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# Genomic alteration of plant germline


## Abstract

Compositions containing chimeric RNA molecules which comprise meristem targeting sequences that are fused to RNA cargo sequences that include gene editing molecules are provided. Methods of using the compositions to efficiently edit plant genomes without intervening tissue culture steps are also provided. The solutions described here relate to engineered RNA molecules useful in producing plants with altered genomes. As such, it relates to substantially purified compositions, vectors, systems, as well as genomes of plants.



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## Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This international patent application claims the benefit of U.S. provisional patent application No. 62/924,542, filed Oct. 22, 2019 and incorporated herein by reference in its entirety.

ELECTRONICALLY REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY
(1) The content of the electronically submitted sequence listing in ASCII text file (Name: 10068_SEQ LST_ST25.txt; Size: 102655 bytes; and Date of Creation: Oct. 22, 2020) filed with the application is incorporated herein by reference in its entirety.
BACKGROUND
(2) Development of new and improved varieties of plants requires a genetically diverse parental pool. Traditional breeding programs are based on genetic variation that originates from exotic germplasm or from random mutagenesis. Selected individuals with potentially advantageous genetic traits are backcrossed into elite germplasm to develop improved varieties.
(3) With a growing understanding of plant genetics, many targets emerge for possible genetic modifications useful in making improved plant varieties. Yet traditional methods of random mutagenesis are time consuming and do not provide a convenient way to explore the full spectrum of potential benefit of genetic variation of candidate loci. Other methods like transgenesis or genome editing are more promising.
(4) A drawback of specific genomic intervention, such as by genome editing, is our limited current ability to directly modify the genome of elite germplasm of the species of interest. Genome editing reagents are most often delivered to transformable rather than elite germplasm, which needs to be followed by prolonged backcrossing into commercial germplasm before the phenotypic impact of individual edits can be assessed. The editing methods often require tissue culture and plant regeneration, which requires specific skills and equipment, and adds significant time and expense to the entire process. The methods are very complicated for most plant species, sometimes requiring use of morphogenic regulators to facilitate successful gene editing reagent delivery using biolistic- or Agrobacterium-mediated methods. This is followed by a long process of selecting the putative edited cells and regenerating the edited plants via a complex tissue culture process that is specific to each genotype for plant species of interest. The dedifferentiation required to produce

regenerable callus using tissue culture often triggers seemingly random epigenetic modifications, which further complicates any phenotypic analyses of primary transformants and their progeny.
(5) A need remains for robust and efficient reagents and methods for performing targeted genetic editing in plants. Ideally, the solutions are broadly applicable or easily adaptable to different species and varieties within each species. Bypassing callus induction and/or tissue culture is preferable, to reduce the time and resources required to produce edited events and to produce many targeted genetic variants plus their combinations in all relevant elite genetic backgrounds.

## SUMMARY

(6) The solutions described here relate to engineered RNA molecules useful in producing plants with altered genomes. As such, it relates to substantially purified compositions, vectors, systems, as well as methods, seeds, pollen, and plants useful at various steps in altering genomes of plants.
(7) In their use, the RNA molecules are often needed in a substantially purified form. The RNAs are generally chimeric, meaning that they are made up of at least two different fused segments. One segment comprises a cargo RNA sequence, and another segment comprises a meristem transport RNA sequence.
(8) The cargo segment is made up of RNA that, once inside meristematic cells, carries out the genome alteration function. In various embodiments, the cargo segment can be made up one or more of different sequences needed for the assembly in the plant cell cytosol of the genome-altering function, i.e. it has one or more DNA-modifying components. The DNA modifying components are typically RNA-guided nuclease components, RNAi, a TALE, zinc finger, or meganuclease sequences. RNA-guided nuclease systems typically require at least one polypeptide nuclease effector and one or more guide RNAs. In some embodiments, the cargo segment has an expressible coding sequence of a polypeptide nuclease effector (e.g. Cas9, Cas12a, or Cas12i), such that the RNA is translated when inside a plant cell. In some embodiments, the cargo segment comprises guide RNAs that are flanked by processing elements designed so that, within a plant cell cytosol, they are excised from the chimeric molecule and function in conjunction with a polypeptide nuclease effector present in the same cell. In some embodiments, the same RNA molecule comprises both the effector polynucleotide-encoding sequence and one or more guide RNAs. In these cases, the guide RNA processing elements can be made up of direct repeat sequences of the bacterial CRISPR array of the RNA-guided polypeptide.
(9) The meristem transport segment is made up of a sequence that allows for transport of a chimeric RNA through the plant (e.g., through the phloem of the vascular system) and into the meristem tissues or meristem cells. The transport segment sequence can occur in any RNA found in the plant vascular system that transits from the tissue/cell of origin to the meristem. In one embodiment, the transport segment sequence is generally based on Flowering Time (FT) genes of plants, and they sometimes correspond to fragments of FT transcripts. Flowering Time (FT) gene products are also referred to as “florigen.” The chimeric RNAs are often arranged so that the meristem transport segment is often located 3′ of the cargo segment. In another embodiment, the chimeric RNAs are arranged so that the meristem transport segment (MTS) is located 3′ of the protein coding segment (e.g., a segment encoding an RNA-guided nuclease) in the chimeric RNA.
(10) The RNAs can be used in methods of producing plants with altered genomes. Accordingly, a subj ect plant is contacted with RNAs as described, so that the RNAs typically reach the phloem of the plant. This step may be carried out at the vegetative stage of the plant life cycle. Germline cells of the treated plant and their progeny will have the genome alterations intended to be made by the introduced RNA. In certain embodiments, germline cells of the treated plant and their daughter cells will have the intended genome alterations encoded by the introduced RNA prior to transitioning to reproductive development.
(11) In certain embodiments, a composition comprising a substantially purified RNA molecule made up of a cargo segment fused to a meristem transport segment is provided. In certain embodiments, the cargo segment comprises a DNA-modifying component. In certain

