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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*
— *of inventorship (Rule 4.17(iv))*

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— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title:** BRASSICA PLANTS WITH INCREASED LOCULES

FIG. 3

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wild type CLV1  GGCTCGATCCCGATCGGAATCGGGAAGATGACGAGCTTAACCACTCTGGATCTCTCCTTCAACGACCTC
                 G  S  I  P  I  G  I  G  K  M  T  S  L  T  T  L  D  L  S  F  N  D  L
sup-c (clv1)    GGCTCGATCCCGATCGGAATCGGGAAGATGACGAACTTAACCACTCTGGATCTCTCCTTCAACGACCTC
                 G  S  I  P  I  G  I  G  K  M  T  N  L  T  T  L  D  L  S  F  N  D  L
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(57) **Abstract:** The disclosure provides mutant Brassica plants that have increased locules and seed production relative to native wild-type plants. Such plants include a point mutation in the clavata 1 gene (CLV1), such as a G->A substitution at position 1745 of the *Brassica rapa* coding sequence, which leads to an S582N substitution in the protein sequence. Equivalent substitutions can be made in any Brassicaceae coding/protein sequence. Also provided are methods of using such plants in breeding programs, as well as parts of such plants (such as seeds), and methods of making commodity products from such plants (*e.g.*, oil). Also provided are mutant CLV1 sequences. Brassica plants harboring the disclosed CLV1 mutation can include other desirable traits, such as herbicide tolerance.

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BRASSICA PLANTS WITH INCREASED LOCULES

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to US Provisional Application No. 63/152,025 filed February 22, 2021, herein incorporated by reference in its entirety.

FIELD

Provided herein are mutant clavata 1 (CLV1) protein and nucleic acid molecules, which include or encode an S582N substitution (or equivalent thereof), as well as plants and plant cells including such nucleic acid molecules and proteins. This mutation can produce a plant with an increased number of locules and/or increase seed production as compared to a native plant or tree of the same species, such as an increase of at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%.

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This invention was made with government support under Grant No. 2021-67013-33797 awarded by USDA/NIFA. The government has certain rights in the invention.

SUMMARY

Provided herein are mutant clavata 1 (CLV1) protein and nucleic acid molecules, which include or encode an S582N substitution (for example due to an adenine (A) nucleotide at position 1745), or its equivalent (*e.g.*, a Ser to Asn substitution in the sequence SLT located in the C-terminal half of the protein, for example between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). Also provided are vectors (such as a plasmid or viral vector) including such nucleic acid molecules. Also provided are plants and plant cells containing such mutant protein and nucleic acid molecules, such those having an S582N mutation in their CLV1 protein (or equivalent mutation). In some examples, the plant is a Brassicaceae plant.

In some examples, this disclosed S582N substitution in CLV1 results in plants with an increased number of locules as compared to a native plant or tree of the same species, such as an increase of at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%, for example as compared to plants with a native CLV1 sequence (*e.g.*, SEQ ID NO: 1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63,

65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99). In some examples, this disclosed S582N substitution in CLV1 results in plants with increased seed production as compared to a native plant or tree of the same species, such as an increase of at least 10%, at least 20%, at least 30%, at least 40%, or at least 50%, for example as compared to plants with a native CLV1
5 sequence (*e.g.*, SEQ ID NO: 1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99). In some examples, combinations of such effects are achieved.

In some examples, a mutant CLV1 nucleic acid molecule comprises at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%
10 identity to SEQ ID NO: 3, and encodes a S582N substitution (or its equivalent, *e.g.*, a Ser to Asn substitution in the sequence SLT located in the C-terminal half of the protein, for example between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

In some examples, a mutant CLV1 protein comprises at least 80%, at least 85%, at least
15 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4, and includes a S582N substitution (or its equivalent, *e.g.*, a Ser to Asn substitution in the sequence SLT located in the C-terminal half of the protein, for example between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

20 The foregoing and other objects and features of the disclosure will become more apparent from the following detailed description, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

25 **FIG. 1** shows an exemplary native *Brassica rapa* CLV1 coding sequence (SEQ ID NO: 1). nt 1745 in bold/underline. This nt can be changed to an A to encode an S582N mutation.

FIG. 2 shows an exemplary native *Brassica rapa* CLV1 protein sequence (SEQ ID NO: 2). S582 in bold/underline. This amino acid can be mutated to an N to cause multilocularity and increase organ number and seed production.

30 **FIG. 3** shows the G to A substitution at nt 175 (codon 582) resulting in a S582N substitution in the CLV1 protein (top sequence nt 1711 to 1779 of SEQ ID NO: 1, aa 571 to 593 of SEQ ID NO: 2, bottom sup-c (clv1) sequence nt 1711 to 1779 of SEQ ID NO: 3, aa 571 to 593 of SEQ ID NO: 4).

FIG. 4A shows the relationship of the diploid and tetraploid members of the *Brassica* genus [figure from Wikimedia Commons]. Other important Brassicaceae oil-seed crops include *Thlaspi arvense* (pennycress) and *Camelina sativa* (false flax, an allohexaploid).

FIG. 4B shows the % sequence identity of the native CLV1 protein sequence for several Brassicaceae plants.

FIGS. 5A and 5B show alignments for several CLV1 proteins (including homologs of SEQ ID NO: 1). Using this information, one skilled in the art can make equivalent S582N substitution in any CLV1 protein (or make an appropriate mutation to the coding sequence). (A) Alignment of Brassicaceae CLV1 proteins from *B. napus* (SEQ ID NOS: 19 and 20), *B. oleracea* (SEQ ID NO: 21), *C. sativa* (SEQ ID NO: 22), *T. arvense* (SEQ ID NO: 23), and *B. rapa* (SEQ ID NO: 24); (B) Alignment of several CLV1 sequences from flowering plants, including important crops, showing Serine 582 is highly conserved. Apple (SEQ ID NOS: 26, 28, 30, 32, 34, 36), peach (SEQ ID NOS: 38, 40, 42), orange (SEQ ID NOS: 44, 46, 48), banana (SEQ ID NO: 50), tomato (SEQ ID NOS: 52, 54), soybean (SEQ ID NOS: 56, 58, 60, 62, 64, 66), bean (SEQ ID NOS: 68, 70), cucumber (SEQ ID NOS: 72, 74), chocolate (SEQ ID NOS: 76, 78), corn (SEQ ID NOS: 80, 82, 84), rice (SEQ ID NOS: 86, 88, 90, 92), sorghum (SEQ ID NOS: 94, 96, 98, 100), and *B. rapa* (SEQ ID NO: 101).

FIGS. 6A-6D show the phenotype of a mutant CLV1 protein containing an S582N substitution. This mutation causes multilocularity and increased organ number and seed production. (A) Top, dried siliques from WT and a CLV1 S582N mutant (sup-c). Locule edges are marked with dots. Bottom, quantification of locule number in 10 siliques. *nrpd1* has no impact on locule number, while sup-c plants have elevated number of locules. (B) Digital image showing sup-c mutants (CLV1 S582N mutants) have increased numbers of petals and stamens. (C) Digital image showing sup-c mutants (CLV1 S582N mutants) have a moderately reduced plant height. (D) Bar graph showing increased seed production in the sup-c mutants (CLV1 S582N mutants) is independent from loss of RdDM. This result was generated from an F2 mapping population, not grown specifically for seed quantification. The difference between genotypes is significant.

FIG. 7. SNP frequency when sup-c BSA-seq reads are mapped to the R500 reference genome.

FIG. 8.are bar graphs from Yang, *et al.* (*Plant Biotechnology Journal* 16 (7): 1322–35, 2018) showing phenotypes of the BnCLV3 mutants. Statistical analysis of the number of seeds per silque and thousand seeds weight in the WT and single and double homozygous mutants of BnCLV3. The data and error bars represent the mean ± SD (n ≥ 15 plants for each genotype) are

provided. Upper-case letters indicate a significant difference at the 0.01 probability level. aa, homozygous mutation of BnA04.CLV3; cc, homozygous mutation of BnC04.CLV3; aacc, double homozygous mutation of BnA04.CLV3 and BnC04.CLV3 (lines that are homozygous for clv3 mutations in both subgenomes of the allotetraploid).

FIG. 9 is a digital image of an *Arabidopsis thaliana* plant expressing a CLV1 S582N transgene, showing this mutation confers multilocularity in other Brassicaceae species.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and single letter code for amino acids, for example as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. The sequence listing generated on February 21, 2022, 582 kB, and filed herewith is incorporated by reference. In the accompanying sequence listing:

SEQ ID NOS: 1 and 2 are an exemplary native *Brassica rapa* CLV1 coding and protein sequences, respectively (A07p048430.1_BraROA).

SEQ ID NOS: 3 and 4 are an exemplary mutant CLV1 coding and protein sequences, respectively, which result in large locules. Nucleotide 1745 is mutated to A (G->A), resulting in an S582N substitution in the protein (underlined)

ATGAGACTTCTGAAAACTCACCTTCTGTTTCTCCATCTTCATTACGTTATCTCGATTTCGCTTCTATGTTTCTCACCATGCCTCGCTTC
CACTGACATGGACCATCTCCTCAACCTCAAATCCTCCATGATTGGTCCCAACGGCAACGGCCTCCACGACTGGGTTCACTCCCCTTC
CCCCACAGCTCACTGTTCTTTCTCCGGCGTTTCCTGCGACGGCGACGCTCGTGTCATCTCCCTCAACGTCTCTTTCACTCCTCTCTTC
GGAACCATCTCCCCGGAGATTGGGATGCTGAACCGTCTTGTGAATCTCACGTTAGCTGCTAATAACTTCTCCGGTATGTTGCCGTT
AGAGATGAAGAGTCTCACTTCTCTAAAGGTTCTCAACATCTCCAACAACGTAAACCTCAACGGAACGTTCCCCGGAGAGATTCTCA
CTCCCATGGTCGACCTCGAAGTCCTCGACGCGTACAACAACAACTTCACAGGCCCATTACCGCCAGAGATCCCCGGGCTCAAGAAA
CTGAGACACCTCTCTCTCGGAGGAAACTTCTTAACCGGAGAGATCCCAGAGAGTTACGGAGATATCCAAAGCTTGGAGTATCTCG
GCCTCAACGGAGCCGGACTCTCCGGTGAATCTCCGGCGTTCTTGTCACGCCTCAAGAATCTTAAAGAAATGTACGTCGGCTACTTC
AACAGCTACACCGGCGGCGTACCGCCGGAGTTCGGTGAATTGACAAACTTAGAAGTCCTCGACATGGCGAGCTGTACTCTCACCG
GAGAGATTCCGACAACACTAAGTAATCTAAAACATTTGCACACTTTGTTTCTCCACATCAACAACTTAACCGGAAACATCCCACCCG
AACTCTCCGGTTTAATCAGCTTAAAATCTCTAGACCTCTCAATAAACCAGCTAACCGGAGAGATTCCTCAGAGCTTCATCTCCCTAG
GGAACATCACTCTCATCAACCTCTTCCGAAACAATCTCCACGGGCCGATACCGGACTTCATCGGAGACATGCCGAACCTCCAAGTC
CTCCAGGTGTGGGAGAACAACTTCACGCTAGAGCTACCGGCGAATCTCGGCCGGAACGGGAATCTGAAAAAGCTCGACGTCTCT
GATAACCATCTCACCGGACTCATCCCCATGGATTTGTGCAGAGGCGGGAAGCTGGAGACGCTGGTGCTCTCCAACAACTTCTTCTT
CGGCTCGATCCCTGAGAAGCTAGGTCAATGCAAATCGCTAAACAAGATCAGAATCGTCAAGAATCTCCTCAACGGCACGGTTCCG
GAGGGCTTATTCAATCTACCGCTCGTAACGATCATCGAGCTCACCGATAACTTCTTCTCCGGGGAGCTTCCGGGGGAGATGTCAG
GCGACGTTCTCGATCATATCTACTTATCTAACAATTGGTTTACCGGTTTAATCCCCCCGGCTATCGGTAATTTTAAAAATCTACAGG
ATTTATTCTTAGACCGGAACCGGTTTAGCGGGAATATTCCGAGAGAAGTTTTCGAGTTGAAGCATCTAACGAAGATCAACACGAG
TGCTAACAACCTAACCGGCGATATCCCTGACTCAATCTCACGTTGCACTTCCTTAATCTCCGTCGATCTCAGCCGTAACCGAATCGG
CGGAGATATCCCTAAAGACATCCACGATGTGATCAATCTCGGAACTCTAAATCTCTCCGGGAATCAACTCACCGGCTCGATCCCGA
TCGGAATCGGGAAGATGACGA**A**CTTAACCACTCTGGATCTCTCCTTCAACGACCTCTCCGGGAGAGTCCCACTCGGCGGCCAGTTC
CTAGTCTTCAACGACACTTCCTTCGCCGGAAACCCTTACCTCTGCCTCCCTCACCACGTCTCGTGCCTTACGCGTCCGGAACAAACCT
CCGATCGTATCCACACGGCTCTCTTCTCTCCGTCGAGGATCGTTATCACGATCGTCGCGGCGATAACGGCGTTGATCCTCATCAGC
GTCGCGATTCGTCAGATGAACAAGAAGAAACACGAGAGGTCTCTCTCGTGGAAGCTAACCGCCTTCCAAAGACTCGATTTCAAAG

CGGAAGACGTCCTCGAGTGTCTCCAGGAAGAGAACATAATCGGCAAAGGCGGAGCTGGGATCGTCTACCGCGGATCCATGCCGA
ACAACGTAGACGTCGCGATCAAACGGTTAGTAGGACGCGGAACAGGGAGGAGCGATCACGGATTCACGGCGGAGATACAAACT
CTAGGGAGAATCCGCCACCGTCATATAGTGAGACTCCTCGGATACGTGGCGAACAAGGACACGAACCTGCTTCTCTACGAGTACA
TGCCTAACGGGAGCCTCGGGGAGCTTTTGCACGGATCTAAAGGAGGTCATCTTCAGTGGGAGACGAGGCACAGAGTAGCCGTGG
AAGCGGCGAAAGGACTGTGTTATCTTCATCATGACTGTTCGCCGTTGATCTTGCATAGAGACGTTAAGTCCAATAACATACTACTG
GACTCTGATTTTGAGGCCCATGTTGCTGATTTTGGGCTTGCTAAGTTCTTAGTGGACGGTGCTGCTTCTGAGTGTATGTCTTCGATA
GCTGGCTCCTATGGATACATCGCTCCAGAGTATGCTTACACTCTCAAAGTGGACGAGAAGAGTGATGTGTATAGTTTCGGAGTGG
TGTTATTGGAACTGATAGCTGGGAAGAAACCGGTTGGTGAGTTTGGGGAAGGAGTGGATATAGTGAGGTGGGTGAGGAACACG
GAGGGTGAGATACCTCAGCCGTCGGATGCAGCTACTGTTGTGGCGATCGTTGACCAGAGGTTGACTGGTTACCCGTTGACTAGTG
TGATTCACGTGTTCAAGATAGCGATGATGTGTGTGGAGGATGAGGCAGCGACAAGGCCGACGATGAGGGAAGTTGTGCACATGC
TCACTAACCCTCCCAAGTCCGTCACTAACTTGATCGCCTTCTGA

MRLLKTHLLFLHLHYVISISLLCFSPCLASTDMDHLLNLKSSMIGPNGNGLHDWVHSPSPTAHCSFSGVSCDGDARVISLNVSFTPLFGTIS
PEIGMLNRLVNLTLAANNFSGMLPLEMKSLTSLKVLNISNNVNLNGTFPGEILTPMVDLEVLDAYNNNFTGPLPPEIPGLKKLRHLSLGG
NFLTGEIPESYGDIQSLEYLGLNGAGLSGESPAFLSRLKNLKEMYVGYFNSYTGGVPPEFGELTNLEVLDMASCTLTGEIPTTLSNLKHLHT
LFLHINNLTGNIPPELSGLISLKSLDLSINQLTGEIPQSFISLGNITLINLFRNNLHGPIPDFIGDMPNLQVLQVWENNFTLELPANLGRNGN
LKKLDVSDNHLTGLIPMDLCRGGKLETLVLSNNFFFGSIPEKLGQCKSLNKIRIVKNLLNGTVPEGLFNLPLVTIIELTDNFFSGELPGEMSG
DVLDHIYLSNNWFTGLIPPAIGNFKNLQDLFLDRNRFSGNIPREVFELKHLTKINTSANNLTGDIPDSISRCTSLISVDLSRNRIGGDIPKDIH
DVINLGTLNLSGNQLTGSIPIGIGKMTNLTTLDLSFNDLSGRVPLGGQFLVFNDTSFAGNPYLCLPHHVSCLTRPEQTSDRIHTALFSPSRI
VITIVAAITALILISVAIRQMNKKKHERSLSWKLTAFQRLDFKAEDVLECLQEENIIGKGGAGIVYRGSMPNNVDVAIKRLVGRGTGRSDH
GFTAEIQTLGRIRHRHIVRLLGYVANKDTNLLLYEYMPNGSLGELLHGSKGGHLQWETRHRVAVEAAKGLCYLHHDCSPLILHRDVKSN
NILLDSDFEAHVADFGLAKFLVDGAASECMSSIAGSYGYIAPEYAYTLKVDEKSDVYSFGVVLLELIAGKKPVGEFGEGVDIVRWVRNTEG
EIPQPSDAATVVAIVDQRLTGYPLTSVIHVFKIAMMCVEDEAATRPTMREVVHMLTNPPKSVTNLIAF

SEQ ID NOS: 5 and 6 are exemplary CLV1a coding and protein sequences from *B. napus*. S562 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NOS: 7 and 8 are exemplary CLV1b coding and protein sequences from *B. napus*. S584 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NOS: 9 and 10 are exemplary CLV1 coding and protein sequences from *B. oleracea*. S584 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NOS: 11 and 12 are exemplary CLV1a coding and protein sequences from *C. sativa*. S589 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NOS: 13 and 14 are exemplary CLV1b coding and protein sequences from *C. sativa*. S590 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NOS: 15 and 16 are exemplary CLV1c coding and protein sequences from *C. sativa*. S857 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NOS: 17 and 18 are exemplary CLV1 coding and protein sequences from *T. arvense*. S586 can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 19 is a fragment of the CLV1a protein (aa 529-593) from *B. napus*.

SEQ ID NO: 20 is a fragment of the CLV1b protein (aa 551-615) from *B. napus*.

SEQ ID NO: 21 is a fragment of the CLV1 protein (aa 551-615) from *B. oleracea*.

SEQ ID NO: 22 is a fragment of the CLV1a (aa 556-620), CLV1b (aa 557-621), and CLV1c (aa 824-888) proteins from *C. sativa*.

SEQ ID NO: 23 is a fragment of the CLV1 protein (aa 553-617) from *T. arvense*.

SEQ ID NO: 24 is a fragment of the CLV1 protein (aa 549-613) from *B. rapa*.

SEQ ID NO: 25 is an exemplary CLV1 protein from *M. domestica (*MDP0000392374*)*. S541 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

5 **SEQ ID NO: 26** is fragment of an CLV1 protein (aa 515-570) from *M. domestica*.

