GENETICALLY MODIFIED PLANTS AND METHODS OF MAKING THE SAME

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/909,074, filed October 1, 2019, which is entirely incorporated herein by reference.

BACKGROUND

[0002] Naturally occurring components in cannabis may impact the efficacy of therapy and any potential side effects. Accordingly, cannabis plants having a modified therapeutic component(s) profile may be useful in the production of cannabis and/or may also be useful in the production of genetically modified cannabis providing a desired drug profile.

SUMMARY

[0002a] In one aspect, provided herein is a transgenic plant that comprises an endonuclease-mediated stably inherited genomic modification of a tetrahydrocannabinol acid synthase (THCAS) gene, the modification resulting in the at least one of the following a-d: a. increased cannabidiol (CBD) in the transgenic plant as compared to a comparable control plant without the modification, b. less than 0.5% by weight of tetrahydrocannabinol (THC) in the transgenic plant as measured by dry weight of the transgenic plant, c. a CBD to THC ratio in the transgenic plant of at least about 25:1, d. reduced or suppressed expression of the THCAS gene in the transgenic plant; wherein the transgenic plant comprises an unmodified endogenous cannabidiolic acid synthase (CBDAS) gene.

[0003] Provided herein is a transgenic plant that comprises an endonuclease-mediated stably inherited genomic modification of a tetrahydrocannabinol acid synthase (THCAS) gene. In some cases, a modification can result in increased cannabidiol (CBD) as compared to a comparable control plant without a modification and wherein the transgenic plant comprises less than 1% of tetrahydrocannabinol (THC) as measured by dry weight. Provided herein is also a transgenic plant comprising an endonuclease mediated genetic modification of a tetrahydrocannabinol acid synthase (THCAS) gene that results in a cannabidiol (CBD) to tetrahydrocannabinol (THC) ratio in the transgenic plant of at least 25: 1 as measured by dry weight. In some cases, a modification reduces or suppresses expression of a THCAS gene.

[0004] In some cases, a transgenic plant described herein comprises a modification that completely reduces or suppresses a CBDAS gene. In some cases, a transgenic plant with increased CBDAS production, comprises an unmodified CBDAS gene. In some cases, a transgenic plant comprises an unmodified endogenous cannabidiolic acid synthase (CBDAS) gene. In some cases, a transgenic plant comprises at least 25% more CBD as measured by dry weight as compared to a comparable control plant without a modification. In some cases, a transgenic plant comprises at least 50% more CBD as measured by dry weight as compared to a comparable control plant without a modification.

[0005] In some instances, a transgenic plant, described herein, contains less than 0.05% of THC as measured by dry weight. In some cases, a transgenic plant comprises a CBD to THC ratio of at least 25:1,

26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 40:1, 45:1, or up to about 50:1 as measured by dry weight. In some cases, a transgenic plant comprises 0% THC or an untraceable amount of THC as measured by dry weight as compared to a comparable control plant without a modification.

[0006] In some cases, a transgenic plant as described herein is modified by use of an endonuclease wherein the endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme, transcription activator-like effector (TALE)-Nuclease, transposon-based nuclease, Zinc finger nuclease, argonaute, meganuclease, or Mega-TAL. In some cases, an endonuclease can be a CRISPR enzyme or argonuate enzyme which can complex with a guide polynucleotide. In some cases, a guide

polynucleotide can be a guide RNA or guide DNA. In some cases, a gRNA or gDNA can comprise a sequence that is complementary to a target sequence, or a sequence on a complementary strand to a target sequence in a THCAS gene. In some cases, a guide polynucleotide binds a THCAS gene sequence. In some cases, a CRISPR enzyme complexed with a guide polynucleotide can be introduced into a transgenic plant as a ribonuclear protein (RNP). In some cases, a guide polynucleotide can be chemically modified. In some cases, a CRISPR enzyme and a guide polynucleotide can be introduced into a transgenic plant by a vector comprising a nucleic acid encoding a CRISPR enzyme and a guide polynucleotide. In some cases, a vector can be a binary vector or a Ti plasmid. In some cases, a vector further comprises a selection marker or a reporter. In some cases, an RNP or vector can be introduced into a transgenic plant via electroporation, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation.

[0007] In some cases, a transgenic plant or cell thereof further comprises a donor polynucleotide. In some cases, a donor polynucleotide comprises homology to sequences flanking a target sequence. In some cases, a donor polynucleotide introduces a stop codon into a THCAS gene. In some cases, a donor polynucleotide comprises a barcode, a reporter, or a selection marker. In some instances, a guide polynucleotide is a single guide RNA (sgRNA). In some cases, a guide polynucleotide can be a chimeric single guide comprising RNA and DNA. In some ~~embodiments~~examples, a target sequence can be at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length. In some cases, a target sequence can be at most 17 nucleotides in length. In some cases, a CRISPR enzyme is Cas9. In some cases, Cas9 recognizes a canonical PAM. In some cases, Cas9 recognizes a non-canonical PAM. In some cases, a guide polynucleotide binds a target sequence from 3-10 nucleotides from a protospacer adjacent motif (PAM). In some cases, a target sequence comprises a sequence complementary to a sequence selected from the group consisting of SEQ ID NOs: 24-34. In some cases, a guide polynucleotide comprises a sequence that comprises at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about 100% identity to a sequence selected from the group consisting of SEQ ID NOs 21-34. In some cases, a modification comprises an insertion, a deletion, a substitution, or a frameshift. In some cases, a modification is in a coding region of a THCAS gene. In some cases, a modification can be in a regulatory region of a THCAS gene. In some instances, a plant is a cannabis plant. In some instances, a modification results in up to about 50% of indel formation. In some cases, a modification results in less than or up to about 25%, less than or up to about 15%, less than or up to about 10%, or less than or up to about 1% of indel formation.