embodiments, the DNA-modifying component is selected from an RNA-guided nuclease component, an RNAi, a TALE, a zinc finger, and a meganuclease. In certain embodiments, the RNA-guided nuclease component comprises an RNA-guided polypeptide encoding sequence. In certain embodiments, the RNA-guided polypeptide encoding sequence can be translated if present in a plant cell cytosol. In certain embodiments, the meristem transport segment comprises an FT-derived sequence. In certain embodiments, the FT-derived sequence is a fragment of an FT transcript. In certain embodiments, the meristem transport segment is located 3′ of the cargo segment. In any of the aforementioned embodiments, the composition further comprise RNase inhibitors. A method of producing a plant with an altered genome, comprising contacting a plant with any of the aforementioned compositions, and retrieving a progeny of the plant, wherein the progeny has an altered genome is provided. In certain embodiments, the contacting comprises phloem loading. In certain embodiments, the contacting with the composition occurs at the vegetative stage of the plant life cycle. Also provided are plants made by the method of producing a plant with an altered genome, comprising contacting a plant with any of the aforementioned compositions, and retrieving a progeny of the plant, wherein the progeny has an altered genome.
(12) A meristem-delivery vector made up of a chimeric RNA having an RNA-guided nuclease component-containing segment and a meristem transport segment is provided.
(13) A recombinant DNA having a sequence capable of producing as a transcript a meristem-delivery vector made up of a chimeric RNA having an RNA-guided nuclease component-containing segment and a meristem transport segment or an RNA that can be purified to form a composition comprising a substantially purified RNA molecule made up of a cargo segment fused to a meristem transport segment is provided.
(14) Also provided are compositions comprising at least one RNA molecule comprising a cargo segment fused to a meristem transport segment (MTS), wherein the cargo segment comprises one or more guide RNAs for an RNA-guided nuclease. Use of the compositions to obtain a plant with an altered genome are provided.
(15) Methods of producing a plant with an altered genome comprising (i) contacting a plant with at least a first composition comprising a cargo segment fused to a meristem transport segment (MTS), wherein the cargo segment comprises one or more guide RNAs for an RNA-guided nuclease; and (ii) retrieving a progeny of the plant, wherein the progeny has an altered genome, are provided. Plants comprising an altered genome made by the method are also provided.

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## Description

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES
(1) FIG. **1**A, B is a diagram of the primary structure of an embodiment of an RNA sequence useful in methods for plant genomic alterations. g=guide RNA. In certain embodiments, the g or guide RNA segment may be made up of a spacer complementary to its genome target, and a crRNA, which is part of the direct repeat sequences of Cas12a and/or Cas12j CRISPR arrays. The various labeled parts are not drawn to scale.
DETAILED DESCRIPTION
(2) The phrase “allelic variant” as used herein refers to a polynucleotide or polypeptide sequence variant that occurs in a different strain, variety, or isolate of a given organism.
(3) The term “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

(4) As used herein, the terms “Cas12a” and “Cpf1” are used interchangeably herein to refer to the same grouping of RNA directed nucleases.
(5) As used herein, the terms “Cas12j” and “CasΦ” are used interchangeably herein to refer to the same grouping of RNA directed nucleases.
(6) The term “fragment” refers to a contiguous set of polynucleotides or polypeptides. In one embodiment, a fragment is at least 10, 15, 20, or greater than 20 contiguous nucleotides. In other embodiments, a fragment is at least 10, 15, 20, or 50 to about 70, 90, 100, 120, 150, or 200 or more continuous nucleotides.
(7) The term “isolated” as used herein means having been removed from its natural environment.
(8) As used herein, the terms “include,” “includes,” and “including” are to be construed as at least having the features to which they refer while not excluding any additional unspecified features.
(9) As used herein, the phrase “operably linked” or “fused” refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. In another non-limiting example, an RNA molecule comprising a “meristem transport sequence” (MTS) is operably linked or fused to a cargo RNA molecule if the MTS provides for delivery of the cargo RNA to meristem cells.
(10) As used herein, the terms “orthologous” or “orthologue” are used to describe genes or the RNAs or proteins encoded by those genes that are from different species but which have the same function (e.g., encode RNAs which exhibit the same meristem transport function). Orthologous genes will typically encode RNAs or proteins with some degree of sequence identity (e.g., at least 40%, 50%, 60%, 70%, 80%, 90%, or 95% sequence identity) and can also exhibit conservation of sequence motifs, and/or conservation of structural features including RNA stem loop structures.
(11) As used herein, the term “plant” includes a whole plant and any descendant, cell, tissue, or part of a plant. The term “plant parts” include any part(s) of a plant, including, for example and without limitation: seed (including mature seed and immature seed); a plant cutting; a plant cell; a plant cell culture; or a plant organ (e.g., pollen, embryos, flowers, fruits, shoots, leaves, roots, stems, and explants). A plant tissue or plant organ may be a seed, protoplast, callus, or any other group of plant cells that is organized into a structural or functional unit. A plant cell or tissue culture may be capable of regenerating a plant having the physiological and morphological characteristics of the plant from which the cell or tissue was obtained, and of regenerating a plant having substantially the same genotype as the plant. Regenerable cells in a plant cell or tissue culture may be embryos, protoplasts, meristematic cells, callus, pollen, leaves, anthers, roots, root tips, silk, flowers, kernels, ears, cobs, husks, or stalks. In contrast, some plant cells are not capable of being regenerated to produce plants and are referred to herein as “non-regenerable” plant cells.
(12) The phrase “substantially purified,” as used herein defines an isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment and means having been increased in purity as a result of being separated from other components of the original composition. The phrase “substantially purified RNA molecule” is used herein to describe an RNA molecule which has been separated from other contaminant compounds including, but not limited to polypeptides, lipids, and carbohydrates. In certain embodiments, a substantially purified RNA is at least 90%, 95%, 97%, 98%, 99%, 99.5%, or 99.9% free of contaminating compounds by weight. A substantially purified RNA molecule can be combined with other compounds including buffers, RNase inhibitors, surfactants, and the like in a composition.
(13) To the extent to which any of the preceding definitions is inconsistent with definitions provided in any patent or non-patent reference incorporated herein by reference, any patent or non-patent reference cited herein, or in any patent or non-patent reference found elsewhere, it is understood that the preceding definition will be used herein.
(14) The reagents and methods described provide a relatively easy and convenient solution for

producing plants with altered genomes, i.e. individuals with designed mutations (i.e., DNA sequence changes including insertions, deletions, and substitutions (Indels)). In most embodiments, the methods and systems rely on RNA molecules produced with established molecular biology techniques. The RNA molecules, which comprise genome-editing reagents, are then introduced into a plant and taken up into meristematic cells. The meristematic cell genomes are thus altered, and the mutations (i.e., DNA sequence changes including Indels) are carried into germline cells and subsequent generations.

(15) Meristem transport segments travel through the plant, typically via the phloem, and are taken up into meristematic tissues. The examples below are sequences from individual species, which sometimes work across species. For example, Arabidopsis FT-based vectors work in Nicotiana benthamiana and Arabidopsis. But, vectors can be designed based on alternative sequences, which can be based either on the species subject to genomic editing, or based on a closely related species.

(16) While the transport segment is based on a plant-transported RNA, its actual sequence may be a fragment determined by characterizing a deletion series to make a smaller sequence retaining the desired transport (phloem mobility and/or meristem cell translocation) capabilities. In certain embodiments, the meristem transport segment is a sub-fragment of a plant transported RNA identified by assaying a deletion series for a smaller sequence retaining the desired transport (phloem mobility and/or meristem cell translocation) function. The initiator methionine codon or translation initiation codon of the base sequence may also be mutated in some cases.

