



(51) International Patent Classification:

A01H 1/06 (2006.01) C12N 9/10 (2006.01)
A01H 5/10 (2018.01)

(21) International Application Number:

PCT/US2019/014178

(22) International Filing Date:

18 January 2019 (18.01.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/619,360 19 January 2018 (19.01.2018) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,

OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available):

ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: LOW GLUCOSINOLATE PENNYCRESS MEAL AND METHODS OF MAKING

(57) Abstract: Pennycress (*Thlaspi arvense*) seed, seed lots, seed meal, and compositions with reduced glucosinolate content as well as plants that yield such seed, seed lots, seed meal, and compositions are provided. Methods of making and using the pennycress plants and/or seed that provide such seed, seed lots, seed meal, and compositions are also provided.

LOW GLUCOSINOLATE PENNYCRESS MEAL AND METHODS OF MAKING

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/619,360, filed January 19, 2018, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERAL FUNDING

[0002] This invention was made with government support under Grant Number 2014-67009-22305 awarded by the National Institute of Food and Agriculture, USDA. The government has certain rights in the invention.

INCORPORATION OF SEQUENCE LISTING

[0003] The sequence listing contained in the file named “CC-9 Low GSL_ST25.txt”, which is 406,450 bytes in size (measured in operating system MS-Windows), contains 236 sequences, and which was created on January 17, 2019, is contemporaneously filed with this specification by electronic submission (using United States Patent Office EFS-Web filing system) and is incorporated herein by reference in its entirety.

BACKGROUND

[0004] Different plants have seed contents that make them desirable for feed compositions. Examples are soybean, canola, rapeseed and sunflower. After crushing the seeds and recovering the oil, the resulting meal has a protein content making the meal useful as a feed ingredient for ruminants and other animals. Nevertheless, there remains a desire for improved plant seeds that can provide additional sources of nutrition to animals.

[0005] Field Pennycress *Thlaspi arvense* L. (common names: fanweed, stinkweed, field pennycress), hereafter referred to as Pennycress or pennycress, is a winter cover crop that helps to protect soil from erosion, prevent the loss of farm-field nitrogen into water systems, and retain nutrients and residues to improve soil productivity. While it is well established that cover crops provide agronomic and ecological benefits to agriculture and environment, only 5% of U.S. farmers today are using them. One reason is economics – it requires on average ~\$30-50/acre to

grow a cover crop on the land that is otherwise idle between two seasons of cash crops such as corn and soy. In the last 5 years, it has been recognized that pennycress could be used as a novel cover crop, because in addition to providing cover crop benefits, it produces harvestable seeds rich in oil and protein having value for feed, food, fuel, and industrial applications. Extensive testing indicates that pennycress can be interseeded over standing corn in early fall and harvested in spring prior to soybean planting (in appropriate climates). As such, its growth and development require minimal incremental inputs (*e.g.*, no/minimum tillage, no/low nitrogen, insecticides or herbicides). Pennycress also does not directly compete with existing crops when intercropped *e.g.*, for energy production, and the recovered oil and meal can provide an additional source of income for farmers.

[0006] Pennycress is a winter annual belonging to the *Brassicaceae* (mustard) family. It's related to cultivated crops, rapeseed and canola, which are also members of the *Brassicaceae* family. Pennycress seeds are smaller than those of canola, but they are also high in oil and protein content. They typically contain 36% oil, which is roughly twice the level found in soybean, and the oil has a very low saturated fat content (~4%). Pennycress represents a clear opportunity for sustainable optimization of agricultural systems. For example, in the U.S. Midwest, ~35M acres that remain idle could be planted with pennycress near the time of corn crop harvest, with pennycress seeds and/or plants harvested before the next soybean crop is planted. Pennycress can serve as an important winter cover crop working within the no/low-till corn and soybean rotation to guard against soil erosion and improve overall field soil nitrogen and pest management.

[0007] Pennycress seeds contain oil that is highly desirable as a feedstock for biofuels and/or chemicals and potentially as a food oil. Once the oil is obtained from pennycress seeds, either from mechanical expeller pressing or hexane extraction, the resulting meal has a high protein level with a favorable amino acid profile that could provide nutritional benefits to animals. However, studies of pennycress processing have consistently demonstrated that the meal produced has a high level of the anti-nutrient compound sinigrin (allyl-glucosinolate or 2-propenyl-glucosinolate), and as a result, without additional treatments, may not be competitive with high-value products like soybean and canola meals, ingredients commonly used in animal feed. Glucosinolates (GSLs) are secondary plant metabolites that are found in all *Brassica* plants such as rapeseed, canola, camelina, carinata and pennycress. Content and composition of GSLs

vary due to plant species, agronomic practices and environmental conditions (Tripathi and Mishra, 2007). Glucosinolates and their breakdown products that are a result of hydrolysis during the processing of the seeds into animal feed can result in negative effects on animal nutrition. The toxicity of glucosinolates for animals has been primarily associated with the metabolites thiocyanates, oxazolidinethiones and nitriles. These compounds interfere with iodine uptake (thiocyanates) and the synthesis of the thyroid hormones T3 and T4 (oxazolidinethiones), leading eventually to hypothyroidism and enlargement of the thyroid gland (EFSA, 2008). The major clinical signs of toxicity described in farm animals include growth retardation, reduction in performance (milk and egg production), impaired reproductive activity, and impairment of liver and kidney functions (EFSA, 2008). A comprehensive review of the effects of glucosinolates in animal nutrition has been published by Tripathi and Mishra (2007) and EFSA (2008).

SUMMARY

[0008] Compositions comprising non-defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight are provided herein.

[0009] Non-defatted pennycress seed meal that comprise less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight are provided herein.

[0010] Defatted pennycress seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight are provided herein.

[0011] Compositions comprising defatted pennycress seed meal that comprise less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight are provided herein.

[0012] Pennycress seed comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight are provided herein.

[0013] Pennycress seed lots comprising pennycress seed with less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight are provided herein.

[0014] In one embodiment, this disclosure provides methods for producing low glucosinolate pennycress seeds and meal. In certain embodiments, the methods comprise genetically modifying pennycress seed (*e.g.*, using gene editing, mutagenesis, or a transgenic approach) to suppress expression of one or more genes involved in sinigrin biosynthesis, transport, and/or hydrolysis. Genetically altered seed lots with lower sinigrin content in comparison to control seed lots that lack the genetic alteration can be obtained by these methods.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The accompanying drawings, which are incorporated in and form a part of the specification, illustrate the embodiments of the present disclosure and together with the description, serve to explain the principles of the disclosure. In the drawings:

[0016] FIG. 1A, B illustrate glucosinolate (GSL) biosynthetic pathways for many *Brassica* plants. Panel A: A schematic diagram of aliphatic GSL pathway which begins with amino acid methionine as the precursor and is relevant for GSL modification in pennycress seed. Panel B: Various GSL forms found in *Brassica* are shown.

[0017] FIG. 2 A, B, C, illustrates pARV1 (SS32_GTR1), *Agrobacterium* CRISPR-Cas9 vector and its gene editing sgRNA cassette, for targeting pennycress homolog of *Glucosinolate transporter 1* (*GTR1* or *Glut1*) gene. Panel A: Plasmid map of pARV1 (SS32_GTR1). Panel B: sgRNA cluster in pARV1, targeting nucleotides 2503-2522 and 2538-2557 of SEQ ID NO: 14. Panel C: Sequence example of one of gRNA cassettes targeting pennycress homolog of *Glucosinolate transporter 1* (*GTR1* or *Glut1*) gene.

[0018] FIG. 3A, B illustrates pDe-SpCas9 and pDe-SaCas9, *Agrobacterium* CRISPR-Cas9 base vectors for editing plant genome. gRNA cassette stuffers are inserted at the multiple cloning site between the Cas9 and HygR cassettes, replacing a small fragment of the vector with synthetic gRNA cassette.

[0019] FIG. 4A, B, C, illustrates pARV145, *Agrobacterium* CRISPR-Cas9 vector and its gene editing sgRNA cassettes, for targeting pennycress homolog of *MYB28* (*HAG1*) gene. Panel A: Plasmid map of pARV145. Panel B: sgRNA cluster in pARV145, targeting nucleotides 719-738 and 793-812 of SEQ ID NO: 20. Panel C: Sequence examples of gRNA cassettes targeting pennycress homolog of *MYB28* (*HAG1*) gene.

[0020] FIG. 5 illustrates pARV187, Agrobacterium CRISPR-FnCpf1 base vector for editing plant genome. gRNA cassette buffers are inserted at the dual *AarI* site, replacing a small fragment of the vector with synthetic gRNA cassette.

[0021] FIG. 6 illustrates pARV190, Agrobacterium CRISPR-SmCms1 base vector for editing plant genome. gRNA cassette buffers are inserted at the dual *AarI* site, replacing a small fragment of the vector with synthetic gRNA cassette.

[0022] FIG. 7 A, B, C, D, E, F, G, H, I, J, K, gRNA cassettes targeting pennycress homologs of multiple genes in glucosinolate biosynthetic/metabolic pathway. FIG. 5A illustrates a gRNA cassette buffer, designed for insertion into the *AarI*-digested plant genome editing vector (such as pARV187 or pARV190) for targeting pennycress *AOP2* gene, nucleotides 2367-2389 and 2419-2441 of SEQ ID NO: 2; FIG. 5B: gRNA cassette buffer for targeting pennycress *BCAT4* gene, nucleotides 2984-3006 and 3048-3070 of SEQ ID NO: 5; FIG. 5C: gRNA cassette buffer for targeting pennycress *BCAT6* gene, nucleotides 1932-1954 and 2387-2409 of SEQ ID NO: 8; FIG. 5D: gRNA cassette buffer for targeting pennycress *CYP79* gene, nucleotides 2914-2936 and 2968-2990 of SEQ ID NO: 11; FIG. 5E: gRNA cassette buffer for targeting pennycress *GTR1* gene, nucleotides 2483-2505 and 2541-2563 of SEQ ID NO: 14; FIG. 5F: gRNA cassette buffer for targeting pennycress *GTR2* gene, nucleotides 2317-2339 and 2404-2426 of SEQ ID NO: 18; FIGs. 5G and 5H: gRNA cassette buffers for targeting pennycress *MYB28* gene, nucleotides 948-970, 1001-1023, 1045-1067 and 1315-1337 of SEQ ID NO: 20; FIG. 5I: gRNA cassette buffer for targeting pennycress *MYB29* gene, nucleotides 2573-2595 and 2625-2647 of SEQ ID NO: 23; FIG. 5J: gRNA cassette buffer for targeting pennycress *MYB76* gene, nucleotides 1539-1561 and 1570-1592 of SEQ ID NO: 26; FIG. 5K: gRNA cassette buffer for targeting pennycress *TFP* gene, nucleotides 2170-2192 and 2559-2581 of SEQ ID NO: 29.

DETAILED DESCRIPTION

[0023] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0024] As used herein, the terms “include,” “includes,” and “including” are to be construed as at least having the features to which they refer while not excluding any additional unspecified features.

[0025] Where a term is provided in the singular, other embodiments described by the plural of that term are also provided.

[0026] To the extent to which any of the preceding definitions is inconsistent with definitions provided in any patent or non-patent reference incorporated herein by reference, any patent or non-patent reference cited herein, or in any patent or non-patent reference found elsewhere, it is understood that the preceding definition will be used herein.

[0027] Reductions in sinigrin content of various pennycress plants, seeds, seed lots, seed meals, and compositions obtained therefrom as well as associated methods of obtaining and using such plants, seeds, seed lots, seed meals, and compositions is provided herein by suppression of certain endogenous pennycress genes. The endogenous pennycress genes that can be suppressed to provide such reductions in sinigrin content include, but are not limited to, endogenous pennycress genes set forth in the following Table 1 and allelic variants of those genes.

[0028] Suppression of certain endogenous pennycress gene expression to provide for reductions in sinigrin content can be affected by a variety of techniques including, but not limited to, loss-of-function (LOF) mutations in endogenous genes, with transgenes, or by using gene-editing- or mutagenesis- mediated genome rearrangements. In certain embodiments, the pennycress plants, seeds, seed lots, seed meals (which can be defatted or non-defatted), and related compositions can comprise one or more LOF mutations that suppress or otherwise alter expression and/or function of one or more genes, coding sequences, and/or proteins, thus resulting in reduced sinigrin content in comparison to control or wild-type pennycress seed, seed lots, and plant lots. Such LOF mutations include, but are not limited to, INDELS (insertions, deletions, and/or substitutions or any combination thereof), translocations, inversions, duplications, or any combination thereof in a promoter, and/or other regulatory elements including enhancers, a 5' untranslated region, coding region, an intron of a gene, and/or a 3' UTR of a gene. Such INDELS can introduce one or more mutations including, but not limited to, frameshift mutations, missense mutations, pre-mature translation termination codons, splice donor and/or acceptor mutations, regulatory mutations, and the like that result in a LOF

mutation. In certain embodiments, the LOF mutation will result in: (a) a reduction in the enzymatic, transport, or other biochemical activity associated with the encoded polypeptide in the plant comprising the LOF mutation in comparison to a wild-type control plant; or (b) both a reduction in the enzymatic, transport or other biochemical activity (*e.g.*, transcription factor) and a reduction in the amount of a transcript (*e.g.*, mRNA) or polypeptide in the plant comprising the LOF mutation in comparison to a wild-type control plant. Such reductions in activity or activity and transcript levels can, in certain embodiments, comprise a reduction of at least 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of activity or activity and transcript levels in the LOF mutant in comparison to the activity or transcript levels in a wild-type control plant. In certain embodiments, the pennycress plants, seeds, seed lots, seed meals (which can be defatted or non-defatted), and related compositions will comprise one or more transgenes or genetic modifications that suppress or otherwise alter expression of one or more genes, coding sequences, and/or proteins, thus resulting in reduced sinigrin content in comparison to control or wild-type pennycress seed lots. Transgenes or genetic modifications that can provide for such suppression or alteration include, but are not limited to, transgenes or genome rearrangements introduced via gene editing or other mutagenesis techniques that produce small interfering RNAs (siRNAs), miRNA, or artificial miRNAs targeting a given gene or gene transcript for suppression. Such genome rearrangements include, but are not limited to, deletions, duplications, insertions, inversions, translocations, and combinations thereof. Useful genome rearrangements include, but are not limited to, rearrangements that place an endogenous promoter and/or transcriptional enhancer in proximity to 3' end of a target gene or coding sequence (*e.g.*, a gene or coding sequence of Table 1) or within the target gene or coding sequence such that the endogenous promoter and/or enhancer drive expression of an siRNA or miRNA that suppresses or otherwise alters expression of the target gene. In certain embodiments, the transgenes or genetic modifications that suppress expression will result in: (a) a reduction in the enzymatic, transport, or other biochemical activity associated with the encoded polypeptide in the plant comprising the transgene or genome rearrangement in comparison to a wild-type control plant; or (b) both a reduction in the enzymatic or other biochemical activity and a reduction in the amount of a transcript (*e.g.*, mRNA) or polypeptide in the plant comprising the transgene or genome rearrangement in comparison to a wild-type control plant. Such reductions in activity and transcript levels can in certain embodiments comprise a reduction

of at least 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of activity and/or transcript levels in the transgenic plant in comparison to the activity or transcript levels in a wild-type control plant. In certain embodiments, certain genes, coding sequences, and/or proteins that can be targeted for introduction of LOF mutations or that are targeted for transgene- or genome rearrangement- mediated suppression are provided in the following Table 1 and accompanying Sequence Listing. In certain embodiments, allelic variants of the wild-type genes, coding sequences, and/or proteins provided in Table 1 and the sequence listing are targeted for introduction of LOF mutations or are targeted for transgene- or genome rearrangement-mediated suppression. Allelic variants found in distinct pennycress isolates or varieties that exhibit wild-type seed sinigrin content can be targeted for introduction of LOF mutations or are targeted for transgene- or genome rearrangement-mediated suppression to obtain seed lots having reduced sinigrin content in comparison to sinigrin content of the control seed lots of wild-type pennycress. Such allelic variants can comprise polynucleotide sequences that have at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity across the entire length of the polynucleotide sequences of the wild-type coding regions or wild-type genes of Table 1 and the sequence listing. Such allelic variants can comprise polypeptide sequences that have at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity across the entire length of the polypeptide sequences of the wild-type proteins of Table 1 and the sequence listing. Pennycress seed lots having reduced sinigrin content as described herein can comprise one or more LOF mutations in one or more genes that encode polypeptides involved in GSL biosynthesis, in GSL transport, in GSL hydrolysis, regulating expression of genes encoding GSL biosynthetic and/or transport genes (*e.g.*, transcription factors) or can comprise transgenes or genome rearrangements that suppress expression of those biosynthetic, transporter, hydrolysis, or expression regulator (*e.g.*, transcription factor) encoding genes. Polypeptides affecting these traits include, without limitation, AOP2, BCAT4, BCAT6, CYP79F1, CYP83A1, GTR1, GTR2, MYB28 (HAG1), MYB29, MYB76, TFP, BHLH05, IMD1, CYP79B3, MAM1, FMO-GS-Ox1 , and UGT74B-1 polypeptides disclosed in Table 1 and allelic variants thereof. In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can comprise one or more LOF mutations found in the pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207* germplasm. Compositions comprising defatted or non-defatted seed meal obtained from

any of the aforementioned seed lots, and seed cakes obtained from any of the aforementioned seed lots are also provided herein. Methods of making any of the aforementioned seed lots, compositions, seed meals, or seed cakes are also provided herein. As used herein, the phrase “seed cake” refers to the material obtained after the seeds are crushed, ground, heated, and expeller pressed or extruded prior to solvent extraction.

[0029] In certain embodiments, reductions in sinigrin content of seed lots, seed meal compositions, seed meal, or seed cake are in comparison to sinigrin content of control or wild-type seed lots, seed meal compositions, seed meal, or seed cake. Such controls include, but are not limited to, seed lots, seed meal compositions, seed meal, or seed cake obtained from control plants that lack the LOF mutations or transgene- or genome rearrangement- mediated gene suppression. In certain embodiments, control plants that lack the LOF mutations or transgene or genome rearrangement mediated gene suppression will be otherwise isogenic to the plants that contain the LOF mutations or transgene- or genome rearrangement- mediated gene suppression. In certain embodiments, the controls will comprise seed lots, seed meal compositions, seed meal, or seed cake obtained from plants that lack the LOF mutations or transgene or genome rearrangement mediated gene suppression and that were grown in parallel with the plants having the LOF mutations or transgene or genome rearrangement- mediated gene suppression. In certain embodiments, the pennycress seed lots, plants, seeds, as well as the defatted or non-defatted seed meals and compositions obtained therefrom, can comprise a less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight.

[0030] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include at least one loss-of-function mutation in a GSL biosynthetic coding sequence or gene (*e.g.*, SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 92, 93, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, or allelic variant thereof) and/or at least one loss-of-function mutation in a GSL transport (SEQ ID NO: 13, 14, 16, 18, or allelic variant thereof), in a GSL hydrolysis (SEQ ID NO: 28, 29, or allelic variant thereof), and/or in an expression regulator (*e.g.*, transcription factor; SEQ ID NO: 19, 20, 22, 23, 25, 26, 159, 160, allelic variant thereof) coding sequence or gene. In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include at least one loss-of-function mutation

in a GSL transport coding sequence or gene (*e.g.*, SEQ ID NO: 13, 14, 16, 18, or allelic variant thereof) and at least one loss-of-function mutation in a GSL hydrolysis (SEQ ID NO: 28, 29, or allelic variant thereof), and/or in an expression regulator (*e.g.*, transcription factor; SEQ ID NO: 19, 20, 22, 23, 25, 26, allelic variant thereof) coding sequence or gene. In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include at least one loss-of-function mutation in a GSL hydrolysis (SEQ ID NO: 28, 29, or allelic variant thereof) coding sequence or gene and/or at least one loss-of-function mutation in an expression regulator (*e.g.*, transcription factor; SEQ ID NO: 19, 20, 22, 23, 25, 26, allelic variant thereof) coding sequence or gene. In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can be obtained from pennycress plants comprising the mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207* germplasm.

[0031] Table 1. Wild-type (WT) coding regions, encoded proteins, and genes that can be targeted for introduction of LOF mutations or transgene- or genome rearrangement- mediated suppression, their mutant variants and representative genetic elements for achieving suppression of gene expression.