SEQ ID NO: 27 is an exemplary CLV1 protein from *M. domestica (*MDP0000523939*)*. S571 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 28 is fragment of an CLV1 protein (aa 545-600) from *M. domestica*.

10 **SEQ ID NO: 29** is an exemplary CLV1 protein from *M. domestica* (MDP0000618819*)*. S570 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 30 is fragment of an CLV1 protein (aa 544-599) from *M. domestica*.

SEQ ID NO: 31 is an exemplary CLV1 protein from *M. domestica* (MDP0000804361*)*.

15 S575 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 32 is fragment of an CLV1 protein (aa 549-604) from *M. domestica*.

SEQ ID NO: 33 is an exemplary CLV1 protein from *M. domestica* (MDP0000804929*)*. S575 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N

20 provided herein).

SEQ ID NO: 34 is fragment of an CLV1 protein (aa 549-604) from *M. domestica*.

SEQ ID NO: 35 is an exemplary CLV1 protein from *M. domestica* (MDP0000897253*)*. S575 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

25 **SEQ ID NO: 36** is fragment of an CLV1 protein (aa 549-604) from *M. domestica*.

SEQ ID NO: 37 is an exemplary CLV1 protein from *Prunus persica* (Prupe.1G363300*)*. S578 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 38 is fragment of an CLV1 protein (aa 552-607) from *Prunus persica*.

30 **SEQ ID NO: 39** is an exemplary CLV1 protein from *Prunus persica* (Prupe.6G163000*)*. S580 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 40 is fragment of an CLV1 protein (aa 554-607) from *Prunus persica*.

SEQ ID NO: 41 is an exemplary CLV1 protein from *Prunus persica* (Prupe.6G212200). S570 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 42 is fragment of an CLV1 protein (aa 544-599) from *Prunus persica*.

5 **SEQ ID NO: 43** is an exemplary CLV1 protein from *Citrus sinensis* (orange1.1g001816m.g). S568 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 44 is fragment of an CLV1 protein (aa 542-597) from *Citrus sinensis*.

SEQ ID NO: 45 is an exemplary CLV1 protein from *Citrus sinensis*

10 (orange1.1g001922m.g). S575 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 46 is fragment of an CLV1 protein (aa 549-604) from *Citrus sinensis*.

SEQ ID NO: 47 is an exemplary CLV1 protein from *Citrus sinensis* (orange1.1g002010m.g). S577 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N

15 (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 48 is fragment of an CLV1 protein (aa 551-606) from *Citrus sinensis*.

SEQ ID NO: 49 is an exemplary CLV1 protein from *Ananas comosus* (Aco030123). S592 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

20 **SEQ ID NO: 50** is fragment of an CLV1 protein (aa 566-621) from *Ananas comosus*.

SEQ ID NO: 51 is an exemplary CLV1 protein from *Solanum lycopersicum* (Solyc01g103530.2). S570 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 52 is fragment of an CLV1 protein (aa 544-599) from *Solanum lycopersicum*.

25 **SEQ ID NO: 53** is an exemplary CLV1 protein from *Solanum lycopersicum* (Solyc02g091840.2). S572 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 54 is fragment of an CLV1 protein (aa 546-601) from *Solanum lycopersicum*.

SEQ ID NO: 55 is an exemplary CLV1 protein from *Glycine max* (Glyma.01G197800).

30 S573 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 56 is fragment of an CLV1 protein (aa 547-602) from *Glycine max*.

SEQ ID NO: 57 is an exemplary CLV1 protein from *Glycine max* (Glyma.05G110400). S568 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 58 is fragment of an CLV1 protein (aa 542-597) from *Glycine max*.

5 **SEQ ID NO: 59** is an exemplary CLV1 protein from *Glycine max* (Glyma.11G043800). S573 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 60 is fragment of an CLV1 protein (aa 547-602) from *Glycine max*.

SEQ ID NO: 61 is an exemplary CLV1 protein from *Glycine max* (Glyma.11G114100).

10 S579 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 62 is fragment of an CLV1 protein (aa 553-608) from *Glycine max*.

SEQ ID NO: 63 is an exemplary CLV1 protein from *Glycine max* (Glyma.12G040000). S579 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N

15 provided herein).

SEQ ID NO: 64 is fragment of an CLV1 protein (aa 553-608) from *Glycine max*.

SEQ ID NO: 65 is an exemplary CLV1 protein from *Glycine max* (Glyma.17G156300). S568 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

20 **SEQ ID NO: 66** is fragment of an CLV1 protein (aa 542-597) from *Glycine max*.

SEQ ID NO: 67 is an exemplary CLV1 protein from *Phaseolus vulgaris* (Phvul.003G231400). S575 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 68 is fragment of an CLV1 protein (aa 549-604) from *Phaseolus vulgaris*.

25 **SEQ ID NO: 69** is an exemplary CLV1 protein from *Phaseolus vulgaris* (Phvul.003G231400). S596 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 70 is fragment of an CLV1 protein (aa 570-625) from *Phaseolus vulgaris*.

SEQ ID NO: 71 is an exemplary CLV1 protein from *Cucumis sativus* (Cucsa.103590).

30 S574 is equivalent to S582 of SEQ ID NO: 12, and can be mutated to N (i.e., is equivalent to S582N provided herein)

SEQ ID NO: 72 is fragment of an CLV1 protein (aa 548-603) from *Cucumis sativus*.

SEQ ID NO: 73 is an exemplary CLV1 protein from *Cucumis sativus* (Cucsa.343120). S581 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 74 is fragment of an CLV1 protein (aa 548-603) from *Cucumis sativus*.

5 **SEQ ID NO: 75** is an exemplary CLV1 protein from *Theobroma cacao* (Thecc1EG000890). S573 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 76 is fragment of an CLV1 protein (aa 547-602) from *Theobroma cacao*.

SEQ ID NO: 77 is an exemplary CLV1 protein from *Theobroma cacao*

10 (Thecc1EG034252). S577 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein)

SEQ ID NO: 78 is fragment of an CLV1 protein (aa 551-606) from *Theobroma cacao*.

SEQ ID NO: 79 is an exemplary CLV1 protein from *Zea mays* (GRMZM2G072569). S574 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N

15 provided herein).

SEQ ID NO: 80 is fragment of an CLV1 protein (aa 548-603) from *Zea mays*.

SEQ ID NO: 81 is an exemplary CLV1 protein from *Zea mays* (GRMZM2G141517). S581 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

20 **SEQ ID NO: 82** is fragment of an CLV1 protein (aa 555-610) from *Zea mays*.

SEQ ID NO: 83 is an exemplary CLV1 protein from *Zea mays* (GRMZM2G300133). S588 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 84 is fragment of an CLV1 protein (aa 562-617) from *Zea mays*.

25 **SEQ ID NO: 85** is an exemplary CLV1 protein from *Oryza sativa* (LOC_Os03g12730). S571 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 86 is fragment of an CLV1 protein (aa 545-600) from *Oryza sativa*.

SEQ ID NO: 87 is an exemplary CLV1 protein from *Oryza sativa* (LOC_Os03g56270).

30 S578 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 88 is fragment of an CLV1 protein (aa 567-607) from *Oryza sativa*.

SEQ ID NO: 89 is an exemplary CLV1 protein from *Oryza sativa* (LOC_Os06g50340). S581 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 90 is fragment of an CLV1 protein (aa 556-610) from *Oryza sativa*.

5 **SEQ ID NO: 91** is an exemplary CLV1 protein from *Oryza sativa* (LOC_Os07g04190). S577 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 92 is fragment of an CLV1 protein (aa 551-606) from *Oryza sativa*.

SEQ ID NO: 93 is an exemplary CLV1 protein from *Sorghum bicolor*

10 (Sobic.001G074000). S580 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 94 is fragment of an CLV1 protein (aa 554-609) from *Sorghum bicolor*.

SEQ ID NO: 95 is an exemplary CLV1 protein from *Sorghum bicolor* (Sobic.001G446400). S565 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e.,

15 is equivalent to S582N provided herein).

SEQ ID NO: 96 is fragment of an CLV1 protein (aa 539-594) from *Sorghum bicolor*.

SEQ ID NO: 97 is an exemplary CLV1 protein from *Sorghum bicolor* (Sobic.002G027600). S579 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

20 **SEQ ID NO: 98** is fragment of an CLV1 protein (aa 553-608) from *Sorghum bicolor*.

SEQ ID NO: 99 is an exemplary CLV1 protein from *Sorghum bicolor* (Sobic.010G267700). S589 is equivalent to S582 of SEQ ID NO: 2, and can be mutated to N (i.e., is equivalent to S582N provided herein).

SEQ ID NO: 100 is fragment of an CLV1 protein (aa 563-618) from *Sorghum bicolor*.

25 **SEQ ID NO: 101** is fragment of an CLV1 protein (aa 556-611) from *B. rapa*.

SEQ ID NOS: 102 and 103 are plasmid sequences used to express CLV1 S582N in *A. thaliana*. The T-DNA is from nt 11,693-9,207 (*i.e.*, nt 11,693-11,743 + 1-9,207). The CLV1 cassette is nt 11-7,672.

30

DETAILED DESCRIPTION

The following explanations of terms and methods are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. As used herein, "comprising" means "including" and the singular forms "a" or "an" or "the" include

plural references unless the context clearly dictates otherwise. For example, reference to "comprising a plant" includes one or a plurality of such plants. The term "or" refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise. For example, the phrase "A or B" refers to A, B, or a combination of
5 both A and B. Furthermore, the various elements, features and steps discussed herein, as well as other known equivalents for each such element, feature or step, can be mixed and matched by one of ordinary skill in this art to perform methods in accordance with principles described herein. Among the various elements, features, and steps some will be specifically included and others specifically excluded in particular examples.

10 Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be
15 limiting. All GenBank® Accession numbers cited herein are incorporated by reference in their entirety for the sequence available on February 22, 2021.

In some examples, the numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth, used to describe and claim certain embodiments are to be understood as being modified in some instances by the term "about" or
20 "approximately." For example, "about" or "approximately" can indicate +/- 20% variation of the value it describes. Accordingly, in some embodiments, the numerical parameters set forth herein are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some examples are approximations, the numerical values set forth in the specific
25 examples are reported as precisely as practicable. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range.

Allele: refers to one or more alternative forms of a gene locus. All of these loci relate to one trait. Sometimes, different alleles can result in different observable phenotypic traits, such as
30 different pigmentation. However, many variations at the genetic level result in little or no observable variation. If a multicellular organism has two sets of chromosomes, i.e. diploid, these chromosomes are referred to as homologous chromosomes. Diploid organisms have one copy of

each gene (and therefore one allele) on each chromosome. If both alleles are the same, they are homozygotes. If the alleles are different, they are heterozygotes.

Backcross: The mating of a hybrid to one of its parents. For example hybrid progeny, for example a first generation hybrid (F_1), can be crossed back one or more times to one of its parents. Backcrossing can be used to introduce one or more single locus conversions (such as one or more desirable traits) from one genetic background into another.

Biomass: Organic matter derived from an organism, such as a plant or part thereof. In some examples, biomass refers to all the above ground plant material at a particular point of time, thus including the leaves, stems and may include flowers (at varying stages of development given the flowering period ranges over a period of time). Biomass can include all vegetative and reproductive material produced by the plant at time of harvest.

Brassicaceae: A family of flowering plants commonly known as mustards, crucifers, or cabbage. The family contains the cruciferous vegetables, including species such as *Brassica oleracea* (*e.g.*, broccoli, cabbage, cauliflower, kale, brussels sprouts, collards), *Brassica rapa* (turnip, Chinese cabbage, rutabaga, bok choy, etc.), *Brassica napus* (rapeseed, etc.), *Raphanus sativus* (common radish), *Armoracia rusticana* (horseradish), Matthiola and *Arabidopsis thaliana* (thale cress). Includes members of the *Brassica*, *Draba*, *Erysimum*, *Lepidium*, *Cardamine*, and *Alyssum* genera. Other specific examples include canola, penny-cress, and camelina.

Cas9: An RNA-guided DNA endonuclease enzyme that can cut DNA. Cas9 has two active cutting sites (HNH and RuvC), one for each strand of a double helix. Catalytically inactive (deactivated) Cas9 (dCas9) is also encompassed by this disclosure. In some examples, a dCas9 includes one or more of the following point mutations: D10A, H840A, and N863A.

Cas9 nucleic acid and protein sequences are publicly available. For example, GenBank® Accession Nos. nucleotides 796693..800799 of CP012045.1 and nucleotides 1100046..1104152 of CP014139.1 disclose Cas9 nucleic acids, and GenBank® Accession Nos. AMA70685.1 and AKP81606.1 disclose Cas9 proteins. In some examples, the Cas9 is a deactivated form of Cas9 (dCas9), such as one that is nuclease deficient (*e.g.*, those shown in GenBank® Accession Nos. AKA60242.1 and KR011748.1). In certain examples, Cas9 has at least 80% sequence identity, for example at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to such sequences, and retains the ability to cut DNA.

Cell. Cell as used herein includes a plant cell, whether isolated, in tissue culture or incorporated in a plant or plant part.

Clavata 1 (CLV1): A receptor kinase with an extracellular leucine-rich domain. Controls shoot and floral meristem size, and contributes to establish and maintain floral meristem identity. Negatively regulated by KAPP (kinase-associated protein phosphatase). CLV3 peptide binds directly CLV1 ectodomain.

5 It is shown herein that introduction of a S582N substitution (or its equivalent, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) in CLV1 can increase the number of locules and/or seed production in a plant.

Native CLV1 sequences are publicly available. For example, GenBank® Accession Nos:
10 CACSHJ010000087.1 and CAA0336861.1 disclose native *Arabidopsis thaliana* CLV1 nucleic acid and protein sequences, respectively. One skilled in the art will appreciate that these and other native CLV1 sequences can be mutated to include a coding sequence that results in a S582N substitution (or equivalent, which can be determined using sequence alignments, e.g., see FIGS. 5A, 5B). For example, S582 of SEQ ID NO: 2 is equivalent to S577 of GenBank CAA0336861.1,
15 S562 of SEQ ID NO: 6, S584 of SEQ ID NOs: 8 and 10, S589 of SEQ ID NO: 12 and 99, S590 of SEQ ID NO; 14, S857 of SEQ ID NO: 16, S586 of SEQ ID NO: 18, S541 of SEQ ID NO: 25, S571 of SEQ ID NO: 27 and 85, S570 of SEQ ID NO: 29, 41, and 51, S575 of SEQ ID NO: 31, 33, 35, 45, and 67, S578 of SEQ ID NO: 37 and 87, S580 of SEQ ID NO: 39 and 93, S568 of SEQ ID NO: 43, 57, and 65, S577 of SEQ ID NO: 47, 77 and 91, S592 of SEQ ID NO: 49, S572 of SEQ ID NO:
20 53, S573 of SEQ ID NO: 55, 59 and 75, S579 of SEQ ID NO: 61, 63 and 97, S596 of SEQ ID NO: 69, S574 of SEQ ID NO: 71 and 79, S581 of SEQ ID NO: 73, 81 and 89, S588 of SEQ ID NO: 83 and S565 of SEQ ID NO: 95.

An exemplary CLV1 protein sequence from *B. rapa* is shown in SEQ ID NO: 2, and a mutant CLV1 S582N sequence in SEQ ID NO: 4. The disclosure thus provides CLV1 proteins
25 having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100 % sequence identity to SEQ ID NO: 3 or 4, which include a S582N mutation (or its equivalent, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). An exemplary CLV1 coding sequence from *B. rapa* is shown
30 in SEQ ID NO: 1, and SEQ ID NO: 3 provides a coding sequence with a mutation at nt 1745 that results in an S582N substitution. The disclosure thus provides CLV1 encoding nucleic acid molecules, including genomic DNA and cDNA having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ

ID NO: 1 or 3 which include a mutation at codon 582 (or its equivalent) that encodes an N amino acid.

Complementarity: The ability of a nucleic acid to form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick base pairing or other non-traditional types. A percent complementarity indicates the percentage of residues in a nucleic acid molecule which can form hydrogen bonds (*e.g.*, Watson-Crick base pairing) with a second nucleic acid sequence (*e.g.*, 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence. "Substantially complementary" as used herein refers to a degree of complementarity that is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% over a region of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, or more nucleotides, or refers to two nucleic acids that hybridize under stringent conditions.

CRISPRs (**C**lustered **R**egularly **I**nter**S**paced **S**hort **P**alindromic **R**epeats): A family of DNA sequences found in the genome of prokaryotic systems, including many bacteria and archaea. These sequences are derived from DNA fragments of bacteriophages that had previously infected the prokaryote. They are used to detect and destroy DNA from similar bacteriophages during subsequent infections. CRISPRs are often associated with cas genes that code for proteins related to CRISPRs (such as Cas9 and Cas13d proteins; exemplary Cas13d proteins can be found in WO 2019/040664). Non-limiting examples of Cas nucleases include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Cas13d, Cpf1, C2c3, C2c2 and C2c1Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Cpf1, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, and Csf4.

CRISPR/Cas nuclease editing systems can be used for nucleic acid targeting, for example to detect a target DNA or RNA, modify a target DNA or RNA at any desired location, or cut the target DNA or RNA at any desired location. Thus, such methods can be used to modify a native CLV sequence, for example by introducing a S582N mutation (or its equivalent, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

By delivering a Cas9 protein and appropriate guide RNAs into a cell, the organism's genome can be cut at any desired location (such as a plant *CLV1* gene). In some examples a CRISPR/Cas9 system is used to introduce a DNA CLV1 S582N mutation (or its equivalent, *e.g.*, a

Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) into a plant or plant cell. In another example, a CRISPR/Cas13d system is used to introduce an RNA CLV1 S582N mutation (or its equivalent, *e.g.*, a Ser to Asn substitution in the sequence SLT
5 located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) into a plant or plant cell.

Cross. Synonymous with hybridize or crossbreed. Includes the mating of genetically different individual plants, such as the mating of two parent plants.

Cross-pollination: Fertilization by the union of two gametes from different plants.

10 **Endogenous**: With reference to a nucleic acid and/or protein refers to the nucleic acid and/or protein in question as found in a plant in its natural form (*i.e.*, without any human intervention).

Exogenous: As used herein with reference to nucleic acid molecule, protein, vector, or cell, refers to any such molecule/cell that does not originate from that particular cell or plant as found in
15 nature. Thus, a non-naturally-occurring nucleic acid or vector is exogenous to a cell once introduced into the cell. An "exogenous" nucleic acid can either not occur in a plant in its natural form, be different from the nucleic acid in question as found in a plant in its natural form, or can be identical to a nucleic acid found in a plant in its natural form, but integrated not within their natural genetic environment. A mutant plant/plant part/cell provided herein in some examples includes an
20 exogenous nucleic acid encoding for a CLV1 S582N mutation (or equivalent thereof).

F_1 hybrid: The first generation progeny of the cross of two stable parents that are nonisogenic or isogenic plants.