(17) The flowering time (FT) mRNA is useful as a meristem transport segment. SEQ ID NO: 2 shows the DNA sequence that encodes the Arabidopsis FT RNA, and SEQ ID NO: 1 is a fraction of SEQ ID NO: 2 that encodes the RNA that functions as a transport segment. Alternative useful FTs may be ZCN8 (encoded by SEQ ID NO: 3), which may work across related monocot species. Alternative useful FTs may be GmFT2a (Sun et al. PLoS One. 2011; 6(12):e29238. doi:10.1371/journal.pone.0029238; Jiang et al. BMC Genomics. 2019; 20(1):230. doi: 10.1186/s12864-019-5577-5; Kong et al. Plant Physiol. 2010 November; 154(3):1220-31. doi: 10.1104/pp.110.160796; Takeshima et al. J Exp Bot. 2019 Aug. 7; 70(15):3941-3953. doi: 10.1093/jxb/erz199), which may work across related dicot species. FT RNA molecules that can be used include: (i) RNAs set forth in SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (ii) allelic variants of SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; and (iii) FT RNAs from various plants set forth in U.S. 20190300890, which is incorporated herein by reference in its entirety, allelic variants thereof, and meristem transport-competent (MTC) orthologs thereof, MTC variants thereof, and/or MTC fragments thereof. FT RNA molecules that can be used include RNAs having at least 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or a meristem transport-competent (MTC) fragment thereof;

(18) More generally, viral and cellular-derived RNA molecules that are useful as part of a transport segment include the mRNAs of FT, GAI, CmNACP, LeT6 a tomato KNOX gene, BEL5, or tRNA-like sequences (Ruiz-Medrano et al., 1999 Phloem long-distance transport of CmNACP mRNA: implications for supracellular regulation in plants. Development 126, 4405-4419; Kim et al., 2001 Developmental changes due to long-distance movement of a homeobox fusion transcript in tomato. Science 293, 287-289; Haywood et al., 2005 Phloem long distance trafficking of GIBBERELLIC ACID-INSENSITIVE RNA regulates leaf development. Plant J. 42, 49-68; and Li et al., 2011 Mobile FT mRNA contributes to the systemic florigen signaling in floral induction. Sci. Rep. 1, 73; Cho et al., 2015, J. Exp. Bot, 66: 6835-6847; Zhang et al., 2016, Plant Cell, 28: 1237-1249; and WO2017178633). GAI RNAs that can be used include: (i) RNAs set forth in SEQ ID NO: 26, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC

fragment thereof; (ii) allelic variants of SEQ ID NO: 26, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; and (iii) RNAs having at least 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 26, or a meristem transport-competent (MTC) fragment thereof. CmNACP RNAs that can be used include: (i) RNAs set forth in SEQ ID NO: 25, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (ii) allelic variants of SEQ ID NO: 25, a MTC variant thereof, and/or a MTC fragment thereof; and (iii) RNAs having at least 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 25, or a meristem transport-competent (MTC) fragment thereof. LeT6 RNAs that can be used include: (i) RNAs set forth in SEQ ID NO: 27, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (ii) allelic variants of SEQ ID NO: 27, a MTC variant thereof, and/or a MTC fragment thereof; and (iii) RNAs having at least 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 27, or a meristem transport-competent (MTC) fragment thereof. BEL5 RNAs that can be used include: (i) RNAs set forth in SEQ ID NO: 28, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (ii) allelic variants of SEQ ID NO: 28, a MTC variant thereof, and/or a MTC fragment thereof; and (iii) RNAs having at least 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 28, or a meristem transport-competent (MTC) fragment thereof. Examples of tRNA-like RNAs that can be used include: (i) RNAs set forth in SEQ ID NO: 29, 30, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (ii) allelic variants of SEQ ID NO: 29, 30, a MTC variant thereof, and/or a MTC fragment thereof, and (iii) RNAs having at least 85%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 29, 30, or a meristem transport-competent (MTC) fragment thereof. In certain embodiments, a TLS sequence, SEQ ID NO: 29 or 30, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or an MTC fragment thereof can comprise an RNA hairpin comprising a first stem of 8 to 12 nucleotides, at least one variable bulge, a second stem of 4 to 7 nucleotides, and a variable loop. TLS sequences suitable for RNA transport and the structural features of such RNAs are set forth in Zhang et al. Plant Cell. 2016 June; 28(6): 1237, doi.org/10.1105/tpc.15.01056.

(19) Further description of biological sequences provided in the sequence listing is set forth in Table 1. RNA molecules set forth in SEQ ID NO: 9-30 are respectively encoded by the DNA molecules set forth in SEQ ID NO: 31-52.

(20) TABLE-US-00001 TABLE 1 Description of biological sequences. SEQ ID NO: TYPE Comments 1 DNA *Arabidopsis thaliana* 2 DNA NM_001334207.1 *Arabidopsis thaliana* PEBP (phosphatidylethanolamine-binding protein) family protein (FT), mRNA 3 DNA EU241924.1 *Zea mays* ZCN8 (ZCN8) mRNA, complete cds 4 DNA GmFT2a CDS, the soy FT ortholog according to Sun et al., 2011 and Cai et al., 2018 (GenBank ID: EU287455) 5 RNA RNA encoded by SEQ ID NO: 1 6 RNA RNA encoded by SEQ ID NO: 2 7 RNA RNA encoded by SEQ ID NO: 3 8 RNA RNA encoded by SEQ ID NO: 4 9 RNA DQ865290.1 *Cucurbita maxima* flowering locus T-like 1 (FTL1) mRNA, complete cds 10 RNA DQ865291.1 *Cucurbita maxima* flowering locus T-like 2 (FTL2) mRNA, complete cds 11 RNA DQ871590.1 *Vitis vinifera* FT-like protein (FT) mRNA, complete cds 12 RNA AB161112.1 *Malus* x *domestica* MdFT1 mRNA for flowering locus T like protein, complete cds 13 RNA AB027456.1 *Citrus unshiu* CiFT mRNA, complete cds 14 RNA AY186735.1: 2002-2199, 2287-2348, 4490-4530, 5586-5818 *Lycopersicon esculentum* SP3D (SP3D) gene, complete cds 15 RNA DQ387859.1 *Populus tremula* flowering locus T-like protein FT1 (FT1) mRNA, complete cds 16 RNA >DQ100327.1: 1332-1532, 1950-2011, 2121-2391 *Hordeum vulgare* subsp. *vulgare* FT-like protein (FT1) gene, complete cds 17 RNA DQ297407.1: 955-1164, 1235-1296, 3672-3712, 3808-4031 *Hordeum vulgare* subsp. *vulgare* FT-like protein (FT2) gene, complete cds 18 RNA AB052944.1 *Oryza sativa* Japonica Group Hd3a mRNA, complete cds, cultivar: Nipponbare 19 RNA AB062676.1 *Oryza sativa* Japonica Group RFT1 mRNA for FT- like protein, complete cds 20 RNA EU178859.1 *Ipomoea nil* FT-like protein (FT1)