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
1	<i>AOP2-CDS</i>	WT Coding region	Plays a role in the secondary modification of aliphatic (methionine-derived) GSLs, namely the conversion of methylsulfinylalkyl GSLs to form alkenyl GSLs, and also influences aliphatic GSL accumulation	ALKENYL HYDROXALKYL PRODUCING 2 - CAPE VERDE ISLANDS, AOP2-CVI, GSL ALK enzyme, AOP (2-oxoglutarate- dependent dioxygenase)
2	<i>AOP2-Genomic locus</i>	WT Gene		
3	<i>AOP2-PRT</i>	WT Protein		
4	<i>BCAT4-CDS</i>	WT Coding region	Involved in the methionine chain elongation pathway that leads to the ultimate biosynthesis of methionine-derived glucosinolates	BCAT4 (BRANCHED-CHAIN AMINOTRANSFERASE 4)
5	<i>BCAT4-Genomic locus</i>	WT Gene		
6	<i>BCAT4-PRT</i>	WT Protein		

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
7	<i>BCAT6-CDS</i>	WT Coding region	Encodes a cytosolic branched-chain aminotransferase that acts on Leu, Ile, Val and also on Met. Together with BCAT4 and BCAT3, it is involved in methionine salvage and glucosinolate biosynthesis	BCAT6 (BRANCHED-CHAIN AMINOTRANSFERASE 6)
8	<i>BCAT6-Genomic locus</i>	WT Gene		
9	<i>BCAT6-PRT</i>	WT Protein		
10	<i>CYP79F1-CDS</i>	WT Coding region	Catalyzes the first committed step in biosynthesis of the core structure of GSLs, Modulates the level of short chain methionine derived aliphatic GSLs	CYP79F1 (CYTOCHROME P450 79F1), BUS1, BUSHY 1, SPS1, SUPERSHOOT 1
11	<i>CYP79F1-Genomic locus</i>	WT Gene		
12	<i>CYP79F1-PRT</i>	WT Protein		
13	<i>GTR1-CDS</i>	WT Coding region	GTR1 encodes high-affinity, proton-dependent GSL-specific transporter essential for the accumulation of GSLs in seeds	GTR1 (Glucosinolate Transporter 1), NPF2.10, NRT1/ PTR FAMILY 2.10
14	<i>GTR1-Genomic locus</i>	WT Gene		
15	<i>GTR1-PRT</i>	WT Protein		
16	<i>GTR2-CDS</i>	WT Coding region	GTR2 encodes high-affinity, proton-dependent GSL-specific transporter essential for the accumulation of GSLs in seeds	GTR2 (Glucosinolate Transporter 2), NPF2.11, NRT1/ PTR FAMILY 2.11
17	<i>GTR2-PRT</i>	WT Protein		
18	<i>GTR2-Genomic locus</i>	WT Gene		
19	<i>MYB28-CDS</i>	WT Coding region	Principal regulator of aliphatic glucosinolate biosynthesis and affects the production of both short- and long-chain aliphatic glucosinolates	HAG1 (High Aliphatic Glucosinolate 1), MYB DOMAIN PROTEIN 28, PMG1 (Production of Methionine-derived Glucosinolate 1)
20	<i>MYB28-Genomic locus</i>	WT Gene		
21	<i>MYB28-PRT</i>	WT Protein		
22	<i>MYB29-CDS</i>	WT Coding region	MYB DOMAIN PROTEIN 29, a Myb transcription factor affects biosynthesis of short-chain aliphatic glucosinolates	HAG3 (High Aliphatic Glucosinolate 3), PMG2 (Production of Methionine-derived Glucosinolate 2), RAO7, (Regulator of Alternative Oxidase 1A 7)
23	<i>MYB29-Genomic locus</i>	WT Gene		
24	<i>MYB29-PRT</i>	WT Protein		

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
25	<i>MYB76-CDS</i>	WT Coding region	MYB DOMAIN PROTEIN 76, a Myb transcription factor affects biosynthesis of short-chain aliphatic glucosinolates.	HAG2 (High Aliphatic Glucosinolate 2), PMG2, (Production of Methionine-derived Glucosinolate 2), RAO7 (Regulator of Alternative Oxidase 1A 7)
26	<i>MYB76-Genomic locus</i>	WT Gene		
27	<i>MYB76-PRT</i>	WT Protein		
28	<i>TFP-CDS</i>	WT Coding region	Promotes the formation of allylthiocyanate as well as the epithionitrile upon myrosinase-catalyzed hydrolysis of allylglucosinolate, the major glucosinolate	Thiocyanate-forming protein (TFP)
29	<i>TFP-Genomic locus</i>	WT Gene		
30	<i>TFP-PRT</i>	WT Protein		
31	AOP2_scaffold_16_11717_0_125481 - CRISPR_64	Oligonucleotide	AOP2 CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
32	AOP2_scaffold_16_11717_0_125481 - Cfp1_226	Oligonucleotide	AOP2 CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
33	AOP2_scaffold_16_11717_0_125481 - Cfp1_627	Oligonucleotide	AOP2 CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
34	AOP2_scaffold_16_11717_0_125481 - CRISPR_66	Oligonucleotide	AOP2 CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
35	BCAT4_scaffold_7_1484_532_149282_2_+ Cfp1_566	Oligonucleotide	BCAT4 CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
36	BCAT4_scaffold_7_1484_532_149282_2_+ CRISPR_184	Oligonucleotide	BCAT4 CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
37	BCAT4_scaf fold_7_1484 532_149282 2_+_CRISP R_185	Oligonucleotide	<i>BCAT4</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
38	BCAT4_scaf fold_7_1484 532_149282 2_+_Cfp1_1 72	Oligonucleotide	<i>BCAT4</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
39	BCAT6_scaf fold_18_731 862_739845 _+_CRISPR _63	Oligonucleotide	<i>BCAT6</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
40	BCAT6_scaf fold_18_731 862_739845 _+_CRISPR _64	Oligonucleotide	<i>BCAT6</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
41	BCAT6_scaf fold_18_731 862_739845 _+_Cfp1_55 8	Oligonucleotide	<i>BCAT6</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
42	BCAT6_scaf fold_18_731 862_739845 _+_Cfp1_57 7	Oligonucleotide	<i>BCAT6</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
43	CYP79F1_sc affold_1_301 8995_30271 06_+_CRISP R_86	Oligonucleotide	<i>CYP79F1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
44	CYP79F1_sc affold_1_301 8995_30271 06_+_Cfp1_493	Oligonucleotide	<i>CYP79F1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
45	CYP79F1_sc affold_1_301 8995_30271 06_+_CRISP R_87	Oligonucleotide	<i>CYP79F1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
46	CYP79F1_sc affold_1_301 8995_30271 06_+_Cfp1_ 495	Oligonucleotide	<i>CYP79F1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
47	GTR1_scaffo ld_63_14688 8_155577_- _Cfp1_88	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
48	GTR1_scaffo ld_63_14688 8_155577_- _Cfp1_92	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
49	GTR1_scaffo ld_63_14688 8_155577_- _Cfp1_506	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
50	GTR1_scaffo ld_63_14688 8_155577_- _Cfp1_525	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
51	GTR1_scaffo ld_63_14688 8_155577_- _Cfp1_574	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
52	GTR1_scaffo ld_63_14688 8_155577_- _Cfp1_214	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
53	GTR2_scaffo ld_0_196442 7_1972843_ +_Cfp1_513	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
54	GTR2_scaffold_0_196442_7_1972843_+ Cfp1_537	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
55	GTR2_scaffold_0_196442_7_1972843_+ Cfp1_174	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
56	GTR2_scaffold_0_196442_7_1972843_+ Cfp1_265	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
57	GTR2_scaffold_0_196442_7_1972843_+ Cfp1_267	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
58	MYB28_scaffold_0_2473_389_248075_8_+ CRISPR_170	Oligonucleotide	<i>MYB28</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
59	MYB28_scaffold_0_2473_389_248075_8_+ CRISPR_172	Oligonucleotide	<i>MYB28</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
60	MYB28_scaffold_0_2473_389_248075_8_+ Cfp1_569	Oligonucleotide	<i>MYB28</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
61	MYB28_scaffold_0_2473_389_248075_8_+ Cfp1_147	Oligonucleotide	<i>MYB28</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
62	MYB28_scaffold_0_2473_389_248075_8_+ Cfp1_573	Oligonucleotide	<i>MYB28</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
63	MYB28_scaf fold_0_2473 389_248075 8_+_Cfp1_1 57	Oligonucleotide	<i>MYB28</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
64	MYB29_scaf fold_3_2545 596_255310 1_- _CRISPR_15 6	Oligonucleotide	<i>MYB29</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
65	MYB29_scaf fold_3_2545 596_255310 1_- _Cfp1_606	Oligonucleotide	<i>MYB29</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
66	MYB29_scaf fold_3_2545 596_255310 1_- _CRISPR_16 1	Oligonucleotide	<i>MYB29</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
67	MYB29_scaf fold_3_2545 596_255310 1_- _Cfp1_247	Oligonucleotide	<i>MYB29</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
68	MYB76_scaf fold_3_2536 681_254389 5_- _CRISPR_55	Oligonucleotide	<i>MYB76</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
69	MYB76_scaf fold_3_2536 681_254389 5_- _Cfp1_493	Oligonucleotide	<i>MYB76</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
70	MYB76_scaf fold_3_2536 681_254389 5_- CRISPR_56	Oligonucleotide	<i>MYB76</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
71	MYB76_scaf fold_3_2536 681_254389 5_- Cfp1_495	Oligonucleotide	<i>MYB76</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
72	Ta_TFP_scaf fold_1_4920 343_492735 6_+ CRISP R_164	Oligonucleotide	<i>TFP</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
73	Ta_TFP_scaf fold_1_4920 343_492735 6_+ Cfp1_482	Oligonucleotide	<i>TFP</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
74	Ta_TFP_scaf fold_1_4920 343_492735 6_+ CRISP R_167	Oligonucleotide	<i>TFP</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
75	Ta_TFP_scaf fold_1_4920 343_492735 6_+ Cfp1_198	Oligonucleotide	<i>TFP</i> CDS targeted for cleavage by Cpf1 enzyme; part of gRNA cassette	
76	GTR1_115	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCAS9 enzyme; part of gRNA cassette	
77	GTR1_116	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCAS9 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
78	GTR2_scaffold_0_1966458_1970958_+_CRISPR_43	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by SpCAS9 enzyme; part of gRNA cassette	
79	GTR2_scaffold_0_1966458_1970958_+_CRISPR_46	Oligonucleotide	<i>GTR2</i> CDS targeted for cleavage by SpCAS9 enzyme; part of gRNA cassette	
80	MYB28-m1-CDS	Mutant Coding region	Mutant <i>hag1-1</i> allele (-G deletion)	<i>hag1-1</i>
81	MYB28-m1-PRT	Mutant Protein	Mutant <i>hag1-1</i> protein (-G deletion)	
82	MYB28-m2-CDS	Mutant Coding region	Mutant <i>hag1-2</i> allele (+A insertion)	<i>hag1-2, 2172A</i>
83	MYB28-m2-PRT	Mutant Protein	Mutant <i>hag1-2</i> protein (+A insertion)	
84	MYB28-m3-CDS	Mutant Coding region	Mutant <i>hag1</i> allele (+G insertion)	
85	MYB28-m3-PRT	Mutant Protein	Mutant <i>hag1</i> protein (+G insertion)	
86	MYB28-m4-CDS	Mutant Coding region	Mutant <i>hag1</i> allele (A -> G mutation)	
87	MYB28-m4-PRT	Mutant Protein	Mutant <i>hag1</i> allele (A -> G mutation)	
88	MYB28-m5-CDS	Mutant Coding region	Mutant <i>hag1</i> allele (+A insertion)	
89	MYB28-m5-PRT	Mutant Protein	Mutant <i>hag1</i> protein (+A insertion)	
90	MYB28-m6-CDS	Mutant Coding region	Mutant <i>hag1</i> allele (-AG deletion)	
91	MYB28-m6-PRT	Mutant Protein	Mutant <i>hag1</i> protein (-AG deletion)	
92	CYP83A1-CDS	WT Coding region	Biosynthetic enzyme and a member of cytochrome P450 family. Catalyzes conversion of aliphatic aldoximes to nitrile oxides or <i>aci</i> -nitro compounds	REF2
93	CYP83A1-Genomic locus	WT Gene		
94	CYP83A1-PRT	WT Protein		

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
95	CYP83A1-m1-CDS	Mutant Coding region	Mutant <i>cyp83a1</i> allele (G insertion)	
96	CYP83A1-m1-PRT	Mutant Protein	Mutant <i>cyp83a1</i> allele (G insertion)	
97	CYP83A1-m2-CDS	Mutant Coding region	Mutant <i>cyp83a1</i> allele (T->G mutation)	
98	CYP83A1-m2-PRT	Mutant Protein	Mutant <i>cyp83a1</i> allele (T->G mutation)	
99	AOP2_sp_P S3	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
100	AOP2_sp_P S1	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
101	AOP2_sp_P S4	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
102	AOP2_sp_P S2	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
103	AOP2_sp_P S6	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
104	AOP2_sa_PS 1	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SaCas9 enzyme; part of gRNA cassette	
105	AOP2_sa_PS 2	Oligonucleotide	<i>AOP2</i> CDS targeted for cleavage by SaCas9 enzyme; part of gRNA cassette	
106	HAG1_513	Oligonucleotide	<i>HAG1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
107	HAG1_sp_P S1_F	Oligonucleotide	<i>HAG1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
108	HAG1/3_sp_R	Oligonucleotide	<i>HAG1/HAG3</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
109	HAG1/2_sa_PS1_F	Oligonucleotide	<i>HAG1/HAG2</i> CDS targeted for cleavage by SaCas9 enzyme; part of gRNA cassette	
110	HAG2_sp_P S1_F	Oligonucleotide	<i>HAG2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
111	HAG2_sp_P S2_F	Oligonucleotide	<i>HAG2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
112	HAG2_sp_P S3_F	Oligonucleotide	<i>HAG2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
113	HAG3_sp_P S2_F	Oligonucleotide	<i>HAG3</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
114	HAG3_431	Oligonucleotide	<i>HAG3</i> CDS targeted for cleavage by SaCas9 enzyme; part of gRNA cassette	
115	HAG3_sp_k knockout_1	Oligonucleotide	<i>HAG3</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
116	HAG3_sp_k knockout_2	Oligonucleotide	<i>HAG3</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
117	CYP83A1_sp_PS3_F	Oligonucleotide	<i>CYP83A1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
118	GTR1_sp_PS1	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
119	GTR1/2_sp_PS1	Oligonucleotide	<i>GTR1/GTR2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
120	GTR1/2_sp_PS2	Oligonucleotide	<i>GTR1/GTR2</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
121	GTR1/2_sa_PS1	Oligonucleotide	<i>GTR1/GTR2</i> CDS targeted for cleavage by SaCas9 enzyme; part of gRNA cassette	
122	GTR1/2_sa_PS2	Oligonucleotide	<i>GTR1/GTR2</i> CDS targeted for cleavage by SaCas9 enzyme; part of gRNA cassette	
123	GTR1_sp_PS2_F	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
124	GTR1_sp_PS3_F	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
125	GTR1_sp_knockout_1	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	
126	GTR1_sp_knockout_2	Oligonucleotide	<i>GTR1</i> CDS targeted for cleavage by SpCas9 enzyme; part of gRNA cassette	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
127	MYB76-m1ARV-CDS	Mutant Coding Region	TAAAGAAAGGAGCAT GGACGT (nt 35-55 of SEQ ID NO:25) → TAAAGAAAGG-GCATGGACGT (nt 35-54 of SEQ ID NO:127)	A427A
128	MYB76-m1ARV-PRT	Mutant Protein	Frameshift caused by 1bp deletion	
129	MYB76-m2ARV-CDS	Mutant Coding Region	CTGTATCGGAGAAGG GTTAAAGAAAGGAGCAT (nt 18-50 of SEQ ID NO:25) → CT-----AT (nt 18-21 of SEQ ID NO:129)	A430B
130	MYB76-m2ARV-PRT	Mutant Protein	Presumed loss of function caused by 27bp deletion	
131	MYB29-m1ARV-CDS	Mutant Coding Region	(nt 86-690 of SEQ ID NO:22) TCCATGAA---598bp deletion---AAGGAACC (nt 72-82 of SEQ ID NO:131)	A264A, A296A, A316B, A329B
132	MYB29-m1ARV-PRT	Mutant Protein	Truncated protein caused by large deletion	
133	MYB29-m2ARV-CDS	Mutant Coding Region	(nt 72-709 of SEQ ID NO:22) ACTCATCT---603bp deletion---ACCGCACTG (nt 72-87 of SEQ ID NO:133)	A361B
134	MYB29-m2ARV-PRT	Mutant Protein	Truncated protein caused by large deletion	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
135	MYB29-m3ARV-CDS	Mutant Coding Region	ATCCATGAACATGGC GAAG (nt 85-103 of SEQ ID NO:22) → ATCCATGAA(A)CATG GCGAAG (nt 85-104 of SEQ ID NO:135), and TCAGCGTCCATGGAA GGAACCTT (nt 670-692 of SEQ ID NO:22) → TCAGCGTCCATGGAA(A)GGAACCTT (nt 670-693 of SEQ ID NO:135)	A262A, A275A
136	MYB29-m3ARV-PRT	Mutant Protein	Frameshift caused by 1bp insertion (second edit also 1bp insertion)	
137	MYB29-m4ARV-CDS	Mutant Coding Region	TCAGCGTCCATGGAA GGAACCTT (nt 670-692 of SEQ ID NO:22) → TCAGCGTCCA--- AAGGAACCTT (nt 670-689 of SEQ ID NO:137)	A261C
138	MYB29-m4ARV-PRT	Mutant Protein	Missing M227, E228->K	
139	MYB29-m5ARV-CDS	Mutant Coding Region	TCAGCGTCCATGGAA GGAACCTT (nt 670-692 of SEQ ID NO:22) → TCAGCGTCC---- AAGGAACCTT (nt 670-689 of SEQ ID NO:139)	A268A
140	MYB29-m5ARV-PRT	Mutant Protein	Frameshift caused by 4bp deletion	
141	MYB29-m6ARV-CDS	Mutant Coding Region	TCAGCGTCCATGGAA GGAACCTT (nt 670-692 of SEQ ID NO:22) → TCAGCGTCCATGGA-GGAACCTT (nt 670-691 of SEQ ID NO:141)	A263A, A347D

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
142	MYB29-m6ARV-PRT	Mutant Protein	Frameshift caused by 1bp deletion	
143	GTR1-m1ARV-CDS	Mutant Coding Region	CCTCTGCGACACTTAC TTTG (nt 321-340 of SEQ ID NO:13) → CCT-----TTTG (nt 321-327 of SEQ ID NO:143)	A382A
144	GTR1-m1ARV-PRT	Mutant Protein	Frameshift caused by 13bp deletion	
145	GTR2-m1ARV-CDS	Mutant Coding Region	AGTGCATTGTGAGAG TGCT (nt 1037-1055 of SEQ ID NO:16) → AGTGCATT(T)GTGAG AGTGCT (nt 1037-1056 of SEQ ID NO:145)	A412A
146	GTR2-m1ARV-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
147	AOP2-m1ARV-CDS	Mutant Coding Region	TTTCCGAGAGTATGG GGATC (nt 275-294 of SEQ ID NO:1) → TTTCCGAGAG(A)TAT GGGGATC (nt 275-295 of SEQ ID NO:147)	A368A
148	AOP2-m1ARV-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
149	AOP2-m2ARV-CDS	Mutant Coding Region	TTTCCGAGAGTATGG GGATC (nt 275-294 of SEQ ID NO:1) → TTTCCGAGA--ATGGGGATC (nt 275-292 of SEQ ID NO:149)	A377A
150	AOP2-m2ARV-PRT	Mutant Protein	Frameshift caused by 2bp deletion	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
151	AOP2-m3ARV-CDS	Mutant Coding Region	TTTCCGAGAGTATGG GGATC (nt 275-294 of SEQ ID NO:1) → TTTCCGAGAGT--GGGGATC (nt 275-292 of SEQ ID NO:151)	A390A
152	AOP2-m3ARV-PRT	Mutant Protein	Frameshift caused by 2bp deletion	
153	AOP2-m4ARV-CDS	Mutant Coding Region	TTTCCGAGAGTATGG GGATC (nt 275-294 of SEQ ID NO:1) → TTTCCGAGAGT----GGATC (nt 275-290 of SEQ ID NO:153)	A402A
154	AOP2-m4ARV-PRT	Mutant Protein	Frameshift caused by 4bp deletion	
155	AOP2-m5ARV-CDS	Mutant Coding Region	TTTCCGAGAGTATGG GGATC (nt 275-294 of SEQ ID NO:1) → TTTCCGAGAGT(T)ATG GGGATC (nt 275-295 of SEQ ID NO:155)	A378A, A379A, A385A, A394A, A403B
156	AOP2-m5ARV-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
157	AOP2-m6ARV-CDS	Mutant Coding Region	TTTCCGAGAGTATGG GGATC (nt 275-294 of SEQ ID NO:1) → TTTC-------TGGGGATC (nt 275-286 of SEQ ID NO:157)	A375A
158	AOP2-m6ARV-PRT	Mutant Protein	Frameshift caused by 8bp deletion	
159	BHLH05-WT-CDS	WT Coding region	basic helix-loop-helix transcription factor05	BHLH05, MYC3, bHLH05