[0008] Provided herein is a method for generating a transgenic plant, the method comprising (a) contacting a plant cell comprising a tetrahydrocannabinol acid synthase (THCAS) gene with an endonuclease or a polynucleotide encoding the endonuclease, wherein the endonuclease introduces a stably inherited genomic modification in the THCAS gene; (b) culturing the plant cell with a modification in THCAS gene thereby generating a transgenic plant, wherein the modification results in increased cannabidiol (CBD) as compared to a comparable control plant without the modification and less than 1% of tetrahydrocannabinol (THC) in the transgenic plant as measured by dry weight. Provided herein is also

a method for generating a transgenic plant, the method comprising (a) contacting a plant cell comprising a THCAS gene with an endonuclease or a polynucleotide encoding the endonuclease, wherein the endonuclease introduces a genetic modification in the tetrahydrocannabinol acid synthase (THCAS) gene; (b) culturing the plant cell with a modification in THCAS gene thereby generating a transgenic plant, wherein the modification results in a cannabidiol (CBD) to tetrahydrocannabinol (THC) ratio in the transgenic plant of at least 25: 1 as measured by dry weight. In some cases, contacting can be via electroporation, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation. In some ~~aspects~~examples, a method further comprises culturing a plant cell in with a modification in THCAS gene to generate a callus, a cotyledon, a root, a leaf, or a fraction thereof of the transgenic plant. In some cases, a modification reduces or suppresses expression of a THCAS gene. In some cases, a modification does not alter a cannabidiolic acid synthase (CBDAS) gene in a transgenic plant. In some cases, a modification results in at least 25% more CBD measured by dry weight in a transgenic plant as compared to a comparable control plant without a modification. In some ~~aspects~~examples, a modification results in at least 50% more CBD as measured by dry weight in a transgenic plant as compared to a comparable control plant without a modification. In some ~~aspects~~examples, a modification results in less than 0.05% of THC in a transgenic plant as measured by dry weight. In some cases, a modification results in a CBD to THC ratio of at least 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 40:1, 45:1, or up to about 50:1 as measured by dry weight. In some instances, a transgenic plant an contain 0% THC or an untraceable amount of THC as measured by dry weight as compared to a comparable control plant without a modification. In some cases, an endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme, transcription activator-like effector (TALE)-nuclease, transposon-based nuclease, Zinc finger nuclease, meganuclease, argonaute, or Mega-TAL. In some cases, an endonuclease can be a CRISPR enzyme or argonaute enzyme complexed with a guide polynucleotide that can be complementary to a target sequence in a THCAS gene. In some cases, a CRISPR enzyme complexed with a guide polynucleotide (RNP) or a CRISPR enzyme and a guide polynucleotide can be contacted with a plant cell. In some instances, a guide polynucleotide can be chemically modified. In some instances, a CRISPR enzyme complexed with a guide polynucleotide can be contacted with a plant cell. In other instances, a plant cell is contacted with a vector comprising a nucleic acid encoding a CRISPR enzyme and a guide polynucleotide. In some cases, a vector can be a binary vector or a Ti plasmid. In some cases, a vector further comprises a selection marker or a reporter. In some cases, a method further comprises contacting a plant cell with a donor polynucleotide. In some cases, a donor polynucleotide comprises homology to sequences flanking a target sequence. In some ~~aspects~~examples, a donor polynucleotide introduces a stop codon into a THCAS gene. In some cases, a donor polynucleotide comprises a barcode, a reporter, or a selection marker. In some cases, a guide polynucleotide can be a single guide RNA (sgRNA). In some cases, a guide polynucleotide can be a chimeric single guide comprising RNA and DNA. In some cases, a target sequence can be at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length. In some cases, a target sequence can be at most 17 nucleotides in length. In some cases, a CRISPR enzyme can be