mRNA, complete cds 21 RNA AB027506.1 *Arabidopsis thaliana* TSF (TWIN SISTER OF FT) mRNA, complete cds 22 RNA LC128590.1: 3049-3243, 3377-3438, 3830-3870, 4102-4322 *Glycine max* FT5a gene for flowering locus T, complete cds, cultivar: Toyoharuka 23 RNA ZmZCN9 NM_001112777.2 *Zea mays* ZCN9 protein (LOC100127520), mRNA 24 RNA ZmZCN10 >EU241926.1 *Zea mays* ZCN10 (ZCN10) mRNA, complete cds 25 RNA CmNACP: >FJ151402.1 *Cucurbita maxima* NAC-domain containing protein (NACP1) mRNA, complete cds 26 RNA GAI: >Y15193.1 *Arabidopsis thaliana* GAI gene 27 RNA LeT6 a tomato KNOX gene: >AF000141.1 *Lycopersicon esculentum* class I knotted-like homeodomain protein (LeT6) mRNA, complete cds 28 RNA BEL5: >NM_001287992.1 *Solanum tuberosum* BEL1-related homeotic protein 5 (BEL5), mRNA 29 RNA AT5G57885.1 (tRNA-Met) 30 RNA AT1G71700 (tRNA-Gly) 31 DNA DQ865290.1 *Cucurbita maxima* flowering locus T-like 1 (FTL1) mRNA, complete cds 32 DNA DQ865291.1 *Cucurbita maxima* flowering locus T-like 2 (FTL2) mRNA, complete cds 33 DNA DQ871590.1 *Vitis vinifera* FT-like protein (FT) mRNA, complete cds 34 DNA AB161112.1 *Malus* x *domestica* MdFT1 mRNA for flowering locus T like protein, complete cds 35 DNA AB027456.1 *Citrus unshiu* CiFT mRNA, complete cds 36 DNA AY186735.1: 2002-2199, 2287-2348, 4490-4530, 5586-5818 *Lycopersicon esculentum* SP3D (SP3D) gene, complete cds 37 DNA DQ387859.1 *Populus tremula* flowering locus T-like protein FT1 (FT1) mRNA, complete cds 38 DNA >DQ100327.1: 1332-1532, 1950-2011, 2121-2391 *Hordeum vulgare* subsp. *vulgare* FT-like protein (FT1) gene, complete cds 39 DNA DQ297407.1: 955-1164, 1235-1296, 3672-3712, 3808-4031 *Hordeum vulgare* subsp. *vulgare* FT-like protein (FT2) gene, complete cds 40 DNA AB052944.1 *Oryza sativa* Japonica Group Hd3a mRNA, complete cds, cultivar: Nipponbare 41 DNA AB062676.1 *Oryza sativa* Japonica Group RFT1 mRNA for FT- like protein, complete cds 42 DNA EU178859.1 *Ipomoea nil* FT-like protein (FT1) mRNA, complete cds 43 DNA AB027506.1 *Arabidopsis thaliana* TSF (TWIN SISTER OF FT) mRNA, complete cds 44 DNA LC128590.1: 3049-3243, 3377-3438, 3830-3870, 4102-4322 *Glycine max* FT5a gene for flowering locus T, complete cds, cultivar: Toyoharuka 45 DNA ZmZCN9 NM_001112777.2 *Zea mays* ZCN9 protein (LOC100127520), mRNA 46 DNA ZmZCN10 >EU241926.1 *Zea mays* ZCN10 (ZCN10) mRNA, complete cds 47 DNA CmNACP: >FJ151402.1 *Cucurbita maxima* NAC-domain containing protein (NACP1) mRNA, complete cds 48 DNA GAI: >Y15193.1 *Arabidopsis thaliana* GAI gene 49 DNA LeT6 a tomato KNOX gene: >AF000141.1 *Lycopersicon esculentum* class I knotted-like homeodomain protein (LeT6) mRNA, complete cds 50 DNA BEL5: >NM_001287992.1 *Solanum tuberosum* BEL1-related homeotic protein 5 (BEL5), mRNA 51 DNA AT5G57885.1 (tRNA-Met) 52 DNA AT1G71700 (tRNA-Gly) 53 PRO FnCas12a (UniProtKB/Swiss-Prot: A0Q7Q2.1); US20160208243; and WO 2017/189308) 54 RNA FnCas12aDR (Fonfara et al. Nature 532, 517-521 (2016). doi.org/10.1038/nature17945; US2016-0208243; WO 2017/189308) 55 PRO LbCpfl (from Lachnospiraceae bacterium ND2006; UniProtKB: A0A182DWE3) 56 RNA LbCpfl DR (from Lachnospiraceae bacterium ND2006; Zetsche et al., doi.org/10.1101/134015) 57 PRO Cas12j-1protein (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337) 58 RNA Cas12j-2 DR sequence (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337) 59 PRO Cas12j-2 protein (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337) 60 RNA Cas12j-2 DR sequence (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337) 61 PRO Cas12j-3 protein (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337) 62 RNA Cas12j-3 DR sequence (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337)

(21) The meristem transport-competence (MTC) potential can be determined for any variants, fragments, and/or orthologs of the aforementioned FT, GAI, CmNACP, LeT6 a tomato KNOX gene, BEL5, or tRNA-like RNAs. A side-by-side comparison with a known MTS as a positive control is useful. As such, a number of configurations can be used. One approach is to fuse candidate sequences to guide sequences of characterized editing potential for a species of interest. RNA sequences can be introduced into the phloem of an individual plant that expresses at least in

the meristem a nuclease capable of associating with the guide sequence and producing the intended genomic alteration. The RNA sequences can be expressed in vitro, and introduced into the phloem as purified molecules. For example, a concentrated solution of RNA molecules of interest can be applied to a mechanically injured plant tissue, such as a cut or abraded leaf, stem, or meristem dome. RNAs can be coated on particles, such as micro or nano-scale particles such as gold or tungsten, for biolistic delivery. Alternatively, the RNA sequences could be incorporated into RNA viruses introduced in the plants (Jackson et al. 2012, Front. Plant Sci. 3, 127; Ali et al. 2015, Mol. Plant 8, 1288-1291; Cody et al. 2017 Plant Physiol. 175, 23-35; Ali et al. 2018, Virus Res. 244, 333-337; Gao et al. 2019, New Phytol. 223, 2120-2133). or the MTC can be assayed by introducing RNAs by grafting, i.e. the RNA molecules can be expressed in the rootstock of a grafted plant, and their effect observed in the scion (Zhang et al., 2016, Plant Cell, 28: 1237-1249; Huang et al, 2018, Plant Physiol. 178:783-794). MTS candidates can be assayed for longer and/or more complex RNA molecules, or mixtures of RNA molecules, that comprise not only guide or processable guide regions, but also nuclease-encoding sequences.

(22) A clear readout of MTC is detection of the expected genomic alterations in progeny plants, which can be done by sequencing of the target genomic region, or even by whole genome sequencing. But alternative readouts can be designed that may be more convenient in some cases. For example, the guide sequences may be directed to disrupt or repair a reporter gene, such as a transgene encoding a fluorescent polypeptide. The expected genetic changes can then be evaluated in the treated plants by measuring changes in the reporter. Another convenient genomic alteration target in many species is phytoene desaturase (PDS), with the albino phenotype serving as a readout.