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
160	BHLH05-WT-Genomic Locus	WT Gene	(bHLH05) transcription factor affects the biosynthesis of glucosinolates	
161	BHLH05-WT-PRT	WT Protein		
162	IMD1-WT-CDS	WT Coding region	ISOPROPYLMALATE DEHYDROGENASE 1 (IMD1) is involved in leucine biosynthesis and methionine chain elongation required for glucosinolate biosynthesis	IMD1, ISOPROPYLMALATE DEHYDROGENASE 1
163	IMD1-WT-Genomic Locus	WT Gene		
164	IMD1-WT-PRT	WT Protein		
165	CYP79B3-WT-CDS	WT Coding region	Encodes cytochrome P450 family 79 and is involved in biosynthesis of glucosinolates	CYP79B3, CYTOCHROME P450, FAMILY 79, SUBFAMILY B, POLYPEPTIDE 3
166	CYP79B3-WT-Genomic Locus	WT Gene		
167	CYP79B3-WT-PRT	WT Protein		
168	MAM1-WT-CDS	WT Coding region	Encodes METHYLTHIOALKYL MALATE SYNTHASE 1 is involved in biosynthesis of glucosinolates	MAM1, METHYLTHIOALKYL MALATE SYNTHASE 1, gsm1
169	MAM1-WT-Genomic Locus	WT Gene		
170	MAM1-WT-PRT	WT Protein		
171	Ta_FMO-GS-Ox1-WT-CDS	WT Coding region	FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 1 catalyzes the conversion of methylthioalkyl glucosinolates to methylsulfinylalkyl glucosinolates	FMO GS-Ox1, FLAVIN-MONOOXYGENASE GLUCOSINOLATE S-OXYGENASE 1,
172	Ta_FMO-GS-Ox1-WT-Genomic Locus	WT Gene		
173	Ta_FMO-GS-Ox1-WT-PRT	WT Protein		
174	Ta_UGT74B1-WT-CDS	WT Coding region	UDP-glucose:thiohydroximate	UGT74B1, UDP-GLUCOSYL

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
175	Ta_UGT74B1-WT-Gemonic Locus	WT Gene	S-glucosyltransferase involved in glucosinolate biosynthesis	TRANSFERASE 74B1
176	Ta_UGT74B1-WT-PRT	WT Protein		
177	Ta_FMO-GS-Ox1-1-CDS	Mutant Coding Region	TTGAGCCTCGTCTAGC TGAA (nt 653-672 of SEQ ID NO:171) →TTGAGCCTC<A>TC TAGCTGAA (nt 653-672 of SEQ ID NO:177)	
178	Ta_FMO-GS-Ox1-1-PRT	Mutant Protein	Amino acid change	
179	Ta_MAM1-1-CDS	Mutant Coding Region	GCAAACATAGAGACA TTGAG (nt 464-483 of SEQ ID NO:168) →GCAAACATA<A>AG ACATTGAG (nt 464-483 of SEQ ID NO:179)	E5 543
180	Ta_MAM1-1-PRT	Mutant Protein	Amino acid change	
181	Ta_MAM1-2-CDS	Mutant Coding Region	TGTGTGTGCTGGAGC AAGAC (nt 891-910 of SEQ ID NO:168) →TGTGTGTGCTGGA<A>CAAGAC (nt 891-910 of SEQ ID NO:181)	D0956
182	Ta_MAM1-2-PRT	Mutant Protein	Amino acid change	
183	Ta_AOP2-like-1MAR-CDS	Mutant Coding Region	CCGAGAGTATGGGGA TCCAG (nt 278-297 of SEQ ID NO:1) →CCGAGAGTATG<A>GGATCCAG (nt 278-297 of SEQ ID NO:183)	E3196, <i>Nutty</i> , <i>aop2-1</i>
184	Ta_AOP2-like-1MAR-PRT	Mutant Protein	Amino acid change	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
185	Ta_bhlh05-1-CDS	Mutant Coding Region	AGAAGGCTGGACCTA CGCGA (nt 189-208 of SEQ ID NO:159) → AGAAGGCTG<A>CCT ACGCGA (nt 189-208 of SEQ ID NO:185)	D3 N13P3
186	Ta_bhlh05-1-PRT	Mutant Protein	Truncated protein caused by premature stop codon	
187	Ta_bhlh05-2-CDS	Mutant Coding Region	CGGAGACAACACAGT GATTCT (nt 246-266 of SEQ ID NO:159) → CGGAGACAAC-CAGTGATTCT (nt 246-265 of SEQ ID NO:187)	E5 202P2
188	Ta_bhlh05-2-PRT	Mutant Protein	Frameshift caused by 1bp deletion	
189	Ta_bhlh05-3-CDS	Mutant Coding Region	GGCGGAACCGGAGTT TCCGA (nt 394-413 of SEQ ID NO:159) → GGCGGAACCG<A>AG TTTCCGA (nt 394-413 of SEQ ID NO:189)	E5 133P2, <i>fad2-2</i>
190	Ta_bhlh05-3-PRT	Mutant Protein	Amino acid change	
191	Ta_myb28-5SED-CDS	Mutant Coding Region	CATCCACGAGCACGG TGAA (nt 84-103 of SEQ ID NO:22) → CATCCACG-GCACGGTGAA (nt 84-102 of SEQ ID NO:191)	
192	Ta_myb28-5SED-PRT	Mutant Protein	Frameshift due to 1bp deletion	
193	myb76-1SED-CDS	Mutant Coding Region	TAAAACGGTGTGGAA AGAG (nt 137-157 of SEQ ID NO:25) → TAAAACGGT(T)GTGG AAAGAG (nt 137-156 of SEQ ID NO:193)	
194	myb76-1SED-PRT	Mutant Protein	Frameshift due to 1bp insertion	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
195	myb29-1SED-CDS	Mutant Coding Region	GCCACTTGCCCCTAG CCCTAGTCCGGCCAC GCTA (nt 381-413 of SEQ ID NO:22) →GCCACTTG----- TCCGGCCACGCT (nt 381-400 of SEQ ID NO:195)	2172A
196	myb29-1SED-PRT	Mutant Protein	Frameshift due to 13bp deletion	
197	myb29-2SED-CDS	Mutant Coding Region	TAGCCCTAGTCCGGC CACGCTC (nt 393-414 of SEQ ID NO:22) →TAGCCCTA----- CCACGCTC (nt 393-408 of SEQ ID NO:197)	2180A
198	myb29-2SED-PRT	Mutant Protein	Presumed loss of function due to 6bp deletion	
199	Ta_imd1-1-CDS	Mutant Coding Region	AGAGCCCAGAGGCAT TAAGA (nt 663-682 of SEQ ID NO:162) → AGAGCCCA<A>AGGC ATTAAGA (nt 663-682 of SEQ ID NO:199)	A7 95, <i>tt4-1</i>
200	Ta_imd1-1-PRT	Mutant Protein	Amino acid change	
201	Ta_imd1-2-CDS	Mutant Coding Region	TCGGTGTATCGGGAC CTGGA (nt 1040-1059 of SEQ ID NO:162) → TCGGTGTAT<T>GGGA CCTGGA (nt 1040-1059 of SEQ ID NO:201)	D3 22
202	Ta_imd1-2-PRT	Mutant Protein	Amino acid change	
203	Ta_cyp79b3-1-CDS	Mutant Coding Region	CTTCCAACGGCTAC AAAAC (nt 412-431 of SEQ ID NO:165) → CTTCCAAC<A>GCTA CAAAAC (nt 412-431 of SEQ ID NO:203)	I87207

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
204	Ta_cyp79b3-1-PRT	Mutant Protein	Amino acid change	
205	Ta_cyp79b3-2-CDS	Mutant Coding Region	GGTCTGATCCACTTA GCTTT (nt 1328-1347 of SEQ ID NO:165) → GGTCTGAT<T>CACTT AGCTTT (nt 1328-1347 of SEQ ID NO:205)	E5 519
206	Ta_cyp79b3-2-PRT	Mutant Protein	Amino acid change	
207	Ta_cyp83a1-1-CDS	Mutant Coding Region	TTCAGGCCCGAGAGG TTTC (nt 1240-1258 of SEQ ID NO:97) → TTCAGGCC<A>AGA GGTTTC (nt 1240-1258 of SEQ ID NO:207)	A7 66
208	Ta_cyp83a1-1-PRT	Mutant Protein	Amino acid change	
209	Ta_cyp83a1-2-CDS	Mutant Coding Region	TTATCATACAAGATA GGAAA (nt 196-215 of SEQ ID NO:97) → TTATCATACAA(A)G ATAGGAAA (nt 196-216 of SEQ ID NO:209)	
210	Ta_cyp83a1-2-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
211	Ta_cyp83a1-3-CDS	Mutant Coding Region	TTATCATACAAGATA GGAAA (nt 196-215 of SEQ ID NO:97) → TTATCATACAA(T)G ATAGGAAA (nt 196-216 of SEQ ID NO:211)	
212	Ta_cyp83a1-3-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
213	Ta_AOP2-like aop2-2SED-CDS	Mutant Coding Region	(nt 270-318 of SEQ ID NO:1) → CGGTCTTT-- 35bp deletion-- TGGACAAA (nt 270-285 of SEQ ID NO:213)	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
214	Ta_AOP2-like aop2-2SED-PRT	Mutant Protein	Presumed loss of function due to 33bp deletion	
215	Ta_AOP2-like aop2-3SED-CDS	Mutant Coding Region	TCCTCATGTTTTGGAC AAAGTTTA (nt 300-323 of SEQ ID NO:1) →TCCTCAT--TTTGGACAAAGTTA (nt 300-319 of SEQ ID NO:215)	
216	Ta_AOP2-like aop2-3SED-PRT	Mutant Protein	Frameshift caused by 2bp deletion	
217	Ta_AOP2-like aop2-4SED-CDS	Mutant Coding Region	TCCTCATGTTTTGGAC AAAGTTTA (nt 300-323 of SEQ ID NO:1) →TCCTCATGTTT-GACAAAGTTTA (nt 300-322 of SEQ ID NO:217)	
218	Ta_AOP2-like aop2-4SED-PRT	Mutant Protein	Frameshift caused by 1bp deletion	
219	Ta_AOP2-like aop2-5SED-CDS	Mutant Coding Region	TCCTCATGTTTTGGAC AAAGTTTA (nt 300-323 of SEQ ID NO:1) →TCCTCATGTTTT(T)G GACAAAGTTTA (nt 300-324 of SEQ ID NO:219)	
220	Ta_AOP2-like aop2-5SED-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
221	Ta_gtr1-1-CDS	Mutant Coding Region	CCGCAGCTCTTGCTTG CAGG (nt 1561-1580 of SEQ ID NO:13) → CCGCAGCTCTTGTT<T>GCAGG (nt 1561-1580 of SEQ ID NO:221)	I87113, <i>gtr1-1</i>
222	Ta_gtr1-1-PRT	Mutant Protein	Amino acid change	

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
223	Ta_gtr1-2-CDS	Mutant Coding Region	TGAAATGCATTGTGA GAGT (nt 1145-1163 of SEQ ID NO:13) → TGAAATGCATGTGTG AGAGT (nt 1145-1164 of SEQ ID NO:223)	3A5K, <i>gtr1-2</i>
224	Ta_gtr1-2-PRT	Mutant Protein	Frameshift caused by 1bp insertion	
225	Ta_gtr2-1-CDS	Mutant Coding Region	AAAGAAAGTGATGAT GATCA (nt 1762-1781 of SEQ ID NO:16) → AAAGAAAGT<A>ATG ATGATCA (nt 1762-1781 of SEQ ID NO:225)	AX17D
226	Ta_gtr2-1-PRT	Mutant Protein	Amino acid change	
227	Ta_gtr2-2-CDS	Mutant Coding Region	AGTGCATTGTGAGAG TGCT (nt 1037-1055 of SEQ ID NO:16) → AGTGCAT(A)TGTGAG AGTGCT (nt 1037-1056 of SEQ ID NO:227)	3A5C, 3A5K, <i>gtr2-2</i> , A427A
228	Ta_gtr2-2-PRT	Mutant Protein	Frameshift caused by 1bp insertion	A427A
229	Ta_gtr2-3-CDS	Mutant Coding Region	AGTGCATTGTGAGAG TGCT (nt 1037-1055 of SEQ ID NO:16) → AGTGCAT(G)TGTGAG AGTGCT (nt 1037-1056 of SEQ ID NO:229)	3A5K, <i>gtr2-3</i>
230	Ta_gtr2-3-PRT	Mutant Protein	Frameshift caused by 1bp insertion	3A5K
231	MYB28-2180A-CDS	Mutant Coding region	Mutant <i>hag1</i> allele (-GT deletion)	2180A
232	MYB28-2180A-PRT	Mutant Protein	Mutant <i>hag1</i> protein (-GT deletion)	2180A
233	MYB28-2172A-CDS	Mutant Coding region	Mutant <i>hag1</i> allele (-TG deletion)	2172A
234	MYB28-2172A-PRT	Mutant Protein	Mutant <i>hag1</i> protein (-TG deletion)	2172A

SEQ ID NO:	Sequence Name	Type	Function / Nature of the mutation	Names Used and/or Representative Pennycress LOF Mutants Disclosed Herein
235	Ta_gtr1-3-CDS	Mutant Coding Region	TGAAATGCATTGTGA GAGT (nt 1145-1163 of SEQ ID NO:13) → TGAAATGCAT-GTGAGAGT (nt 1145-1164 of SEQ ID NO:235)	3A5C, <i>gtr1-3</i>
236	Ta_gtr1-3-PRT	Mutant Protein	Frameshift caused by 1bp deletion	3A5C

[0032] In certain embodiments, pennycress plant seeds, seed lots, seed meal, and compositions having reduced sinigrin content as described herein can be obtained from the *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207* pennycress mutant lines provided herein, from progeny derived from those mutant lines, from hybrids derived from those mutant lines, or from germplasm from the mutants that provide seed or seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight. In certain embodiments, germplasm from the mutants that provides seed or seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight can be obtained by outcrossing the *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207* pennycress mutant lines to other pennycress lines with wild-type sinigrin levels, selfing progeny of the cross, and selecting for progeny of the self that provide seed or seed meal having less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight. In certain embodiments, germplasm from the mutants that provides seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram can be introgressed into the genetic background of a second pennycress line with wild-type sinigrin levels by using the second pennycress line as a recurrent parent in a series of backcrosses followed by selfs, where progeny of the selfs that seed or seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight are selected and carried forward into additional crosses to the recurrent parent. In certain embodiments, the pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, and/or *I87207* germplasm that provides seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight can be combined in a pennycress plant to provide pennycress plant seeds, seed lots, seed meal, and compositions having reduced

sinigrin content as described herein. In certain embodiments, the pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, and/or *I87207* germplasm can provide pennycress plant seeds, seed lots, seed meal, and compositions comprising 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 μmol sinigrin/gm dw (gram dry weight). Germplasm combinations comprising any of: (i) *E3 196* and *E5 444P1* germplasm; (ii) *E3 196* and *I87113* or *E5 444P1* germplasm; (iii) *E3 196* and *I87207* or *E5 444P1* germplasm; (iv) *I87113* and *I87207* or *E5 444P1* germplasm; (iv) *E3 196*, *I87113*, *E5 444P1*, and *I87207* germplasm; (v) *E5 356P5* and *E5 543* germplasm; or (vi) any combination of *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, and/or *I87207* germplasm that provide seed or seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight are provided herein. Also provided herein is the combination of any of the germplasms of the *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, and/or *I87207* pennycress mutant lines that provides for reduced sinigrin content or any of the aforementioned germplasm combinations of (i), (ii), (iii), (iv), or (v) with germplasm comprising loss-of function mutations in a GSL biosynthetic coding sequence or gene (*e.g.*, SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 92, 93, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, or allelic variant thereof), at least one loss-of-function mutation in a GSL transport coding sequence or gene (SEQ ID NO: 13, 14, 16, 18, or allelic variant thereof), in a GSL hydrolysis coding sequence or gene (SEQ ID NO: 28, 29, or allelic variant thereof), and/or in an expression regulator (*e.g.*, transcription factor; SEQ ID NO: 19, 20, 22, 23, 25, 26, 159, 160, allelic variant thereof) coding sequence or gene.

[0033] A representative wild-type (WT) pennycress MYB28 (*HAG1*) coding sequence is as shown in sequence listing (SEQ ID NO: 19). The terms “MYB28” and “HAG1” are used interchangeably herein. In certain embodiments, a WT pennycress MYB28 coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO: 19), and is referred to as an allelic variant sequence. In certain embodiments, a MYB28 coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 19. A representative wild-type pennycress MYB28 polypeptide is shown in sequence listing (SEQ ID NO: 21). In certain embodiments, a WT pennycress MYB28 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO: 21), and is referred to as an allelic variant sequence. In certain embodiments, a WT pennycress MYB28 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO: 21), referred to herein as an allelic

variant sequence, provided the polypeptide maintains its wild-type function. For example, a MYB28 polypeptide can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99) percent sequence identity to SEQ ID NO: 21. A MYB28 polypeptide of an allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO: 21.

[0034] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include at least one loss-of-function modification in a MYB28 gene (*e.g.*, in a MYB28 coding sequence, in a MYB28 regulatory sequence including the promoter, 5' UTR, intron, 3' UTR, or in any combination thereof) or a transgene or genome rearrangement that suppresses expression of the MYB28 gene. As used herein, a loss-of-function mutation in a MYB28 gene can be any modification that is effective to suppress MYB28 polypeptide expression or MYB28 polypeptide function. In certain embodiments, suppressed MYB28 polypeptide expression and/or MYB28 polypeptide function can comprise elimination or a reduction in such expression or function in comparison to a wild-type plant (*i.e.*, can be complete or partial). Examples of genetic modifications that can provide for a loss-of-function mutation include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, or any combination thereof. In certain embodiments, any of the aforementioned loss-of-function (LOF) modifications in the *MYB28* gene can be combined with a loss-of-function modification in a *MYB29* gene or allelic variant thereof, and/or a loss-of-function modification in a MYB76 gene or allelic variant thereof to obtain pennycress plant seeds, seed lots, seed meal, and compositions having reduced sinigrin content described herein. Plants, germplasm, seed, seed lots, seed meal, and compositions comprising: (i) MYB28 and MYB29 LOF modifications; (ii) MYB28 and MYB76 LOF modifications; (iii) MYB29 and MYB76 LOF modifications; and (iii) MYB28, MYB29 and MYB76 LOF modifications are also provided herein.

[0035] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a deletion (*e.g.*, a single base-pair deletion) relative to the WT pennycress MYB28 coding sequence. In certain embodiments, a modified MYB28 coding sequence can include a single base-pair deletion of the guanine (G) at nucleotide residue 20 in a WT pennycress MYB28 coding sequence (*e.g.*, SEQ ID NO: 19 or an allelic variant thereof). For example, a single base-pair

deletion of the guanine (G) at nucleotide residue at nucleotide residue 20 in a WT pennycress MYB28 coding sequence thereby producing a premature stop codon. A representative modified pennycress MYB28 coding sequence having a loss-of-function single base pair deletion is presented in SEQ ID NO: 80.

[0036] A modified or mutated pennycress MYB28 coding sequence having a loss-of-function single base pair deletion mutation (*e.g.*, SEQ ID NO: 80) can encode a modified MYB28 polypeptide (*e.g.*, a modified MYB28 polypeptide having suppressed MYB28 polypeptide expression and/or reduced MYB28 polypeptide function). For example, a modified pennycress MYB28 coding sequence having a single base-pair deletion (*e.g.*, SEQ ID NO:80) can encode a modified MYB28 polypeptide. In certain embodiments, a modified MYB28 polypeptide can include a truncation resulting from the introduction of a stop codon at codon position 20 within the MYB28 open reading frame (*e.g.*, SEQ ID NO:19). A representative truncated pennycress MYB28 polypeptide is presented in SEQ ID NO:81. The aforementioned loss-of-function modifications in a MYB28 encoding gene or a transgene or genome rearrangement that suppresses expression of the *MYB28* gene thus include loss-of-function modifications in a gene encoding an *MYB28* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *MYB28* allelic variant gene.

[0037] A representative WT pennycress *CYP83A1* coding region is presented in SEQ ID NO:92. Two protospacer locations and adjacent protospacer-adjacent motif (PAM) sites that can be targeted by, for example, CRISPR-SpCAS9, correspond to nucleotides 190 - 209 (protospacer) and 210 - 212 (PAM site).

[0038] In certain embodiments, a WT pennycress *CYP83A1* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:92), and is referred to as an allelic variant sequence. In certain embodiments, a *CYP83A1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:92. A representative WT pennycress CYP83A1 polypeptide is presented in SEQ ID NO:94.

[0039] In certain embodiments, a WT pennycress CYP83A1 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:94), and is referred to as an allelic variant sequence, provided the polypeptide maintains its wild-type function. For example, a CYP83A1 polypeptide can have at least 80, at least 85, at least 90, at least 95, at least

98, or at least 99 percent sequence identity to SEQ ID NO:94. A CYP83A1 polypeptide can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:94.