comprises a sequence that binds a THCAS gene sequence. In some cases, a CRISPR enzyme complexed with a guide polynucleotide forms an RNP and is introduced into a genetically modified cell. In some cases, a guide polynucleotide is a chemically modified. In some cases, a CRISPR enzyme and a guide polynucleotide are introduced into a cell by a vector comprising a nucleic acid encoding a CRISPR enzyme and a guide polynucleotide. In an ~~aspect~~example, a vector is a binary vector or a Ti plasmid. In an ~~aspect~~example, a vector further comprises a selection marker or a reporter. In an ~~aspect~~example, an RNP or vector is introduced into a cell via electroporation, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation. In an ~~aspect~~example, a cell further comprises a donor polynucleotide. In some cases, a donor polynucleotide comprises homology to sequences flanking the target sequence. In some cases, a donor polynucleotide introduces a stop codon into the THCAS gene. In some cases, a donor polynucleotide comprises a barcode, a reporter, or a selection marker. In some cases, a guide polynucleotide can be a single guide RNA (sgRNA). In some cases, a guide polynucleotide is a chimeric single guide comprising RNA and DNA. In some cases, a target sequence is at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length. In some cases, a target sequence is at most 17 nucleotides in length. In some cases, a CRISPR enzyme can be a Cas9. In an ~~aspect~~example, Cas9 recognizes a canonical protospacer adjacent motif (PAM). In an ~~aspect~~example, Cas9 recognizes a non-canonical PAM. In some cases, a guide polynucleotide binds a target sequence 3-10 nucleotides from PAM. In some cases, a guide polynucleotide hybridizes with a target sequence within the THCAS gene selected from the group consisting of SEQ ID NOs 21-34 or a complementary thereof. In some cases, a guide polynucleotide comprises a sequence that comprises at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about 100% identity to a sequence selected from the group consisting of SEQ ID NOs 21-34. In some cases, a modification comprises an insertion, a deletion, a substitution, or a frameshift. In some cases, a modification is in a coding region of the THCAS gene. In some cases, a modification is in a regulatory region of the THCAS gene. In some cases, a modification results in at least or up to about 50% of indel formation. In some cases, a modification results in less than or up to about 25%, less than or up to about 15%, less than or up to about 10%, or less than or up to about 1% of indel formation.

[0010] Provided herein is a tissue comprising the genetically modified cell of any one of the claims 78-119. In an ~~aspect~~example, a tissue is a cannabis plant tissue. In an ~~aspect~~example, a tissue is a callus tissue. In an ~~aspect~~example, a tissue contains less than 1% of THC. In an ~~aspect~~example, a tissue contains less than 0.05% of THC. In an ~~aspect~~example, a tissue contains 0% THC or an untraceable amount thereof. In some cases, a tissue comprises at least 25% more CBD measured by dry weight as compared to a comparable control tissue without a modification. In some cases, a tissue comprises at least 50% more CBD measured by dry weight as compared to a comparable control tissue without a modification.

[0011] Provided herein is a plant comprising a tissue. In some cases, a plant comprises at least 25% more CBD measured by dry weight as compared to a comparable control plant without a modification. In some cases, a plant comprises at least 50% more CBD measured by dry weight as compared to a comparable control plant without a modification. In some cases, a plant is a cannabis plant.

[0012] Provided herein is a method for increasing cannabidiol (CBD) production in a plant cell, the method comprising introducing an endonuclease mediated genomic modification into a tetrahydrocannabinol acid synthase (THCAS) gene of the plant cell, thereby minimizing THCAS expression and increasing CBD production of the plant cell as compared to a comparable control cell without the modification. In some cases, a modification reduces or suppresses expression of a THCAS gene. In some cases, a plant comprises an unmodified endogenous CBDAS gene. In some cases, a modification results in at least 25% more CBD in a plant cell as compared to a comparable control cell without a modification. In some cases, a modification results in a CBD to THC ratio of at least 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 40:1, 45:1, or up to about 50:1 in a plant cell. In some cases, a modification results in at least 99% reduction of THC in a plant cell as compared to a comparable control cell without a modification. In some cases, a modification results in at least 99.9% reduction of THC in a plant cell as compared to a comparable control cell without a modification. In an ~~aspect~~example, an endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme, transcription activator-like effector (TALE)-nuclease, transposon-based nuclease, Zinc finger nuclease, argonaute, meganuclease, or Mega-TAL. In an ~~aspect~~example, an endonuclease is a CRISPR enzyme or argonaute enzyme complexed with a guide polynucleotide that comprises a sequence that binds a target sequence within or adjacent to a THCAS gene. In some cases, a guide polynucleotide binds a portion of a THCAS sequence. In some cases, a guide polynucleotide comprises a sequence that binds a THCAS gene sequence. In some cases, a CRISPR enzyme complexed with a guide polynucleotide forms an RNP that can be introduced into a plant cell. In some cases, a guide polynucleotide is a chemically modified. In some cases, a CRISPR enzyme and a guide polynucleotide are introduced into a plant cell by a vector comprising a nucleic acid encoding a CRISPR enzyme and a guide polynucleotide. In some cases, a vector is a binary vector or a Ti plasmid. In some cases, a vector further comprises a selection marker or a reporter. In an ~~aspect~~example, an RNP or vector can be introduced into a plant cell via electroporation, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation. In some cases, a method further comprises introducing a donor polynucleotide into a plant cell. In an ~~aspect~~example, a donor polynucleotide comprises homology to sequences flanking a target sequence. In some cases, a donor polynucleotide introduces a stop codon into a THCAS gene. In some cases, a donor polynucleotide comprises a barcode, a reporter, or a selection marker. In some cases, a guide polynucleotide is a single guide RNA (sgRNA). In an ~~aspect~~example, a guide polynucleotide is a chimeric single guide comprising RNA and DNA. In some cases, a target sequence is at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length. In some cases, a target sequence is at most 17 nucleotides in length. In some cases, a CRISPR enzyme can be a Cas9. In some cases, Cas9 recognizes a canonical PAM. In some cases, Cas9 recognizes a non-canonical PAM. In some cases, a guide polynucleotide binds a target sequence from 3-10 nucleotides from a PAM. In some cases, a guide polynucleotide binds a target sequence within a THCAS gene, or binds a sequence complementary to a target sequence within a THCAS gene. In some cases, a guide polynucleotide comprises a sequence comprising from about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about