(23) The cargo segments of the engineered RNA deliver the genome-editing components. In general, these will be based on CRISPR-Cas systems, but some alternatives are possible. The alternatives include RNAi for heritable knock-down as affected by DNA methylation status, a TALEN, a zinc finger nucleases (ZFN), and a meganuclease.

(24) In certain embodiments, an RNA molecule comprising a RNA segment encoding a ZFN (e.g., a zinc finger nuclease or zinc finger nickase) that is operably linked to an RNA segment comprising an MTS to provide for ZFN-mediated gene editing in a plant meristem. Zinc-finger nucleases are site-specific endonucleases comprising two protein domains: a DNA-binding domain, comprising a plurality of individual zinc finger repeats that each recognize between 9 and 18 base pairs, and a DNA-cleavage domain that comprises a nuclease domain (typically Fokl). The cleavage domain dimerizes in order to cleave DNA; therefore, a pair of ZFNs are required to target non-palindromic target polynucleotides. In certain embodiments, zinc finger nuclease and zinc finger nickase design methods which have been described (Urnov et al. (2010) Nature Rev. Genet., 11:636-646; Mohanta et al. (2017) Genes vol. 8,12: 399; Ramirez et al. Nucleic Acids Res. (2012); 40(12): 5560-5568; Liu et al. (2013) Nature Communications, 4: 2565) can be adapted for use in the methods set forth herein. The zinc finger binding domains of the zinc finger nuclease or nickase provide specificity and can be engineered to specifically recognize any desired target DNA sequence. The zinc finger DNA binding domains are derived from the DNA-binding domain of a large class of eukaryotic transcription factors called zinc finger proteins (ZFPs). The DNA-binding domain of ZFPs typically contains a tandem array of at least three zinc “fingers” each recognizing a specific triplet of DNA. A number of strategies can be used to design the binding specificity of the zinc finger binding domain. One approach, termed “modular assembly”, relies on the functional autonomy of individual zinc fingers with DNA. In this approach, a given sequence is targeted by identifying zinc fingers for each component triplet in the sequence and linking them into a multifinger peptide. Several alternative strategies for designing zinc finger DNA binding domains have also been developed. These methods are designed to accommodate the ability of zinc fingers to contact neighboring fingers as well as nucleotide bases outside their target triplet. Typically, the engineered zinc finger DNA binding domain has a novel binding specificity, compared to a naturally-occurring

zinc finger protein. Engineering methods include, for example, rational design and various types of selection. Rational design includes, for example, the use of databases of triplet (or quadruplet) nucleotide sequences and individual zinc finger amino acid sequences, in which each triplet or quadruplet nucleotide sequence is associated with one or more amino acid sequences of zinc fingers which bind the particular triplet or quadruplet sequence. See, e.g., U.S. Pat. Nos. 6,453,242 and 6,534,261, both incorporated herein by reference in their entirety. Exemplary selection methods (e.g., phage display and yeast two-hybrid systems) can be adapted for use in the methods described herein. In addition, enhancement of binding specificity for zinc finger binding domains has been described in U.S. Pat. No. 6,794,136, incorporated herein by reference in its entirety. In addition, individual zinc finger domains may be linked together using any suitable linker sequences. Examples of linker sequences are publicly known, e.g., see U.S. Pat. Nos. 6,479,626; 6,903,185; and 7,153,949, incorporated herein by reference in their entirety. The nucleic acid cleavage domain is non-specific and is typically a restriction endonuclease, such as FokI. This endonuclease must dimerize to cleave DNA. Thus, cleavage by FokI as part of a ZFN requires two adjacent and independent binding events, which must occur in both the correct orientation and with appropriate spacing to permit dimer formation. The requirement for two DNA binding events enables more specific targeting of long and potentially unique recognition sites. FokI variants with enhanced activities have been described and can be adapted for use in the methods described herein; see, e.g., Guo et al. (2010) J. Mol. Biol., 400:96-107.

(25) In certain embodiments, an RNA molecule comprising a RNA segment encoding a TALEN (e.g., a TALE nuclease or nickase) that is operably linked to an RNA segment comprising an MTS to provide for TALEN-mediated gene editing in a plant meristem. Transcription activator like effectors (TALEs) are proteins secreted by certain Xanthomonas species to modulate gene expression in host plants and to facilitate the colonization by and survival of the bacterium. TALEs act as transcription factors and modulate expression of resistance genes in the plants. Recent studies of TALEs have revealed the code linking the repetitive region of TALEs with their target DNA-binding sites. TALEs comprise a highly conserved and repetitive region consisting of tandem repeats of mostly 33 or 34 amino acid segments. The repeat monomers differ from each other mainly at amino acid positions 12 and 13. A strong correlation between unique pairs of amino acids at positions 12 and 13 and the corresponding nucleotide in the TALE-binding site has been found. The simple relationship between amino acid sequence and DNA recognition of the TALE binding domain allows for the design of DNA binding domains of any desired specificity. TALEs can be linked to a non-specific DNA cleavage domain to prepare genome editing proteins, referred to as TAL-effector nucleases or TALENs. As in the case of ZFNs, a restriction endonuclease, such as FokI, can be conveniently used. Methods for use of TALENs in plants have been described and can be adapted for use in the methods described herein, see Mahfouz et al. (2011) Proc. Natl. Acad. Sci. USA, 108:2623-2628; Mahfouz (2011) GM Crops, 2:99-103; and Mohanta et al. (2017) Genes vol. 8,12: 399). TALE nickases have also been described and can be adapted for use in methods described herein (Wu et al.; Biochem Biophys Res Commun. (2014); 446(1):261-6; Luo et al; Scientific Reports 6, Article No.: 20657 (2016)).

(26) Plants comprising the RNA molecules that comprise cargo segments that are operably linked to MTS sequences are also provided herein. In certain embodiments, such RNA molecules will be present at detectable concentrations in the plants for only a certain period of time following. For example, the concentrations of RNA molecules comprising guide RNAs separated by processing elements comprising direct repeats (DR, i.e., pre-crRNAs comprising a full-length direct repeat (full-DR-crRNA)) which are capable of being processed (i.e., cleaved) by an RNA-guided nuclease are expected to decrease over time when the RNA-guided nuclease is also present in the plant. The concentrations of RNA molecules comprising guide RNAs separated by processing elements comprising direct repeats which are capable of being processed by an RNA-guided nuclease are also expected to be decreased in tissues where the RNA-guided nuclease is located. Nonetheless,

the unprocessed RNA molecules can be detected by a variety of techniques that include reverse transcriptase PCR (RT-PCR) assays where oligonucleotide primers and optionally detection probes which specifically amplify and detect the unprocessed RNA molecule comprising the cargo segments that are operably linked to MTS sequences are used. Such plants can comprise any of the RNA molecules or combinations of RNA molecules present in the compositions provided herein that are used to contact the plants. In certain embodiments, an active form of the RNA guided nuclease is predominantly localized in meristem tissue of the plant. In certain embodiments, the RNA-guided nuclease can be encoded by an RNA molecule that is optionally further comprises an operably linked MTS sequence. In certain embodiments, the RNA-guided nuclease can be encoded by DNA that is operably linked to promoters that include a meristem-preferred or meristem-specific promoter which is active in meristem cells. DNA encoding the RNA-guided nuclease can be provided in a transgene that is stably integrated in the genome of the plant, in DNA that is not integrated into the plant genome, or in DNA provided in a viral vector (e.g., a geminivirus replicon). Geminivirus DNA replicons suitable for delivery of DNA molecules encoding an RNA-guided nuclease to plants include a Beet Yellow Dwarf Virus replicon (Baltes, Nicholas J. et al. Plant Cell vol. 26,1 (2014): 151-63. doi:10.1105/tpc.113.119792).