[0040] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *CYP83A1* gene (*e.g.*, in a *CYP83A1* coding sequence) or a transgene or genome rearrangement that suppresses expression of the *CYP83A1* gene. As used herein, a loss-of-function mutation in a *CYP83A1* gene can be any modification that is effective to suppress CYP83A1 polypeptide expression or CYP83A1 polypeptide function. In certain embodiments, suppressed CYP83A1 polypeptide expression and/or CYP83A1 polypeptide function can comprise elimination or a reduction in such expression (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof. The aforementioned loss-of-function modifications in a CYP83A1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *CYP83A1* gene thus include loss-of-function modifications in a gene encoding an *CYP83A1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of an *CYP83A1* allelic variant gene.

[0041] In certain embodiments, a WT pennycress *AOP2* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:1 or 2), and is referred to as an allelic variant sequence. In certain embodiments, a *AOP2* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:1 or 2. In certain embodiments, a WT pennycress *AOP2* polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:3), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a *AOP2* polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:3. An *AOP2* polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:3.

[0042] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *AOP2* encoding gene or a transgene or genome rearrangement that

suppresses expression of the *AOP2* gene. As used herein, a loss-of-function mutation in a *AOP2* gene can be any modification that is effective to reduce *AOP2* polypeptide expression or *AOP2* polypeptide function. In certain embodiments, suppressed *AOP2* polypeptide expression and/or *AOP2* polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof. The aforementioned loss-of-function modifications in an *AOP2* encoding gene or a transgene or genome rearrangement that suppresses expression of the *AOP2* gene thus include loss-of-function modifications in a gene encoding an *AOP2* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of an *AOP2* allelic variant gene.

[0043] In certain embodiments, a WT pennycress *BCAT4* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:4), and is referred to as an allelic variant sequence. In certain embodiments, a *BCAT4* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:4. In certain embodiments, a WT pennycress *BCAT4* polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:6), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a *BCAT4* polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:6. A *BCAT4* polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:76.

[0044] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *BCAT4* encoding gene or a transgene or genome rearrangement that suppresses expression of the *BCAT4* gene. As used herein, a loss-of-function mutation in a *BCAT4* gene can be any modification that is effective to reduce *BCAT4* polypeptide expression or *BCAT4* polypeptide function. In certain embodiments, suppressed *BCAT4* polypeptide expression and/or *BCAT4* polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof. The aforementioned loss-of-function modifications in

a *BCAT4* encoding gene or a transgene or genome rearrangement that suppresses expression of the *BCAT4* gene thus include loss-of-function modifications in a gene encoding a *BCAT4* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *BCAT4* allelic variant gene.

[0045] In certain embodiments, a WT pennycress *BCAT6* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:7), and is referred to as an allelic variant sequence. In certain embodiments, a *BCAT6* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:7. In certain embodiments, a WT pennycress *BCAT6* polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:9), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a *BCAT6* polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:9. A *BCAT6* polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:9.

[0046] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *BCAT6* encoding gene or a transgene or genome rearrangement that suppresses expression of the *BCAT6* gene. As used herein, a loss-of-function mutation in a *BCAT6* gene can be any modification that is effective to reduce *BCAT6* polypeptide expression or *BCAT6* polypeptide function. In certain embodiments, suppressed *BCAT6* polypeptide expression and/or *BCAT6* polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof. The aforementioned loss-of-function modifications in a *BCAT6* encoding gene or a transgene or genome rearrangement that suppresses expression of the *BCAT6* gene thus include loss-of-function modifications in a gene encoding a *BCAT6* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *BCAT6* allelic variant gene.

[0047] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-

function modification in a CYP79F1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *CYP79F1* gene. As used herein, a loss-of-function mutation in a *CYP79F1* gene can be any modification that is effective to reduce CYP79F1 polypeptide expression or CYP79F1 polypeptide function. In certain embodiments, suppressed CYP79F1 polypeptide expression and/or CYP79F1 polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0048] In certain embodiments, a WT pennycress *CYP79F1* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:10), and is referred to as an allelic variant sequence. In certain embodiments, a *CYP79F1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:10. In certain embodiments, a WT pennycress CYP79F1 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:46), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. In certain embodiments, a CYP79F1 polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:12. A CYP79F1 polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:12. Loss-of-function modifications in a CYP79F1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *CYP79F1* gene thus include loss-of-function modifications in a gene encoding a *CYP79F1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *CYP79F1* allelic variant gene.

[0049] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a GTR1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *GTR1* gene. As used herein, a loss-of-function mutation in a *GTR1* gene can be any modification that is effective to reduce GTR1 polypeptide expression or GTR1 polypeptide function. In certain embodiments, suppressed GTR1 polypeptide expression and/or GTR1 polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without

limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0050] In certain embodiments, a WT pennycress *GTR1* coding sequence can have a sequence that deviates from the coding sequence set forth above (e.g., SEQ ID NO:13), and is referred to as an allelic variant sequence. In certain embodiments, a *GTR1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:13. In certain embodiments, a WT pennycress GTR1 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:15), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. In certain embodiments, a GTR1 polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:15. A GTR1 polypeptide allelic variant can have one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (e.g., substitutions) relative to SEQ ID NO:15. The aforementioned loss-of-function modifications in a GTR1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *GTR1* gene thus include loss-of-function modifications in a gene encoding a *GTR1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *GTR1* allelic variant gene.

[0051] In certain embodiments, pennycress seed lots, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content can include a complete or partial loss-of-function modification in a GTR2 encoding gene or a transgene or genome rearrangement that suppresses expression of the *GTR2* gene. As used herein, a loss-of-function mutation in a *GTR2* gene can be any modification that is effective to reduce GTR2 polypeptide expression or *GTR2* polypeptide function. In certain embodiments, suppressed GTR2 polypeptide expression and/or GTR2 polypeptide function can comprise elimination or a reduction in such expression or function (i.e., can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0052] In certain embodiments, a WT pennycress *GTR2* coding sequence can have a sequence that deviates from the coding sequence set forth above (e.g., SEQ ID NO:16), and is referred to as an allelic variant sequence. In certain embodiments, a *GTR2* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence

identity to SEQ ID NO:16. In certain embodiments, a WT pennycress GTR2 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:17), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. In certain embodiments, a GTR2 polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:17. A GTR2 polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:17. The aforementioned loss-of-function modifications in a GTR2 encoding gene or a transgene or genome rearrangement that suppresses expression of the *GTR2* gene thus include loss-of-function modifications in a gene encoding a *GTR2* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *GTR2* allelic variant gene.

[0053] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content can include a complete or partial loss-of-function modification in a TFP encoding gene or a transgene or genome rearrangement that suppresses expression of the *TFP* gene. As used herein, a loss-of-function mutation in a *TFP* gene can be any modification that is effective to reduce TFP polypeptide expression or TFP polypeptide function. In certain embodiments, suppressed TFP polypeptide expression and/or TFP polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0054] In certain embodiments, a WT pennycress *TFP* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:28), and is referred to as an allelic variant sequence. In certain embodiments, a *TFP* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:28. In certain embodiments, a WT pennycress TFP polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:30), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. In certain embodiments, a TFP polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:30. A TFP polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino

acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:30. The aforementioned loss-of-function modifications in a *TFP* encoding gene or a transgene or genome rearrangement that suppresses expression of the *TFP* gene thus include loss-of-function modifications in a gene encoding a *TFP* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *TFP* allelic variant gene.

[0055] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *BHLH05* encoding gene or a transgene or genome rearrangement that suppresses expression of the *BHLH05* gene. As used herein, a loss-of-function mutation in a *BHLH05* gene can be any modification that is effective to reduce *BHLH05* polypeptide expression or *BHLH05* polypeptide function. In certain embodiments, suppressed *BHLH05* polypeptide expression and/or *BHLH05* polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0056] In certain embodiments, a WT pennycress *BHLH05* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:159 or 160), and is referred to as an allelic variant sequence. In certain embodiments, a *BHLH05* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO:159 or 160. In certain embodiments, a WT pennycress *BHLH05* polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:161), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a *BHLH05* polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 161. An *BHLH05* polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:161. The aforementioned loss-of-function modifications in a *BHLH05* encoding gene or a transgene or genome rearrangement that suppresses expression of the *BHLH05* gene thus include loss-of-function modifications in a gene encoding a *BHLH05* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *BHLH05* allelic variant gene.

[0057] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *IMD1* encoding gene or a transgene or genome rearrangement that suppresses expression of the *IMD1* gene. As used herein, a loss-of-function mutation in a *IMD1* gene can be any modification that is effective to reduce *IMD1* polypeptide expression or *IMD1* polypeptide function. In certain embodiments, suppressed *IMD1* polypeptide expression and/or *IMD1* polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0058] In certain embodiments, a WT pennycress *IMD1* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO: 162 or 163), and is referred to as an allelic variant sequence. In certain embodiments, a *IMD1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 162 or 163. In certain embodiments, a WT pennycress *IMD1* polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO: 164), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a *IMD1* polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 164. An *IMD1* polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO: 164. The aforementioned loss-of-function modifications in a *IMD1* encoding gene or a transgene or genome rearrangement that suppresses expression of the *IMD1* gene thus include loss-of-function modifications in a gene encoding a *IMD1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *IMD1* allelic variant gene.

[0059] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *CYP79B3* encoding gene or a transgene or genome rearrangement that suppresses expression of the *CYP79B3* gene. As used herein, a loss-of-function mutation in a *CYP79B3* gene can be any modification that is effective to reduce *CYP79B3* polypeptide expression or *CYP79B3* polypeptide function. In certain embodiments, suppressed *CYP79B3*

polypeptide expression and/or CYP79B3 polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0060] In certain embodiments, a WT pennycress *CYP79B3* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO: 165 or 166), and is referred to as an allelic variant sequence. In certain embodiments, a *CYP79B3* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 165 or 166. In certain embodiments, a WT pennycress CYP79B3 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:167), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a CYP79B3 polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 167. A CYP79B3 polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:167. The aforementioned loss-of-function modifications in a CYP79B3 encoding gene or a transgene or genome rearrangement that suppresses expression of the *CYP79B3* gene thus include loss-of-function modifications in a gene encoding a *CYP79B3* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *CYP79B3* allelic variant gene.

[0061] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a MAM1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *MAM1* gene. As used herein, a loss-of-function mutation in a *MAM1* gene can be any modification that is effective to reduce MAM1 polypeptide expression or MAM1 polypeptide function. In certain embodiments, suppressed MAM1 polypeptide expression and/or MAM1 polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0062] In certain embodiments, a WT pennycress *MAM1* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO:168 or 169), and is referred to as an allelic variant sequence. In certain embodiments, a *MAM1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 168 or 169. In certain embodiments, a WT pennycress *MAM1* polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:170), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a *MAM1* polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 170. A *MAM1* polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:170. The aforementioned loss-of-function modifications in a *MAM1* encoding gene or a transgene or genome rearrangement that suppresses expression of the *MAM1* gene thus include loss-of-function modifications in a gene encoding a *MAM1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *MAM1* allelic variant gene.

[0063] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a *FMO-GS-Ox1* encoding gene or a transgene or genome rearrangement that suppresses expression of the *FMO-GS-Ox1* gene. As used herein, a loss-of-function mutation in a *FMO-GS-Ox1* gene can be any modification that is effective to reduce *FMO-GS-Ox1* polypeptide expression or *FMO-GS-Ox1* polypeptide function. In certain embodiments, suppressed *FMO-GS-Ox1* polypeptide expression and/or *FMO-GS-Ox1* polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0064] In certain embodiments, a WT pennycress *FMO-GS-Ox1* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO: 171 or 172), and is referred to as an allelic variant sequence. In certain embodiments, a *FMO-GS-Ox1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 171 or 172. In certain embodiments, a WT pennycress *FMO-GS-Ox1* polypeptide can have a sequence that deviates from the polypeptide

sequence set forth above (SEQ ID NO:173), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a FMO-GS-Ox1 polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 173. An FMO-GS-Ox1 polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions) relative to SEQ ID NO:173. The aforementioned loss-of-function modifications in a FMO-GS-Ox1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *FMO-GS-Ox1* gene thus include loss-of-function modifications in a gene encoding a *FMO-GS-Ox1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *FMO-GS-Ox1* allelic variant gene.

[0065] In certain embodiments, pennycress seed lots, plants, seeds, as well as the seed meals and compositions obtained therefrom, all having reduced sinigrin content, can include a loss-of-function modification in a UGT74B1 encoding gene or a transgene or genome rearrangement that suppresses expression of the *UGT74B1* gene. As used herein, a loss-of-function mutation in a *UGT74B1* gene can be any modification that is effective to reduce UGT74B1 polypeptide expression or UGT74B1 polypeptide function. In certain embodiments, suppressed UGT74B1 polypeptide expression and/or UGT74B1 polypeptide function can comprise elimination or a reduction in such expression or function (*i.e.*, can be complete or partial). Examples of genetic modifications include, without limitation, deletions, insertions, substitutions, translocations, inversions, duplications, and any combination thereof.

[0066] In certain embodiments, a WT pennycress *UGT74B1* coding sequence can have a sequence that deviates from the coding sequence set forth above (*e.g.*, SEQ ID NO: 174 or 175), and is referred to as an allelic variant sequence. In certain embodiments, a *UGT74B1* coding sequence allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 174 or 175. In certain embodiments, a WT pennycress UGT74B1 polypeptide can have a sequence that deviates from the polypeptide sequence set forth above (SEQ ID NO:176), and is referred to as an allelic variant sequence provided the polypeptide maintains its wild-type function. For example, a UGT74B1 polypeptide allelic variant can have at least 80, at least 85, at least 90, at least 95, at least 98, or at least 99 percent sequence identity to SEQ ID NO: 176. An UGT74B1 polypeptide allelic variant can have one or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) amino acid modifications (*e.g.*, substitutions)

relative to SEQ ID NO:176. The aforementioned loss-of-function modifications in a *UGT74B1* encoding gene or a transgene or genome rearrangement that suppresses expression of the *UGT74B1* gene thus include loss-of-function modifications in a gene encoding a *UGT74B1* allelic variant gene, or a transgene or genome rearrangement that suppresses expression of a *UGT74B1* allelic variant gene.

[0067] In certain embodiments, the pennycress seeds, seed lots, seed meals (defatted and non-defatted), compositions comprising those seed meals, and pennycress plants provided herein can comprise loss-of-function mutation(s), transgene(s), and/or genomic rearrangement(s) that suppress expression and/or activity of at least two of any of the aforementioned endogenous pennycress genes or allelic variants thereof (*e.g.*, *MYB28*, *MYB29*, *MYB76*, *CYP83A1*, *AOP2*, *BCAT4*, *BCAT6*, *CYP79F1*, *GTR1*, *GTR2*, *TFP*, *BHLH05* *IMD1*, *CYP79B3*, *MAM1*, *FMO-GS-Ox1*, and/or *UGT74B1*) or encoded polypeptides). In one embodiment, the loss-of-function mutation(s), genomic rearrangement(s), and/or transgene(s) can suppress expression of both a *GTR1* gene (*e.g.*, of SEQ ID NO:15 or an allelic variant thereof) and a *GTR2* gene (*e.g.*, of SEQ ID NO:17 or an allelic variant thereof). In one embodiment, the loss-of-function mutation(s), genomic rearrangement(s), and/or transgene(s) can suppress expression and/or activity of both a *MYB28* gene (*e.g.*, of SEQ ID NO:21 or an allelic variant thereof) and a *MYB29* gene (*e.g.*, of SEQ ID NO:24 or an allelic variant thereof). In one embodiment, the loss-of-function mutation(s), transgene(s), and/or genomic rearrangement(s) can suppress expression and/or activity of both a *GTR1* gene (*e.g.*, of SEQ ID NO:15 or an allelic variant thereof) and a *MYB29* gene (*e.g.*, of SEQ ID NO:24 or an allelic variant thereof). In certain embodiments, suppression of gene expression and/or activity provided by the loss-of-function mutation(s), transgene(s), and/or genomic rearrangement(s) is partial. In certain embodiments, such partial suppression in the any of the aforementioned embodiments can comprise a reduction of at least 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% of activity and/or transcript levels of the endogenous pennycress gene (*e.g.*, *MYB28*, *MYB29*, *MYB76*, *CYP83A1*, *AOP2*, *BCAT4*, *BCAT6*, *CYP79F1*, *GTR1*, *GTR2*, *TFP*, *BHLH05* *IMD1*, *CYP79B3*, *MAM1*, *FMO-GS-Ox1*, and/or *UGT74B1*) in the plant or a part of the plant (*e.g.*, seed) comprising the loss-of-function mutation(s), transgene(s), and/or genomic rearrangement(s) in comparison to the activity and/or transcript levels in a wild-type control plant lacking the loss-of-function mutation(s), transgene(s), and/or genomic rearrangement(s).

[0068] In certain embodiments, a genome editing system such as a CRISPR-Cas9 system can be used to introduce one or more loss-of-function mutations into genes such as the glucosinolate biosynthesis, transporters and related regulatory genes (*i.e.*, transcription factors) provided herewith in Table 1 and the sequence listing to obtain pennycress plants, seeds, seed lots, and compositions with reduced seed sinigrin content. For example, a CRISPR-Cas9 vector can include at least one guide sequence specific to a pennycress *GTR2* sequence (see, *e.g.*, SEQ ID NO:16) and/or at least one guide sequence specific to a pennycress *GTR2* sequence (see, *e.g.*, SEQ ID NO:17). A Cas9 enzyme will bind to and cleave within the gene when the target site is followed by a PAM sequence. For example, the canonical SpCAS9 PAM site is the sequence 5'-NGG-3', where N is any nucleotide followed by two guanine (G) nucleotides. The Cas9 component of a CRISPR-Cas9 system designed to introduce one or more loss-of-function modifications described herein can be any appropriate Cas9. In certain embodiments, the Cas9 of a CRISPR-Cas9 system described herein can be a *Streptococcus pyogenes* Cas9 (SpCas9). One example of a SpCas9 is described in Fauser et al., 2014.

[0069] The LOF mutations in any of the genes of coding sequences of Table 1 can be introduced by a variety of methods. Methods for introduction of the LOF mutations include, but are not limited to, traditional mutagenesis (*e.g.*, Ethyl Methane Sulfonate (EMS), fast neutrons (FN), or gamma rays), TILLING, meganucleases, zinc finger nucleases, transcription activator-like effector nucleases, clustered regularly interspaced short palindromic repeat (CRISPR)-associated nuclease (*e.g.*, Cas9, Cpf1, Cms1, *S.aureus* Cas9 variants, eSpCas9), targetrons, and the like. Various tools that can be used to introduce mutations into genes have been disclosed in Guha et al., 2017. Methods for modifying genomes by use of Cpf1 or Csm1 nucleases are disclosed in US Patent Application Publication 20180148735, which is incorporated herein by reference in its entirety, can be adapted for introduction of the LOF mutations disclosed herein. Methods for modifying genomes by use of CRISPR-CAS systems are disclosed in US Patent Application Publication 20180179547, which is incorporated herein by reference in its entirety, can be adapted for introduction of the LOF mutations disclosed herein. The genome editing reagents described herein can be introduced into a pennycress plant by any appropriate method. In certain embodiments, nucleic acids encoding the genome editing reagents can be introduced into a plant cell using *Agrobacterium*- or *Ensifer* mediated transformation, particle bombardment, liposome delivery, nanoparticle delivery, electroporation, polyethylene glycol

(PEG) transformation, or any other method suitable for introducing a nucleic acid into a plant cell. In certain embodiments, the Site-Specific Nuclease (SSN) or other expressed gene editing reagents can be delivered as RNAs or as proteins to a plant cell and the RT, if one is used, can be delivered as DNA.

[0070] Also provided herein are defatted pennycress seed meal with reduced sinigrin content in comparison to defatted pennycress seed meal obtained from wild-type pennycress seed lots. Defatted-pennycress seed meal is a product obtained from high-pressure crushing of seed, or from a pre-press solvent extraction process, which removes oil from the whole seed. Solvents used in such extractions include, but are not limited to, hexane or mixed hexanes. The meal is the material that remains after most of the oil has been removed. The typical range of sinigrin in meal made from wild-type pennycress seed is greater than 190 micromoles sinigrin per gram meal by dry weight ($\mu\text{mol/gm dw}$). To be useful as a high protein animal feed, and competitive with other protein feedstuffs, the level of sinigrin level in meal should be less than 30 micromoles sinigrin per gram by dry weight of the meal. In certain embodiments, defatted pennycress seed meal having a sinigrin content of less than 30, 28, 25, or 15 $\mu\text{mol sinigrin/gm dw}$ are provided. In certain embodiments, defatted pennycress seed meal having a sinigrin content of about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 $\mu\text{mol sinigrin/gm dw}$ is provided herein. Compositions comprising such defatted pennycress seed meal are also provided herein. Such seed meal or compositions can comprise polynucleotides encoding any of the aforementioned LOF mutations. Such seed meal or compositions can also comprise any marker that is characteristic of the pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207* germplasm. In certain embodiments, such biomarkers include a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*. Mutations in the pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207* can be identified by sequencing the genomic DNA or pertinent genes (*e.g.*, genes of Table 1) and comparing those sequences to the corresponding sequences of the parent pennycress lines from which they were obtained.