Gene editing: A type of gene modification in which a nucleic acid molecule, such as DNA, is inserted, deleted or replaced in a native genome of a cell (such as a plant cell) using engineered
25 nucleases, which create site-specific double-strand breaks (DSBs) at desired locations in the genome, and resulting in a non-native genomic sequence. Thus, the resulting genome is one that does not occur in nature. In some examples, a gene editing results in the introduction of an exogenous transgene (*e.g.*, one that does not occur naturally in the cell into which it is introduced) into the genome. Such edited plants, plant parts, and cells can be referred to as gene-edited plants,
30 gene-edited plant parts, and gene-edited plant cells, respectively. The induced double-strand breaks are repaired through nonhomologous end-joining (NHEJ) or homologous recombination (HR), resulting in targeted mutations or repairs. CRISPR/Cas methods can be used to edit the sequence of one or more target genes, such as *CLV1*. In addition, other nucleases can be used to edit the

sequence of one or more target genes, such as *CLV1,* such as TALEN nucleases, meganucleases, and zinc finger nucleases. In one example, homology directed repair methods are used to edit the sequence of one or more target genes, such as *CLV1*. For example, gene editing in a plant can be used to confer a desirable trait to the plant, such as increased locule number and/or seed production.

5 **Genotype**. The genetic constitution of a cell, an organism, or an individual (*i.e.*, the specific allele makeup of the individual) usually with reference to a specific character under consideration.

Guide sequence: A polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific 10 binding of a Cas protein (such as Cas9) to the target sequence (such as *CLV1*). In some examples, the guide sequence is RNA. In some examples, the guide sequence is DNA. The guide nucleic acid can include modified bases or chemical modifications (*e.g.*, see Latorre *et al.*, *Angewandte Chemie* 55:3548-50, 2016). In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence (*e.g.*, a native CLV1 sequence), when optimally 15 aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97.5%, 99%, or more. Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (*e.g.*, the Burrows Wheeler Aligner), ClustalW, Clustal X, BLAT, Novoalign 20 (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net). In some embodiments, a guide sequence is about, or at least, about 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, a guide sequence is less than about 75, 50, 45, 40, 35, 30, 25, 20, 15, 12, or fewer nucleotides in length. In 25 some embodiments, a guide sequence is 15-25 nucleotides (such as 18-22 or 18 nucleotides).

The ability of a guide sequence to direct sequence-specific binding of a CRISPR complex to a target sequence may be assessed by a suitable assay. For example, the components of a CRISPR system sufficient to form a CRISPR complex, including the guide sequence to be tested, may be provided to a host cell having the corresponding target sequence, such as by transfection with 30 vectors encoding the components of the CRISPR sequence, followed by an assessment of preferential cleavage within the target sequence, such as by Surveyor assay. Similarly, cleavage of a target polynucleotide sequence may be evaluated in a test tube by providing the target sequence, components of a CRISPR complex, including the guide sequence to be tested and a control guide

sequence different from the test guide sequence, and comparing binding or rate of cleavage at the target sequence between the test and control guide sequence reactions.

Increase or Decrease: A statistically significant positive or negative change, respectively, in quantity from a control value. An increase is a positive change, such as an increase at least 10%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 100%, at least 200%, at least 300%, at least 400% or at least 500% as compared to the control value. A decrease is a negative change, such as a decrease of at least 20%, at least 25%, at least 50%, at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% decrease as compared to a control value. In some examples the decrease is less than 100%, such as a decrease of no more than 90%, no more than 95% or no more than 99%. In some examples, the control value is a value or range of values expected for the same plant with a native CLV1 sequence, *e.g.*, a wild-type plant (*e.g.*, without an S582N substitution, *e.g.*, SEQ ID NO: 1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99).

Isolated: An "isolated" biological component (such as a protein, nucleic acid, guide sequence, or cell) has been substantially separated, produced apart from, or purified away from other biological components in the cell or tissue of a plant in which the component occurs, such as other cells, chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids and proteins. Isolated nucleic acid molecules (such as guide nucleic acids or a vector comprising such), or cells containing such, in some examples are at least 50% pure, such as at least 75%, at least 80%, at least 90%, at least 95%, at least 98%, or at least 100% pure.

Non-naturally occurring or engineered: Terms used herein as interchangeably and indicate the involvement of the hand of man. The terms, when referring to nucleic acid molecules or polypeptides indicate that the nucleic acid molecule or the polypeptide is at least substantially free from at least one other component with which they are naturally associated in nature and as found in nature. In addition, the terms can indicate that the nucleic acid molecules or polypeptides have a sequence not found in nature.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a nucleic acid

sequence (such as a guide nucleic acid sequence) if the promoter affects the transcription or expression of the nucleic acid sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Plant: Includes reference to an immature or mature whole plant, including a plant from which seed, roots, or leaves have been removed. Seed or embryo that will produce the plant is also considered to be the plant. The present disclosure also includes seeds produced by the plants provided herein, wherein the seeds can include a nucleic acid encoding a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). In one embodiment, the seeds can develop into plants having increased locule number and/or seed production, as compared to a wild-type variety of the plant seed. As used herein, a "plant cell" includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant.

Any commercially or scientifically valuable plant can be used in accordance with this disclosure. Exemplary plants include plants belonging to the super family Viridiplantae, such as monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub, such as *Acacia spp., Acer spp., Actinidia spp., Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chacoomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Dibeteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehraffia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia vi/losa, Pagopyrum spp., Feijoa sellowlana, Fragaria spp., Flemingia spp, Freycinetia banksli, Geranium thunbergii, GinAgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemaffhia altissima, Heteropogon contoffus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hypeffhelia dissolute, Indigo incamata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lo tonus bainesli, Lotus spp., Macro tyloma axillare, Malus spp., Manihot esculenta, Medicago*

saliva, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canadensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativam, Podocarpus totara, Pogonarthria fleckii, Pogonaffhria squarrosa, 5 *Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys vefficillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp,* 10 *Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays*, amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, maize, wheat, barley, rye, oat, peanut, 15 pea, lentil and alfalfa, cotton, rapeseed, canola, pepper, sunflower, tobacco, eggplant, switchgrass, Miscanthus, Setaria, fescue, eucalyptus, a tree, an ornamental plant, a perennial grass and a forage crop. In a specific example, the plant is a Brassicaceae plant, such as a *B. rapa*, *B. napus*, or *B. oleracea* plant. Other exemplary Brassicaceae are provided herein. In one example the plant is a flowering plant. In one example the plant is one listed in FIG. 5A or 5B.

20 **Plant parts:** Includes protoplasts, leaves, stems, roots, root tips, anthers, pistils, seed, embryo, pollen, stamen, ovule, microspore, protoplast, sporophyte, gametophyte, cotyledon, hypocotyl, flower, shoot, tissue, petiole, cells, meristematic cells and the like. Includes plant cells of a tissue culture from which plants can be regenerated. In one example a plant part is a plant cell.

Progeny: Offspring; descendants.

25 **Promoter:** An array of nucleic acid control sequences which direct transcription of a nucleic acid. A promoter includes necessary nucleic acid sequences near the start site of transcription. A promoter also optionally includes distal enhancer or repressor elements. A "constitutive promoter" is a promoter that is continuously active and is not subject to regulation by external signals or molecules. In contrast, the activity of an "inducible promoter" is regulated by an 30 external signal or molecule (for example, a transcription factor). In some examples, a promoter used for recombinant expression of a nucleic acid molecule is not naturally occurring in the cell into which it is introduced, is not native to the nucleic acid molecule to which it is attached, or both.

In one example, a promoter used is not endogenous (*i.e.,* is exogenous) to the plant in which it is introduced.

Recombinant or host cell: A cell that has been genetically altered, or is capable of being genetically altered by introduction of an exogenous polynucleotide, such as a recombinant plasmid or vector, such as one that expresses one or more exogenous nucleic acid molecules that encode a CLV1 S582N mutation (or equivalent thereof, *e.g*., a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). Typically, a host cell is a cell in which a vector can be propagated and its nucleic acid expressed. In specific examples, such cells are plant cells, such as from a monocot or dicot. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Regeneration: The development of a plant from tissue culture. The cells may, or may, not have been genetically modified. Plant tissue culture relies on the fact that all plant cells have the ability to generate a whole plant (totipotency). Single cells (protoplasts), pieces of leaves, or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones.

Self-pollination: The transfer of pollen from the anther to the stigma of the same plant.

Sequence identity/similarity: The similarity between amino acid (or nucleotide) sequences is expressed in terms of the similarity between the sequences, otherwise referred to as sequence identity. Sequence identity is frequently measured in terms of percentage identity (or similarity or homology); the higher the percentage, the more similar the two sequences are.

Methods of alignment of sequences for comparison are well known. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet *et al*., *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444, 1988. Altschul *et al*., *Nature Genet*. 6:119, 1994, presents a detailed consideration of sequence alignment methods and homology calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al*., *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the internet, for use in connection with the sequence

analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Variants of protein sequences known and disclosed herein are typically characterized by possession of at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity counted over the full length alignment with the amino acid sequence using the NCBI Blast 2.0, gapped blastp set to default parameters. For comparisons of amino acid sequences of greater than about 30 amino acids, the Blast 2 sequences function is employed using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1). When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologs and variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or at least 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. These sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologs could be obtained that fall outside of the ranges provided.

Thus, in some examples, a native CLV1 protein has at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 2, 6, 8, 10, 12, 14, 16, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99, and includes a Ser at position 582 or equivalent thereof (*e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). In some examples, a mutant CLV1 protein with an S582N substitution has at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99 and includes an S582N substitution or equivalent thereof (*e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

Variants of the disclosed nucleic acid sequences are typically characterized by possession of at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity counted over the full length alignment with the nucleic acid sequence using the NCBI Blast 2.0, gapped blastn set to default parameters. Thus, in some examples, a nucleic acid encoding a native CLV1 protein has at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 1, 5, 7, 9, 11, 13, 15, or 17, and encodes a protein with a Ser at position 582 or equivalent thereof (*e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). In some examples, a nucleic acid encoding a mutant CLV1 protein with an S582N substitution has at least about 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% sequence identity to SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, 15, or 17 and encodes an S582N substitution or equivalent thereof (*e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is possible that sequences coding for a CLV1 S582N mutation and CLV1 S582N protein sequences (or equivalent thereof) could be obtained that fall outside of the ranges provided.

Single locus converted (conversion) plant: Plants developed by backcrossing and/or by genetic transformation, wherein essentially all of the desired morphological and physiological characteristics of a particular variety (such as number of locules and/or seed production) are recovered in addition to the characteristics of the single locus transferred into the variety via the backcrossing technique and/or by genetic transformation.

Tissue culture: A composition that includes isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. In some examples, the tissue culture includes a homogenous population of plant cells. In some examples, the tissue culture includes a callus tissue. In some examples, the tissue culture includes an anther culture. In some examples, the tissue culture includes a hairy root cultures.

Tolerance and resistance: These terms are used interchangeably to refer to any type of increase in resistance or tolerance to, or any type of decrease in susceptibility. A "tolerant plant" or "tolerant plant variety" need not possess absolute or complete tolerance. Instead, a "tolerant plant," "tolerant plant variety," or a plant or plant variety with "improved tolerance" will have a level of resistance or tolerance which is higher than that of a comparable susceptible plant or variety.

Transformation: The introduction of exogenous genetic material (*e.g.*, nucleic acids and vectors containing such) into cells, for example a plant cell. Exemplary mechanisms that can be used to transfer DNA into plant cells include (but not limited to) electroporation, microprojectile bombardment, *Agrobacterium*-mediated transformation and direct DNA uptake by protoplasts.

5 **Transgene:** An exogenous gene or other genetic material (*e.g.*, nucleic acid molecules that encode a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) and vectors containing such) that has been transferred into the genome of a plant or plant cell, for example by genetic
10 engineering methods. Exemplary transgenes include cDNA (complementary DNA) segment, which is a copy of mRNA (messenger RNA), and the gene itself residing in its original region of genomic DNA. In one example, describes a segment of DNA containing a gene sequence that is introduced into the genome of a Brassiceae plant or plant cell. This non-native segment of DNA may retain the ability to produce RNA or protein in the transgenic plant, or it may alter the normal
15 function of the transgenic plant's genetic code. In general, the transferred nucleic acid is incorporated into the plant's germ line. Transgene can also describe any DNA sequence, regardless of whether it contains a gene coding sequence or it has been artificially constructed, which has been introduced into a plant or vector construct in which it was previously not found.

Under conditions sufficient for: A phrase that is used to describe any environment that
20 permits a desired activity. In one example the desired activity is expression of a nucleic acid molecule that encode a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), in combination with other necessary elements, for example to increase locule number and or seed production.

25 **Vector**: A nucleic acid molecule into which a foreign nucleic acid molecule can be introduced without disrupting the ability of the vector to replicate and/or integrate in a host cell. In one example, a vector is not native to the cell into which it is introduced. Vectors include, but are not limited to, nucleic acid molecules that are single-stranded, double-stranded, or partially double-stranded; nucleic acid molecules that comprise one or more free ends, no free ends (*e.g.*, circular);
30 nucleic acid molecules that comprise DNA, RNA, or both; and other varieties of polynucleotides known in the art.

A vector can include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector can also include one or more selectable marker genes and other

genetic elements. An integrating vector is capable of integrating itself into a host nucleic acid. An expression vector is a vector that contains the necessary regulatory sequences to allow transcription and translation of inserted gene or genes.

One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally-derived DNA or RNA sequences are present in the vector for packaging into a virus (*e.g.*, retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses).

Overview

Provided herein are mutant CLV1 nucleic acids and proteins that include or encode a S582N (or equivalent) substitution. In some examples such mutant CLV1 nucleic acids and proteins that include or encode a S582N (or equivalent) substitution are isolated or purified. Such mutant CLV1 nucleic acids and proteins can be introduced into a plant (or the mutation introduced into the plant, plant part or plant cell, for example by genetic engineering) to increase the number of locules and/or seed production in a plant, a plant part, or a plant cell. The methods can include introducing one or more exogenous nucleic acid molecules that encode a CLV1 S582N (or equivalent) substitution or introduce a CLV1 S582N (or equivalent) encoding substitution. This generates a plant, plant part, or plant cell comprising the exogenous nucleic acid (which in some examples are transgenic plants, transgenic plant parts, or transgenic plant cells that include an exogenous, non-native nucleic acid molecule).

One skilled in the art will appreciate that for the CLV1 sequence from some plant species, the position of the Ser to be mutated to an Asn might not be at position 582. However, nucleic acids encoding such, proteins containing such, and plants expressing such, are encompassed by this disclosure. For example, SEQ ID NO: 1 from *Brassica rapa* shows a native G at position 1745, which results in a Ser at position 582 in SEQ ID NO: 2. The resulting mutation of nt 1745 generating an Asn at position 582 is shown in SEQ ID NOS: 3 and 4. Although the specific location of the Ser to be mutated, can vary depending on the particular CLV1 sequence, the Ser to be mutated to Asn is located in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B. For example, S582 of SEQ ID NO: 2 is equivalent to S577 of GenBank CAA0336861.1, S562 of SEQ ID NO: 6, S584 of SEQ ID NOs: 8 and 10, S589 of SEQ ID NO: 12

and 99, S590 of SEQ ID NO; 14, S857 of SEQ ID NO: 16, S586 of SEQ ID NO: 18, S541 of SEQ ID NO: 25, S571 of SEQ ID NO: 27 and 85, S570 of SEQ ID NO: 29, 41, and 51, S575 of SEQ ID NO: 31, 33, 35, 45, and 67, S578 of SEQ ID NO: 37 and 87, S580 of SEQ ID NO: 39 and 93, S568 of SEQ ID NO: 43, 57, and 65, S577 of SEQ ID NO: 47, 77 and 91, S592 of SEQ ID NO: 49, S572 of SEQ ID NO: 53, S573 of SEQ ID NO: 55, 59 and 75, S579 of SEQ ID NO: 61, 63 and 97, S596 of SEQ ID NO: 69, S574 of SEQ ID NO: 71 and 79, S581 of SEQ ID NO: 73, 81 and 89, S588 of SEQ ID NO: 83 and S565 of SEQ ID NO: 95.

Also provided are plants, plant parts, and plant cells (such as recombinant) having/containing one or more exogenous nucleic acids that express a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), such as one encoding a protein having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4 retaining the S582N mutation, such as a nucleic acid molecule having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 3 retaining a codon at 582 (or equivalent thereof) that encodes N, wherein expression of the CLV1 S582N protein increases the number of locules and/or seed production by the plant in comparison to a wild type plant, wild type plant part, or wild type plant cell. In some examples such plants, plant parts, and plant cells are isolated or purified.

The provided plants, plant parts, and plant cells can further include one or more additional exogenous nucleic acid(s) encoding a protein(s) that confers upon the plant, plant part, or plant cell a desired trait, such as one or more of herbicide tolerance, drought tolerance, heat tolerance, low or high soil pH level tolerance, salt tolerance, resistance to an insect, resistance to a bacterial disease, resistance to a viral disease, resistance to a fungal disease, resistance to a nematode, resistance to a pest, male sterility, abiotic stress tolerance, modified phosphorus characteristics, modified antioxidant characteristics, modified essential seed amino acid characteristics, decreased phytate, modified fatty acid metabolism, and modified carbohydrate metabolism.

Methods of producing a commodity plant product are provided. Such methods can include collecting or producing the commodity plant product from a plant, plant part, or plant cell provided herein (*e.g.*, one that expresses a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof)). For example, such a method can include growing the plant, removing the harvestable parts (such as leaves, seeds, or oils) from the plant, and producing the product from or

by the harvestable parts of the plant. Also provided are commodity plant product produced by such methods, wherein in come examples the commodity plant product includes the at least one exogenous nucleic acid molecule, a nucleic acid molecule that encodes a CLV1 S582N mutation, and/or a CLV1 S582N protein (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). Exemplary commodity products include a protein concentrate, protein isolate, leaves, extract, and oil.

Methods of producing plant seed are provided herein. Such methods can include comprising crossing a plant provided herein (*e.g.*, one that expresses a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B)) with itself or a second plant. In some examples, the second plant is recombinant. Also provided are F_1 seed produced by such a method, and a plant or part thereof produced by growing the seed. Such methods can further include (a) crossing a plant grown from said seed with itself or a different plant to produce a seed of a progeny plant of a subsequent generation; (b) growing a progeny plant of a subsequent generation from said seed of a progeny plant of a subsequent generation and crossing the progeny plant of a subsequent generation with itself or a second plant to produce a progeny plant of a further subsequent generation; and (c) repeating steps (a) and (b) using said progeny plant of a further subsequent generation from step (b) in place of the plant grown from said seed in step (a), wherein steps (a) and (b) are repeated with sufficient inbreeding to produce an inbred plant derived from the plant.

The disclosed plants, plant parts, and plant cells (*e.g.*, one that expresses a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B)) can further include a single locus conversion, such as a transgene, for example a single locus that confers a desired trait. Examples of such traits include male sterility, herbicide tolerance, insect resistance, pest resistance, disease resistance, modified fatty acid metabolism, abiotic stress resistance, altered seed amino acid composition, and modified carbohydrate metabolism.

