use different auxin proteins to achieve herbicide resistance.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-V inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of number 4 above: claims 14-15, 26-30 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).