(27) It is understood that for all systems, the use of a nuclease activity for cutting DNA followed by repair by the endogenous cell machinery is one solution to generate useful mutants. The nuclease activity can be eliminated or altered, as in dCas or nCas, TALE or ZF versions of the polypeptides. The inactivated nucleases can be useful for targeting the desired DNA sequence, while editing can be performed by nucleobase editors attached to the altered nucleases. Examples are included in WO2018176009 and U.S. Pat. No. 10,113,163, incorporated herein by reference.

(28) CRISPR-based RNA-guided nuclease systems typically require an effector polypeptide, and one or more guide RNAs. The guide RNAs are generally made up of an effector-binding region and a target DNA recognition region, and in some embodiments include tracrRNAs. Useful CRISPR-based RNA-guided nuclease systems have been described and are known from the literature as Cas9, Cas12a (Cpf1), Cas12e (CasX), Cas12d (CasY), C2c1, C2c2, and C2c3, (see WO2018176009) Cas12h, Cas12i (see Yan et al. 2019, Science Vol. 363, Issue 6422, pp. 88-91) and Cas12j (Pausch et al., 2020 Science 17 Jul. 2020: Vol. 369, Issue 6501, pp. 333-337).

(29) The Cas nuclease or effector polypeptide is intended to be translated inside a plant meristem cell. As such, it is typically embedded within an mRNA component. A 5′ cap and polyA tail are also useful in stabilizing the RNA. A 5′ UTR has translation initiation sequences upstream of the Cas coding sequence. For example, an mRNA can comprise a 5′UTR comprising a 7-methylguanosine cap at its 5′ terminus followed by an untranslated sequence and terminated by the translation initiation codon of the coding sequence (e.g., the CAS coding sequence).

(30) Cargo containing guide RNA can be part of the same RNA (mRNA) capable of expressing the Cas nuclease. In one embodiment, one or more guide RNAs are flanked by direct repeats (DR) of the CRISPR array from which the Cas effector polypeptide was first isolated. For example, a translated and expressed active Cas12a nuclease can process the DR-flanked spacers of the cargo RNA to make guide RNAs. In certain embodiments, a translated and expressed active Cas12j nuclease can process Cas12j DR-flanked spacers of the cargo RNA to make guide RNAs. Alternatively, guide RNA suitable for matching expressed effector polypeptide can be flanked by processing elements, so that functional guide RNAs are excised inside the cells. Exemplary processing elements include hammerhead ribozymes, Csy4, and tRNAs (see Mikami et al, Plant Cell Physiol. 2017 November; 58(11): 1857-1867, and U.S. Pat. No. 10,308,947).

(31) In certain embodiments, an MTS is operably linked to a cargo segment comprising an array of a plurality of guide RNAs (e.g., 2, 3, 4, or more guide RNAs) separated by processing elements to provide for gene editing at a plurality of genomic locations targeted by each guide RNA. In certain embodiments, the plurality of guide RNAs are separated by processing elements comprising direct repeats (DR; i.e., pre-crRNAs comprising a full-length direct repeat (full-DR-crRNA)) which are

capable of being processed (i.e., cleaved) by an RNA-guided nuclease. Examples of such DRs include the Cas12a DR (e.g., SEQ ID NO: 54 or 56) which can be cleaved by a Cas12a guided nuclease (e.g., SEQ ID NO: 53 or 55, respectively). Cleavage of RNAs comprising Cas12a DRs by Cas12a has been described (Fonfara et al. Nature 532, 517-521 (2016). doi.org/10.1038/nature17945); U.S. 20160208243; WO 2017/189308). Other examples of such DRs include the Cas12j DRs (e.g., SEQ ID NO: 58, 60, or 62) which can be cleaved by a Cas12j guided nuclease ((e.g., SEQ ID NO: 57, 59, or 61, respectively). In such embodiments, the crRNA portion of the DR can remain as a part of the gRNA after processing and can be recognized by the RNA guided nuclease to provide for editing of genomic DNA recognized via hybridization of the gRNA to the targeted genomic site.

(32) Compositions comprising: (i) RNA molecules comprising an MTS is operably linked to a cargo segment; (ii) nucleic acids encoding RNA guided nucleases; and/or (iii) donor DNA templates can further comprise components that include: (a) solvents (e.g., water, dimethylsulfoxide, dimethylformamide, acetonitrile, N-pyrrolidine, pyridine, hexamethylphosphoramide, alcohols, alkanes, alkenes, dioxanes, polyethylene glycol, and other solvents miscible or emulsifiable with water or that will dissolve phosphonucleotides in non-aqueous systems); (b) fluorocarbons (e.g., perfluorodecalin, perfluoromethyldecalin); (c) glycols or polyols (e.g., propylene glycol, polyethylene glycol); (d) surfactants, including cationic surfactants, anionic surfactants, non-ionic surfactants, and amphiphilic surfactants, e.g., alkyl or aryl sulfates, phosphates, sulfonates, or carboxylates; primary, secondary, or tertiary amines; quaternary ammonium salts; sultaines, betaines; cationic lipids; phospholipids; tallowamine; bile acids such as cholic acid; saponins or glycosylated triterpenoids or glycosylated sterols (e.g., saponin commercially available as catalogue number 47036-50 g-F, Sigma-Aldrich, St. Louis, MO); long chain alcohols; organosilicone surfactants including nonionic organosilicone surfactants such as trisiloxane ethoxylate surfactants or a silicone polyether copolymer such as a copolymer of polyalkylene oxide modified heptamethyl trisiloxane and allyloxypolypropylene glycol methylether (commercially available as SILWET L-77™ brand surfactant having CAS No. 27306-78-1 and EPA Number CAL. REG. No. 5905-50073-AA, Momentive Performance Materials, Inc., Albany, N.Y.); specific examples of useful surfactants include sodium lauryl sulfate, the Tween series of surfactants, Triton-X100, Triton-X114, CHAPS and CHAPSO, Tergitol-type NP-40, Nonidet P-40; (e) lipids, lipoproteins, lipopolysaccharides; (f) acids, bases, caustic agents; buffers; (g) peptides, proteins, or enzymes (e.g., cellulase, pectolyase, maceroenzyme, pectinase), including cell-penetrating or pore-forming peptides (e. g., (BO100)2K8, Genscript; poly-lysine, poly-arginine, or poly-homoarginine peptides; gamma zein, see U.S. Patent Application publication 2011/0247100, incorporated herein by reference in its entirety; transcription activator of human immunodeficiency virus type 1 (“HIV-1 Tat”) and other Tat proteins, see, e. g., www[dot]lifetein[dot]com/Cell_Penetrating_Peptides[dot]html and Järver (2012) Mol. Therapy-Nucleic Acids, 1:e27,1-17); octa-arginine or nona-arginine; poly-homoarginine (see Unnamalai et al. (2004) FEBS Letters, 566:307-310); see also the database of cell-penetrating peptides CPPsite 2.0 publicly available at crdd[dot]osdd[dot]net/raghava/cppsite/ (h) RNase inhibitors; (i) cationic branched or linear polymers such as chitosan, poly-lysine, DEAE-dextran, polyvinylpyrrolidone (“PVP”), or polyethylenimine (“PEI”, e. g., PEI, branched, MW 25,000, CAS #9002-98-6; PEI, linear, MW 5000, CAS #9002-98-6; PEI linear, MW 2500, CAS #9002-98-6); (j) dendrimers (see, e. g., U.S. Patent Application Publication 2011/0093982, incorporated herein by reference in its entirety); (k) counter-ions, amines or polyamines (e. g., spermine, spermidine, putrescine), osmolytes, buffers, and salts (e. g., calcium phosphate, ammonium phosphate); (l) polynucleotides (e. g., non-specific double-stranded DNA, salmon sperm DNA); (m) transfection agents (e. g., Lipofectin®, Lipofectamine®, and Oligofectamine®, and Invivofectamine® (all from Thermo Fisher Scientific, Waltham, MA), PepFect (see Ezzat et al. (2011) Nucleic Acids Res., 39:5284-5298), TransIt® transfection reagents (Mirus Bio, LLC, Madison, WI), and poly-lysine, poly-