Methods for breeding a plant that expresses a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or

about aa 540 to 860, see for example FIGS. 5A, 5B) are provided. Such methods can include crossing a plant provided herein (*e.g.*, one that expresses a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B)) with a second plant, thereby generating plants with increased number of locules and/or seed production. The method can further include obtaining seed from the crossing; planting the seeds and growing the seeds to plants; and selecting from said plants those with increased number of locules and/or seed production.

Also provided are containers (such as a paper, plastic or glass container, such as a bag, envelope, clamshell container, vial, or box), which includes dried, frozen, or fresh leaves (or sprouts or microgreens) of a plant provided herein (*e.g.*, one that expresses a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B)); an oil extract of a plant provided herein, or combinations thereof.

In some examples, expression of CLV1 S582N (or equivalent thereof) in the plant, plant part, or plant cell increases number of locules and/or seed production by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 100%, at least 200%, at least 300%, at least 400%, or at least 500%, for example as compared to a plant, cell, or plant part of the same plant type that expresses CLV1 S582 or equivalent thereof (e.g., wild type CLV1, such as a Ser in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

In some examples, the plant is a flowering plant. In some examples, the plant is a member of the family Brassicaceae. In some examples, the plant, plant part, or plant cell is or is from the genus *Brassica*, the genus *Camelina*, or the genus *Thlaspi*. In some examples, the plant, plant part, or plant cell is or is *B. rapa*, *B. napus*, *B. oleracea*, *C. sativa*, *Thlaspi arvense,* or any other listed in FIGS. 3, 4, 5A, 5B. In some examples, the plant, plant part, or plant cell is or is from canola, camelina, pennycress, grape, cantaloupe, cucumber, pumpkin, squash, watermelon, hops, soybean, spinach, or sunflower. In some examples, the plant, plant part, or plant cell is or is from a monocot. In some examples, the plant, plant part, or plant cell is or is from a dicot.

In some examples, the plant part is a protoplast, leaf, stem, root, root tips, anther, pistil, stamen, seed, embryo, pollen, ovule, microspore, protoplast, sporophyte, gametophyte, cotyledon, hypocotyl, flower, shoot, tissue, petiole, or meristematic cell.

Multilocularity in oil seed-type Brassica crops is associated with increased seed production. The tetralocular phenotype in *B. rapa* R-o-18 (see Example 1) is caused by a recessive mutation in a secreted peptide produced from BraA.CLV3. CLV3 binds to the CLV1 receptor kinase to signal in meristem size control. A multilocular phenotype in the tetraploid *Brassica juncea* (an allotetraploid of *B. rapa* and *B. nigra*) is due to recessive mutations in homologs of the peptide receptor CLV1, but none are S582N.

Cas9-based mutation of CLAVATA genes by another group in the tetraploid *Brassica napus* (canola, an allotetraploid of *B. rapa* and *B. oleracea*) (Yang et al., Plant Biotechnology Journal 16 (7): 1322–35, 2018) resulted in created clv3 alleles producing increased seeds per silique and 1000 seed weight (FIG. 8). Total seed yield was not reported. The clv1 alleles created are reported in this publication to be "unstable" and result in only a proportion of siliques showing a multilocular phenotype. Therefore, the *B. rapa* clv1 allele disclosed herein can serve as germplasm for breeding multilocularity into *B. rapa* and other plants, and in breeding multilocular resynthesized *B. napus* and other plants.

Breeding New Varieties with Increase Number of Locules and/or Seed Production

Methods for crossing one or more of the disclosed plants, such as a plant (*e.g.*, Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof), with itself or a second plant are provided, as are the seeds and plants produced by such methods. Such methods can be used for propagation of a new plant variety, or can be used to produce hybrid seeds and the plants grown therefrom. Hybrid plants can be used, for example, in the commercial production of commodity products (including leaves, fruit, biomass, oil, and extracts) or in breeding programs for the production of novel varieties. A hybrid plant can also be used as a recurrent parent at any given stage in a backcrossing protocol during the production of a single locus conversion (for example introduction of one or more desirable traits) of a plant provided herein.

Methods of producing plants and/or seed are provided. Such methods can include crossing one or more of the disclosed plants, such as a plant (*e.g.*, Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such

as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), with itself or a second plant and harvesting a resulting seed, such as an F_1 hybrid seed. The resulting plant can be grown, resulting in a plant or part thereof (such as an F1 plant).

In one example methods of producing an inbred plant derived from a plant provided herein, such as a plant *e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). In one example such methods include (a) generating a progeny plant derived from plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), by crossing such a plant with a second variety; (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation; (c) growing a progeny plant of a subsequent generation from said seed and crossing the progeny plant of a subsequent generation with itself or a second plant; and (d) repeating steps (b) and (c) for an additional at least 2 generations (such as at least 3, at least 4, at least 5, at least 6, at least 7, at least 8 at least 9, at least 10, at least 15 or at least 20, such as 2 to 10, 3 to 10, or 3 to 15 generations) with sufficient inbreeding to produce an inbred plant derived from a plant provided herein.

The second plant crossed with a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), for the purpose of developing novel varieties, is typically a plant which either itself exhibits one or more desirable characteristics or which exhibits one or more desired characteristic(s) when in hybrid combination. In one example, the second plant is recombinant. Exemplary desired characteristics include, but are not limited to: increased seed yield, increased seedling vigor, modified maturity date, desired plant height, high anthocyanin content, high phenolic content, herbicide tolerance or resistance, drought tolerance or resistance, heat tolerance or resistance, low or high soil pH level tolerance, salt tolerance or resistance, resistance to an insect, resistance to a bacterial disease, resistance to a viral disease, resistance to a fungal disease,

resistance to a nematode, resistance to a pest, male sterility, abiotic stress tolerance, and increased number of locules and/or seed production.

When a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g*., a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) is crossed with another different variety, first generation (F_1) progeny are produced. The hybrid progeny are produced regardless of characteristics of the two varieties produced. As such, an F_1 hybrid plant can be produced by crossing a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g*., a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), with any second plant. The second plant can be genetically homogeneous (*e.g*., inbred) or can itself be a hybrid. Therefore, the disclosure provides any F_1 hybrid plant produced by crossing a plant provided herein, such as a plant (*e.g.* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g*., a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), with a second plant (such as a plant having one or more genes that confer to the plant one or more desired characteristics).

Plants can be crossed by either natural or mechanical techniques. Natural pollination occurs by self-pollination or natural cross pollination, which typically is aided by pollinating organisms. In either natural or artificial crosses, flowering and flowering time can be a consideration.

Sensitivity to day length can be a consideration when genotypes are grown outside of their area of adaptation. When genotypes adapted to tropical latitudes are grown in the field at higher latitudes, they may not mature before frost occurs. Plants can be induced to flower and mature earlier by creating artificially short days or by grafting. Plants can be grown in winter nurseries located at sea level in tropical latitudes where day lengths are shorter than their critical photoperiod. The short day lengths and warm temperatures encourage early flowering and seed maturation. Early flowering can be useful for generation advance when only a few self-pollinated seeds per plant are desired, but usually not for artificial hybridization because the flowers self-pollinate before they are large enough to manipulate for hybridization. Artificial lighting can be used to extend the natural day length to about 14.5 hours to obtain flowers suitable for hybridization and to

increase yields of self-pollinated seed. The effect of a short photoperiod on flowering and seed yield can be partly offset by altitude. At tropical latitudes, varieties adapted to the northern U.S. perform more like those adapted to the southern U.S. at high altitudes than they do at sea level. The light level for delay of flowering can be dependent on the quality of light emitted from the source and the genotype being grown. For example, blue light with a wavelength of about 480 nm typically needs more than about 30 times the energy to inhibit flowering as red light with a wavelength of about 640 nm (Parker *et al*. 1946. *Bot. Gaz.* 108:1-26).

Temperature can also affect the flowering and development of plants. It can influence the time of flowering and suitability of flowers for hybridization. Artificial hybridization is typically successful between about 26ºC and about 32ºC.

Self-pollination can occur naturally with no manipulation of the flowers. In some examples, the crossing of two plants is accomplished using artificial hybridization. In artificial hybridization, the flower used as a female in a cross is manually cross pollinated prior to maturation of pollen from the flower, thereby preventing self-fertilization, or alternatively, the male parts of the flower are emasculated using known methods. Exemplary methods for emasculating the male parts of a flower include physical removal of the male parts, use of a cytoplasmic or genetic factor conferring male sterility, and application of a chemical gametocide to the male parts.

For artificial hybridization employing emasculation, flowers that are expected to open the following day are selected on the female parent. The buds are swollen and the corolla is just visible through the calyx or has begun to emerge. Usually no more than two buds on a parent plant are prepared, and all self-pollinated flowers or immature buds are removed, for example with forceps. Immature buds, such as those hidden under the stipules at the leaf axil, are removed. The calyx is removed, for example by grasping a sepal with the forceps, pulling it down and around the flower, and repeating the procedure until the five sepals are removed. The exposed corolla is removed, for example by grasping it just above the calyx scar, then lifting and wiggling the forceps simultaneously. The ring of anthers is visible after the corolla is removed, unless the anthers were removed with the petals. Cross-pollination can then be performed using, for example, petri dishes or envelopes in which male flowers have been collected. Desiccators containing calcium chloride crystals are used in some environments to dry male flowers to obtain adequate pollen shed.

Emasculation is not necessary to prevent self-pollination (Walker et al. 1979. *Crop Sci.* 19:285-286). When emasculation is not used, the anthers near the stigma can be removed to make the stigma visible for pollination. The female flower is usually hand-pollinated immediately after it is prepared; although a delay of several hours does not reduce seed set. Pollen shed typically

begins in the morning and can end when temperatures are above about 30°C. Pollen shed can also begin later and continue throughout much of the day with more moderate temperatures.

Pollen is available from a flower with a recently opened corolla, but the degree of corolla opening associated with pollen shed can vary during the day. In many environments, collection and use of male flowers immediately without storage can be conducted. In the southern U.S. and other humid climates, pollen shed occurs in the morning when female flowers are more immature and difficult to manipulate than in the afternoon, and the flowers can be damp from heavy dew. In those circumstances, male flowers are collected into envelopes or petri dishes in the morning, and the open container is typically placed in a desiccator for about 4 hours at a temperature of about 25°C. The desiccator can be taken to the field in the afternoon and kept in the shade to prevent excessive temperatures from developing within it. Pollen viability can be maintained in flowers for up to about 2 days when stored at about 5°C. In a desiccator at about 3°C, flowers can be stored successfully for several weeks; however, varieties can differ in the percentage of pollen that germinates after long-term storage.

Either with or without emasculation of the female flower, hand pollination can be carried out by removing the stamens and pistil from a flower of the male parent and gently brushing the anthers against the stigma of the female flower. Access to the stamens can be achieved by removing the front sepal and keel petals, or piercing the keel with closed forceps and allowing them to open to push the petals away. Brushing the anthers on the stigma causes them to rupture, and high percentages of successful crosses are typically obtained when pollen is clearly visible on the stigma. Pollen shed can be checked by tapping the anthers before brushing the stigma. Several male flowers can be used to obtain suitable pollen shed when conditions are unfavorable, or the same male can be used to pollinate several flowers with good pollen shed.

When male flowers are not collected and dried in a desiccator, the parents of a cross can be planted adjacent to each other. Plants are typically grown in rows about 65 cm to about 100 cm apart, but plant densities for seed production fields can be significantly higher in density without compromising fertilization and seed quality. Yield of self-pollinated seed from an individual plant can range from a few seeds to more than about 1,000 as a function of plant density. A density of about 30 plants/m of row can be used when about 30 or fewer seeds per plant is adequate, about 10 plants/m can be used to obtain about 100 seeds/plant, and about 3 plants/m usually results in a high seed production per plant. Densities of about 12 plants/m or less are commonly used for artificial hybridization.

Multiple planting dates about 7 days to about 14 days apart can typically be used to match parents of different flowering dates. When differences in flowering dates are extreme between parents, flowering of the later parent can be hastened by creating an artificially short day. Alternatively, flowering of the earlier parent can be delayed by use of artificially long days or delayed planting. For example, crosses with genotypes adapted to the southern U.S. are made in northern U.S. locations by covering the late genotype with a box, large can, or similar container to create an artificially short photoperiod of about 12 hours for about 15 days beginning when there are three nodes with trifoliate leaves on the main stem. Plants induced to flower early tend to have flowers that self-pollinate when they are small and can be difficult to prepare for hybridization. Grafting can be used to hasten the flowering of late flowering genotypes.

Plants Having One or More Heritable Traits

The disclosure provides plants (*e.g.,* Brassicaceae plants) having increased number of locules and/or increased seed production relative to a native plant of the same species, which can be further modified to include one or more additional desired heritable traits. In some examples, such plants can be developed using backcrossing or genetic engineering (for example by introducing one or more transgenes into a plant provided herein, such as a plant (*e.g.,* Brassicaceae plants) having increased number of locules and/or increased seed production relative to a native plant of the same species, wherein the transgenes encode one or more desired traits), wherein essentially all of the desired morphological and physiological characteristics of a disclosed plant are recovered (such as increased number of locules and/or seed production) in addition to a genetic locus transferred into the plant via the backcrossing technique. Plants developed using such methods can be referred to as a single locus converted plant.

In one example, the method of introducing one or more desired traits into one or more of the plants provided herein, such as a plant (*e.g.* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof), with a second plant having one or more desired traits to produce F_1 progeny plants; (b) selecting F_1 progeny plants that have the one or more desired traits to produce selected F_1 progeny plants; (c) crossing the selected progeny plants with at least a first plant of the variety to produce backcross progeny plants; (d) selecting backcross progeny plants that have the one or more desired traits and physiological and morphological characteristics of the variety to produce selected backcross progeny plants; and (e) repeating steps (c) and (d) one or more times in succession to produce selected second or higher backcross progeny plants that have the one or more desired traits and the physiological and

morphological characteristics of a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), when grown in the same environmental conditions.

Backcrossing methods can be used to improve or introduce a characteristic into a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof). The parental plant, which contributes the locus for the desired characteristic, is termed the "nonrecurring" or "donor" parent. This terminology refers to the fact that the nonrecurring parent is used one time in the backcross protocol and therefore does not recur. The parental plant to which the locus or loci from the nonrecurring parent are transferred is known as the recurrent parent as it is used for several rounds in the backcrossing protocol (Poehlman and Sleper. 1995. "Breeding Field Crops" Ames, Iowa: Iowa State University Press; Sprague and Dudley, eds. 1988. Corn and Improvement, 3rd edition). In a typical backcross protocol, the original variety of interest (e.g., plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B)) is crossed to a second variety (nonrecurring parent) that carries the single locus of interest (such as a desirable trait) to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent and the process is repeated until a plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent parent (*e.g.*, a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B)) are recovered (such as increased number of locules and/or seed production) in the converted plant, in addition to the single transferred locus from the nonrecurring parent.

A backcross protocol alters or substitutes a single trait or characteristic in the original variety, such as a plant provided herein, such as a (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof). To accomplish this, a single locus of the recurrent variety is modified or substituted with the desired locus from the

nonrecurrent parent, while retaining essentially all of the rest of the desired genetic, and therefore the desired physiological and morphological constitution of the original variety. The choice of the particular nonrecurrent parent can depend on the purpose of the backcross; for example, to add a commercially desirable, agronomically important trait to the plant. The exact backcrossing protocol can depend on the characteristic or trait being altered to determine an appropriate testing protocol. Although backcrossing methods are simplified when the characteristic being transferred is a dominant allele, a recessive allele can also be transferred. In this instance, it can be useful to introduce a test of the progeny to determine if the desired characteristic has been successfully transferred.

In a backcross where the desired characteristic being transferred to the recurrent parent is controlled by a major gene which can be readily evaluated during the backcrossing, it is common to conduct enough backcrosses to avoid testing individual progeny for specific traits such as yield in extensive replicated tests. In general, four or more backcrosses are used when there is no evaluation of the progeny for specific traits, such as yield. As in this example, lines with the phenotype of the recurrent parent can be composited without the usual replicated tests for traits such as yield, in the individual lines.

Varieties can also be developed from more than two parents, for example using modified backcrossing, which uses different recurrent parents during the backcrossing. Modified backcrossing can be used to replace the original recurrent parent with a variety having certain more desirable characteristics, or multiple parents can be used to obtain different desirable characteristics from each.

Many single locus traits are known that are not regularly selected for in the development of a new inbred but that can be improved by backcrossing techniques. Single locus traits can be, but are not necessarily, recombinant. Examples of these traits include, but are not limited to, male sterility, herbicide resistance, abiotic stress tolerance (such as tolerance or resistance to drought, heat, cold, low or high soil pH level, and/or salt), resistance to bacterial, fungal, or viral disease, insect resistance, restoration of male fertility, enhanced nutritional quality, modified phosphorus characteristics, modified antioxidant characteristics, yield stability, and yield enhancement. These comprise genes generally inherited through the nucleus. Thus plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) or progeny thereof, which include a single locus conversion

(such as one that confers a desired trait, such as increased number of locules and/or seed production).

Direct selection can be applied where the single locus acts as a dominant trait. An example of a dominant trait is herbicide resistance (such as glyphosate resistance). For the selection process, the progeny of the initial cross are sprayed with an herbicide (such as RoundUp® herbicide) prior to the backcrossing. The spraying eliminates any plants which do not have the desired herbicide resistance characteristic; only those plants which have the herbicide resistance gene are used in the subsequent backcross. This process is then repeated for all additional backcross generations.

Selection of plants for breeding may not be dependent on the phenotype of a plant and instead can be based on genetic investigations. For example, a suitable genetic marker can be used which is genetically-linked to a desired trait. One of these markers can therefore be used to identify the presence or absence of a trait in the offspring of a particular cross, and hence can be used in selection of progeny for continued breeding. This technique is referred to as marker assisted selection. Any other type of genetic marker or other assay which is able to identify the relative presence or absence of a trait of interest in a plant can also be useful for breeding. Procedures for marker assisted selection applicable to plant breeding are well known. Such methods can be useful in the case of recessive traits and variable phenotypes, or where conventional assays are more expensive, time consuming, or otherwise disadvantageous. Types of genetic markers which can be used, but are not limited to, Simple Sequence Length Polymorphisms (SSLPs), Randomly Amplified Polymorphic DNAs (RAPDs), DNA Amplification Fingerprinting (DAF), Sequence Characterized Amplified Regions (SCARs), Arbitrary Primed Polymerase Chain Reaction (AP-PCR), Amplified Fragment Length Polymorphisms (AFLPs) (EP 534 858, which is incorporated herein by reference in its entirety), and Single Nucleotide Polymorphisms (SNPs).

Qualitative characteristics can also be useful as phenotype-based genetic markers in plants; however, some or many may not differ among varieties commonly used as parents. Exemplary genetic markers include flower color, differences in maturity, height, and pest resistance.