100% identity to a sequence selected from the group consisting of SEQ ID NOs 21-34. In an ~~aspect~~example, a modification comprises an insertion, a deletion, a substitution, or a frameshift. In an ~~aspect~~example, a modification is in a coding region of the THCAS gene. In an ~~aspect~~example, a modification is in a regulatory region of the THCAS gene. In an ~~aspect~~example, a plant cell is a cannabis plant cell. In some cases, a method further comprises culturing a plant cell to generate a plant tissue. In some cases, a method further comprises culturing a plant tissue to generate a plant. In some cases, a plant contains less than 0.01% of THC measured by dry weight. In some cases, a plant comprises a ratio of CBD to THC of at least 25:1 measured by dry weight. In some cases, a plant comprises at least 25% more CBD measured by dry weight as compared to a comparable control plant without a modification. In some cases, a modification results in at least or up to about 50% of indel formation. In an ~~aspect~~example, a modification results in less than or up to about 25%, less than or up to about 15%, less than or up to about 10%, or less than or up to about 1% of indel formation.

[0013] Provided herein is a composition comprising an endonuclease or a polynucleotide encoding an endonuclease, wherein an endonuclease preferentially binds a tetrahydrocannabinol acid synthase (THCAS) gene over a cannabidiolic acid synthase (CBDAS) gene and is capable of introducing a modification into a THCAS gene, wherein a modification reduces or abrogates expression of a THCAS gene. In some cases, a modification reduces or suppresses expression of the THCAS gene. In an ~~aspect~~example, a modification comprises an insertion, a deletion, a substitution, or a frameshift. In an ~~aspect~~example, a modification is in a coding region of the THCAS gene. In some cases, a modification is in a regulatory region of the THCAS gene. In some cases, an endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme, transcription activator-like effector (TALE)-nuclease, transposon-based nuclease, Zinc finger nuclease, argonaute, meganuclease, or Mega-TAL. In some cases, an endonuclease is a CRISPR enzyme or argonaute enzyme complexed with a guide polynucleotide that comprises a sequence that binds a target sequence within or adjacent to a THCAS gene. In some cases, a guide polynucleotide binds a portion of a THCAS sequence. In some cases, a guide polynucleotide comprises less than 50% identity to a CBDAS gene. In some cases, a CRISPR enzyme complexed with a guide polynucleotide forms a ribonuclear protein (RNP). In some cases, a guide polynucleotide are encoded by a vector. A vector can be a binary vector or a Ti plasmid. In some instances, a vector further comprises a selection marker or a reporter. In some instances, an RNP or vector can be introduced into a plant cell provided herein via electroporation, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation. In some cases, composition provided herein further comprises a donor polynucleotide. In some cases, a donor polynucleotide comprises homology to sequences flanking the target sequence. In some cases, a donor polynucleotide introduces a stop codon into a THCAS gene. In some cases, a donor polynucleotide comprises a barcode, a reporter, or a selection marker. In some cases, a guide polynucleotide is a single guide RNA (sgRNA). In some cases, a guide polynucleotide is a chimeric single guide comprising RNA and DNA. In some cases, a target sequence is at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length. In some cases, a target sequence is at most 17 nucleotides in length. In an ~~aspect~~example, a

CRISPR enzyme can be Cas9. In some cases, Cas9 recognizes a canonical PAM. In some cases, Cas9 recognizes a non-canonical PAM. In some cases, a guide polynucleotide binds a target sequence from 3-10 nucleotides from a PAM. A target sequence can comprise a sequence complementary to a sequence selected from the group consisting of SEQ ID NOs 21-34. In some cases, a guide polynucleotide comprises a sequence comprising from about 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about 100% identity to a sequence selected from the group consisting of SEQ ID NOs 21-34. In some cases, a modification comprises an insertion, a deletion, a substitution, or a frameshift. In some cases, a modification is in a coding region of the THCAS gene. In some cases, a modification is in a regulatory region of the THCAS gene.

[0014] Provided herein is a kit for genome editing comprising a composition provided herein.

[0015] Provided herein is a cell comprising a composition provided herein. A cell can be a plant cell, an agrobacterium cell, a E. coli cell, or a yeast cell. In some cases, a cell is a plant cell. In some cases, a cell is a cannabis plant cell. In some cases, a cell is a callus cell, a protoplast, an embryonic cell, a leaf cell, a seed cell, a stem cell, or a root cell.

[0016] Provided herein is a plant comprising a cell provided herein.

[0017] Provided herein is a pharmaceutical composition comprising a transgenic plant or a derivative or extract thereof. Also provided herein is a genetically modified cell and/or a tissue. In some cases, a pharmaceutical composition further comprises a pharmaceutically acceptable excipient, diluent, or carrier. A pharmaceutically acceptable excipient can be a lipid.

[0018] Provided herein is a nutraceutical composition comprising a transgenic plant or a derivative or extract thereof. Provided herein is also a nutraceutical composition comprising a genetically modified cell or a tissue.

[0019] Provided herein is a food supplement comprising a transgenic plant or a derivative or extract thereof. Provided herein is also a genetically modified cell or a tissue. In some ~~aspects~~examples a nutraceutical composition or a food supplement can be in an oral form, a transdermal form, an oil formulation, an edible food, or a food substrate, an aqueous dispersion, an emulsion, a solution, a suspension, an elixir, a gel, a syrup, an aerosol, a mist, a powder, a tablet, a lozenge, a gel, a lotion, a paste, a formulated stick, a balm, a cream, or an ointment.