homoarginine, and poly-arginine molecules including octo-arginine and nono-arginine as described in Lu et al. (2010) J. Agric. Food Chem., 58:2288-2294); (n) antibiotics, including non-specific DNA double-strand-break-inducing agents (e. g., phleomycin, bleomycin, talisomycin); (o) antioxidants (e. g., glutathione, dithiothreitol, ascorbate); and/or (p) chelating agents (e. g., EDTA, EGTA).

(33) Compositions comprising: (i) RNA molecules comprising an MTS is operably linked to a cargo segment; (ii) nucleic acids encoding RNA guided nucleases; and/or (iii) donor DNA templates can be delivered to the plant and/or meristem cells of the plant by particle mediated delivery, and any other direct method of delivery, such as but not limiting to, Agrobacterium-mediated transformation, polyethylene glycol (PEG)-mediated transfection to protoplasts, whiskers mediated transformation, electroporation, particle bombardment, and/or by use of cell-penetrating peptides.

(34) In certain embodiments, plants are contacted either simultaneously or sequentially with one, two, three or more RNA molecules in one or more compositions where at least one of the RNA molecules comprises an MTS operably linked to a cargo segment comprising at least one guide RNA. In certain embodiments, one of the RNA molecules comprises an MTS operably linked to a cargo segment comprising at least one guide RNA and the other RNA molecule encoding an RNA guided nuclease and optionally an MTS, where the RNA guided nuclease can process the RNA comprising the guide RNA to release a functional guide RNA. In certain embodiments, one of the RNA molecules comprises an MTS operably linked to a cargo segment comprising at least one guide RNA and the other RNA molecule comprises an RNA guided nuclease and optionally an MTS, where the RNA guided nuclease cannot process the RNA comprising the guide RNA to release a functional guide RNA (e.g., processing elements present in the RNA molecule comprising the gRNA and the MTS are not recognized by the RNA-guided nuclease). In certain embodiments, guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements (e.g., DRs) which are processed by different RNA-guided nuclease (e.g., a Cas12a nuclease can process the first RNA molecule and a Cas12j nuclease can process the second RNA molecule). In certain embodiments, the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the cargo segment second RNA molecule. Such distinct gRNAs provided by the first RNA molecule can provide for genome editing at one or more first genomic sites in a meristem cell while the distinct gRNAs provided by the second RNA molecule can provide for genome editing at one or more second genomic sites in a meristem cell. Such contacting the plant with RNA molecules in a composition can occur sequentially such that the first gRNA(s) are delivered, allowed sufficient time (e.g., about 6, 12, 18 or 20 to about 24, 30, or 36 hours) to effect desired genome edits, followed by contacting the plant with the second RNA molecules in a second composition to deliver the second gRNA(s) to effect additional desired genome edits, where such desired genome edits are effected by providing the gRNA(s) and an RNA guided nuclease in at least the meristem cell. Without seeking to be limited by theory, it is believed that cutting chromosomes at multiple location simultaneously is cytotoxic and that such cytotoxicity can be mitigated by delivering a limited number of guide RNAs at different times (e.g., about 6, 12, 18 or 20 to about 24, 30, or 36 hours apart). In certain embodiments, a plant can be contacted by one or more RNA molecules that comprise at least one gRNA operably linked to an MTS, optionally along with an RNA encoding RNA guided nuclease, permitted a sufficient period of time to accumulate the RNA molecule in the meristem cells (e.g., about 6, 12, 18 or 20 to about 24, 30, or 36 hours apart), and then contacted with a different mixture of one or more RNA molecules that comprise at least one different gRNA operably linked to an MTS, optionally along with an RNA encoding RNA guided nuclease, where the RNA guided nuclease can process the RNA comprising the guide RNA to release a functional guide RNA and/or effect a desired genomic edit with the gRNA in the meristem cells.

(35) In certain embodiments, the RNA molecules comprising at least one gRNA fused to an MTS