Useful or desirable traits can be introduced by backcrossing, as well as directly into a plant by genetic transformation methods. Genetic transformation can therefore be used to insert a selected transgene into a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or

progeny thereof, or can, alternatively, be used for the preparation of transgenes which can be introduced by backcrossing. Thus, the disclosure provides methods of producing a plant of a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof, that includes one or more added desired traits, for example that include introducing a transgene(s) conferring the one or more desired traits into a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof (for example by transformation with a transgene that confers upon the plant the desired trait), thereby producing a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof that includes the one or more added desired traits.

Exemplary methods for introducing a desired nucleic acid molecule (*e.g.,* transgene), such as DNA or RNA, which can be employed for the genetic transformation of a plant include, but are not limited to, electroporation, microprojectile bombardment, *Agrobacterium*-mediated transformation and direct DNA uptake by protoplasts.

To effect transformation by electroporation, friable tissues, such as a suspension culture of cells or embryogenic callus, can be used. Alternatively, immature embryos or other organized tissue can be transformed directly. In this technique, the cell walls of target cells can be partially degraded by exposing them to pectin-degrading enzymes (pectolyases) or mechanically wound tissues in a controlled manner. Protoplasts can also be employed for electroporation transformation of plants (Bates. 1994. *Mol. Biotechnol.* 2(2):135-145; Lazzeri. 1995. *Methods Mol. Biol.* 49:95-106).

In microprojectile bombardment, particles (such as those comprised of tungsten, platinum, or gold) are coated with nucleic acids and delivered into cells by a propelling force. For the bombardment, cells in suspension are concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells can be arranged on solid culture medium.

The cells to be bombarded are positioned at an appropriate distance below the macroprojectile stopping plate. An exemplary method for delivering DNA into plant cells by acceleration is the Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a surface covered with target plant cells. The screen disperses the particles so that they are not delivered to the recipient cells in large aggregates. A screen intervening between the projectile apparatus and the cells to be bombarded can reduce the size of projectiles aggregate and contribute to a higher frequency of transformation by reducing the damage inflicted on the recipient cells by projectiles that are too large.

Agrobacterium-mediated transfer is a method for introducing gene loci into plant cells. DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations (Klee *et al*. 1985. *Bio. Tech.* 3(7):637-342). Moreover, vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate the construction of vectors capable of expressing various polypeptide coding genes. Such vectors have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes. Additionally, *Agrobacterium* containing both armed and disarmed Ti genes can be used for transformation. *Agrobacterium*-mediated plant integrating vectors can be used to introduce DNA into plant cells i (*e.g*., Fraley *et al*. 1985. *Bio. Tech.* 3(7):629-635; U.S. Pat. No. 5,563,055). Briefly, plant tissue (often leaves) is cut into small pieces, *e.g*. 10mm x 10mm, and soaked for 10 minutes in a fluid containing suspended *Agrobacterium*. Some cells along the cut will be transformed by the bacterium, which inserts its DNA into the cell, which is placed on selectable rooting and shooting media, allowing the plants to regrow. Some plants can be transformed just by dipping the flowers into suspension of *Agrobacterium* and then planting the seeds in a selective medium.

Transformation of plant protoplasts can also be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (*e.g.*, Potrykus *et al*. 1985. *Mol. Gen. Genet.* 199(2):169-177; Omirulleh *et al.* 1993. *Plant Mol. Biol*. 21(3):415-428; Fromm et al. 1986. *Nature*. 319(6056):791-739; Uchimiya *et al.* 1986. *Mol. Gen. Genet.* 204(2):207-207; Marcotte *et al.* 1988. *Nature* 335(6189):454-457).

In one example, such methods can also be used to introduce transgenes for the production of proteins in plant cells. The resulting produced protein can be harvested from the plant. The transgene can be harvested from the plants that are originated or are descended from a plant

provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g*., a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), a seed of such a plant, or a hybrid progeny of such a plant.

Numerous different genes are known and can be introduced into a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof), or progeny thereof. Non-limiting examples of particular genes and corresponding phenotypes that can be chosen for introduction into a plant are provided herein.

Included among various plant transformation techniques are methods permitting the site-specific modification of a plant genome. These modifications can include, but are not limited to, site-specific mutations, deletions, insertions, and replacements of nucleotides. These modifications can be made anywhere within the genome of a plant, for example, in genomic elements, including, among others, coding sequences, regulatory elements, and non-coding DNA sequences. Any number of such modifications can be made and the modifications may be made in any order or combination, for example, simultaneously, all together, or one after another. Such methods may be used to modify a particular trait conferred by a locus. Techniques for making such modifications by genome editing include, for example, use of CRISPR-Cas systems, zinc-finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), among others.

A transgene need not be directly transformed into a plant, as techniques for the production of stably transformed corn plants that pass single loci to progeny by Mendelian inheritance is known. Such loci may therefore be passed from parent plant to progeny plants by standard plant breeding techniques.

Herbicide Resistance

Herbicide resistance genes can be used with the methods and plants provided herein. In particular examples, a herbicide resistance gene confers tolerance to an herbicide comprising glyphosate, sulfonylurea, imidazalinone, dicamba, glufosinate, phenoxy proprionic acid, cyclohexone, triazine, benzonitrile, broxynil, L-phosphinothricin, cyclohexanedione, chlorophenoxy acetic acid, or combinations thereof.

In one example the herbicide resistance gene is a gene that confers resistance to an herbicide that inhibits the growing point or meristem, such as an imidazalinone or a sulfonylurea.

Exemplary genes in this category code for mutant ALS and AHAS enzyme as described, for example, by Lee *et al.* (1988. *Embryo J.* 7:1241-8) and Miki *et al.* (1990. *Theoret. Appl. Genet.* 80:449-458). In one non-limiting example, the herbicide resistance gene is a gene that confers resistance to the sulfonylurea herbicide nicosulfuron.

5 Resistance genes for glyphosate (*e.g.*, resistance conferred by mutant 5-enolpyruvl-3 phosphikimate synthase (EPSPS) enzyme and aroA genes) and other phosphono compounds such as glufosinate (phosphinothricin acetyl transferase (PAT) and *Streptomyces hygroscopicus* phosphinothricin-acetyl transferase (bar) genes) can be used (*e.g.*, see U.S. Pat. No. 4,940,835). Examples of specific EPSP transformation events conferring glyphosate resistance are described,
10 for example, in U.S. Pat. No. 6,040,497 and 7,632,985. The MON89788 event disclosed in U.S. Pat. No. 7,632,985 can be used to confer glyphosate tolerance in combination with an increase in average yield relative to prior events. Exemplary PAT sequences are provided in RE44962.

DNA molecules encoding a mutant aroA gene can be used with the methods and plants provided herein (*e.g.*, ATCC accession number 39256 and U.S. Pat. No. 4,769,061), as are
15 sequences for glutamine synthetase genes, which confer resistance to herbicides such as L-phosphinothricin (*e.g.*, U.S. Pat. No. 4,975,374), phosphinothricin-acetyltransferase (*e.g.*, U.S. Pat. No. 5,879,903). DeGreef et al. (1989. *Bio/Technology* 61-64) describe the production of plants that express chimeric bar genes coding for phosphinothricin acetyl transferase activity. Exemplary genes conferring resistance to phenoxy propionic acids and cyclohexones, such as sethoxydim and
20 haloxyfop are the Acct-S1, Accl-S2 and Acct-S3 genes described by Marshall *et al.* (1992. *Theor Appl Genet.* 83:435-442).

Exemplary genes conferring resistance to an herbicide that inhibits photosynthesis include triazine (psbA and gs+genes) and benzonitrile (nitrilase gene) (see Przibilla *et al.*, 1991. *Plant Cell.* 3:169-174). Nucleotide sequences for nitrilase genes are disclosed in U.S. Pat. No. 4,810,648, and
25 DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441, and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes *et al.* (1992. *Biochem. J.* 285:173).

U.S. Patent Publication No: 20030135879 describes dicamba monooxygenase (DMO) from *Pseuodmonas maltophilia,* which is involved in the conversion of a herbicidal form of the herbicide
30 dicamba to a non-toxic 3,6-dichlorosalicylic acid and thus can be used for producing plants tolerant to this herbicide.

Genes or plasmids that contribute to the metabolism of chlorophenoxyacetic acids, such as, 2,4-D herbicide and which can be used with the methods and plants provided herein are described,

for example, by Muller *et al*. (2006. *Appl. Environ. Microbiol*. 72(7):4853-4861), Don and Pemberton (1981. *J Bacteriol* 145(2):681-686), Don *et al*. (1985. *J Bacteriol* 161(1):85-90) and Evans *et al*. (1971. *Biochem J* 122(4):543-551).

Genes are also known that confer resistance to herbicides that inhibit photosynthesis such as, for example, triazine herbicides (psbA and gs+ genes) and benzonitrile herbicides (nitrilase gene). In one non-limiting example, a gene confers resistance to the benzonitrile herbicide bromoxynil. Przibila *et al.* (*Plant Cell,* 3:169, 1991) describe the transformation of *Chlamydomonas* with plasmids encoding mutant psbA genes. Nucleotide sequences for nitrilase genes are disclosed in U.S. Pat. No. 4,810,648, and DNA molecules containing these genes are available under ATCC Accession Nos. 53435, 67441, and 67442. Cloning and expression of DNA coding for a glutathione S-transferase is described by Hayes, *et al.* (*Biochem. J.,* 285:173, 1992). 4-hydroxyphenylpyruvate dioxygenase (HPPD) is a target of the HPPD-inhibiting herbicides, which deplete plant plastoquinone and vitamin E pools. Rippert, *et al.* (*Plant Physiol.,* 134:92, 2004) describes an HPPD-inhibitor resistant tobacco plant that was transformed with a yeast-derived prephenate dehydrogenase (PDH) gene. Protoporphyrinogen oxidase (PPO) is the target of the PPO-inhibitor class of herbicides; a PPO-inhibitor resistant PPO gene was recently identified in *Amaranthus tuberculatus* (Patzoldt, *et al*., *PNAS,* 103(33):12329, 2006). The herbicide methyl viologen inhibits CO_2 assimilation. Foyer, et al. (*Plant Physiol.,* 109:1047, 1995) describe a plant overexpressing glutathione reductase (GR) that is resistant to methyl viologen treatment.

Siminszky (*Phytochemistry Reviews,* 5:445, 2006) describes plant cytochrome P450-mediated detoxification of multiple, chemically unrelated classes of herbicides. Modified bacterial genes have been successfully demonstrated to confer resistance to atrazine, an herbicide that binds to the plastoquinone-binding membrane protein Q_B in photosystem II to inhibit electron transport. For example, Cheung, et al. (*PNAS,* 85:391, 1988) describe tobacco plants expressing the chloroplast psbA gene from an atrazine-resistant biotype of *Amaranthus hybridus* fused to the regulatory sequences of a nuclear gene, and Wang, *et al.* (*Plant Biotech. J.,* 3:475, 2005) describe transgenic alfalfa, *Arabidopsis*, and tobacco plants expressing the atzA gene from *Pseudomonas* sp. that were able to detoxify atrazine.

Bayley, *et al.* (*Theor. Appl. Genet.,* 83:645, 1992) describe the creation of 2,4-D-resistant transgenic tobacco and cotton plants using the 2,4-D monooxygenase gene tfdA from *Alcaligenes eutrophus* plasmid pJP5. U.S. Patent Application Publication No. 20030135879 describes the isolation of a dicamba monooxygenase (DMO) gene from *Pseudomonas maltophilia* that is

involved in the conversion of dicamba to a non-toxic 3,6-dichlorosalicylic acid and thus may be used for producing plants tolerant to this herbicide.

Other examples of herbicide resistance have been described, for example, in U.S. Pat. Nos. 6,803,501; 6,448,476; 6,248,876; 6,225,114; 6,107,549; 5,866,775; 5,804,425; 5,633,435; 5,463,175.

Disease Resistance

Plant defenses are often activated by specific interaction between the product of a disease resistance gene (R) in the plant and the product of a corresponding avirulence (Avr) gene in the pathogen. A plant, such as a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof), or progeny thereof, can be transformed with cloned resistance gene to engineer plants that are resistant to specific pathogen strains. See, for example Jones *et al.* (1994. *Science* 266:789) (tomato Cf-9 gene for resistance to *Cladosporium fulvum*); Martin *et al.* (1993. *Science* 262(5138):1432-1436) (tomato Pto gene for resistance to *Pseudomonas syringae pv.*); and Mindrinos *et al.* (1994. *Cell* 78:1089-1099) (*Arabidopsis* RSP2 gene for resistance to *Pseudomonas syringae*).

A viral-invasive protein or a complex toxin derived therefrom can also be used for viral disease resistance in a plant provided herein. For example, the accumulation of viral coat proteins in transformed plant cells imparts resistance to viral infection and/or disease development effected by the virus from which the coat protein gene is derived, as well as by related viruses. See Beachy *et al.* (1990. *Annu Rev Phytopathol* 28:451-474). Coat protein-mediated resistance has been conferred upon transformed plants against alfalfa mosaic virus, cucumber mosaic virus, tobacco streak virus, potato virus X, potato virus Y, tobacco etch virus, tobacco rattle virus and tobacco mosaic virus.

A virus-specific antibody can also be used. See, for example, Tavladoraki *et al.* (1993. *Nature* 366:469-472), which shows that transgenic plants expressing recombinant antibody genes are protected from virus attack. Additional means of inducing whole-plant resistance to a pathogen include modulation of the systemic acquired resistance (SAR) or pathogenesis related (PR) genes, for example genes homologous to the *Arabidopsis thaliana* NIM1/NPR1/SAI1, and/or by increasing salicylic acid production.

The barley ribosome-inactivating gene described by Logemann *et al.* (1992. *Bio/Technology* 10:305-308) can be used to increase resistance to fungal disease. Plant defensins

may be used to provide resistance to fungal pathogens (Thomma *et al.*, *Planta,* 216:193, 2002). Other examples of fungal disease resistance are provided in U.S. Pat. Nos. 6,653,280; 6,573,361; 6,506,962; 6,316,407; 6,215,048; 5,516,671; 5,773,696; 6,121,436; 6,316,407; and 6,506,962.

5 **Insect Resistance**

One example of an insect resistance gene is one that encodes a *Bacillus thuringiensis* (Bt) protein (a Cry toxin), a derivative thereof or a synthetic polypeptide modeled thereon (*e.g.,* see Geiser *et al.*, 1986. *Gene* 48:109, discloses a Bt Δendotoxin gene) can be used with the methods and plants provided herein. Moreover, DNA molecules encoding Δ-endotoxin (*e.g.*, ATCC 10 Accession Nos. 40098, 67136, 31995 and 31998), or lectin (*e.g.*, Van Damme *et al.* (1994. *Plant Mol Biol* 24(5):825-830), which discloses several *Clivia miniata* mannose-binding lectin genes) can be used with the methods and plants provided herein. Another example is a. . A vitamin-binding protein can also be used with the methods and plants provided herein, such as avidin (e.g., WO 1994/000992, which teaches the use of avidin and avidin homologues as larvicides against insect 15 pests). In one example, the *Bacillus thuringiensis* (Bt) protein is a member of the Cry1 class, and is active primarily against larval stages of the order Lepidoptera. Examples include Cry1Ab (Bt11), Cry1Ac, and Cry1F (*e.g.*, Cry1Fa2 (TC1507)), as well as variants and truncations thereof that provide insect resistance. In one example, the *Bacillus thuringiensis* (Bt) protein is a member of the Cry2 class or the Cy3 class (such as Cy34Ab1, Cry35ab1).

20 In one example the insect resistance gene used with the methods and plants provided herein is an enzyme inhibitor, for example, a protease, proteinase inhibitor, or an α-amylase inhibitor. See, for example, Abe *et al.* (1987. *J. Biol. Chem.* 262:16793-7; discloses a rice cysteine proteinase inhibitor), Genbank Accession Nos. Z99173.1 and DQ009797.1 which disclose proteinase inhibitor coding sequences, and Sumitani *et al.* (1993. *Plant Mol. Biol.* 21:985; discloses *Streptomyces* 25 *nitrosporeus* α-amylase inhibitor). An insect-specific hormone or pheromone, such as juvenile hormone esterase, can also be used with the methods and plants provided herein. See, for example, Hammock *et al.* (1990. *Nature* 344:458-461).

An insect-specific hormone or pheromone may also be used. For example, Hammock *et al.* (Nature, 344:458, 1990) describe baculovirus expression of cloned juvenile hormone esterase, an 30 inactivator of juvenile hormone. Further, Gade and Goldsworthy (Eds., Physiological Systems in Insects, Elsevier Academic Press, Burlington, Mass., 2007) describe allostatins and their potential use in pest control, and Palli *et al.* (*Vitam. Horm.,* 73:59, 2005) describes the use of ecdysteroid and

ecdysteroid receptor in agriculture. Additionally, Price *et al.*, (*Insect Mol. Biol.,* 13:469, 2004) identified the diuretic hormone receptor (DHR) as a candidate target of insecticides.

Still other examples include an insect-specific antibody or an immunotoxin derived therefrom and a developmental-arrestive protein. See Taylor *et al.* (1994. Seventh Intl. Symposium on Molecular Plant-Microbe Interactions (Edinburgh Scotland), Abstract #497), who described enzymatic inactivation in transgenic tobacco via production of single-chain antibody fragments.

Nematode resistance has been described, for example, in U.S. Pat. No. 6,228,992, and bacterial disease resistance has been described, for example, in U.S. Pat. No. 5,516,671.

Male Sterility

Genetic male sterility can increase the efficiency with which hybrids are made, in that it can eliminate the need to physically emasculate the plant used as a female in a given cross (Brim and Stuber. 1973. *Crop Sci.* 13:528-530). Herbicide-inducible male sterility systems (*e.g.*, U.S. Pat. No. 6,762,344) can be used with the methods and plants provided herein.

Where use of male-sterility systems is desired, it can be beneficial to also utilize one or more male-fertility restorer genes. For example, where cytoplasmic male sterility (CMS) is used, hybrid seed production involves three inbred lines: (1) a cytoplasmically male-sterile line having a CMS cytoplasm; (2) a fertile inbred with normal cytoplasm, which is isogenic with the CMS line for nuclear genes ("maintainer line"); and (3) a distinct, fertile inbred with normal cytoplasm, carrying a fertility restoring gene ("restorer" line). The CMS line is propagated by pollination with the maintainer line, with all of the progeny being male sterile, as the CMS cytoplasm is derived from the female parent. These male sterile plants can then be efficiently employed as the female parent in hybrid crosses with the restorer line, without the need for physical emasculation of the male reproductive parts of the female parent.

The presence of a male-fertility restorer gene results in the production of fully fertile F_1 hybrid progeny. If no restorer gene is present in the male parent, male-sterile hybrids are obtained. Such hybrids are useful where the vegetative tissue of the plant is utilized. However, in many cases, the seeds are considered to be a valuable portion of the crop, thus, it is desirable to restore the fertility of the hybrids in these crops. Therefore, the disclosure provides a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof), comprising a genetic locus capable of restoring male fertility in an otherwise male-sterile plant. Examples of male-sterility genes and

corresponding restorers which can be employed are found in , e.g., U.S. Pat. No. 3,861,709, 3,710,511, 4,654,465, 4,727,219, 5,530,191 5,684,242 and 5,625,132.