[0020] Provided herein is a method of treating a disease or condition comprising administering a pharmaceutical composition, a nutraceutical composition, or a food supplement to a subject in need thereof. In some cases, a disease or condition is selected from the group consisting of anorexia, emesis, pain, inflammation, multiple sclerosis, Parkinson's disease, Huntington's disease, Tourette's syndrome, Alzheimer's disease, epilepsy, glaucoma, osteoporosis, schizophrenia, cardiovascular disorders, cancer, and obesity.

CLAIMS

WHAT IS CLAIMED IS:

1. A transgenic plant that comprises an endonuclease-mediated stably inherited genomic modification of a tetrahydrocannabinol acid synthase (THCAS) gene, ~~said modification resulting in increased cannabidiol (CBD) as compared to a comparable control plant without said modification and less than 0.5% of tetrahydrocannabinol (THC) in said transgenic plant as measured by dry weight.~~ the modification resulting in the at least one of the following a-d:

~~2. A transgenic plant comprising an endonuclease mediated genetic modification of a tetrahydrocannabinol acid synthase (THCAS) gene, said modification resulting in a cannabidiol (CBD) to tetrahydrocannabinol (THC) ratio in said plant of at least 25: 1 as measured by dry weight.~~

a. ~~The transgenic plant of claim 2, wherein said modification resulting in~~ increased cannabidiol (CBD) in the transgenic plant as compared to a comparable control plant without the modification,

~~3.~~b. less than ~~1%~~ 0.5% by weight of tetrahydrocannabinol (THC) in ~~said~~the transgenic plant as measured by dry weight~~.~~ of the transgenic plant,

c. ~~The~~a CBD to THC ratio in the transgenic plant of ~~claim~~ at least about 25:1,

~~4. reduced~~ or ~~2, wherein said modification reduces or suppresses~~suppressed expression of the THCAS gene~~.~~

d. ~~The~~ in the transgenic plant ~~of any one of claims 1-4 comprising~~ ;

~~5.~~ wherein the transgenic plant comprises an unmodified endogenous cannabidiolic acid synthase (CBDAS) gene.

~~6.~~2. The transgenic plant of ~~any one of the preceding claims~~claim 1, comprising at least 25%, or at least 50% more CBD as measured by dry weight of the transgenic plant as compared to an amount of CBD as measured by dry weight of a comparable control plant without ~~said~~the endonuclease-mediated stably inherited genomic modification~~.~~ of the THCAS gene.

~~7. The transgenic plant of claim 6 comprising at least 50% more CBD as measured by dry weight as compared to a comparable control plant without said modification.~~

~~8.~~3. ~~The transgenic plant of any one of the preceding claims~~1, comprising a CBD to THC ratio of at least about: 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 40:1, 45:1~~,~~; or up to about 50:~~1as measured by dry weight.~~1.

~~9.~~4. The transgenic plant of ~~any one of claims~~claim 1~~-8~~, containing 0% THC or an untraceable amount thereof as measured by dry weight ~~as compared to a comparable control plant without said modification~~of the transgenic plant.

~~10.~~5. The transgenic plant of ~~any one~~claim 1, wherein the endonuclease-mediated stably inherited genomic modification of ~~the preceding claims,~~a tetrahydrocannabinol acid synthase (THCAS) gene comprises an endonuclease wherein ~~said~~the endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme~~, transcription activator-like effector (TALE)-nuclease, transposon-based nuclease, argonaute, Zinc finger nuclease, meganuclease, or Mega-TAL~~.

~~11.~~6. The transgenic plant of claim ~~10, wherein said endonuclease is a CRISPR enzyme or argonaute enzyme complexed with a guide polynucleotide~~5, wherein the ~~guide polynucleotide~~ endonuclease comprises ~~a sequence that binds a target sequence within or adjacent to said THCAS gene.~~ Cas9.

~~12. The transgenic plant of claim 11, wherein said guide polynucleotide binds said THCAS gene sequence or portion thereof.~~

7. The transgenic plant of claim ~~11 or 12~~1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene comprises an insertion.

8. The transgenic plant of claim 1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene comprises a deletion.

~~13.~~9. The transgenic plant of claim 1, wherein ~~said CRISPR enzyme complexed with said guide polynucleotide is introduced into said transgenic plant as a ribonuclear protein (RNP).~~ the endonuclease-mediated stably inherited genomic modification of the THCAS gene comprises a substitution.

~~14. The transgenic plant of claim 13, wherein said guide polynucleotide is chemically modified.~~

~~15. The transgenic plant of claim 11 or 12, wherein said CRISPR enzyme complexed with said guide polynucleotide is introduced into said transgenic plant by a vector comprising a nucleic acid encoding said CRISPR enzyme and said guide polynucleotide.~~

10. The transgenic plant of claim ~~15~~1, wherein ~~said vector~~the endonuclease-mediated stably inherited genomic modification of the THCAS gene comprises a frame shift.

~~16.~~11. The transgenic plant of claim 1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene is ~~a binary vector or~~in a ~~Ti plasmid~~coding region of the THCAS gene.

12. The transgenic plant of claim ~~16, wherein said vector~~1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene is in a regulatory region of the THCAS gene.