[0071] Non-defatted pennycress seed meal having less sinigrin than non-defatted control pennycress seed meal obtained from wild-type pennycress seed is provided herein. In certain embodiments, the sinigrin content of non-defatted pennycress seed meal and compositions comprising the same that are provided herein is reduced from about 1.25-, 1.5-, 2-, or 3-fold to

about 4-, 5-, 6-, 7-, 10-, 20-, 40-, 50-, 60-, 70-, 80-, 100-, 120-, 140-, 160-, 180-, or 200- fold in comparison to control non-defatted pennycress seed meal and compositions comprising the same obtained from control wild-type pennycress seeds. In certain embodiments, the non-defatted pennycress seed meal is obtained from pennycress seeds that have been crushed, ground, macerated, expelled, extruded, or any combination thereof. Typically, the level of sinigrin in wild-type pennycress seed and non-defatted seed meal obtained therefrom varies from about 70 to about 150 μmol sinigrin /gm dw. To be useful as a high protein animal feed, and competitive with other protein feedstuffs, the sinigrin level in non-defatted meal should be less than 30 μmol sinigrin /gm dw of the meal. In certain embodiments, non-defatted pennycress seed meal having a sinigrin content of less than 30, 28, 25, 16, or 15 μmol sinigrin /gm dw are provided herein. In certain embodiments, non-defatted pennycress seed meal having a sinigrin content of about less than 15, 14, or 12 μmol sinigrin/gm dw is provided herein. In certain embodiments, non-defatted pennycress seed meal having a sinigrin content of 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 μmol sinigrin/gm dw are provided herein. Compositions comprising such non-defatted pennycress seed meal are also provided herein. Such seed meal or compositions can comprise polynucleotides encoding any of the aforementioned LOF mutations.

[0072] The disclosure will be further described in the following examples, which do not limit the scope of the disclosure described in the claims.

Examples

[0073] *Example 1: Generation of transgenic pennycress lines harboring the CRISPR-Cas9 or CRISPR-Cpf1 or CRISPR-Cms1 constructs.*

Materials and Methods

[0074] *Construction of the *Thlaspi arvense* (pennycress) AOP2, BCAT4, BCAT6, CYP79F1, CYP83A1, GTR1, GTR2, MYB28 (HAG1), HAG3 (MYB29), MYB76 and TFP gene-specific CRISPR genome-editing vectors.*

[0075] The constructs and cloning procedures for generation of the *Thlaspi arvense* (pennycress) AOP2, BCAT4, BCAT6, CYP79F1, CYP83A1, GTR1, GTR2, MYB28 (HAG1), HAG3 (MYB29), MYB76 and TFP-specific CRISPR-SpCas9 and CRISPR-SaCas9 constructs were adapted in part from the following two publications that describe general procedures for use of SaCas9 in plants: Steinert J, et. al. (2015) and Fauser F, et. al. (2014).

[0076] The plant selectable markers (formerly *NPT*) in the original pDe-SpCas9 and pDe-SaCas9 binary vectors were swapped for hygromycin resistance (*Hygromycin phosphotransferase*, or *HPT*) or fluorescent protein marker (FP) gene.

[0077] Vector transformation into *Agrobacterium*

[0078] The pDe-SpCas9_Hyg, pDe-SaCas9_Hyg, pARV145, containing the *Streptococcus pyogenes Cas9* (*SpCas9*) and the *Staphylococcus aureus Cas9* (*SaCas9*) cassettes, or related vectors represented in Figures 1-7, with the corresponding sequence-specific protospacers were transformed into *Agrobacterium tumefaciens* strain GV3101 using the freeze/thaw method (Holsters et al, 1978).

[0079] The transformation product was plated on 1% agar Luria Broth (LB) plates with gentamycin (50 µg/ml) rifampicin (50 µg/ml) and spectinomycin (75 µg/ml). Single colonies were selected after two days of growth at 28°C.

[0080] Plant transformation - *pennycress floral dip*

[0081] DAY ONE:

[0082] 5mL of LB + 5uL with appropriate antibiotics (Rifampin (50), Spectinomycin (75), and/or Gentamycin (50)) were inoculated with *Agrobacterium*. The cultures were allowed to grow, with shaking, overnight at 28°C.

[0083] DAY TWO (early morning):

[0084] 25mL of Luria Broth + 25uL appropriate antibiotics (Rifampin (50), Spectinomycin (75), and/or Gentamycin (50)) were inoculated with the initial culture from day one. The cultures were allowed to grow, with shaking, overnight at 28°C.

[0085] DAY TWO (late afternoon):

[0086] 250mL of Luria Broth + 250uL appropriate antibiotic (Rifampin (50), Spectinomycin (75), and/or Gentamycin (50)) were inoculated with 25mL culture. The cultures were allowed to grow, with shaking, overnight at 28°C.

[0087] DAY THREE:

[0088] When the culture had grown to an OD₆₀₀ of ~1 (or looks thick and silky), the culture was decanted into large centrifuge tubes (all evenly weighted with analytical balance), and spun at 3,500 RPM at room temperature for 10 minutes to pellet cells. The supernatant was decanted off. The pelleted cells were resuspended in a solution of 5% sucrose and 0.02% Silwet L-77. The suspension was poured into clean beakers and placed in a vacuum chamber.

[0089] Newly flowering inflorescences of pennycress were fully submerged into the beakers, and subjected to a vacuum pressure of ~30 inches mercury (~14.7 psi) for 5 to 10 minutes.

[0090] After racemes of pennycress plants (W0011 variety; these plants were 5 generations removed from seeds) were dipped, they were covered loosely with Saran wrap to maintain humidity and kept in the dark overnight before being uncovered and placed back in the environmental growth chamber.

[0091] ***Screening transgenic plants and growth condition***

[0092] Pennycress seeds were surface sterilized by first rinsing in 70% ethanol then incubating 10 minutes in a 30% bleach, 0.05% SDS solution before being rinsed two times with sterile water and plated on selective plates (0.8% agar/one half-strength Murashige and Skoog salts with hygromycin B selection (40 U/ml) or glufosinate (18 µg/ml). Plates were wrapped in parafilm and kept in an environmental growth chamber at 21°C, 16:8 day/night for 8 days until antibiotic or herbicide selection was apparent.

[0093] Surviving hygromycin or glufosinate-resistant T₁-generation seedlings were transplanted into autoclaved Redi-Earth soil mix and grown in an environmental growth chamber set to 16-hour days/8-hour nights at 21°C and 50% humidity. T₂-generation seeds were planted, and ~1.5 mg of leaf tissue from each T₂-generation plant was harvested with a 3-mm hole punch, then processed using the Thermo Scientific™ Phire™ Plant Direct PCR Kit (Catalog # F130WH) as per manufacturer's instructions. PCR (20 µl volume) was performed.

[0094] ***Example 2: Generation and Characterization of EMS-mutagenized low sinigrin mutant lines E3 196, E5 444P1, I87113 and I87207.***

[0095] Mutants carrying domestication enabling low glucosinolate trait were isolated from two mutant populations independently created using chemical mutagenesis (ethyl methanesulfonate, EMS) protocol essentially as described in the Materials and Methods section below.

[0096] In other embodiments, pennycress plants exhibiting domestication enabling traits such as reduced seed glucosinolate content and loss-of-function mutations in domestication genes can be identified in mutant populations created using fast neutrons (FN), gamma rays (γ rays) or other methods of introducing genetic diversity into genomic DNA.

Materials and Methods

[0097] Solutions:

- A) 0.2 M sodium phosphate monobasic ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) 6.9g/250mL
B) 0.2 M sodium phosphate dibasic (NaH_2PO_4 anhydrous) 7.1g/250mL

[0098] For 50 mL of 0.1 M sodium phosphate buffer at pH 7:

9.75 mL A
15.25 mL B
25.0 mL dH₂O

[0099] 0.2% EMS in buffer:

20 mL 0.1M Sodium Phosphate Buffer, pH 7
40 µL EMS liquid (Sigma #M0880-5G)

[00100] 0.1 M sodium thiosulfate at pH 7.3:

12.4 g sodium thiosulfate in 500 mL

[00101] Primary Seed Surface Sterilization

[00102] In the Set #1 experiments, wild-type pennycress (*Thlaspi arvense*) seeds (W0011 ecotype) were surface sterilized for 10 minutes in a 30% bleach, 0.05% SDS solution before being rinsed 3X with sterile water. Sterilized seeds were immediately subjected to EMS treatment.

[00103] Ethyl Methane Sulfonate (EMS) Treatment of Pennycress Seeds

[00104] In the Set #1 experiments, sterilized pennycress seeds (41 g) were agitated in distilled water overnight. Four 250 mL Erlenmeyer flasks with 10 g seed each, and 1 g in a separate small flask as a control, were agitated. The water was decanted.

[00105] 25 mLs of 0.2% EMS in 0.1M sodium phosphate buffer (pH 7) was added. The control received only phosphate buffer with no EMS. The flasks were shaken in fume hood for 18 hours. The EMS solution was decanted off into an EMS waste bottle.

[00106] To rinse the seeds, 25 ml of dH₂O was added to each flask, and the flasks were shaken for 20 minutes. The rinse water was decanted into the EMS waste bottle.

[00107] To deactivate the EMS, seeds were washed for 20 minutes in 0.1M sodium thiosulfate (pH 7.3). The sodium thiosulfate solution was decanted into the EMS waste bottle.

[00108] The seeds were rinsed 4 times with dH₂O for 15 minutes.

[00109] The seeds were suspended in 0.1% agarose, and germinated directly in autoclaved Redi-Earth soil mix at a density of approximately 10 seeds per 4-inch pot.

[00110] In the Set #2 experiments, 42 grams of seeds derived from pennycress accession MN106 were collected as described elsewhere (Dorn et al., 2013), and were treated with 180 ml 0.2% ethyl methanesulfonate (EMS) in a chemical flow hood. The solution and seeds were kept mixed on a rotating platform for 14 hours at room temperature. The seeds were thereafter extensively rinsed with distilled water to remove all traces of the EMS. The seeds were then dried for 24 hours on filter paper in a chemical flow hood. These seeds were considered to be the progenitors of the M1-generation of plants.

[00111] ***Plant Growth Conditions***

[00112] In the Set #1 experiments, EMS-treated pennycress seeds were germinated and grown in an environmental growth chamber at 21°C, 16:8 6400K fluorescent light/dark, 50% humidity. Approximately 14 days after planting, plants were thinned and transplanted to a density of 4 plants per 4-inch pot. These M1-generation plants showed telltale chlorotic leaf sectors that are indicative of a successful mutagenesis.

[00113] After dry-down, these M1-generation W0011 plants were catalogued and harvested. The M2- and M3-generation seeds were surface sterilized, planted and grown according to the protocols previously described.

[00114] In the Set #2 experiments, the MN106 mutagenized seeds were sowed into two small field plots. These plots were allowed to grow over the winter. The following spring abundant albino sectors were noted on the flowering plants as an indication of a successful mutagenesis.

[00115] ***Identification and characterization of low seed sinigrin mutant lines***

[00116] In the Set #1 experiments, seeds (M3-generation) from putative M2-generation mutants were planted and grown in potting soil-containing 4-inch pots in a growth chamber, harvested and the sinigrin content in the seed was assessed upon its desiccation to a moisture level of 7-9%. EMS mutagenesis typically introduces single-nucleotide transition mutations (*e.g.*, G to A, or C to T) into plant genomes.

[00117] In the Set #2 experiments, seeds were collected from mature M1-generation MN106 plants. M2-generation seeds from batches of 10 M1-generation plants were pooled together. In all, 500 pools representing 5000 mutagenized M1-generation plants were collected. In August, each pool was sowed in a field into an individual row. Robust growth was noted in October.

During the following June, M3-generation seeds were collected from approximately 8,000 mature M2-generation individual plants and stored in individual packets.

[00118] In both Sets #1 and #2 experiments, NIR spectral analysis was used to make preliminary identification of lines with reduced glucosinolate in M3-generation seeds from each packet. These seeds were scanned using a Metrohm NIRS XDS MultiVial Analyzer or a Pertent DA7250 NIR Spectroscopy Analyzer to assess the amount of sinigrin as described elsewhere (Sidhu et. al., 2014; Golebiowski et. al, 2005; Riu et. al., 2006; Xin et. al., 2014). These analyses captured information related to the approximate levels of total glucosinolate and were used to identify low sinigrin candidates. Seeds showing a significant predicted reduction were used in a wet lab analysis to confirm or determine the sinigrin amount with better accuracy.

[00119] Near infrared (NIR) spectroscopic analysis was used to determine the sinigrin content of selected seed lines *E3 196*, *E5 444P1*, *I87113* and *I87207* and to compare the obtained values to the range of sinigrin in corresponding wild type seeds. These mutant lines showed sinigrin content significantly below population average and along with some other representative lines and controls were further analyzed using a method adapted from (Kliebenstein et.al., 2001). Results presented in Table 2 indicate that sinigrin levels in the seeds of these mutant lines are significantly lower and are outside of the corresponding ranges found in control parental seeds.

[00120] **Table 2.** Sinigrin content in seeds from selected pennycress lines with low glucosinolates content was measured using high throughput ion-exchange chromatography-based method. A minimum of three biological replicates each consisting of 20 mg (~20 seeds) per replicate was used. Each biological replicate was split into two technical replicates that were loaded on the mini-column and treated independently after seed extraction process. Last column represents standard error of the mean for glucosinolates (primarily sinigrin) content in each line.

	Line ID	Origin	Biological Reps	Technical Reps	Sinigrin, Mean μ moles/g seed	Std Error, Mean μ moles/g
1	E3 196	MN106-derived	6	2	15	1.6
2	E5 444P1	MN106-derived	6	2	23	3.5
3	I87207	W0011-derived	3	2	25	4.1
4	I87113	W0011-derived	6	2	30	4.5
5	I87102	W0011-derived	3	2	94	8.0
6	I87383	W0011-derived	3	2	96	10.7
7	E5 051 P1	MN106-derived	3	2	99	8.9
8	I87256	W0011 wild	3	2	110	9.2

	Line ID	Origin	Biologic al Reps	Technica l Reps	Sinigrin, Mean μmoles/g seed	Std Error, Mean μmoles/g
		type				
9	E5 101 P1	MN106-derived	3	2	102	10.1
10	E5 484P6	MN106-derived	3	2	106	10.4
11	1120/1062 1-13	ARV breeding	3	2	101	12.1
12	1082/1008 3-12-1	ARV breeding	3	2	106	12.2
13	1053/1023 2-5-1	ARV breeding	3	2	112	5.9
14	Y1067	ARV low fiber	3	2	129	9.4
15	Y1126	ARV low fiber	3	2	128	10.2
16	Beecher (WT parent)	USDA	120	2	103	2.5
17	W0011 (WT parent)	WIU/ISU	6	2	102	6.4
18	MN106 (WT parent)	UMN	6	2	116	8.5

[00121] Example 3. Identification of underlying gene mutations in EMS-generated low seed sinigrin mutant lines.

[00122] Genomic DNA was extracted from each mutant, and each sample was subjected to whole-genome sequencing (adapted from Zhang, X., et al., 2018) and extensive bioinformatic analysis to identify induced mutations resulting in amino acid substitutions. For every gene target described in Table 1, a sequence from the mutant line was compared to a WT sequence from the parental line. If the EMS-induced change resulted in a non-silent mutation (amino acid change or a stop codon), the mutation was considered to be a candidate for the low sinigrin phenotype. Once the mutation was identified, a co-segregation analysis in the F2 single seeds or F3 families derived from each of these mutants was performed. This whole-genome sequencing (WGS) revealed that *E3 196 (Nutty)* line contains a mutation in a predicted pennycress ALKNYL HYDROXALKYL PRODUCING (AOP) polypeptide involved in the last step of sinigrin biosynthesis, while the *I87113* line carries a homozygous mutation in the *GTR1* gene which encodes a glucosinolate transporter.

[00123] Mutation in the AOP2-like gene co-segregates with low glucosinolate phenotype in seeds and vegetative tissues of mutant E3 196 (Nutty) pennycress line.

[00124] In order to demonstrate that the mutation in the *AOP2* gene discovered in the *E3 196 (Nutty)* mutant is responsible for the low sinigrin phenotype, a segregating F2 population from

the cross of homozygous *Nutty* mutant with WT MN106 parental line was performed. The results are presented in Table 3.

[00125] Table 3. Glucosinolates content in seeds and vegetative tissues from the segregating population created using mutant pennycress line *E3 196 (Nutty)*. Each line was genotyped for the presence of G97R mutation found in *AOP2* gene variant in *E3 196 (Nutty)* using standard sequencing. Moisture and sinigrin content in seeds were measured using NIRS, whereas total glucosinolates content in fresh tissue was determined using a wet-lab method described in Chopra et al. (2019).

	NIR sample #	Genotype, G97R	Moisture, %	Sinigrin, μ moles/g seed	Glucosinolates μ moles/g tissue
1	15	wt	7.3	115.4	26.1
2	23	wt	7.6	98.3	23.9
3	29	wt	7.1	101.1	20.7
4	34	wt	7.1	108.1	9.7
5	35	wt	7.3	111.3	24.6
6	37	wt	7.4	115.1	13.8
7	38	wt	7.3	106.0	7.8
8	8	homo	7.5	4.9	0.7
9	12	homo	7.5	9.2	0.5
10	17	homo	7.0	6.9	1.4
11	24	homo	7.7	13.7	0.4
12	28	homo	7.4	7.4	0.3
13	41	homo	6.9	2.1	2.1
14	1	het	6.9	107.7	19.0
15	6	het	7.1	106.3	21.5
16	7	het	7.1	102.0	23.9
17	10	het	7.7	110.0	25.6
18	13	het	7.3	95.4	28.6

	NIR sample #	Genotype, G97R	Moisture, %	Sinigrin, μ moles/g seed	Glucosinolates μ moles/g tissue
19	14	het	7.5	100.4	17.2
20	19	het	7.4	89.8	17.7
21	22	het	7.4	108.1	24.4
22	26	het	7.4	103.5	23.3
23	27	het	7.6	99.6	23.7
24	32	het	7.0	114.3	n/a
25	33	het	7.2	103.6	23.6
	Average	WT		107.9	18.1
	Average	HET		103.4	22.6
	Average	HOMO		7.4	0.9

[00126] The results presented in Table 3 strongly indicate that the G97R mutation present in the *AOP2* gene variant in mutant line *E3 196 (Nutty)* mutant line results in ~20-fold reduction of total glucosinolates content in dry seeds and vegetative tissues of the mutant plant.

[00127] *Mutation in the homolog of GTR1 gene results in low glucosinolate phenotype in seeds and vegetative tissues of mutant I87113 pennycress line.*

[00128] Using a WGS approach, the *I87113* line was found to carry a homozygous mutation believed to confer a L491F amino acid change in GTR1, a glucosinolate transporter and a member of a major facilitator superfamily. In 98 *Embryophyta* sequences this position is in a conserved transmembrane helical region and is populated exclusively with small hydrophobic AAs, suggesting that the L491F-causing mutation results in at least a partial loss of function. Indeed, in a separate set of NIRS and wet-lab experiments, the progeny of the *I87113* mutant has consistently demonstrated a significant reduction in glucosinolates levels in dry seeds (~30% of the WT level).

[00129] **Table 4.** Sinigrin content in seeds of *gtr1-1* mutant *I87113* as determined using a wet-lab method described in Chopra et.al. (2019).

Line ID	Generation/Type	Sinigrin, Mean μmoles/g seed	Std Error, Mean μmoles/g
I87113	M3	25	4
I87113	M3	30	4
I87113	M4	33	2
W0011	Control	98	4
Beecher	Control	101.4	7

[00130] Example 4: Discovery and Characterization of Other Mutant Lines with Low Sinigrin Content in Seeds.

[00131] In the process of whole genome sequencing (WGS) of multiple EMS-mutagenized lines segregating for useful traits (flowering, pod-shattering, oil, protein and fiber content, etc.) mutations in other genes described as potential targets for suppression in Table 1 were identified. In these cases, mutations were present almost exclusively in a heterozygous form, consistent with the fact that they were not selected based on a low glucosinolate phenotype which typically requires a homozygous LOF mutation. Instead, they were identified using this opportunistic approach because the original seed stock was very heavily mutagenized (with an estimated 1,000-2,000 mutations per haploid genome), which makes the presence of more than one potentially useful mutation in the same line relatively likely. Because these lines were selected exclusively based on presence of non-silent mutations, most are expected to be in non-conserved regions and have little or no impact on corresponding gene functions. Nevertheless, these lines were subjected to NIRS and wet-lab assays in order to determine the effects of the identified mutations on glucosinolate content in seeds. The results are summarized in Table 5.