Modified Fatty Acid, Phytate, and Carbohydrate Metabolism

5 Genes conferring modified fatty acid metabolism can be introduced into a plant provided herein, such as antisense stearoyl acyl carrier protein (ACP) desaturase genes (EC 1.14.99.6) (*e.g.*, Knutzon *et al.* 1992. *PNAS* 89:2624-2628). Fatty acid desaturases can be introduced into a plant provided herein, such as *Saccharomyces cerevisiae* OLE1 gene encoding Δ9-fatty acid desaturase, an enzyme which forms the monounsaturated palmitoleic (16:1) and oleic (18:1) fatty acids from 10 palmitoyl (16:0) or stearoyl (18:0) CoA (McDonough *et al.*, 1992. *J Biol Chem* 267(9):5931-5936); a gene encoding a stearoyl-acyl carrier protein -9 desaturase from castor (Fox *et al.* 1993. *PNAS* 90(6):2486-2490); Δ6- and Δ12-desaturases from the cyanobacteria *Synechocystis* responsible for the conversion of linoleic acid (18:2) to gamma-linolenic acid (18:3 gamma) (Reddy *et al.*, 1993. *Plant Mol Biol* 22(2):293-300); a gene from *Arabidopsis thaliana* that encodes an omega-3 15 desaturase (Arondel *et al.* 1992. *Science* 258:1353-5); plant Δ9-desaturases (WIPO Publication No. WO 1991/013972) and corn and *Brassica* Δ15 desaturases (European Patent Application Publ. No. EP 0616644).

Phytate metabolism can also be modified by introduction of a phytase-encoding gene to enhance breakdown of phytate, adding more free phosphate to the transformed plant. For example, 20 see Van Hartingsveldt *et al.* (1993. *Gene* 127:87-94), for an *Aspergillus niger* phytase gene. In corn, this, for example, could be accomplished by cloning and then reintroducing DNA associated with the single allele which is responsible for corn mutants characterized by low levels of phytic acid. See Raboy et al. (2000, *Plant Physiol*. 124(1):355-68).

A number of genes can be used to alter carbohydrate metabolism. For example, plants can 25 be transformed with a gene coding for an enzyme that alters the branching pattern of starch. See Shiroza *et al.* (1988. *J Bacteriol* 170(2):810-816) (*Streptococcus* fructosyltransferase gene), Steinmetz *et al.* (1985. Mol Gen Genet. 200:220-228) (*Bacillus subtilis* levansucrase gene), Pen *et al.* (1992. *BioTechnology* 10:292) (*Bacillus lichenifonnis* α-amylase), Elliot *et al.* (1993. *Plant Mol. Biol* 21:515) (tomato invertase genes), Sergaard *et al.* (1993. *J. Biol. Chem*. 268:22480) (site-30 directed mutagenesis of barley α-amylase gene), and Fisher *et al.* (1993. *Plant Physiol* 102:1045) (maize endosperm starch branching enzyme II). The Z10 gene encoding a 10 kD zein storage protein from maize can also be used to alter the quantities of 10 kD zein in the cells relative to other components (Kirihara *et al.,* 1988. *Mol Gen Genet*. 211:477-484).

U.S. Pat. No. 6,930,225 describes corn cellulose synthase genes and methods of use thereof.

Resistance to Abiotic Stress

Abiotic stress tolerance in a plant provided herein can include, but is not limited to, tolerance to stress induced by, for example, flowering, ear and seed development, enhancement of nitrogen utilization efficiency, altered nitrogen responsiveness, drought resistance or tolerance, cold resistance or tolerance, heat resistance or tolerance, low or high soil pH level resistance or tolerance, submergence tolerance, tolerance of exposure to heavy metals, oxidative stress tolerance, and salt resistance or tolerance. Such abiotic stress tolerance can increase yield under stress.

Delta-pyrroline-5-carboxylate synthetase (P5CS) from mothbean has been used to provide protection against general osmotic stress. Mannitol-1-phosphate dehydrogenase (mt1D) from *E. coli* has been used to provide protection against drought and salinity. Choline oxidase (codA from *Arthrobactor globiformis*) can protect against cold and salt. *E. coli* choline dehydrogenase (betA) provides protection against salt. Additional protection from cold can be provided by omega-3-fatty acid desaturase (fad7) from *Arabidopsis thaliana*. Trehalose-6-phosphate synthase and levan sucrase (SacB) from yeast and *Bacillus subtilis*, respectively, can provide protection against drought (summarized from Annex II Genetic Engineering for Abiotic Stress Tolerance in Plants, Consultative Group On International Agricultural Research Technical Advisory Committee). Overexpression of superoxide dismutase can be used to protect against superoxides, as described in U.S. Pat. No. 5,538,878.

Additional Traits

Additional traits can be introduced into the disclosed plants. A non-limiting example of such a trait is a coding sequence that decreases RNA and/or protein levels. The decreased RNA and/or protein levels may be achieved through RNAi methods, such as those described in U.S. Pat. No. 6,506,559.

Modifications can also include site-specific recombination; modified antioxidant characteristics; modified essential seed amino acid characteristics, or the like, or any combination thereof. Merely by way of example, FRT sites and/or Lox sites can be introduced into a plant. FRT sites can be used in the FLP/FRT system. Lox sites can be used in the Cre/Loxp system. Modifications can be made to a plant to introduce modified antioxidant characteristics (*e.g.,* content or composition, such as alteration of tocopherol or tocotrienols) and/or modified essential seed amino acid characteristics (*e.g.*, increasing accumulation of essential amino acids in seeds).

Exemplary useful genes and traits for transgenic modification of the variety are disclosed in, for example, U.S. Pat. Nos. 7,687,686, 7,649,127 and 7,645,923.

In addition to the modification of oil, fatty acid, or phytate content described above, it may additionally be beneficial to modify the amounts or levels of other compounds. For example, the
5 amount or composition of antioxidants can be altered. For example, U.S. Pat. Nos. 6,787,618 and 7,154,029 and International Patent Application Publication No. WO 00/68393 disclose manipulation of antioxidant levels, and International Patent Application Publication No. WO 03/082899 discloses manipulation of an antioxidant biosynthetic pathway.

Additionally, seed amino acid content may be manipulated. U.S. Pat. No. 5,850,016 and
10 International Patent Application Publication No. WO 99/40209 disclose alteration of the amino acid compositions of seeds. U.S. Pat. Nos. 6,080,913 and 6,127,600 disclose methods of increasing accumulation of essential amino acids in seeds.

U.S. Pat. No. 5,559,223 describes synthetic storage proteins in which the levels of essential amino acids can be manipulated. International Patent Application Publication No. WO 99/29882
15 discloses methods for altering amino acid content of proteins. International Patent Application Publication No. WO 98/20133 describes proteins with enhanced levels of essential amino acids. International Patent Application Publication No. WO 98/56935 and U.S. Pat. Nos. 6,346,403, 6,441,274, and 6,664,445 disclose plant amino acid biosynthetic enzymes. International Patent Application Publication No. WO 98/45458 describes synthetic seed proteins having a higher
20 percentage of essential amino acids than wildtype.

U.S. Pat. No. 5,633,436 discloses plants comprising a higher content of sulfur-containing amino acids; U.S. Pat. No. 5,885,801 discloses plants comprising a high threonine content; U.S. Pat. No. 5,885,802 discloses plants comprising a high methionine content; U.S. Pat. No. 5,912,414 discloses plants comprising a high methionine content; U.S. Pat. No. 5,990,389 discloses plants
25 comprising a high lysine content; U.S. Pat. No. 6,459,019 discloses plants comprising an increased lysine and threonine content; International Patent Application Publication No. WO 98/42831 discloses plants comprising a high lysine content; International Patent Application Publication No. WO 96/01905 discloses plants comprising a high threonine content; and International Patent Application Publication No. WO 95/15392 discloses plants comprising a high lysine content.

30

Tissue Cultures and In Vitro Regeneration of Plants

Tissue cultures of one or more of the plants provided herein, such as a plant (*e.g.*, Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or

equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), are provided. A tissue culture includes isolated cells of the same or a different type or a collection of such cells organized into parts of a plant. Exemplary types of tissue cultures include protoplasts, calli and plant cells that are intact in plants or parts of plants, such as embryos, pollen, flowers, leaves, roots, root tips, anthers, meristematic cells, pistil, seed, petiole, stein, ovule, cotyledon, hypocotyl, shoot or stem, and the like. In a particular example, the tissue culture includes embryos, protoplasts, meristematic cells, pollen, leaves or anthers of the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B). Also provided are plants regenerated from such tissue cultures, wherein the regenerated plant expresses the physiological and morphological characteristics of a new plant disclosed herein.

Methods for preparing tissue cultures of regenerable plant cells and regenerating plants therefrom are known, such as those disclosed in U.S. Pat. Nos. 4,992,375; 5,015,580; 5,024,944, and 5,416,011. Tissue culture provides the capability to regenerate fertile plants. This can allow, for example, transformation of the tissue culture cells followed by regeneration of plants. For transformation to be efficient and successful, DNA can be introduced into cells that give rise to plants or germ-line tissue.

Plants can be regenerated using organogenesis or somatic embryogenesis. Organogenesis is the process of shoot meristem organization and development. Shoots grow out from a source tissue and are excised and rooted to obtain an intact plant. During somatic embryogenesis, an embryo (similar to the zygotic embryo), containing both shoot and root axes, is formed from somatic plant tissue. An intact plant rather than a rooted shoot results from the germination of the somatic embryo.

Organogenesis and somatic embryogenesis are different processes and the specific route of regeneration is primarily dependent on the explant source and media used for tissue culture manipulations. While the systems are different, both systems show variety-specific responses where some lines are more responsive to tissue culture manipulations than others. A line that is highly responsive in organogenesis may not generate many somatic embryos, while lines that produce large numbers of embryos during an "induction" step (typically, exposure of the plant material to a specific regimen of plant growth regulators) may not give rise to rapidly-growing

proliferative cultures. In addition to line-specific responses, proliferative cultures can be observed with both shoot morphogenesis and somatic embryogenesis. Proliferation allows a single, transformed cell to multiply to the point that it can contribute to germ-line tissue.

Organogenesis is a system whereby shoots are obtained de novo from cotyledonary nodes of seedlings (Wright *et al.,* 1986. *Plant Cell Reports* 5:150-154). The shoot meristems form subepidermally and morphogenic tissue can proliferate on a medium containing benzyl adenine (BA). This system can be used for transformation if the subepidermal, multicellular origin of the shoots is recognized and proliferative cultures are utilized. Tissue that can give rise to new shoots are targeted and proliferated within the meristematic tissue to lessen problems associated with chimerism.

Somatic embryogenesis is a system in which embryogenic tissue is obtained from the zygotic embryo axis (Christianson *et al.,* 1983. *Science* 222:632-634). The embryogenic cultures are proliferative and the proliferative embryos are of apical or surface origin with a small number of cells contributing to embryo formation. The origin of primary embryos (the first embryos derived from the initial explant) is dependent on the explant tissue and the auxin levels in the induction medium (Hartweck *et al.,* 1988. *In Vitro Cell. Develop. Bio.* 24:821-828). With proliferative embryonic cultures, single cells or small groups of surface cells of the "older" somatic embryos form the "newer", more recently developed somatic embryos.

Embryogenic cultures can also be used for regeneration, including regeneration of plants.

Methods of Making Plant Extracts

Extracts can be generated from the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof. Such extracts can be used, for example as a commodity, such as oil. In some examples, the extract includes genetic material, proteins, or both, from the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof).

In one example, plants of the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for

example FIGS. 5A, 5B), or any above-ground part of the plant (such as seed), are harvested, for example, after at least 20 days, at least 30 days, at least 45 days, at least 60 days, at least 70 days, at least 90 days, at least 100 days, or at least 120 days of growth (such as after 45 to 100 days, 60 to 100 days, or 50 to 90 days, such as after 60 days or 90 days of growth). For example, canola oil is made by slightly heating and then crushing the seed, and can be extracted using hexane solvent which is recovered at the end of processing. Canola oil cam be refined using water precipitation and organic acid to remove gums and free fatty acids, filtering to remove color, and deodorizing using steam distillation.

The remainder of the seeds can be used for animal feed.

The plant from which an extract is generated can be field-grown, greenhouse grown or grown in pots, sacs and containers, and cut at any height above the soil, and the plant distilled fresh or partially dried to obtain the oil. Plants can be cut once per growing season, but can be harvested (or cut) once or twice or more per growing season, provided it is grown with ample water, nutrients and under environmental conditions that result in plant growth and development.

Products

The disclosure provides products obtained from one or more of the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof. Exemplary products include a biomass or part thereof, such as an extract, oil, protein isolate, protein concentrate, oil extract, or leaves. For example, a dried biomass and/or leaves of the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof can be used as part of food, beverage, or aroma-based product. In some examples, the product includes at least one cell, DNA, and/or protein of a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

The disclosure provides containers, such as a glass, paper, or plastic container, which includes seeds or oil of a plant provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B).

Provided herein are personal consumer items, which include leaves, oil extract, and/or biomass of one or more of the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B), or progeny thereof.

Oil extracts of one or more of the plants provided herein, such as a plant (*e.g.,* Brassicaceae plant) expressing a nucleic acid molecule that encodes a CLV1 S582N mutation (or equivalent thereof, *e.g.*, a Ser to Asn substitution in the sequence SLT located between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860, see for example FIGS. 5A, 5B) or progeny thereof are provided, and in one example are formulated into a spray.

Example 1

Identification of CLV1 S582N Mutation that Increases Locule Number

To understand factors linking epigenetic modification with seed production, a suppressor screen of the nrpd1 mutant, which produces virtually no seeds, was performed. One suppressor mutant that arose from this mutant screen (sup-c) has increased number of locules (seed-containing compartments in the fruit/silique) and results in significantly higher seed production compared to the nrpd1 background. The mutant also has increased floral organs (FIGS. 6A-6D).

Genetic mapping was used to identify this mutation. The background containing the sup-c mutation is R-o-18, which normally has 4 locules due to a CLV3 mutation. The sup-c mutant was crossed to the R500 background, which has 2 locules. In the F2 mapping population, the following phenotypes were observed:

Phenotype	number	conclusion
2 locules	221 (61%)	$CLV3^{R500}$; no sup-c phenotype
4 locules	64 (17.6%)	$CLV3^{R\text{-}o\text{-}18}$; no sup-c phenotype

5-10 locules	46 (12.6%)	sup-c phenotype
Failure to develop mature flowers	33 (9.1%)	Likely effect of secondary mutation from original screen; cannot determine sup-c state

This analysis confirmed that the sup-c mutation is recessive and heritable.

Because the CLV3 gene is associated with locule number, it was also confirmed that sup-c was segregating from the CLV3 gene in the F2 population. Among the 46 sup-c F2 plants, 9 $CLV3^{R500}/CLV3^{R500}$, 17 $CLV3^{R500}/CLV3^{R\text{-}o\text{-}18}$, and 20 $CLV3^{R\text{-}o\text{-}18}/CLV3^{R\text{-}o\text{-}18}$ were observed. This confirms that sup-c is not an allele of CLV3 and indicates that sup-c is linked to CLV3 (approximately 38 cM away).

It was determined whether the sup-c phenotype required the nrpd1 mutation, and observed 12 NRPD1/NRPD1, 27 NRPD1/nrpd1, and 7 nrpd1/nrpd1. This confirms that the multilocule phenotype of sup-c is independent from (and unlinked from) the nrpd1 mutation.

Bulked Segregant Analysis sequencing (BSA-seq) was performed on the 46 F2 sup-c individuals. 207 million paired-end reads were obtained, of which 201 million mapped to the reference genomes. Analysis of SNP frequency indicates that the sup-c mutation is near the end of chromosome 7 (FIG. 7). This is consistent with its weak linkage to CLV3.

Reads were then aligned to the R-o-18 genome to identify putative mutations in a 4.15 Mb mapping interval. Various filtering steps narrowed the list to 2 putative mutations, one in a homolog of CLV1 and the second in a phosphoenolpyruvate carboxylase family protein.

Because of the role of CLAVATA (CLV) signaling in meristem size and locule number, it was concluded that the mutation in CLV1 is responsible for the multilocular phenotype of sup-c.

Example 2

An S582N Mutation in CLV1 Increases Locule Number in *Arabidopsis*

To determine whether the CLV1 S582N mutation conferred multilocularity in other Brassicaceae species, a transgene carrying this mutation was transformed into *Arabidopsis thaliana* that lacked functional CLV1. The *Brassica rapa* CLV1 genomic sequence (3.2 kb) with or without the S582N mutation was inserted between the Arabidopsis CLV1 promoter (4.0 kb) and the AtUBQ10 transcription terminator (0.4 kb) in a binary plasmid carrying an herbicide resistance gene (SEQ ID NOS: 102 and 103). These plasmids were independently transformed into *Agrobacterium*, and the resulting strains were used to transform the *Arabidopsis clv1-11* T-DNA

mutant via floral dip. Herbicide-resistant transformants were selected and analyzed for silique morphology.

As shown in FIG. 9, plants carrying the CLV1-S582N transgene, but not the wild-type CLV1 transgene, had higher number of locules than the untransformed control. This confirms that 5 the BrCLV1 with S582N mutation (or its equivalent) confers multilocularity to other Brassicaceae species.

In view of the many possible embodiments to which the principles of the disclosed invention may be applied, it should be recognized that the illustrated embodiments are only examples of the invention and should not be taken as limiting the scope of the invention. Rather, 10 the scope of the invention is defined by the following claims. I therefore claim as my invention all that comes within the scope and spirit of these claims.

I claim:

1. An isolated mutant clavata 1 (CLV1) nucleic acid molecule, comprising
at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, 5, 7, 9, 11, 13, 15, or 17, wherein the mutant CLV1 nucleic acid molecule encodes an Asn (N) amino acid at codon 582 of SEQ ID NO: 3 or an equivalent codon, for example includes an adenine (A) nucleotide at position 1745 of SEQ ID NO: 3, such as codon AAT or AAC at codon 582.

2. A recombinant nucleic acid vector comprising the isolated mutant CLV1 nucleic acid molecule of claim 1, such as a plasmid or viral vector.

3. A plant cell, bacterial cell, plant or bacterium comprising the isolated mutant CLV1 nucleic acid molecule of claim 1, or the recombinant nucleic acid vector of claim 2.

4. The bacterial cell or bacterium of claim 3, wherein the bacterial cell or bacterium is an *Agrobacterium* cell or, such as *Agrobacterium tumefaciens*.

5. The plant cell of plant of claim 3, wherein the plant cell or plant is in the family Brassicaceae, the genus *Brassica*, the genus *Camelina*, the genus *Thlaspi*, such as *B. rapa*, *B. napus*, *B. oleracea*, *C. sativa*, *Thlaspi arvense,* or any other listed in FIGS. 3, 4, 5A, 5B.