13. The transgenic plant of claim 1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene comprises an introduced stop codon.

14. The transgenic plant of claim 1, that is a cannabis plant.

15. The transgenic plant of claim 1, further ~~comprises~~ comprising a barcode.

16. The transgenic plant of claim 1, further comprising a reporter.

17. The transgenic plant of claim 1, further comprising a selection marker ~~or a reporter~~.

~~18.~~ The transgenic plant of ~~any one of claims 13-17~~claim 1, wherein ~~said RNP or vector is introduced into said transgenic plant via electroporation, agrobacterium~~ the endonuclease-mediated ~~transformation, biolistic particle bombardment, or protoplast transformation.~~

~~19.~~18. ~~The transgenic plant of any one of claims 13-18, further comprising~~stably inherited genomic modification of the THCAS gene modification results in a ~~donor polynucleotide~~.

~~20.~~ The transgenic plant of claim ~~19~~1, wherein ~~said donor polynucleotide comprises homology to sequences flanking~~ the ~~target sequence.~~

~~21. The transgenic plant of claim 19 or 20, wherein said donor polynucleotide introduces a stop codon into the THCAS gene.~~

~~22. The transgenic plant of any one of claims 19-21, wherein said donor polynucleotide comprises a barcode, a reporter, or a selection marker.~~

~~23. The transgenic plant of any one of claims 11-22, wherein said guide polynucleotide is a single guide RNA (sgRNA).~~

~~24. The transgenic plant of any one of claims 11-23, wherein said guide polynucleotide is a chimeric single guide comprising RNA and DNA.~~

~~25. The transgenic plant of any one of claims 11-24, wherein said target sequence is at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length.~~

~~26. The transgenic plant of any one of claims 11-25, wherein said target sequence is at most 17 nucleotides in length.~~

~~27. The transgenic plant of any one of claims 11-26, wherein said CRISPR enzyme is Cas9.~~

~~28. The transgenic plant of claim 27, wherein said Cas9 recognizes a canonical protospacer adjacent motif (PAM).~~

~~29. The transgenic plant of claim 28, wherein said Cas9 recognizes a non-canonical PAM.~~

~~30. The transgenic plant of claim 28 or 29, wherein said guide polynucleotide binds said target sequence from 3-10 nucleotides from said PAM.~~

~~31. The transgenic plant of any one of claims 25-30, wherein said target sequence comprises a sequence complementary to a sequence selected from the group consisting of SEQ ID NOs: 21-34 and 77-86.~~

32. The transgenic plant of any one of claims 25-31, wherein said guide polynucleotide comprises a sequence that comprises at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about 100% identity to a sequence selected from the group consisting of SEQ ID NOs: 21-34 and 77-86.

33. The transgenic plant of any one of the preceding claims, wherein said modification comprises an insertion, a deletion, a substitution, or a frameshift.

34. The transgenic plant of claim 33, wherein said modification is in a coding region of the THCAS gene.

35. The transgenic plant of claim 34, wherein the modification is in a regulatory region of the THCAS gene.

36. The transgenic plant of any one of the preceding claims, wherein said plant is a cannabis plant.

37. The transgenic plant of any one of the preceding claims, wherein said modification results in up to about 50% of indel formation.

38. The transgenic plant of any one of claims 1-37, wherein said modification results in up to about 25%, 15%, 10%, or 1% of indel formation.

39. A method for generating a transgenic plant, said method comprising

19. (a) contacting a plant cell comprising a tetrahydrocannabinol acid synthase (THCAS) gene with an endonuclease or a polynucleotide encoding said endonuclease, wherein said endonuclease introduces a mediated stably inherited genomic modification inof the THCAS gene; modification results in a and b.

(b) culturing said plant cell with said genomic modification in said THCAS gene to generate a The transgenic plant,

wherein said modification results in increased cannabidiol (CBD) as compared to a comparable control plant without said modification and less than 1% of tetrahydrocannabinol (THC) in said transgenic plant as measured by dry weight.

40. A method for generating a transgenic plant, said method comprising

(a) contacting a plant cell comprising a tetrahydrocannabinol acid synthase (THCAS) gene with an of claim 1, wherein the endonuclease or a polynucleotide encoding said endonuclease, wherein said endonuclease introduces a genetic modification in the THCAS gene;

(b) culturing said plant cell with said genomic modification in said THCAS gene to generate a transgenic plant,

wherein said modification results in a cannabidiol (CBD) to tetrahydrocannabinol (THC) ratio in said transgenic plant of at least 25: 1 as measured by dry weight.

41.20. The method of claims 39 or 40, wherein said contacting is via electroporation, agrobacterium - mediated transformation, biolistic particle bombardment, or protoplast transformationstably inherited genomic modification of the THCAS gene modification results in a, b, and c.

42. The method of any one of claims 39-41, further comprising culturing saidtransgenic plant cell with saidof claim 1, wherein the endonuclease-mediated stably inherited genomic modification in saidof the THCAS gene to generate a callus, a cotyledon, a hypocotyl, a protoplast, a root, a leaf, or a fraction thereof.

43.21. The method of any one of claims 39-42, wherein said modification reduces or supresses expression of said THCAS gene. results in a, b, c, and d.

22. The transgenic plant of claim 1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene modification results in a and c.