[00132] Table 5. Sinigrin content in seeds of the segregating populations created using mutant pennycress lines identified via WGS. The genotypes of each mother line were determined using standard sequencing. Moisture and sinigrin content in seeds were measured using NIRS whereas, total glucosinolates content in dry seeds was determined using a wet-lab procedure described in (Chopra *et.al.*, 2019).

	Line Name	Gene Affected	Moisture %	NIRS Sinigrin, μ moles/g seed	Wet-Lab Glucosinolate μ moles/g seed	Genotype of the mother line
1	A7 11	<i>FMO GS-OXI</i>	7.6	103.3	113.5	HET
2	A7 66 - CYP83A1 Mut	<i>CYP83A1</i>	7.3	101.0	123.5	HOM
3	A7 66 - CYP83A1 WT	<i>CYP83A1</i>	8.1	85.3	119.2	WT
4	A7 95	<i>IMD1</i>	4.9	115.5	115.1	HOM
5	D3 22	<i>IMD1</i>	8.3	96.6	120.0	HET
6	D3 N13P3 - F2 (Mut) - 16	<i>bHLH05 (MYC3)</i>	17.1	81.1	66.0	HOM
7	D3 N13P3 - F2 (Mut) - 22	<i>bHLH05 (MYC3)</i>	12.3	80.2	91.4	HOM
8	D3 N13P3 - F2 (Wt) -11	<i>bHLH05 (MYC3)</i>	13.7	119.1	125.1	WT
9	D3 N13P3 - F2 (Wt) -12	<i>bHLH05 (MYC3)</i>	16.8	122.8	118.8	WT
10	E5 133P2-1	<i>bHLH05 (MYC3)</i>	7.5	63.6	86.0	unknown
11	E5 133P2-2	<i>bHLH05 (MYC3)</i>	8.1	90.3	107.9	unknown
12	E5 133P2-3	<i>bHLH05 (MYC3)</i>	7.9	57.9	94.9	unknown
13	E5 356P5	<i>FMO GS-OXI</i>	7.5	92.5	96.7	HET
14	E5 519 - CYP79B3 Mut 309	<i>CYP79B3</i>	7.9	91.8	110.2	HOM
15	E5 519 - CYP79B3 Mut 311	<i>CYP79B3</i>	8.1	80.9	108.4	HOM

	Line Name	Gene Affected	Moisture %	NIRS Sinigrin, $\mu\text{moles/g}$ seed	Wet-Lab Glucosinolate $\mu\text{moles/g}$ seed	Genotype of the mother line
16	E5 543	<i>MAMI</i>	6.3	57.9	89.7	HET
17	MN106 #33	Wt	8.2	91.2	106.6	WT
18	A7 137	Wt	7.7	99.8	104.0	WT
19	E5 301P1	Wt	8.3	94.5	99.0	WT

[00133] This analysis suggested that some of the mutations (such as in FMO-GS-Ox1 and MAM1 genes) may have at least a partial impact on corresponding protein function. To test this hypothesis, the seeds from the progeny of the original heterozygous lines (segregating in a typical 1:2:1 Mendelian ratio) were subjected to a single-seed wet-lab assay and PCR-based genotyping. The results summarized in Table 6 suggest that mutations in FMO-GS-Ox1 and MAM1 may result in reduction of glucosinolates in dry seeds of homozygous mutant lines (40-60% of WT level).

[00134] **Table 6. Glucosinolates** content in seeds of the segregating populations created using mutant pennycress lines. Total glucosinolates content ($\mu\text{moles/g}$) in single seeds was determined using a wet-lab method described in Chopra *et al.* (2019).

	Gene	WT	Mutant
1	CYP83A1	138 ($\pm$ 12.22)	113 ($\pm$ 19.72)
2	FMO-GS-Ox1	106 ($\pm$ 10.4)	64 ($\pm$ 3.98)
3	MAM1	127 ($\pm$ 8.82)	51 ($\pm$ 4.67)

Example 5. Identification and characterization of CRISPR-induced mutations in target genes related to glucosinolate pathway and seed accumulation.

[00135] Gene editing using Cas9, Cpf1 and Cms1 nucleases typically introduces a double-stranded break into a targeted genome area in close proximity to the nuclease's PAM site. During non-homologous end-joining process (NHEJ) double-stranded breaks are repaired, at times resulting in the introduction of INDELS-type mutations at the repair location in targeted

genomes. To identify plants with small INDELS in targeted genes of interest, standard Sanger sequencing and/or T7 endonuclease assays (Guschin et. al., 2010) were employed. Standard PCR protocols followed by Sanger sequencing were used to identify and characterize larger (several hundred base pairs) deletions. Sequence analyses revealed that multiple guide RNAs/CRISPR nuclease combinations were effective in generating loss-of-function (LOF) mutations in gene targets described in Table 1. Plants carrying LOF mutations were grown to the next generation and the phenotypes in seeds or vegetative tissues were confirmed using analytical methods.

[00136] Multiple mutations in the MYB28 (*HAG1*) gene were identified, including frameshift mutations likely conferring complete loss of gene function, but no reduction in sinigrin was observed in any of the corresponding homozygous mutant lines (Table 7). On the other hand, mutations in another MYB family member, *MYB29* (*HAG3*), did result in sinigrin reduction, on average, by 35-50% (Tables 7-9). However, in vegetative tissues of *myb28/myb29* (*hag1/hag3*) mutations stack, a dramatic reductions in glucosinolate content relative to WT controls were observed, suggesting a redundancy in the *MYB28* and *MYB29* gene functions.

[00137] **Table 7.** Sinigrin levels as determined using a wet-lab method described in Chopra *et al.* (2019), in homozygous lines generated using CRISPR-induced mutagenesis in selected gene targets described in Table 1.

	Gene Name(s)	Line Name Genotype	Gene ratio n	Sinigrin, μmoles/g seed	Glucosinolates μmoles/g fresh tissue	% Control
1	WT Control (Beecher)	WT-Beecher		105	46.1	n/a
2	WT Control (W0011)	WT-W0011		94.3	40.1 ± 5.7	n/a
3	<i>MYB28</i> (<i>HAG1</i>)	<i>hag1-1</i> (homozygous-G deletion)	T2	98.1		98%
4	<i>MYB28</i> (<i>HAG1</i>)	<i>hag1-2</i> (homozygous+ A insertion)	T3	100.7		101%
5	<i>MYB28</i> (<i>HAG1</i>) <i>MYB29</i> (<i>HAG3</i>) Stack	2180A (<i>hag1</i> het -2bp; <i>hag3</i> - 2 homo -6 bp)	T1		26.0 ± 3.1	65%

	Gene Name(s)	Line Name Genotype	Gene ratio n	Sinigrin, μmoles/g seed	Glucosinolates μmoles/g fresh tissue	% Control
6	<i>MYB28 (HAG1)</i> <i>/MYB29 (HAG3)</i> stack	2172A (<i>hag1</i> biallelic -2bp, +A; <i>hag3-1</i> homo -13 bp)	T1		1.1 ± 0.3	3%
7	<i>GTR1/GTR2</i> stack	3A5K (<i>gtr1-2</i> homo +G, <i>gtr2-3</i> chimeric +G, +A, WT)	T2	20.6		21%
8	<i>GTR1/GTR2</i> stack	3A5C (<i>gtr1-3</i> het -T, <i>gtr2-2</i> homo +A)	T2	48.9		49%

[00138] Table 8. Sinigrin levels in single T2-generation seeds obtained from selected biallelic/homozygous *MYB29 (HAG3)*-edited lines (wet-lab method, normalized to μmoles/g seed).

Seed #	WT Control	Line A263A	Line A264A	Line A269A
1	117.9	70.8	121.5	77.4
2	106.7	58.8	103.5	84.1
3	119.6	46.5	94.5	60.6
4	124.6	42.1	70.5	56.3
5	130.3	64.5	84.7	56.2
6	123.9	51.3	86.1	54.6
7	111.7	56.4	94.1	62.1
8	126.5	45.4	89.6	41.9
9	127.5	52.1	114.0	57.1
10	125.1	45.9	83.5	51.5
11	124.5	44.0	71.3	63.3
12	116.1	49.8	68.7	57.2

Seed #	WT Control	Line A263A	Line A264A	Line A269A
13	126.1	75.7	113.4	85.7
14	128.1	53.0	73.2	61.5
15	115.9	46.7	84.9	87.3
16	114.4	46.2	74.6	69.2
17	103.2	55.3	101.6	86.8
18	114.5	54.3	99.6	81.5
19	101.4	47.6	116.8	56.2
20	150.0	98.4	62.6	41.5
21	127.1	48.1	71.1	63.7
22	135.0	58.6	101.3	60.8
23	133.7	70.3	78.3	48.1
24	126.9	51.5	83.3	65.7
AVE, μ moles/g	122.1	55.5	89.3	63.8
STDEV	10.8	12.8	16.8	13.6
% Control	100%	45%	73%	52%

[00139] Table 9. Sinigrin levels in vegetative tissues from selected 4-weeks old T2-generation plants grown from biallelically modified *MYB29* (*HAG3*) CRISPR-mutated line A269A (wet-lab method, normalized to μ moles/g fresh tissue punch).

BioRep #	WT Control	A269A, line #							
		13	16	21	22	11	14	19	24
1	19.8	4.0	7.5	3.0	3.4	3.9	8.3	7.9	8.2
2	19.9	4.1	5.6	3.1	5.7	2.7	6.9	5.6	8.4
3	16.4	3.4	5.0	3.6	5.8	2.8	7.0	6.4	7.8
AVERAGE	18.7	3.8	6.0	3.2	5.0	3.1	7.4	6.6	8.1

STDEV	2.0	0.4	1.3	0.3	1.4	0.7	0.8	1.2	0.3
% Control	100%	20%	32%	17%	27%	17%	40%	35%	43%

[00140] Table 10. Sinigrin levels in vegetative tissues from selected T1-generation seedlings grown from biallelically modified AOP2 lines (wet-lab method, normalized to $\mu\text{moles}/4.3 \text{ mg}$ fresh tissue punch). Tissue samples were harvested from cauline leaves when plants were setting pods (wet-lab method, normalized to $\mu\text{moles}/\text{g}$ fresh tissue punch). T1 plants are generally chimeric for the edits, resulting in overestimated sinigrin numbers and increased variability.

BioRep #	2032 WT control	A370A	A379A	A381A	A380A
1	7.9	0.2	1.4	-0.3	0.3
2	4.6	2.1	0.6	0.1	0.3
3	4.1	0.4	0.1	6.9	0.4
4	4.0	-0.4	2.9	7.2	-0.6
5	4.2	3.5	0.5	0.1	-0.4
6	1.6	4.1	0.9	0.1	-0.4
AVERAGE	4.4	1.7	1.1	2.3	-0.1
STDEV	2.0	1.9	1.0	3.6	0.4
% Control	100%	38%	24%	53%	-1%

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OTHER EMBODIMENTS

[00141] It is to be understood that while certain embodiments have been described in conjunction with the detailed description thereof and examples, the foregoing description is intended to illustrate and not limit the scope of the disclosure. Other aspects, advantages, and modifications are within the scope of the following embodiments and claims.

[00142] Embodiment 1. A composition comprising non-defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00143] Embodiment 2. The composition of embodiment 1, wherein said seed meal comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00144] Embodiment 3. The composition of any one of embodiments 1 or 2, wherein said composition has an oil content of about 30% or 35% to about 40% or 50% by dry weight.

[00145] Embodiment 4. The composition of any one of embodiments 1 to 3, wherein said composition further comprises a preservative, a dust preventing agent, a bulking agent, a flowing agent, or any combination thereof.

[00146] Embodiment 5. The composition of any one of embodiments 1 to 4, wherein said pennycress seed meal is obtained from pennycress seeds that have been crushed, ground, macerated, expelled, extruded, or any combination thereof.

[00147] Embodiment 6. The composition of any one of embodiments 1 to 5, wherein said composition comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

[00148] Embodiment 7. A non-defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00149] Embodiment 8. The seed meal of embodiment 7, wherein said seed meal comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00150] Embodiment 9. The seed meal of embodiment 7 or 8, wherein said composition has an oil content of 30% or 35% to 40% or 50% by dry weight.

[00151] Embodiment 10. The seed meal of any one of embodiments 7 to 9, wherein said seed meal comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 356P5*, *I87113*, or *E5 543*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 356P5*, *I87113*, *E5 543*, or germplasm therefrom.

[00152] Embodiment 11. A pennycress seed comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00153] Embodiment 12. The pennycress seed of embodiment 11, wherein the seed comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00154] Embodiment 13. The pennycress seed of embodiment 11 or 12, wherein the seed comprises: (i) at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; (ii) at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; or (iii) seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, *I87207*, or germplasm therefrom.

[00155] Embodiment 14. The pennycress seed of any one of embodiments 11 to 13, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof.

[00156] Embodiment 15. The pennycress seed of any one of embodiments 11 to 14, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress gene

encoding a sinigrin biosynthetic enzyme and/or at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin transporter.

[00157] Embodiment 16. The pennycress seed of embodiment 15, wherein: (i) the sinigrin biosynthetic enzyme comprises a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 21, 24, 27, 94, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; or (ii) the pennycress seed comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 21 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

[00158] Embodiment 17. The pennycress seed of embodiment 15 or 16, wherein: (i) the sinigrin transporter comprises a polypeptide selected from the group consisting of SEQ ID NO: 15, 17 and allelic variants thereof; (ii) the pennycress seed comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 17 or an allelic variant thereof; or (iii) the pennycress seed comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

[00159] Embodiment 18. A seed lot comprising a population of pennycress seeds comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00160] Embodiment 19. The seed lot of embodiment 18, wherein the pennycress seeds comprise 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00161] Embodiment 20. The seed lot of embodiment 18 or 19, wherein the seed comprises: (i) at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; or (ii) at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; or (ii) seed of pennycress mutant lines *E3 196*, *E5 356P5*, *I87113*, *E5 543*, or germplasm therefrom.

[00162] Embodiment 21. The seed lot of any one of embodiments 18 to 20, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof.

[00163] Embodiment 22. The seed lot of any one of embodiments 18 to 20, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin biosynthetic enzyme and/or at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin transporter.

[00164] Embodiment 23. The seed lot of embodiment 22, wherein: (i) the sinigrin biosynthetic enzyme comprises a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 21, 24, 27, 94, 164, 167, 170, 173, 176, and allelic variants thereof; or (ii) the pennycress seed lot comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 21 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

[00165] Embodiment 24. The seed lot of embodiment 22 or 23, wherein: (i) the sinigrin transporter comprises a polypeptide selected from the group consisting of SEQ ID NO: 15, 17 and allelic variants thereof; (ii) the pennycress seed lot comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 17 or an allelic variant thereof; or (iii) the pennycress seed lot comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

[00166] Embodiment 25. The seed lot of any one of embodiments 18 to 24, wherein said population of pennycress seeds comprise seeds having at least one loss-of-function mutation in an endogenous pennycress gene that encodes SEQ ID NO:2 or an allelic variant thereof.

[00167] Embodiment 26. The seed lot of any one of embodiments 18 to 25, wherein the loss-of-function mutation in the gene encoding SEQ ID NO:2 or the allelic variant thereof comprises an insertion, deletion, or substitution of one or more nucleotides.

[00168] Embodiment 27. The seed lot of embodiment 26, wherein the loss-of-function mutation in the gene encoding SEQ ID NO:2 or the allelic variant thereof comprises a mutation that introduces a pre-mature stop codon or frameshift mutation at codon positions 1-108 of SEQ ID NO:1 or an allelic variant thereof.

[00169] Embodiment 28. The seed lot of embodiment 26, wherein the loss-of-function mutation is in a polynucleotide encoding MYB28, MYB29, MYB76, or any combination thereof.

[00170] Embodiment 29. The seed lot of any one of embodiments 18 to 28, wherein the population comprises at least 10 seeds comprising less than 25 micromoles sinigrin per gram by dry weight or 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00171] Embodiment 30. The seed lot of any one of embodiments 18 to 29, wherein at least 95% of the pennycress seeds in the seed lot are seeds comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00172] Embodiment 31. The seed lot of any one of embodiments 18 to 30, wherein less than 5% of the seeds in said seed lot have greater than 25 or 30 micromoles sinigrin per gram by dry weight.

[00173] Embodiment 32. The seed lot of any one of embodiments 18 to 31, wherein said seeds further comprise an agriculturally acceptable excipient or adjuvant.

[00174] Embodiment 33. The seed lot of any one of embodiments 18 to 32, wherein said seeds further comprise a fungicide, a safener, or any combination thereof.

[00175] Embodiment 34. A method of making non-defatted pennycress seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight, comprising the step of grinding, macerating, extruding, and/or crushing the seed lot of any one of embodiments 18 to 32 thereby obtaining the non-defatted seed meal.

[00176] Embodiment 35. A method of making defatted pennycress seed meal comprising less than 30 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight, comprising the steps of solvent

extracting the seed lot of any one of embodiments 18 to 32, and separating the extracted seed meal from the solvent, thereby obtaining the defatted seed meal.

[00177] Embodiment 36. Pennycress seed meal comprising less than 30, 28, or micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight, wherein the seed meal is defatted.

[00178] Embodiment 37. The seed meal of embodiment 36, wherein said seed meal has an oil content of about 0% or 0.5% to about 12% or 15% by dry weight.

[00179] Embodiment 38. The pennycress seed meal of embodiments 36 or 37, wherein said meal comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

[00180] Embodiment 39. The pennycress seed meal of any one of embodiments 36 to 38, wherein said meal comprises ground and/or macerated seed of a population of pennycress seeds comprising seeds having at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof.

[00181] Embodiment 40. The pennycress seed meal of any one of embodiments 36 to 39, wherein said meal comprises ground and/or macerated seed of a population of pennycress seeds comprising seeds having at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof.

[00182] Embodiment 41. The pennycress seed meal of any one of embodiments 36 to 40, wherein said meal comprises ground and/or macerated seed of a population of pennycress seeds comprising seeds having at least one transgene or genome rearrangement that suppresses

expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof.

[00183] Embodiment 42. A composition comprising defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00184] Embodiment 43. The composition of embodiment 42, wherein said seed meal comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight.

[00185] Embodiment 44. The composition of embodiments 42 or 43, wherein said composition has an oil content of about 0% or 0.5% to about 12% or 15% by dry weight.

[00186] Embodiment 45. The composition of any one of embodiments 42 to 44, wherein said composition further comprises a preservative, a dust preventing agent, a bulking agent, a flowing agent, or any combination thereof.

[00187] Embodiment 46. The composition of any one of embodiments 42 to 45, wherein said composition comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

[00188] Embodiment 47. Pennycress seed cake comprising 30 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight.

[00189] Embodiment 48. The seed cake of embodiment 47, wherein said seed cake has an oil content of about 0% or 0.5% to about 12% or 15% by dry weight.

[00190] Embodiment 49. The pennycress seed cake of embodiment 47, wherein the cake comprises crushed or expelled seed of the seed lot of any one of embodiments 18 to 33.

[00191] Embodiment 50. The pennycress seed cake of any one of embodiments 47 to 49, wherein the cake comprises: (i) a detectable amount of a polynucleotide comprising at least one

loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) seed cake obtained from seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

[00192] Embodiment 51. A method of making a pennycress seed lot comprising the steps of:

- (a) introducing at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof;
- (b) selecting germplasm that is homozygous for said loss-of-function mutation; and,
- (c) harvesting seed from the homozygous germplasm, thereby obtaining a seed lot, wherein said seed lot comprises a population of pennycress seed having less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00193] Embodiment 52. The method of embodiment 51, wherein the harvested seed of the seed lot comprise 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00194] Embodiment 53. The method of embodiment 51 or 52, wherein said harvested seed of the seed lot comprises the seed lot of any one of embodiments 18 to 33.

[00195] Embodiment 54. A method of making a pennycress seed lot comprising the steps of:

- (a) introducing at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof into a pennycress plant genome;
- (b) selecting a transgenic plant line that comprises said transgene or genome rearrangement; and,
- (c) harvesting seed from the transgenic plant line, thereby obtaining a seed lot, wherein said seed lot comprises a population of pennycress seed having less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

[00196] Embodiment 55. The method of embodiment 54, wherein the harvested seed of the seed lot comprise 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

[00197] Embodiment 56. The method of embodiment 54 or 55, wherein said harvested seed comprise a seed lot of any one of embodiments 18 to 33.