6. The plant cell of plant of claim 3, wherein the plant cell or plant is a crop plant, such as a fruit tree cell or fruit tree (e.g., apple cell or apple tree (e.g., *Malus*), peach cell or peach tree, orange cell or orange tree, banana cell or banana tree (e.g., *Musa*), plum cell or plum tree), vegetable cell or vegetable plant (e.g., cell or plant of a tomato, soybean (e.g., Glycine max), green bean, cucumber, corn, rice (e.g., Oryza sativa or Oryza glaberrima), or sorghum), pepper or chili (e.g., *Capsicum*), canola, camelina, pennycress, or chocolate cell or chocolate tree (e.g., Theobroma cacao).

7. The plant or tree of any one of claims 3-6, wherein the plant or tree has increased locules as compared to a native plant or tree of the same species.

8. The plant or tree of any one of claims 3-7, wherein the plant or tree has increased seed production as compared to a native plant or tree of the same species.

5 9. The isolated mutant CLV1 nucleic acid molecule of claim 1, encoding a mutant CLV1 protein comprising:

at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 25,
10 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99 wherein the mutant CLV1 protein comprises an Asn (N) amino acid at position 582 of SEQ ID NO: 4 or an equivalent position.

10. An isolated mutant clavata 1 (CLV1) protein, comprising:
15 at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99, wherein the mutant CLV1 protein comprises an Asn
20 (N) amino acid at position 582 of SEQ ID NO: 4 or an equivalent position.

11. A plant cell or plant comprising the isolated mutant CLV1 protein of claim 10.

12. The plant cell or plant of claim 11, wherein the plant cell or plant is in the family
25 Brassicaceae, the genus *Brassica*, the genus *Camelina*, the genus *Thlaspi*, such as *B. rapa*, *B. napus*, *B. oleracea*, *B. balearica*, *B. carinata*, *B. pervirdis*, *B. rupestris, B. nigra*, *B. juncea*, *C. sativa*, *Thlaspi arvense,* or any other listed in FIGS. 3, 4, 5A, 5B.

13. The plant cell or plant of claim 11, wherein the plant cell or plant is a crop plant, such as a
30 fruit tree cell or fruit tree (e.g., apple cell or apple tree (e.g., *Malus*), peach cell or peach tree, orange cell or orange tree, banana cell or banana tree (e.g., *Musa*), plum cell or plum tree), vegetable cell or vegetable plant (e.g., cell or plant of a tomato, soybean (e.g., Glycine max), green bean, cucumber, corn, rice (e.g., Oryza sativa or Oryza glaberrima), or sorghum), pepper or chili

(e.g., *Capsicum*), grape, cantaloupe, , pumpkin, squash, watermelon, hops, spinach, sunflower, canola, camelina, pennycress, or chocolate cell or chocolate tree (e.g., Theobroma cacao).

14. The plant or tree of any one of claims 11-13, wherein the plant or tree has increased locules as
5 compared to a native plant or tree of the same species.

15. The plant or tree of any one of claims 11-14, wherein the plant or tree has increased seed production as compared to a native plant or tree of the same species.

10 16. A method for increasing a number of locules and/or seed production in a plant, comprising:
mutating a CLV1 nucleic acid molecule in the plant to include an Asn (N) amino acid at codon 582 or an equivalent codon, for example includes an adenine (A) nucleotide at position 1745 of SEQ ID NO: 3, such as codon AAT or AAC at codon 582, for example resulting in a mutant CLV1 protein comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at
15 least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99 and comprises an Asn (N) amino acid at position 582 or equivalent position, thereby increasing the number of locules and/or seed production in the resulting plant.

20

17. The method of claim 16, wherein mutating the CLV1 nucleic acid molecule comprises using CRISPER/Cas9 technology.

18. A plant, plant part, or plant cell comprising a mutant CLV1 protein comprising at least 80%,
25 at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4, , 6, 8, 10, 12, 14, 16, 18, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, or 99wherein the mutant CLV1 protein comprises an Asn (N) amino acid at
30 position 582 or equivalent position and increases the number of locules and/or seed production.

19. Any plant or plant cell in a prior claim, further comprising one or more exogenous nucleic acid(s) encoding a protein(s) that confers upon the plant, plant part, or plant cell a desired trait,

wherein the desired trait is one or more of herbicide tolerance, drought tolerance, heat tolerance, low or high soil pH level tolerance, salt tolerance, resistance to an insect, resistance to a bacterial disease, resistance to a viral disease, resistance to a fungal disease, resistance to a nematode, resistance to a pest, male sterility, abiotic stress tolerance, modified phosphorus characteristics, 5 modified antioxidant characteristics, modified essential seed amino acid characteristics, decreased phytate, modified fatty acid metabolism, and modified carbohydrate metabolism.

20. A method of producing a commodity plant product, comprising collecting or producing the commodity plant product from any plant or plant cell recited in a prior claim.

10

21. A commodity plant product produced by the method of claim 20, wherein the commodity plant product comprises the mutant CLV1 nucleic acid molecule and/or mutant CLV1 protein.

22 The method of claim 20 or the commodity plant product of claim 21, wherein the 15 commodity product comprises a protein concentrate, protein isolate, leaves, extract, or oil.

23. A method of producing plant seed, comprising crossing any plant or plant cell in a prior claim with itself or a second plant or second plant cell.

20 24. Any plant or plant cell recited in a prior claim, further comprising a single locus conversion.

25. Seed obtained from any plant or plant cell recited in a prior claim.

26. A method for breeding a plant with an increased number of locules and/or seed production 25 relative to a native plant of the same species, comprising:

crossing any plant recited in a prior claim with a second plant;

obtaining seed from the crossing;

planting the seeds and growing the seeds to plants; and

selecting from said plants those with an increased number of locules and/or seed production 30 relative to a native plant of the same species.

27. A method of generating a plant an increased number of locules and/or seed production relative to a native plant of the same species comprising:

crossing any plant recited in a prior claim with a second plant with a second plant, thereby generating plants an increased number of locules and/or seed production relative to a native plant of the same species.

5 28. A container, comprising

a plant part of any plant recited in a prior claim; or

an oil extract of any plant recited in a prior claim.

29. Any plant or plant cell recited in a prior claim wherein the plant or plant cell is a monocot.

10

30. Any plant or plant cell recited in a prior claim wherein the plant or plant cell is a dicot.

31. A plant part from any plant or plant cell recited in a prior claim, such as a protoplast, leaf, stem, root, root tips, anther, pistil, stamen, seed, embryo, pollen, ovule, microspore, protoplast,

15 sporophyte, gametophyte, cotyledon, hypocotyl, flower, shoot, tissue, petiole, or meristematic cell.

32. A plasmid that does not occur in plants comprising

the isolated nucleic acid molecule of claim 1 optionally operably linked to an exogenous promoter, and

20 optionally further a Cas9 coding sequence.

33. Any proceeding claim, wherein

the equivalent of codon S582 is in the sequence SLT located in the C-terminal half of the protein, for example between aa 500 and 900 of a native CLV1 sequence, such as between about aa

25 540 and 600 or about aa 540 to 860;

the equivalent of position 582 is the S in the sequence SLT located in the C-terminal half of the protein, for example between aa 500 and 900 of a native CLV1 sequence, such as between about aa 540 and 600 or about aa 540 to 860;

the equivalent of codon S582 is S562 of SEQ ID NO: 6, S584 of SEQ ID NOs: 8 and 10,

30 S589 of SEQ ID NO: 12 and 99, S590 of SEQ ID NO; 14, S857 of SEQ ID NO: 16, S586 of SEQ ID NO: 18, S541 of SEQ ID NO: 25, S571 of SEQ ID NO: 27 and 85, S570 of SEQ ID NO: 29, 41, and 51, S575 of SEQ ID NO: 31, 33, 35, 45, and 67, S578 of SEQ ID NO: 37 and 87, S580 of SEQ ID NO: 39 and 93, S568 of SEQ ID NO: 43, 57, and 65, S577 of SEQ ID NO: 47, 77 and 91, S592

of SEQ ID NO: 49, S572 of SEQ ID NO: 53, S573 of SEQ ID NO: 55, 59 and 75, S579 of SEQ ID NO: 61, 63 and 97, S596 of SEQ ID NO: 69, S574 of SEQ ID NO: 71 and 79, S581 of SEQ ID NO: 73, 81 and 89, S588 of SEQ ID NO: 83 or S565 of SEQ ID NO: 95; and/or

the equivalent of position 582 is 562 of SEQ ID NO: 6, 584 of SEQ ID NOs: 8 and 10, 589 of SEQ ID NO: 12 and 99, 590 of SEQ ID NO; 14, 857 of SEQ ID NO: 16, 586 of SEQ ID NO: 18, 541 of SEQ ID NO: 25, S571 of SEQ ID NO: 27 and 85, 570 of SEQ ID NO: 29, 41, and 51, 575 of SEQ ID NO: 31, 33, 35, 45, and 67, 578 of SEQ ID NO: 37 and 87, 580 of SEQ ID NO: 39 and 93, 568 of SEQ ID NO: 43, 57, and 65, 577 of SEQ ID NO: 47, 77 and 91, S592 of SEQ ID NO: 49, 572 of SEQ ID NO: 53, 573 of SEQ ID NO: 55, 59 and 75, 579 of SEQ ID NO: 61, 63 and 97, 596 of SEQ ID NO: 69, 574 of SEQ ID NO: 71 and 79, 581 of SEQ ID NO: 73, 81 and 89, 588 of SEQ ID NO: 83 or 565 of SEQ ID NO: 95.

FIG. 1

Brassica rapa CLV1 coding sequence (A07p048430.1 BraROA)

ATGAGACTTCTGAAAACTCACCTTCTGTTTCTCCATCTTCATTACGTTATCTCGATTTCGCTTCTATGTTTCTCACCATGCCTCGCTTCCACTGACATG
GACCATCTCCTCAACCTCAAATCCTCCATGATTGGTCCCAACGGCAACGGCCTCCACGACTGGGTTCACTCCCCTTCCCCCACAGCTCACTGTTCTTTC
TCCGGCGTTTCCTGCGACGGCGACGCTCGTGTCATCTCCCTCAACGTCTCTTTCACTCCTCTCTTCGGAACCATCTCCCCGGAGATTGGGATGCTGAAC
CGTCTTGTGAATCTCACGTTAGCTGCTAATAACTTCTCCGGTATGTTGCCGTTAGAGATGAAGAGTCTCACTTCTCTAAAGGTTCTCAACATCTCCAAC
AACGTAAACCTCAACGGAACGTTCCCCGGAGAGATTCTCACTCCCATGGTCGACCTCGAAGTCCTCGACGCGTACAACAACAACTTCACAGGCCCATTA
CCGCCAGAGATCCCCGGGCTCAAGAAACTGAGACACCTCTCTCTCGGAGGAAACTTCTTAACCGGAGAGATCCCAGAGAGTTACGGAGATATCCAAAGC
TTGGAGTATCTCGGCCTCAACGGAGCCGGACTCTCCGGTGAATCTCCGGCGTTCTTGTCACGCCTCAAGAATCTTAAAGAAATGTACGTCGGCTACTTC
AACAGCTACACCGGCGGCGTACCGCCGGAGTTCGGTGAATTGACAAACTTAGAAGTCCTCGACATGGCGAGCTGTACTCTCACCGGAGAGATTCCGACA
ACACTAAGTAATCTAAAACATTTGCACACTTTGTTTCTCCACATCAACAACTTAACCGGAAACATCCCACCCGAACTCTCCGGTTTAATCAGCTTAAAA
TCTCTAGACCTCTCAATAAACCAGCTAACCGGAGAGATTCCTCAGAGCTTCATCTCCCTAGGGAACATCACTCTCATCAACCTCTTCCGAAACAATCTC
CACGGGCCGATACCGGACTTCATCGGAGACATGCCGAACCTCCAAGTCCTCCAGGTGTGGGAGAACAACTTCACGCTAGAGCTACCGGCGAATCTCGGC
CGGAACGGGAATCTGAAAAAGCTCGACGTCTCTGATAACCATCTCACCGGACTCATCCCCATGGATTTGTGCAGAGGCGGGAAGCTGGAGACGCTGGTG
CTCTCCAACAACTTCTTCTTCGGCTCGATCCCTGAGAAGCTAGGTCAATGCAAATCGCTAAACAAGATCAGAATCGTCAAGAATCTCCTCAACGGCACG
GTTCCGGAGGGCTTATTCAATCTACCGCTCGTAACGATCATCGAGCTCACCGATAACTTCTTCTCCGGGGAGCTTCCGGGGGAGATGTCAGGCGACGTT
CTCGATCATATCTACTTATCTAACAATTGGTTTACCGGTTTAATCCCCCCGGCTATCGGTAATTTTAAAAATCTACAGGATTTATTCTTAGACCGGAAC
CGGTTTAGCGGGAATATTCCGAGAGAAGTTTTCGAGTTGAAGCATCTAACGAAGATCAACACGAGTGCTAACAACCTAACCGGCGATATCCCTGACTCA
ATCTCACGTTGCACTTCCTTAATCTCCGTCGATCTCAGCCGTAACCGAATCGGCGGAGATATCCCTAAAGACATCCACGATGTGATCAATCTCGGAACT
CTAAATCTCTCCGGGAATCAACTCACCGGCTCGATCCCGATCGGAATCGGGAAGATGACGAGCTTAACCACTCTGGATCTCTCCTTCAACGACCTCTCC
GGGAGAGTCCCACTCGGCGGCCAGTTCCTAGTCTTCAACGACACTTCCTTCGCCGGAAACCCTTACCTCTGCCTCCCTCACCACGTCTCGTGCCTTACG
CGTCCGGAACAAACCTCCGATCGTATCCACACGGCTCTCTTCTCTCCGTCGAGGATCGTTATCACGATCGTCGCGGCGATAACGGCGTTGATCCTCATC
AGCGTCGCGATTCGTCAGATGAACAAGAAGAAACACGAGAGGTCTCTCTCGTGGAAGCTAACCGCCTTCCAAAGACTCGATTTCAAAGCGGAAGACGTC
CTCGAGTGTCTCCAGGAAGAGAACATAATCGGCAAAGGCGGAGCTGGGATCGTCTACCGCGGATCCATGCCGAACAACGTAGACGTCGCGATCAAACGG
TTAGTAGGACGCGGAACAGGGAGGAGCGATCACGGATTCACGGCGGAGATACAAACTCTAGGGAGAATCCGCCACCGTCATATAGTGAGACTCCTCGGA
TACGTGGCGAACAAGGACACGAACCTGCTTCTCTACGAGTACATGCCTAACGGGAGCCTCGGGGAGCTTTTGCACGGATCTAAAGGAGGTCATCTTCAG
TGGGAGACGAGGCACAGAGTAGCCGTGGAAGCGGCGAAAGGACTGTGTTATCTTCATCATGACTGTTCGCCGTTGATCTTGCATAGAGACGTTAAGTCC
AATAACATACTACTGGACTCTGATTTTGAGGCCCATGTTGCTGATTTTGGGCTTGCTAAGTTCTTAGTGGACGGTGCTGCTTCTGAGTGTATGTCTTCG
ATAGCTGGCTCCTATGGATACATCGCTCCAGAGTATGCTTACACTCTCAAAGTGGACGAGAAGAGTGATGTGTATAGTTTCGGAGTGGTGTTATTGGAA
CTGATAGCTGGGAAGAAACCGGTTGGTGAGTTTGGGGAAGGAGTGGATATAGTGAGGTGGGTGAGGAACACGGAGGGTGAGATACCTCAGCCGTCGGAT
GCAGCTACTGTTGTGGCGATCGTTGACCAGAGGTTGACTGGTTACCCGTTGACTAGTGTGATTCACGTGTTCAAGATAGCGATGATGTGTGTGGAGGAT
GAGGCAGCGACAAGGCCGACGATGAGGGAAGTTGTGCACATGCTCACTAACCCTCCCAAGTCCGTCACTAACTTGATCGCCTTCTGA

FIG. 2

Brassica rapa CLV1 protein sequence (A07p048430.1 BraROA)

MRLLKTHLLFLHLHYVISISLLCFSPCLASTDMDHLLNLKSSMIGPNGNGLHDWVHSPSPTAHCSFS
GVSCDGDARVISLNVSFTPLFGTISPEIGMLNRLVNLTLAANNFSGMLPLEMKSLTSLKVLNISNNV
NLNGTFPGEILTPMVDLEVLDAYNNNFTGPLPPEIPGLKKLRHLSLGGNFLTGEIPESYGDIQSLEY
LGLNGAGLSGESPAFLSRLKNLKEMYVGYFNSYTGGVPPEFGELTNLEVLDMASCTLTGEIPTTLSN
LKHLHTLFLHINNLTGNIPPELSGLISLKSLDLSINQLTGEIPQSFISLGNITLINLFRNNLHGPIP
DFIGDMPNLQVLQVWENNFTLELPANLGRNGNLKKLDVSDNHLTGLIPMDLCRGGKLETLVLSNNFF
FGSIPEKLGQCKSLNKIRIVKNLLNGTVPEGLFNLPLVTIIELTDNFFSGELPGEMSGDVLDHIYLS
NNWFTGLIPPAIGNFKNLQDLFLDRNRFSGNIPREVFELKHLTKINTSANNLTGDIPDSISRCTSLI
SVDLSRNRIGGDIPKDIHDVINLGTLNLSGNQLTGSIPIGIGKMTSLTTLDLSFNDLSGRVPLGGQF
LVFNDTSFAGNPYLCLPHHVSCLTRPEQTSDRIHTALFSPSRIVITIVAAITALILISVAIRQMNKK
KHERSLSWKLTAFQRLDFKAEDVLECLQEENIIGKGGAGIVYRGSMPNNVDVAIKRLVGRGTGRSDH
GFTAEIQTLGRIRHRHIVRLLGYVANKDTNLLLYEYMPNGSLGELLHGSKGGHLQWETRHRVAVEAA
KGLCYLHHDCSPLILHRDVKSNNILLDSDFEAHVADFGLAKFLVDGAASECMSSIAGSYGYIAPEYA
YTLKVDEKSDVYSFGVVLLELIAGKKPVGEFGEGVDIVRWVRNTEGEIPQPSDAATVVAIVDQRLTG
YPLTSVIHVFKIAMMCVEDEAATRPTMREVVHMLTNPPKSVTNLIAF