44. The methodtransgenic plant of any one of claims 39-43claim 1, wherein saidthe endonuclease-mediated stably inherited genomic modification does not alter a cannabidiolic acid synthase (CBDAS)of the THCAS gene in said transgenic plant.

45. The method of any one of claims 39-44, wherein said modification results in at least 25% more CBD as measured by dry weight in said transgenic plant as compared to a comparable control plant without said modification.

46.23. The method of claim 45, wherein said modification results in at least 50% more CBD as measured by dry weight in said transgenic plant as compared to a comparable control plant without said modificationa and d.

47. The method of any one of claims 39-46, wherein said modification results in less than 0.05% of THC in said transgenic plant as measured by dry weight.

48. The method of any one of claims 39-47, wherein said modification results in a CBD to THC ratio of at least 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 40:1, 45:1, or up to about 50:1 as measured by dry weight.

49. The transgenic plant of any one of claims 39-44 containing 0% THC or an untraceable amount thereof as measured by dry weight as compared to a comparable control plant without said modification.

50. The method of any one of claims 39-48, wherein said endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme, transcription activator-like effector (TALE) nuclease, transposon-based nuclease, Zinc finger nuclease, meganuclease, argonaute, or Mega-TAL.

51. The method of claim 49, wherein said endonuclease is a CRISPR enzyme or argonaute enzyme complexed with a guide polynucleotide, wherein the guide polynucleotide comprises a sequence that binds a target sequence within or adjacent to said THCAS gene.

52. The method of claim 50, wherein said guide polynucleotide comprises a sequence that binds said THCAS gene sequence or a portion thereof.

53. The method of claim 50 or 51, wherein said CRISPR enzyme complexed with said guide polynucleotide is contacted with the plant cell as a ribonuclear protein (RNP) to generate said plant cell with said genomic modification in said THCAS gene.

54. The method of claim 521, wherein said guide polynucleotide is chemically modified.

55. The method of claim 49 or 50, wherein said plant cell is contacted with a vector comprising a nucleic acid encoding said CRISPR enzyme and said guide polynucleotide thereby generating said plant cell with saidthe endonuclease-mediated stably inherited genomic modification in said THCAS gene.

56. The method of claim 53, wherein said vector is a binary vector or a Ti plasmid.

57. The method of claim 55, wherein said vector further comprises a selection marker or a reporter.

58. The method of any one of claims 52-56, further comprising contacting said plant cell from (a) with a donor polynucleotide.

59. The method of claim 57, wherein said donor polynucleotide comprises homology to sequences flanking the target sequence.

60. The method of claim 58, wherein said donor polynucleotide introduces a stop codon into the THCAS gene.

61. The method of any one of claims 5-60, wherein said donor polynucleotide comprises a barcode, a reporter, or a selection marker.

62. The method of any one of claims 51-61, wherein said guide polynucleotide is a single guide RNA (sgRNA).

63. The method of any one of claims 51-61, wherein said guide polynucleotide is a chimeric single guide comprising RNA and DNA.

64. The method of any one of claims 51-63, wherein said target sequence is at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, or at least 22 nucleotides in length.

65. The method of any one of claims 51-63, wherein said target sequence is at most 17 nucleotides in length.

66. The method of any one of claims 51-65, wherein said CRISPR enzyme is Cas9.

67. The method of claim 66, wherein said Cas9 recognizes a canonical protospacer adjacent motif (PAM).

68. The method of claim 66, wherein said Cas9 recognizes a non-canonical PAM.

69. The method of claim 67 or 68, wherein said guide polynucleotide binds said target sequence from 3-10 nucleotides from a PAM.

70. The method of any one of claims 51-69, wherein said target sequence comprises a sequence complementary to a sequence selected from the group consisting of SEQ ID NOs:21-34 and 77-86.

71. The method of any one of claims 51-70, wherein said guide polynucleotide comprises a sequence that comprises at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or up to about 100% identity to a sequence selected from the group consisting of SEQ ID NOs:21-34 and 77-86.

72. The method of any one of claims 39-69, wherein the modification comprises an insertion, a deletion, a substitution, or a frameshift.

73.24. The method of claim 72, wherein the modification is in a coding region of the THCAS gene modification results in a, c and d.

74. The methodtransgenic plant of claim 721, wherein the modification is in a regulatory region of the THCAS gene.

75. The method of any one of claims 39-74, wherein said plant is a cannabis plant.

76. The method of any one of claims 39-75, wherein said modification results in up to about 50% of indel formation.

77. The method of any one of claims 39-75, wherein said modification results in up to about 25%, 15%, 10%, or 1% of indel formation.

78. A pharmaceutical composition comprising the transgenic plant of any one of claims 1-38 or a derivative or extract thereof.

79. The pharmaceutical composition of claim 78, further comprising a pharmaceutically acceptable excipient, diluent, or carrier.

80. The pharmaceutical composition of claim 79, wherein said pharmaceutically acceptable excipient is a lipid.

81. A nutraceutical composition comprising the transgenic plant of any one of claims 1-38 or a derivative or extract thereof.

82. A food supplement comprising the transgenic plant of any one of claims 1-38 or a derivative or extract thereof.

83. The pharmaceutical composition of any one of claims 77-79, the nutraceutical composition of claim 80, or the food supplement of claim 81 in an oral form, a transdermal form, an oil formulation, a edible food, or a food substrate, an aqueous dispersion, an emulsion, a solution, a suspension, an elixir, a gel, a syrup, an aerosol, a mist, a powder, a tablet, a lozenge, a gel, a lotion, a paste, a formulated stick, a balm, a cream, or an ointment

84. A method of treating a disease or condition comprising administering the pharmaceutical composition of any one of claims 78-80, the nutraceutical composition of claim 81, or the food supplement of claim 82 to a subject in need thereof.