What Is Claimed Is:

1. A composition comprising non-defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.
2. The composition of claim 1, wherein said seed meal comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.
3. The composition of claim 1 or 2, wherein said composition has an oil content of about 30% or 35% to about 40% or 50% by dry weight.
4. The composition of claim 1 to 2, wherein said composition further comprises a preservative, a dust preventing agent, a bulking agent, a flowing agent, or any combination thereof.
5. The composition of claim 1 or 2, wherein said pennycress seed meal is obtained from pennycress seeds that have been crushed, ground, macerated, expelled, extruded, or any combination thereof.
6. The composition of claim 1 or 2, wherein said composition comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.
7. A non-defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

8. The seed meal of claim 7, wherein said seed meal comprises about 1, 2.5, 5, or 10 to about 15, 20, or 25 micromoles sinigrin per gram by dry weight.
9. The seed meal of claim 7 or 8, wherein said composition has an oil content of 30% or 35% to 40% or 50% by dry weight.
10. The seed meal of claim 7 or 8, wherein said seed meal comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.
11. A pennycress seed comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.
12. The pennycress seed of claim 11, wherein the seed comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.
13. The pennycress seed of claim 11 or 12, wherein the seed comprises: (i) at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; (ii) at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; or (iii) seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

14. The pennycress seed of claim 11 or 12, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof.

15. The pennycress seed of claim 11 or 12, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin biosynthetic enzyme and/or at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin transporter.

16. The pennycress seed of claim 15, wherein: (i) the sinigrin biosynthetic enzyme comprises a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 21, 24, 27, 94, 164, 167, 170, 173, 176, and allelic variants thereof; or (ii) the pennycress seed comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 21 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

17. The pennycress seed of claim 15, wherein: (i) the sinigrin transporter comprises a polypeptide selected from the group consisting of SEQ ID NO: 15, 17 and allelic variants thereof; (ii) the pennycress seed comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 17 or an allelic variant thereof; or (iii) the pennycress seed comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

18. A seed lot comprising a population of pennycress seeds comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

19. The seed lot of claim 18, wherein the pennycress seeds comprise 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

20. The seed lot of claim 18 or 19, wherein the seed comprises: (i) at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; (ii) at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof; or (iii) seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

21. The seed lot of claim 18 or 19, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof.

22. The seed lot of claim 18 or 19, wherein the seed comprises at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin biosynthetic enzyme and/or at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a sinigrin transporter.

23. The seed lot of claim 22, wherein: (i) the sinigrin biosynthetic enzyme comprises a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 21, 24, 27, 94, 164, 167, 170, 173, 176, and allelic variants thereof; or (ii) the pennycress seed lot comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 21 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

24. The seed lot of claim 22, wherein: (i) the sinigrin transporter comprises a polypeptide selected from the group consisting of SEQ ID NO: 15, 17 and allelic variants thereof; (ii) the pennycress seed lot comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 17 or an allelic variant thereof; or (iii) the pennycress seed lot comprises a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of SEQ ID NO: 15 or an allelic variant thereof and a loss-of-function mutation in an endogenous pennycress gene encoding the polypeptide of a SEQ ID NO: 24 or an allelic variant thereof.

25. The seed lot of claim 18 or 19, wherein said population of pennycress seeds comprise seeds having at least one loss-of-function mutation in an endogenous pennycress gene that encodes SEQ ID NO:2 or an allelic variant thereof.

26. The seed lot of claim 25, wherein the loss-of-function mutation in the gene encoding SEQ ID NO:2 or the allelic variant thereof comprises an insertion, deletion, or substitution of one or more nucleotides.

27. The seed lot of claim 25, wherein the loss-of-function mutation in the gene encoding SEQ ID NO:2 or the allelic variant thereof comprises a mutation that introduces a pre-mature stop codon or frameshift mutation at codon positions 1-108 of SEQ ID NO:1 or an allelic variant thereof.

28. The seed lot of claim 27, wherein the loss-of-function mutation is in a polynucleotide encoding MYB28, MYB29, MYB76, or any combination thereof.

29. The seed lot of claim 18 or 19, wherein the population comprises at least 10 seeds comprising less than 25 micromoles sinigrin per gram by dry weight or 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

30. The seed lot of claim 18 or 19, wherein at least 95% of the pennycress seeds in the seed lot are seeds comprising less than 25 micromoles sinigrin per gram by dry weight or 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.
31. The seed lot of claim 18 or 19, wherein less than 5% of the seeds in said seed lot have greater than 25 or 30 micromoles sinigrin per gram by dry weight.
32. The seed lot of claim 18 or 19, wherein said seeds further comprise an agriculturally acceptable excipient or adjuvant.
33. The seed lot of claim 18 or 19, wherein said seeds further comprise a fungicide, a safener, or any combination thereof.
34. A method of making non-defatted pennycress seed meal comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight or 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight, comprising the step of grinding, macerating, extruding, and/or crushing the seed lot of claim 18 or 19, thereby obtaining the non-defatted seed meal.
35. A method of making defatted pennycress seed meal comprising less than 30 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight, comprising the steps of solvent extracting the seed lot of claim 18 or 19, and separating the extracted seed meal from the solvent, thereby obtaining the defatted seed meal.
36. Pennycress seed meal comprising less than 30 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight, wherein the seed meal is defatted.
37. The seed meal of claim 25, wherein said seed meal has an oil content of about 0% or 0.5% to about 12% or 15% by dry weight.

38. The pennycress seed meal of claim 36 or 37, wherein said meal comprises a detectable amount of a polynucleotide comprising: (i) at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed; ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

39. The pennycress seed meal of claim 36 or 37, wherein said meal comprises ground and/or macerated seed of a population of pennycress seeds comprising seeds having at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof.

40. The pennycress seed meal of claim 36 or 37, wherein said meal comprises ground and/or macerated seed of a population of pennycress seeds comprising seeds having at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof.

41. The pennycress seed meal of claim 36 or 37, wherein said meal comprises ground and/or macerated seed of a population of pennycress seeds comprising seeds having at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof.

42. A composition comprising defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

43. The composition of claim 42, wherein said seed meal comprises about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight.

44. The composition of claim 42 or 43, wherein said composition has an oil content of about of about 0% or 0.5% to about 12% or 15% by dry weight.

45. The composition of claim 42 or 43, wherein said composition further comprises a preservative, a dust preventing agent, a bulking agent, a flowing agent, or any combination thereof.

46. The composition of claim 42 or 43, wherein said composition comprises: (i) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) crushed, ground, and/or macerated seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

47. Pennycress seed cake comprising 30 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight.

48. The seed cake of claim 47, wherein said seed cake has an oil content of about 0% or 0.5% to about 12% or 15% by dry weight.

49. The pennycress seed cake of claim 48, wherein the cake comprises crushed or expelled seed of the seed lot of claim 18 or 19.

50. The pennycress seed cake of claim 18 or 19, wherein the cake comprises a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in at least one endogenous pennycress coding sequence or gene comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 4, 5, 7, 8, 10, 11, 13, 14, 16, 18, 19, 20, 22, 23, 25, 26, 28, 29, 92, 93, 159, 160, 162, 163, 165, 166, 168, 169, 171, 172, 174, 175, and allelic variants thereof; (ii) a detectable amount of a polynucleotide comprising at least one loss-of-function mutation in pennycress mutant *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *E5 543*, or *I87207*; or (iii) seed cake obtained from seed of pennycress mutant lines *E3 196*, *E5 444P1*, *E5 356P5*, *I87113*, *I87207*, *E5 543*, or germplasm therefrom.

51. A method of making a pennycress seed lot comprising the steps of:

- (a) introducing at least one loss-of-function mutation in at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176, and allelic variants thereof;
- (b) selecting germplasm that is homozygous for said loss-of-function mutation; and,
- (c) harvesting seed from the homozygous germplasm, thereby obtaining a seed lot, wherein said seed lot comprises a population of pennycress seed having less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

52. The method of claim 51, wherein the harvested seed of the seed lot comprise 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

53. The method of claim 51, wherein said harvested seed of the seed lot comprises the seed lot of claim 18 or 19.

54. A method of making a pennycress seed lot comprising the steps of:

- (a) introducing at least one transgene or genome rearrangement that suppresses expression of at least one endogenous pennycress gene encoding a polypeptide selected from the group consisting of SEQ ID NO: 3, 6, 9, 12, 15, 17, 21, 24, 27, 30, 94, 161, 164, 167, 170, 173, 176 and allelic variants thereof into a pennycress plant genome;

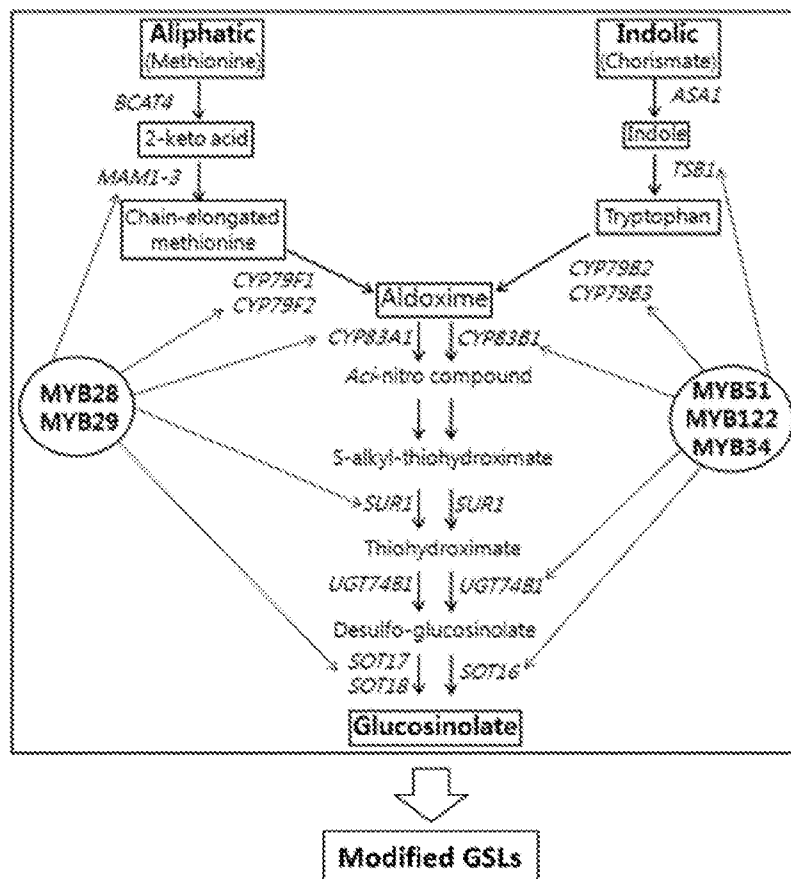
(b) selecting a transgenic plant line that comprises said transgene or genome rearrangement; and,
(c) harvesting seed from the transgenic plant line, thereby obtaining a seed lot, wherein said seed lot comprises a population of pennycress seed having less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

55. The method of claim 54, wherein the harvested seed of the seed lot comprise 1, 2.5, 5, or 10 to 15, 16, 18, 20, or 25 micromoles sinigrin per gram by dry weight.

56. The method of claim 54, wherein said harvested seed comprise a seed lot of claim 18 or 19.

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A.



B.

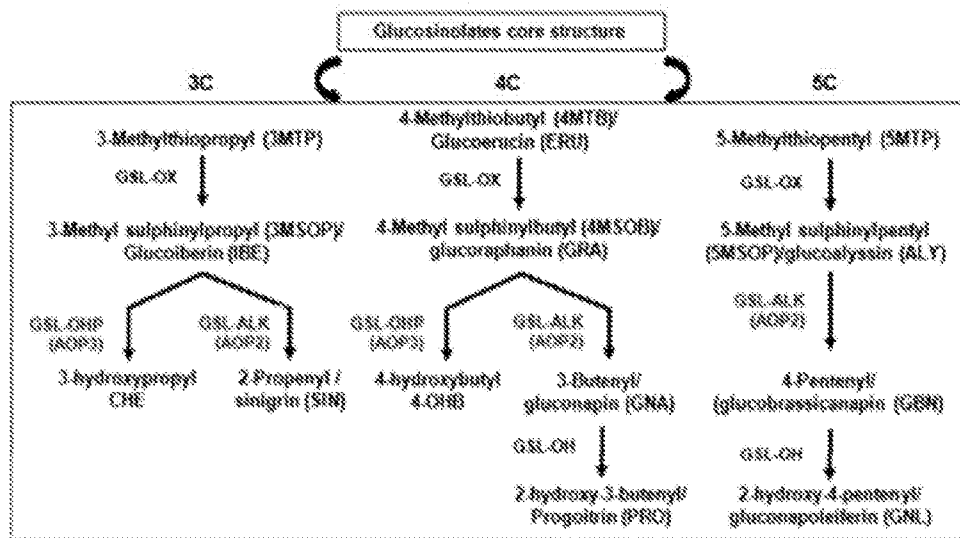
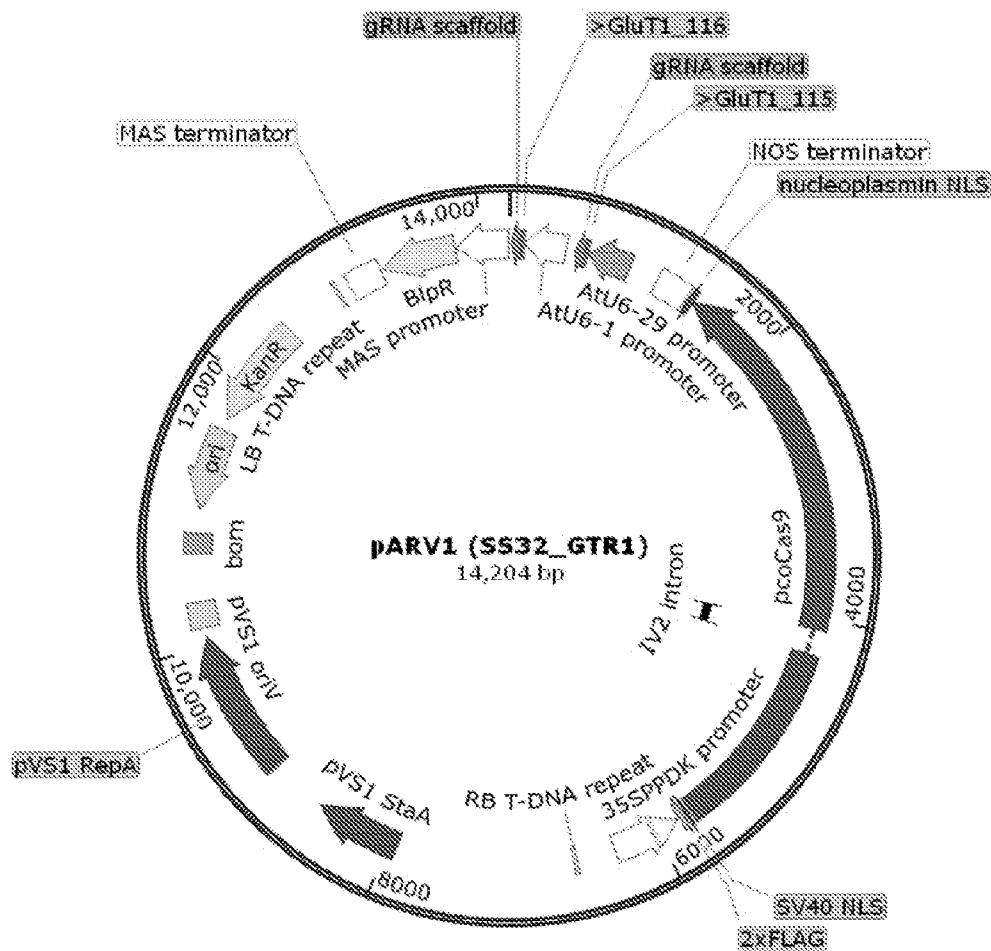
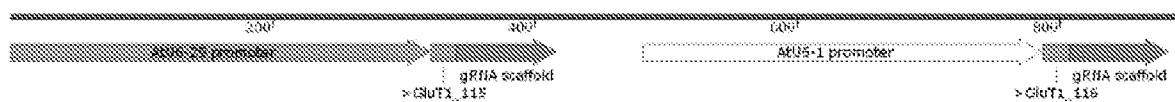


FIGURE 1A, B

A.



B.



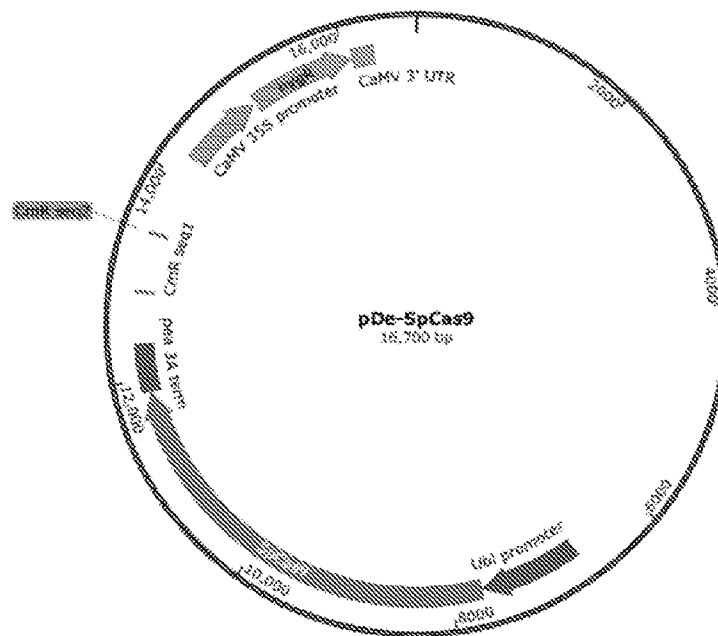
C.

[illegible]

FIGURE 2A, B, C

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A.



B.