FIG. 3

```
wild type CLV1  GGCTCGATCCCGATCGGAATCGGGAAGATGACGAGCTTAACCACTCTGGATCTCTCCTTCAACGACCTC
                 G  S  I  P  I  G  I  G  K  M  T  S  L  T  T  L  D  L  S  F  N  D  L
sup-c (clv1)    GGCTCGATCCCGATCGGAATCGGGAAGATGACGAACTTAACCACTCTGGATCTCTCCTTCAACGACCTC
                 G  S  I  P  I  G  I  G  K  M  T  N  L  T  T  L  D  L  S  F  N  D  L
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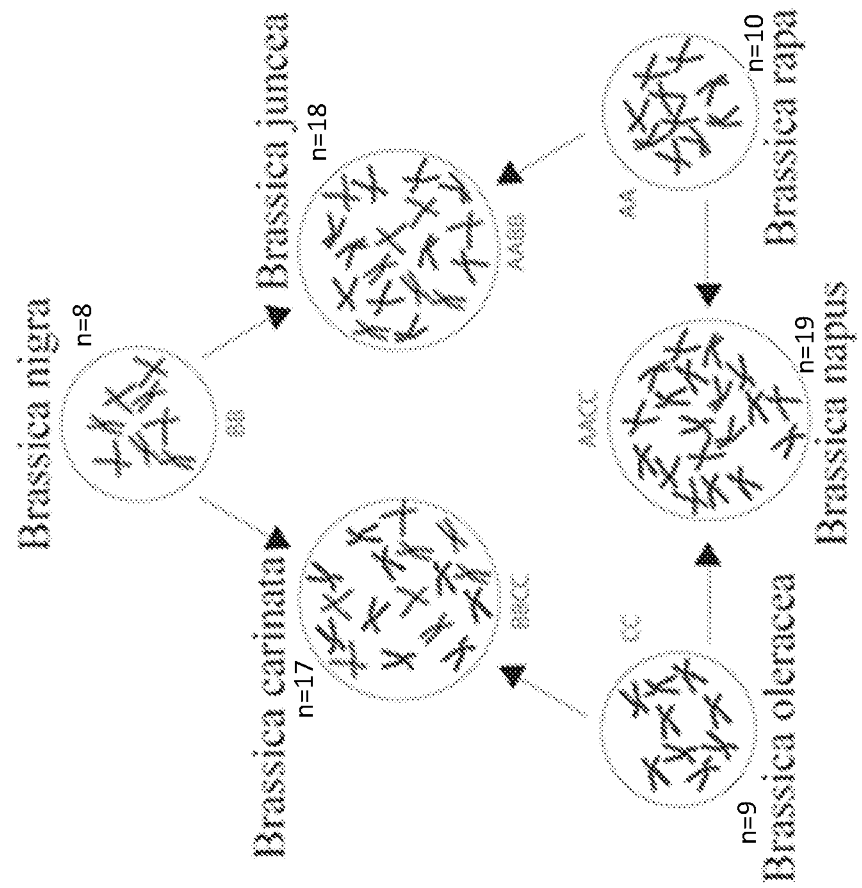


FIG. 4A

FIG. 4B

% identity between CLV1 protein sequences among important Brassicaceae oil-seed crops

	B. napus a	B. napus b	B. oleracea	C. sativa a	C. sativa b	C. sativa c	T. arvense
B. rapa CLV1	97.8	97.2	98.0	89.1	89.5	88.8	90.5
B. napus a		95.3	98.9	88.4	88.7	87.8	90.0
B. napus b			95.5	86.8	87.1	86.2	88.3
B. oleracea				88.3	88.6	87.7	90.1
C. sativa a					97.7	97.2	89.9
C. sativa b						97.7	90.8
C. sativa c							90.6

FIG. 5A

B. napus a IPKDIHDVMNLGTLNLSGNQLTGSIPIGIGKMTSLTTLDLSFNDLSGRVPLGGQFLVFNDTSFAG
B. napus b IPKDIHDVINLGTLNLSGNQLTGSIPIGIGKMTSLTTLDLSFNDLSGRVPLGGQFLVFNDTSFAG
B. oleracea IPKDIHDVINLGTLNLSGNQLTGSIPIGIGKMTSLTTLDLSFNDLSGRVPLGGQFLVFNDTSFAG
C. sativa a IPKEINNVINLGTLNLSGNQLTGSIPTGIGNMTSLTTLDLSFNDLSGRVPLGGQFMVFNDTSFAG
C. sativa b IPKEINNVINLGTLNLSGNQLTGSIPTGIGNMTSLTTLDLSFNDLSGRVPLGGQFMVFNDTSFAG
C. sativa c IPKEINNVINLGTLNLSGNQLTGSIPTGIGNMTSLTTLDLSFNDLSGRVPLGGQFMVFNDTSFAG
T. arvense IPKEIHNVINLGTLNLSGNQLTGPIPTGIGNMTSLTTLDLSFNDLSGRVPLGGQFMVFNDTSFAG
B. rapa CLV1 IPKDIHDVINLGTLNLSGNQLTGSIPIGIGKMTSLTTLDLSFNDLSGRVPLGGQFLVFNDTSFAG

Serine 582

final LRR repeat in extracellular domain

6/12

apple
peach
orange
banana
tomato
soybean
bean
cucumber
chocolate
corn
rice
sorghum
B. rapa CLV1

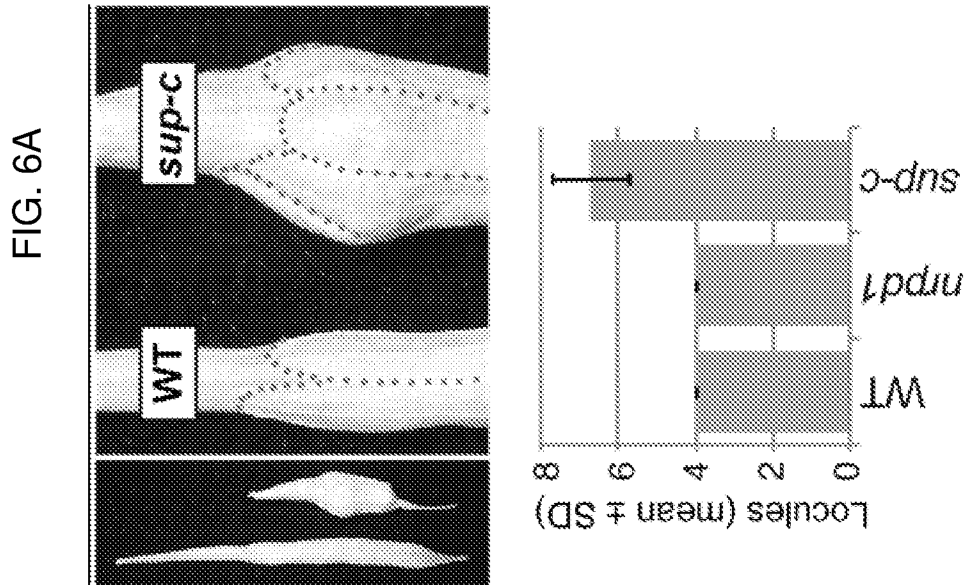
Serine 582

final LRR repeat in extracellular domain

FIG. 5B

FIG. 6B

FIG. 6A



SUBSTITUTE SHEET (RULE 26)

FIG. 6D

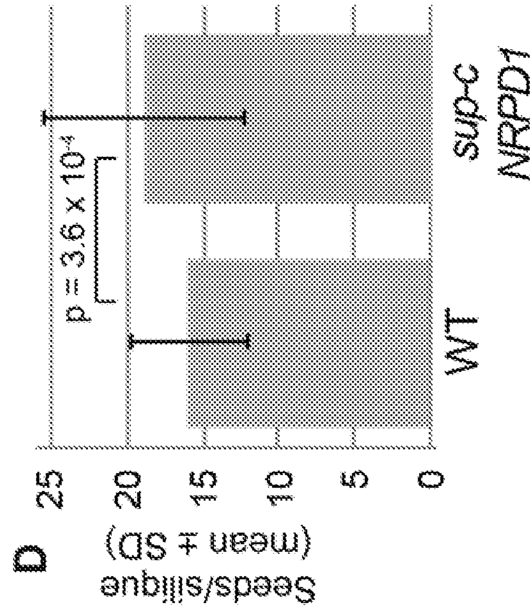
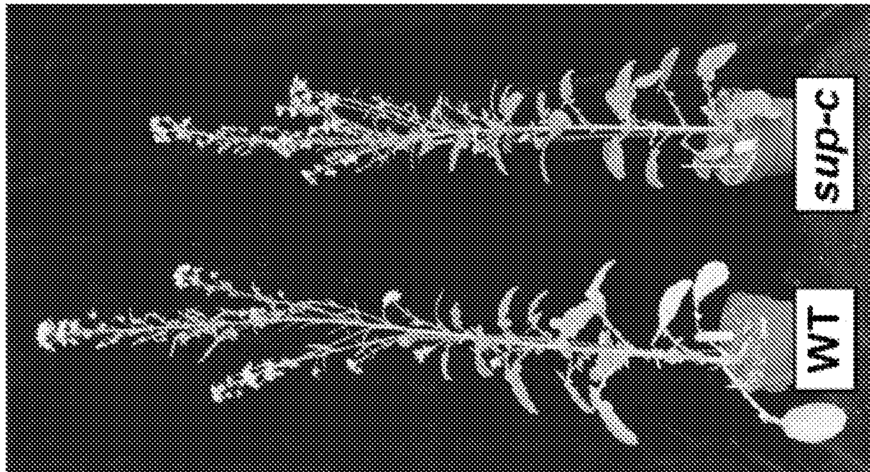


FIG. 6C



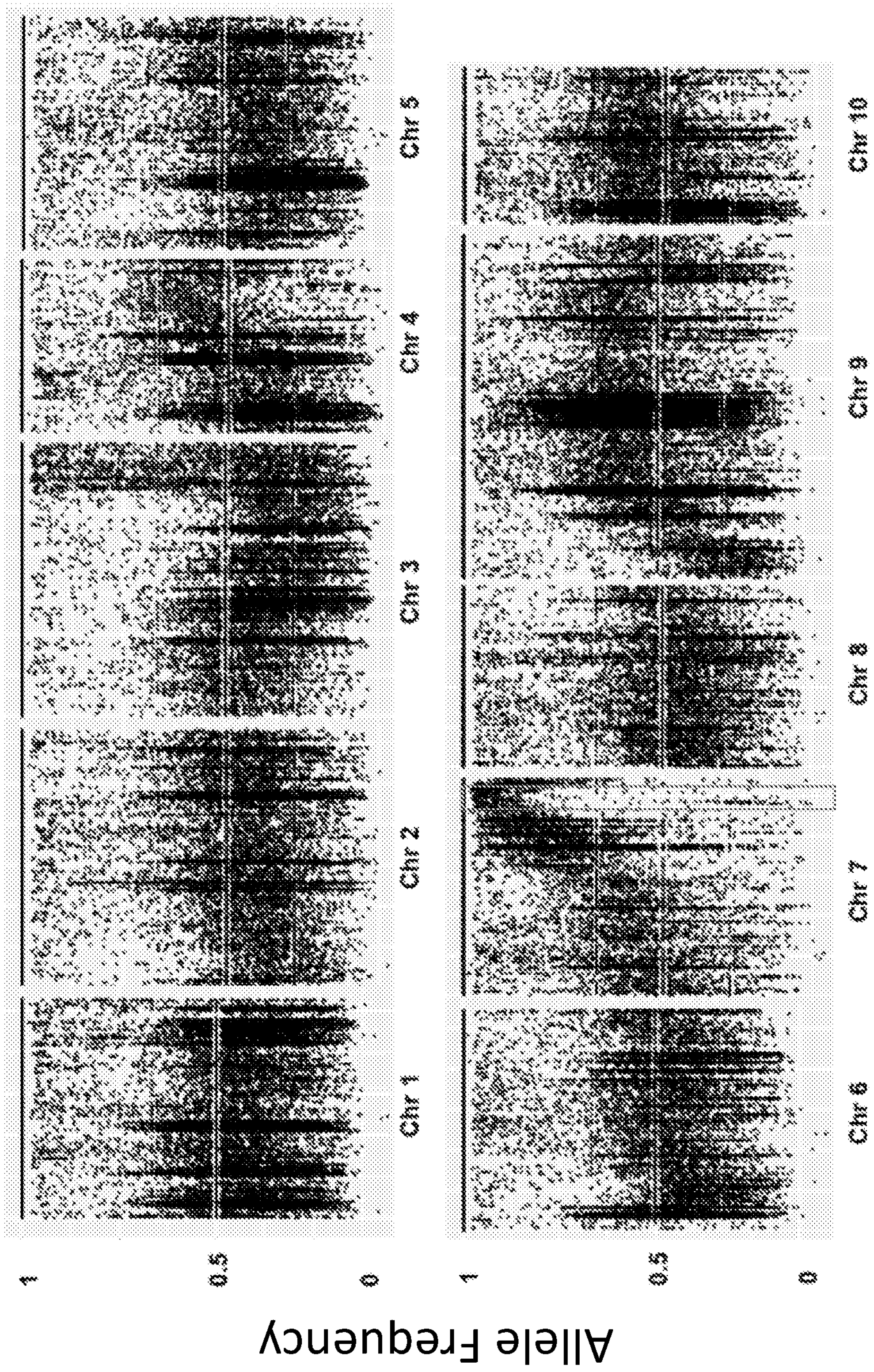


FIG. 7

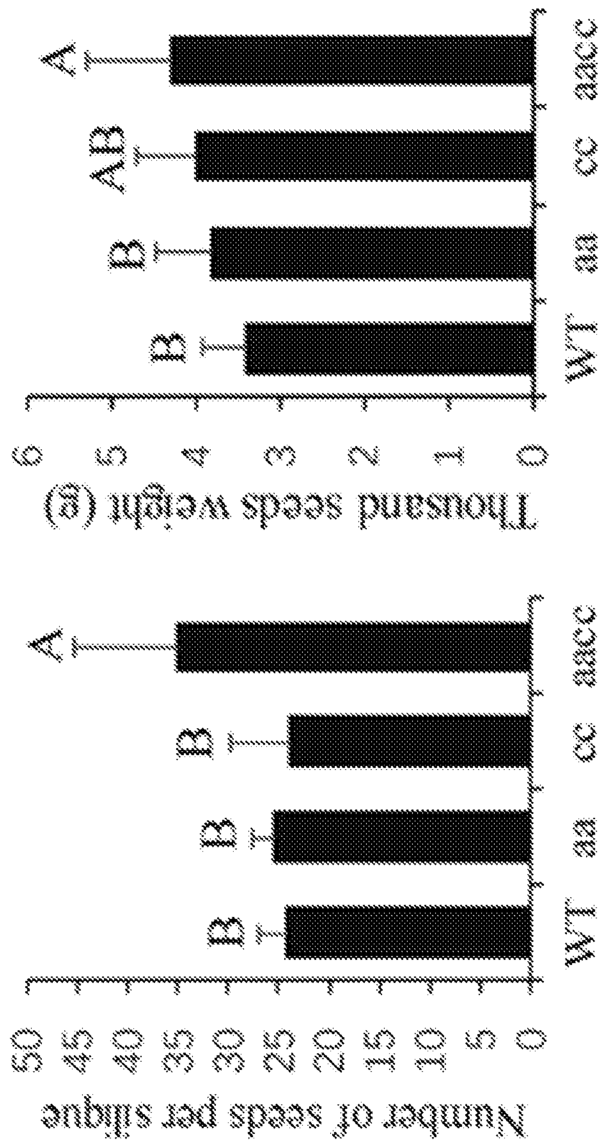


FIG. 8

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/17153

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A01H 1/00, C12N 15/82 (2022.01)

CPC - C12N 15/8294, C12N 15/8285, Y02A 40/146

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2008/062049 A1 (CROPDESIGN N.V.) 29 May 2008 (29.05.2008); pg 7, para 2; pg 80, para 3; pg 84, para 1; claim 1, 49	1-6, 9-14, 16-18, 32
A	GenBank Accession No. FX693195, TSA: Brassica rapa subsp. pekinensis RNA, contig: CF3_10396, transcribed RNA sequence, 17 Decmeber 2014 [online]. [Retrieved on 20 June 2022]. Retrieved from the internet: <URL: https://www.ncbi.nlm.nih.gov/nuccore/FX693195>; Entire document	1-6, 9-14, 16-18, 32
A	US 2011/0016586 A1 (SANZ MOLINERO et al.) 20 January 2011 (08.01.2013); para [0380]; claim 24	1-6, 9-14, 16-18, 32

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 2022	JUL 07 2022

Name and mailing address of the ISA/US	Authorized officer
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/17153

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:
      - [X] in the form of an Annex C/ST.25 text file.
      - [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:
      - [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      - [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 22/17153

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.: 7-8, 15, 19-31, 33
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:

---See Supplemental Box ---

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.:
1-6, 9-14, 16-18, 32 limited to SEQ ID NOs: 3 and 4

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.

Box No. III Observations where unity of invention is lacking

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

Groups I+: Claims 1-6, 9-14, 16-18, 32, drawn to an isolated mutant clavata 1 (CLV1) nucleic acid molecule. The composition will be searched to the extent that the CLV1 encompasses SEQ ID NO: 3 (nucleic acid) and SEQ ID NO: 4 (amino acid). It is believed that claims 1-6, 9-14, 16-18, 32 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 3 and 4. Additional CLV1 polypeptide(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected CLV1 polypeptide(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 a CLV1 polypeptide encompassing SEQ ID NO: 5 (nucleic acid) and SEQ ID NO: 6 (amino acid) (Claims 1-6, 9-14, 16-18, 32).

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

No technical features are shared between the nucleic acid sequences and amino acid sequences Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of including: an isolated mutant clavata 1 (CLV1) nucleic acid molecule, these shared technical features are previously disclosed by WO 2008/062049 A1 to Cropdesign N.V. (hereinafter "Cropdesign").

Cropdesign discloses (instant claim 1) an isolated mutant clavata 1 (CLV1) nucleic acid molecule (pg 7, para 2 - "an isolated nucleic acid sequence encoding a Clavata1 protein"; claim 49 - "wherein said nucleic acid sequence encoding a CLV1 polypeptide encodes any one of the CLV1 polypeptides as given in Table C"; pg 80, para 3 - "A preferred method for increasing expression of a nucleic acid sequence encoding a CLV1 polypeptide with a non-functional C-terminal domain, is by introducing and expressing in a plant a nucleic acid sequence encoding a CLV1 polypeptide with a non-functional C-terminal domain as defined below"; pg 84, para 1 - "Mutation(s) within this site can be introduced to abolish (or reduce) kinase activity, which is one method of disrupting the biological function the C-terminal domain of a CVL1 polypeptide useful in performing the methods of the invention"), comprising at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 5 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3 (Figure 13, pg 80/96, 4, SEQ ID NO: 213 Brassica napus Brana-LRR-RLK nucleic acid sequence AY283519; Note, SEQ ID NO: 213 is 92% match to SEQ ID NO: 3 of instant claim.).

Cropdesign discloses (instant claim 16) a method for increasing a number of locules and/or seed production in a plant (claim 1 - "Method for increasing seed yield in plants relative to control plants"), comprising: mutating a CLV1 nucleic acid molecule in the plant (pg 84, para 1 - "Mutation(s) within this site can be introduced to abolish (or reduce) kinase activity, which is one method of disrupting the biological function the C-terminal domain of a CVL1 polypeptide useful in performing the methods of the invention"), resulting in a mutant CLV1 protein comprising at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 4 (Figure 13, pg 81/96, SEQ ID NO: 214 Brassica napus Brana-LRR-RLK translated amino acid sequence; Note, SEQ ID NO: 214 is 97% match to SEQ ID NO: 4 of instant claim.).

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

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued):
Claims 7-8, 15, 19-31, 33 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).