85. The method of claim 84, wherein said disease or condition is selected from the group consisting of anorexia, emesis, pain, inflammation, multiple sclerosis, Parkinson's disease, Huntington's disease, Tourette's syndrome, Alzheimer's disease, epilepsy, glaucoma, osteoporosis, schizophrenia, cardiovascular disorders, cancer, and obesity.

86. A method for generating a transgenic plant, said method comprising:

(a) contacting a plant cell comprising a gene with an endonuclease or a polynucleotide encoding said endonuclease, wherein said endonuclease introduces a genetic modification in the gene; and

25. (b) culturing said plant cell with said-mediated stably inherited genomic modification inof the THCAS gene to generate a transgenic plantmodification results in c and d.

87. The methodtransgenic plant of claim 86, wherein said1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene is tetrahydrocannabinol acid synthase (THCAS) gene.

88.26. The method of claim 86, wherein said genetic modification results in a cannabidiol (CBD) to tetrahydrocannabinol (THC) ratio in said transgenic plant of at least 25: 1 as measured by dry weight. b and c.

27. The transgenic plant of claim 1, wherein the endonuclease-mediated stably inherited genomic modification of the THCAS gene modification results in b and d.

89. The method transgenic plant of claim 861, wherein said contacting is via electroporation, agrobacterium mediated transformation, biolistic particle bombardment, or protoplast transformation.

90.28. The method of any one the endonuclease-mediated stably inherited genomic modification of claims 86-89, further comprising culturing said plant cell with said genomic modification in said the THCAS gene to generate a callus, a cotyledon, a hypocotyl, a protoplast, a root, a leaf, or a fraction thereofmodification results in b, c and d.

91.29. The methodtransgenic plant of any one of claims 86-90claim 1, wherein saidthe endonuclease-mediated stably inherited genomic modification reduces or supresses expression of saidof the THCAS gene. results in d.

92. The method of any one of claims 86-91, wherein said modification does not alter aThe transgenic plant of claim 1, which has increased cannabidiolic acid synthase (CBDAS) gene(CBDA) in saidthe transgenic plant.

93. The method of any one of claims 86-92, wherein said modification results in at least 25% more CBD as measured by dry weight in said transgenic plant as compared to a comparable control plant without said modification.

94. The method of claim 93, wherein said modification results in at least 50% more CBD as measured by dry weight in said transgenic plant as compared relative to a comparable control plant without saidthe endonuclease-mediated stably inherited genomic modification.

95. The method of any one of claims 86-94, wherein said modification results in less than 0.05% of THC in said transgenic plant as measured by dry weight.

96. The method of any one of claims 86-95, wherein said modification results in a CBD to THC ratio of at least 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 31:1, 32:1, 33:1, 34:1, 35:1, 40:1, 45:1, or up to about 50:1 as measured by dry weight.

97. The method of any one of claims 86-96, wherein the transgenic plant containing 0% THC or an untraceable amount thereof as measured by dry weight as compared to a comparable control plant without said modification.

98. The method of any one of claims 86-97, wherein said endonuclease comprises a clustered regularly interspaced short palindromic repeats (CRISPR) enzyme, transcription activator-like effector (TALE) nuclease, transposon-based nuclease, Zinc finger nuclease, meganuclease, argonaute, or Mega-TAL.

99. The method of claim 98, wherein said endonuclease is a CRISPR enzyme or argonaute enzyme complexed with a guide polynucleotide, wherein the guide polynucleotide comprises a sequence that binds a target sequence within or adjacent to said THCAS gene.

100.30. The method of claim 99, wherein said guide polynucleotide comprises a sequence that binds said of the THCAS gene sequence or a portion thereof.

101. The method of claim 98 or 99, wherein said CRISPR enzyme complexed with said guide polynucleotide is contacted with the plant cell as a ribonuclear protein (RNP) to generate said plant cell with said genomic modification in said THCAS gene.

102. The method of claim 98 or 99, wherein said plant cell is contacted with a vector comprising a nucleic acid encoding said CRISPR enzyme and said guide polynucleotide thereby generating said plant cell with said genomic modification in said THCAS gene.

103. The method of any one of claims 86-102, further comprising contacting said plant cell from (a) with a donor polynucleotide.

104. The method of claim 103, wherein said donor polynucleotide comprises homology to sequences flanking the target sequence.

105. The method of claim 104, wherein said donor polynucleotide introduces a stop codon into the THCAS gene.

106. A method for generating a transgenic plant, said method comprising:

(a) contacting an immature female flower with a solution comprising a vector that contains a nucleotide sequence encoding an endonuclease to introduce a genetic modification in the female flower;

(b) contacting the female flower with a sufficient amount of pollen to produce one or more seeds that comprise the genetic modification; and

(c) culturing said seed to generate a transgenic plant.

107. The method of claim 40, claim 86, or claim 106, wherein the plant is a cannabis plant.

108. The method of claim 107, wherein the plant is *Cannabis sativa*.

109. A method for generating a transgenic plant of any one of the above claims.