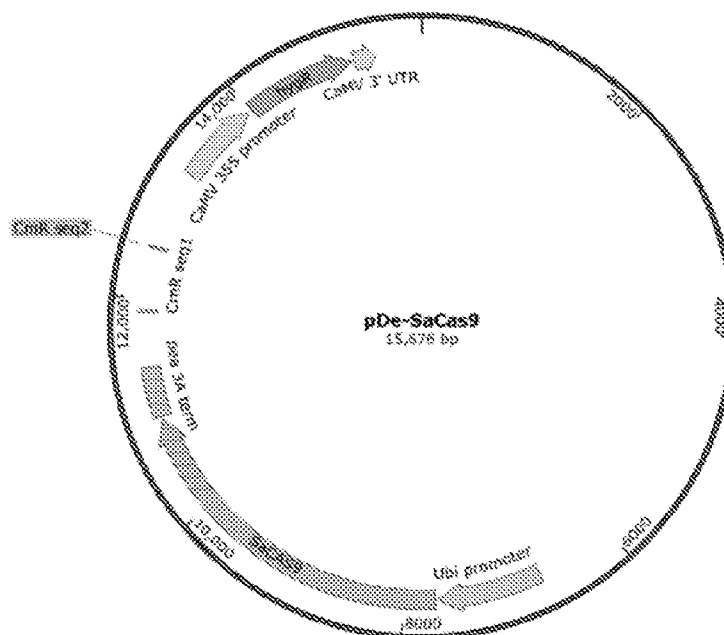


FIGURE 3A,B



FIGURE 4A, B, C

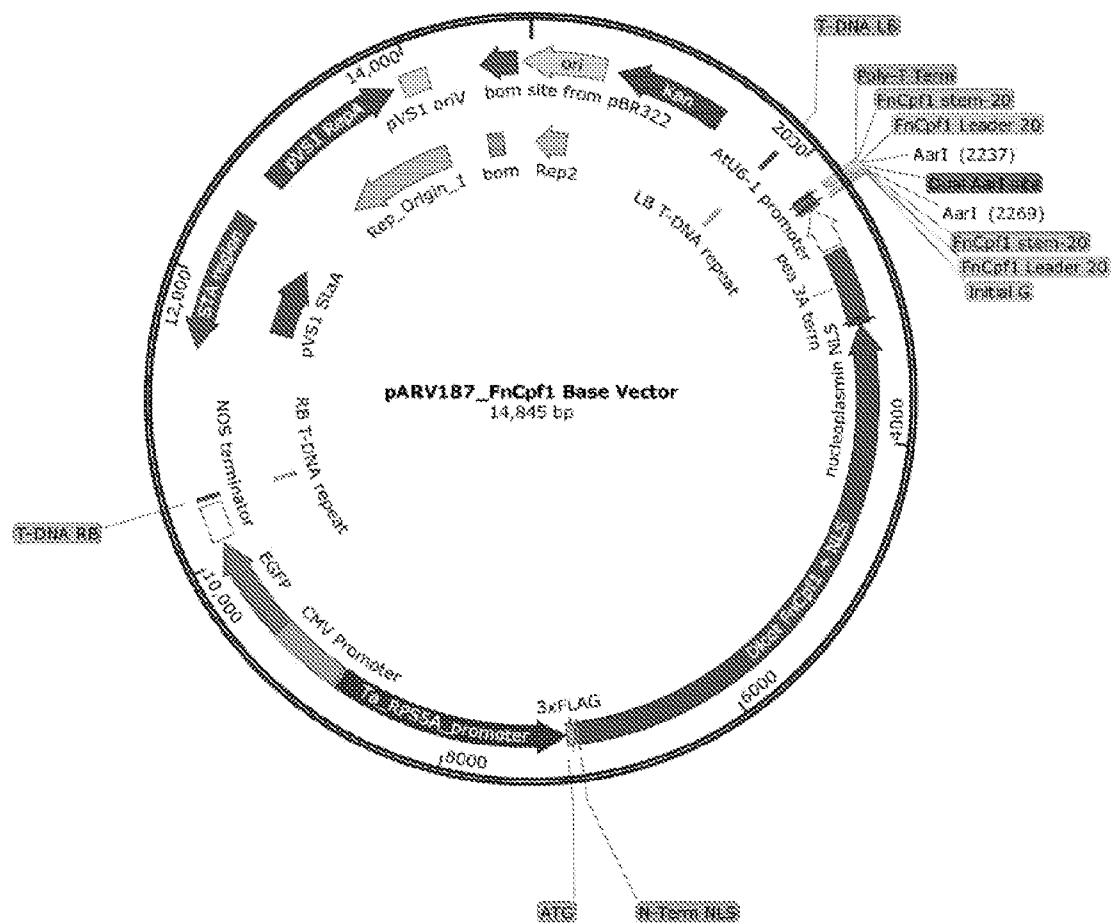


FIGURE 5

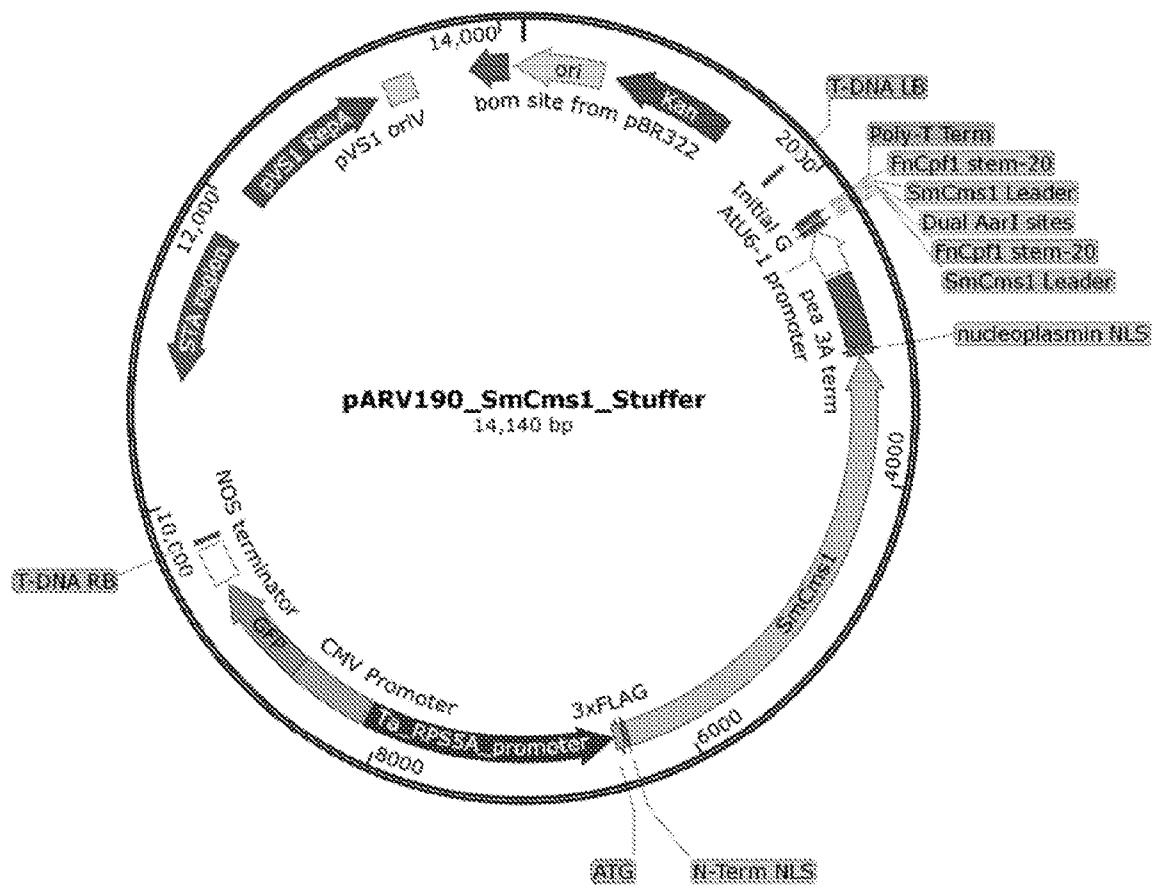
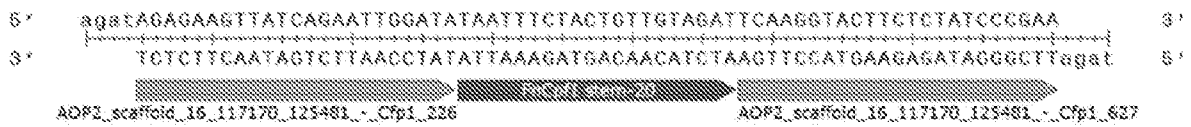
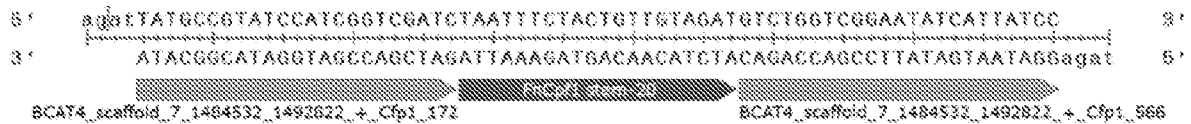
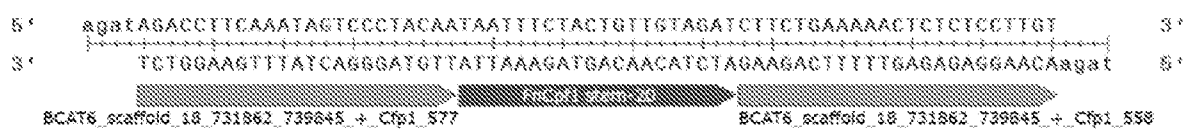
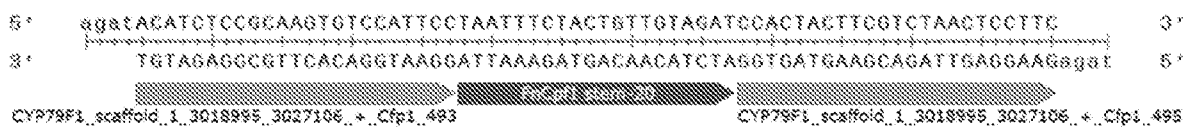
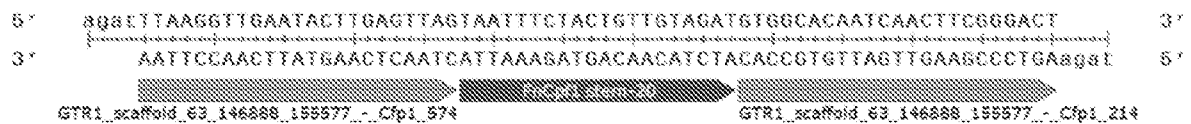
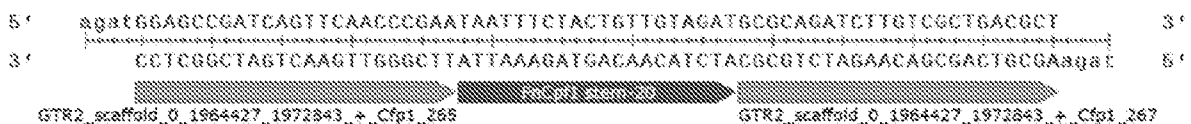
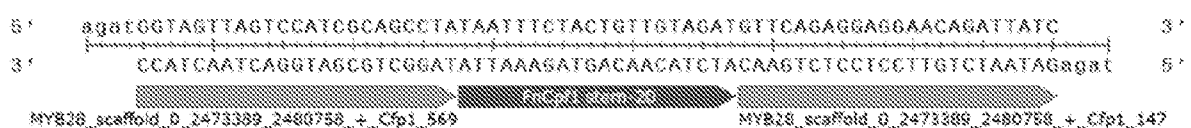
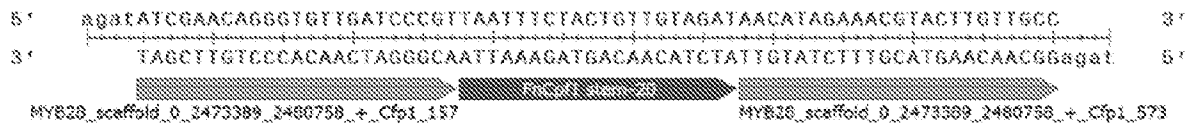


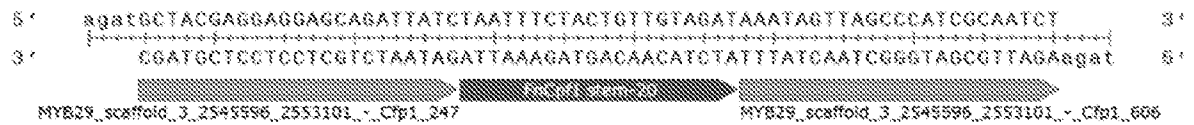
FIGURE 6

A.**B.****C.****D.****E.****F.****G.****FIGURE 7A, B, C, D, E, F, G**

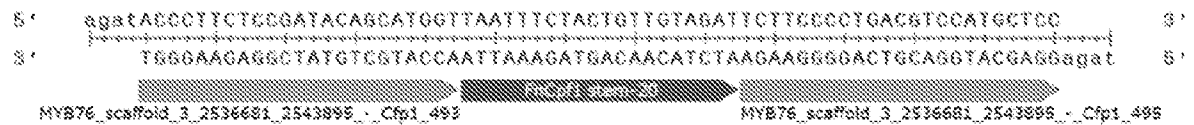
H.



I.



J.



K.

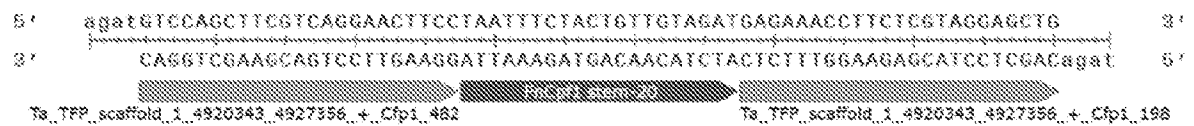


FIGURE 7H, I, J, K

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/14178

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - A01H 1/06, A01H 5/10, C12N 9/10 (2019.01)
 CPC - A01H 1/06, A01H 5/10, C12N 9/1029, A01H 6/20

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 --- Y --- A	IN 00363DE2012 A (NATIONAL INSTITUTE OF PLANT GENOME RESEARCH) 10 April 2015 (10.04.2015) Especially pg 3, 17, 18, 28	11-12, 18-19, 29-31 ----- 1-3, 5, 7-9, 14-15, 22, 32-33 ----- 6, 10, 13, 16, 20-21, 23, 25-27
Y --- A	US 2015/0005172 A1 (MPT MUSTARD PRODUCTS & TECHNOLOGIES INC.) 1 January 2015 (01.01.2015) Especially para [0041], [0045], [0047]	1-3, 5, 7-9 ----- 6, 10
Y --- A	ZHANG et al., Three genes encoding AOP2, a protein involved in aliphatic glucosinolate biosynthesis, are differentially expressed in Brassica rapa, Journal of experimental botany, 17 July 2015, Vol 66, No 20, pp 6205-6218, Especially pg 6206, 6211, 6214, Fig. 3	14-15, 22 ----- 6, 10, 16, 21, 23
Y	US 2009/0306060 A1 (WATRIN et al.) 10 December 2009 (10.12.2009) Especially Abstract, para [0018]	32-33
A	US 7,250,559 B2 (QUIROS et al.) 31 July 2007 (31.07.2007) Especially Claim 7, col 31, SEQ ID Nos: 2, 4	10, 13, 16, 20-21, 23, 25-27

☐ 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

"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

6 May 2019

Date of mailing of the international search report

17 MAY 2019

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 W. Young

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/14178

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.: 4, 49, 53, 56
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:
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.

-----Please see continuation in 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.: 1-3, 5-16, 18-23, 25-27 and 29-33 limited to AOP2 (SEQ ID NOs: 1-3)

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

International application No.

PCT/US 19/14178

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

Group I+, claims 1-3, 5-33 and 36-48, 50 directed to a pennycress seed meal, composition comprising the same, pennycress seed, seed lot, or seed cake, comprising a mutant sinigrin biosynthetic enzyme pennycress sequence. The meal, composition, seed, seed lot or seed cake will be searched to the extent that the mutant sinigrin biosynthetic enzyme pennycress sequence encompass a loss-of-function mutation in the AOP2 polynucleotide sequences SEQ ID NO: 1 and 2, and corresponding polypeptide sequence SEQ ID NO: 3 [see applicant Table 1 in specification p. 10]. It is believed that claims 1-3, 5-16, 18-23, 25-27 and 29-33 encompass this first named invention, and thus these claims will be searched without fee to the extent that the mutant pennycress sequence(s) encompass a loss-of-function mutation in AOP2 (SEQ ID NOs: 1-3). Additional mutant pennycress sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected mutant pennycress sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be mutant pennycress sequences comprising a loss-of-function mutation in BCAT4 (SEQ ID NOs: 4-6), (claims 1-3, 5-16, 18-23, 29-33). [note, claims 17 and 24 are excluded from the first embodiment because they require a different mutant pennycress sequence, a sinigrin transporter]

Group II, claims 34 and 35, directed to a method of making defatted or non-defatted pennycress seed meal.

Group III, claims 51, 52, 54 and 55, directed to a method of making a pennycress seed lot.

The inventions listed as Groups I+, II and III 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 pennycress seed meal, composition comprising the same, pennycress seed, seed lot, or seed cake comprising a mutant pennycress sequence, that is not required by Group II or III.

Group II has the special technical feature of a method of making a seed meal comprising grinding, macerating, extruding, and/or crushing the seed lot, or solvent extracting the seed lot and separating the extracted seed meal from the solvent, that is not required by Group I+ or III.

Group III has the special technical feature of making a pennycress seed lot comprising introducing at least one loss-of-function mutation in at least one endogenous pennycress gene, selecting germ plasm that is homozygous for said loss-of-function mutation, and harvesting seed from the homozygous germplasm, that is not required by Group I+ or II.

The inventions of Group I+ each include the special technical feature of a unique nucleic acid and corresponding amino acid sequence. Each nucleic acid and corresponding amino acid sequence encodes a unique peptide, and is considered a distinct technical feature.

Common technical features

The inventions of Group I+, II and III share the common technical features of a pennycress seed meal or seed lot comprising a population of pennycress seeds that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight.

No technical features are shared between the mutant pennycress nucleic acid and corresponding amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Group I+ were considered to share the technical features of including: a composition comprising defatted or non-defatted pennycress seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight, and a Pennycress seed, seed lot comprising a population of pennycress seeds, or seed cake comprising 30 micromoles sinigrin per gram by dry weight or about 1, 2.5, 5, or 10 to about 15, 16, 18, 20, 25, 28, or 30 micromoles sinigrin per gram by dry weight, these shared technical features are previously taught by IN00363DE2012 A to National Institute Of Plant Genome Research (hereinafter 'NIGPR'), US 2015/0005172 A1 to MPT Mustard Products & Technologies Inc. (hereinafter 'MPT'), and WO 2017/091891 A1 to Agrisoma Biosciences Inc. (hereinafter 'Agrisoma').

NIGPR discloses a seed lot comprising a population of Brassicales seeds comprising less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight (pg 3, para 3 - "B. juncea varieties particularly grown in India contains high amount of glucosinolate (>100 .mu.mol/g dry seed weight)"; pg 17, para 1-3 - "The present invention further provides a transgenic brassica plant having reduced level of glucosinolate compared to its level in a control non-transgenic brassica plant...The invention is further directed to transgenic Brassica seed produced from the transgenic Brassica plant of the invention...the invention relates to other plants including any member of the order Brassicales that can be transformed with one or more genetic constructs of the present invention..."; pg 28, para 2-pg 29, para 1 - "sinigrin was also reduced significantly in the T1 seeds of the transgenic lines ranging from 1.09 to 4.13 .mu.mol/g seed"). NIGPR does not specifically teach the Brassicales seeds are pennycress. However, since NIGPR teaches application of the invention to any Brassicales plants (pg 17, para 2-3 - "the invention relates to other plants including any member of the order Brassicales that can be transformed with one or more genetic constructs of the present invention"), it would have been obvious to one of ordinary skill in the art to have applied the NIGPR methods to any known Brassicales plants, including pennycress during the ordinary course of experimentation, in order to optimize plant properties.

-----Please see continuation in next extra sheet-----

Continuation of Previous Extra Sheet

NIGPR further discloses a composition comprising Brassicales seed meal that comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight (pg 17, para 1-3 - "The present invention further provides a transgenic brassica plant having reduced level of glucosinolate compared to its level in a control non-transgenic brassica...the invention relates to other plants including any member of the order Brassicales that can be transformed with one or more genetic constructs of the present invention..."; pg 18, para 1 - "Our results clearly showed that the controlled silencing of a transcriptional regulator BjMYB28 has an enormous potential for reducing the total aliphatic glucosinolate pools in *B. juncea* and improving its meal quality."; pg 28, para 2-pg 29, para 1 - "sinigrin was also reduced significantly in the T1 seeds of the transgenic lines ranging from 1.09 to 4.13 $\mu\text{mol/g}$ seed..."), but does not teach the Brassicales seed meal is non-defatted.

MPT teaches a non-defatted seed meal from mustard plants (para [0041] - "Plants comprising glucosinolates that may be used in accordance herewith include plants belonging to the families of Brassicaceae (Cruciferae)"; para [0047] - "the processed plant material used is a seed meal...prepared from mustard plants...The next step in the process is largely dependent on the oil (also known as "lipid" or "fat") content of the mustard meal that is desired. If a "full fat" meal is desired than the kernels are subjected to a process that does not result in oil extraction."). Since it was commonly in the art that pennycress belongs to the mustard family Brassicaceae, it would have been obvious to one of ordinary skill in the art that the pennycress seed meal can be non-defatted because a full fat meal can be prepared depending on the desired use.

Agrisoma discloses a composition comprising defatted pennycress seed meal (para [0032] - "the present invention provides the process described herein for removing at least one glucosinolate from a meal fraction of oilseed...comprising a slurry of defatted meal in water..."; para [0044] - the present invention provides the process described herein for removing at least one glucosinolate from a meal fraction of oilseed... from oilseed of...*Thlaspi arvense*."; note, *Thlaspi* is a synonym for pennycress), but does not teach the defatted pennycress seed meal comprises less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight. However, Agrisoma does teach a method of reducing sinigrin content in pennycress seed meal and the undesirable taste and health impacts of sinigrin (para [0044] - "the present invention provides the process described herein for removing at least one glucosinolate from a meal fraction of oilseed... from oilseed of...*Thlaspi arvense*."; para [0063] - "The glucosinolates found in meal samples of oilseeds include sinigrin...These compounds and their metabolites can impact the taste of the meal, reducing its palatability can also adversely impact the health of an animal that has consumed plant material containing glucosinolates.").

Agrisoma further discloses a pennycress seed cake (para [0044] - "the present invention provides the process described herein for removing at least one glucosinolate from a meal fraction of oilseed... from oilseed of...*Thlaspi arvense*."; para [0209] - "the processes described herein further comprise a treatment of a seed meal cake containing glucosinolate").

Since NIGPR teaches a method of reducing sinigrin content in seed meal from plants of the order Brassicales to less than 30 micromoles per gram by dry weight (pg 3, para 3; pg 17, para 2-3; pg 18, para 1; pg 28, para 2-pg 29, para 1), and it was commonly known in the art that pennycress belongs to the order Brassicales, it would have been obvious to one of ordinary skill in the art to produce a defatted pennycress seed meal or a pennycress seed cake according to Agrisoma having less than 30, 28, 25, 16, or 15 micromoles sinigrin per gram by dry weight, in order to improve the palatability and nutrient value of pennycress seed meals for food and feedstock application.

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+, II and III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, claims 18 and 19 lack antecedent basis for the "pennycress seed cake" of claim 50. For the purposes of the present international search and opinion, claim 50 is considered dependent from claim 47.

NOTE, continuation of Item 4 above: claims 4, 49, 53, 56 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).

Note, claim 25 lacks antecedent basis for the "seed meal" of claim 37. For the purposes of the present international search and opinion, claim 37 is considered dependent from claim 36.

Note, claim 7 or 8 lacks antecedent basis for the "said composition" of claim 9. For the purposes of the present international search and opinion, claim 9 is considered dependent from claim 3.