are provided in combination with the RNA guided nuclease and a donor DNA template to effect insertions of DNA elements in the donor DNA template at the target editing site in the plant genome by homology dependent repair (HDR), non-homologous end joining (NHEJ), or microhomology-mediated end joining (MMEJ). Donor DNA template molecules used in the methods provided herein include DNA molecules comprising, from 5′ to 3′, a first homology arm, a replacement DNA, and a second homology arm, wherein the homology arms containing sequences that are partially or completely homologous to genomic DNA (gDNA) sequences flanking a target site-specific endonuclease cleavage site in the gDNA. In certain embodiments, the replacement DNA can comprise an insertion, deletion, or substitution of 1 or more DNA base pairs relative to the target gDNA. In one embodiment, the donor DNA template molecule is double-stranded and perfectly base-paired through all or most of its length, with the possible exception of any unpaired nucleotides at either terminus or both termini. In another embodiment, the donor DNA template molecule is double-stranded and includes one or more non-terminal mismatches or non-terminal unpaired nucleotides within the otherwise double-stranded duplex. In an embodiment, the donor DNA template molecule that is integrated at the site of at least one double-strand break (DSB) includes between 2-20 nucleotides in one (if single-stranded) or in both strands (if double-stranded), e. g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides on one or on both strands, each of which can be base-paired to a nucleotide on the opposite strand of the targeted integration site (in the case of a perfectly base-paired double-stranded polynucleotide molecule). Such donor DNA templates can be integrated in genomic DNA containing blunt and/or staggered double stranded DNA breaks by homology-directed repair (HDR) or microhomology-mediated end joining (MMEJ). In certain embodiments, a donor DNA template homology arm can be about 20, 50, 100, 200, 400, or 600 to about 800, or 1000 base pairs in length. In certain embodiments, a donor DNA template molecule can be delivered to a plant cell in a circular (e.g., a plasmid or a viral vector including a geminivirus vector) or a linear DNA molecule. In certain embodiments, a circular or linear DNA molecule that is used can comprise a modified donor DNA template molecule comprising, from 5′ to 3′, a first copy of the target sequence-specific endonuclease cleavage site sequence, the first homology arm, the replacement DNA, the second homology arm, and a second copy of the target sequence-specific endonuclease cleavage site sequence. In other embodiments, DNA templates suitable for NHEJ insertion will lack homology arms that are partially or completely homologous to genomic DNA (gDNA) sequences flanking a target site-specific endonuclease cleavage site in the gDNA. Compositions comprising the donor templates can be delivered to the plant and/or meristem cells of the plant by particle mediated delivery, and any other direct method of delivery, such as but not limiting to, Agrobacterium-mediated transformation, polyethylene glycol (PEG)-mediated transfection to protoplasts, whiskers mediated transformation, electroporation, particle bombardment, and/or by use of cell-penetrating peptides. The donor DNA templates may be present transiently in the cell or it could be introduced via a viral replicon (e.g, a geminivirus replicon). Geminivirus DNA replicons suitable for delivery of donor DNA templates to plants include a Beet Yellow Dwarf Virus replicon (Baltes, N.J. et al. Plant Cell vol. 26,1(2014): 151-63. doi:10.1105/tpc.113.119792).

(36) RNA guided nucleases can be provided to at least the meristem cell by a variety of methods that include stable expression with an integrated transgenes, expression from a viral vector, or transient expression such as by introducing an RNA that encodes the RNA guided nuclease or an that RNA that encodes the RNA guided nuclease that is operably linked an MTS. In certain embodiments, an active form of the RNA guided nuclease is predominantly localized in meristem tissue of the plant. Delivery of RNAs encoding the RNA guided nucleases or DNAs then encode those RNAs to the plant and/or meristem cells of the plant can be achieved by particle mediated delivery, and any other direct method of delivery, such as but not limiting to, Agrobacterium-mediated transformation, polyethylene glycol (PEG)-mediated transfection to protoplasts, whiskers mediated transformation, electroporation, particle bombardment, and/or by use of cell-penetrating

peptides. In certain embodiments, such predominant localization of the RNA guided nuclease can result in at least about 60%, 70%, 80%, 90%, 95%, 98%, or 99% of the active form of the RNA guided nuclease in the plant being localized in the meristem. In certain embodiments, the nucleic acid encoding the RNA guided nuclease can be delivered directly to the meristem by methods that include use of biolistic devices (e.g., as in U.S. 20200123554). In certain embodiments, the RNA guided nuclease can be operably linked to a vegetative stage, meristem-preferred or meristem-specific promoter including: (i) a pAt.Erecta, At.PNH, At.AN3, or At.MYB17 promoter or functional fragment thereof from Arabidopsis; (ii) a promoter or functional fragment thereof from a Glyma10g38730, Glyma09g27950, Glyma06g05900, or Glyma17g34380 soybean gene; or (iii) receptor like kinase (RLK) gene promoters from a PGSC0003DMP400032802 or PGS C0003DMP400054040 gene of potato. Such vegetative stage, meristem-preferred or meristem-specific promoters are set forth in U.S. 20190300890, which is incorporated herein by reference in its entirety. In certain embodiments, expression of the RNA guided nuclease can increased in floral meristems of maize plants by operable linkage to a floral meristem-enhanced promoters that include Zap1a, Zap1b, ZLF1, ZLF2, or ZMM4 endogenous genes (Dong et al. 2012 PLoS ONE 7(8):e43450). Alternatively, the RNA guided nuclease can be expressed meristems and tissues other than the vascular tissues to mitigate cleavage of an RNA molecule comprising the gRNA and the MTS during transit from the site of contact to the meristem.

(37) In some embodiments, a plant expressing transgenically a Cas polypeptide may be genome edited by delivery of a cargo containing only guide RNAs suitable for the transgenically expressed Cas polypeptide.

(38) The RNA sequences are generally made and assembled at first in DNA form as RNA expressing vectors using recombinant DNA technology. RNA expression is done in vitro, and purified according to well established methods. Addition of RNA 5′ caps and polyA tails to mRNAs can be performed according to methods established in the literature. Alternatively, some RNAs designed as described can be purchased from commercial providers.

(39) A substantially purified RNA composition is understood to comprise a high concentration of an RNA molecule of interest, although in some cases it may comprise two distinct RNAs. For example, one RNA may comprise a Cas nuclease while another may comprise a corresponding guide or guide array. In addition, a substantially purified RNA composition may comprise other added components, such as a pH buffer, salt, surfactants, and/or RNase inhibitors.

(40) Plants can be effectively contacted with the RNA vectors in many ways. Often it will be convenient to load them into the phloem of plants through the leaves, for example by nicking a leaf and submerging the injured tissue into a solution of substantially purified RNAs. Other avenues are also possible, such as by injection into the stems with a needle or use of a handheld biolistics device. In some embodiments, a surfactant is added to the purified RNA, and the liquid is applied to a tissue like embryonic shoot, leaf, stem, or inflorescence, with or without slight injury such as scratching.

(41) The RNAs are often applied at the vegetative stage of the life cycle of a plant, so as to reach vegetative meristems before they convert to floral meristems. In some cases, however, it may be convenient to apply the vectors, RNA molecules, or compositions comprising the RNA molecules or vectors, to floral meristems, especially at early stages of differentiation. In certain embodiments, a soybean plant is contacted at the vegetative stage with a composition comprising the RNA molecules or vectors at vegetative stage Ve, V1, or V2 to about the V4 V(n) stage where 1, 2, 3, 4, or n is the number of trifoliate leaves (Soybean Growth and Development, M. Licht, 2014, Iowa State University Extension and Outreach, PM 1945). In certain embodiments, a maize plant is contacted at the vegetative stage with a composition comprising the RNA molecules or vectors at vegetative stage Ve, V1, or V2 to about the V4 V(n) stage (Corn Growth Stages, M. Licht, Iowa State University Extension and Outreach, on the https interne site “crops.extension.iastate.edu/encyclopedia/corn-growth-stages”).

(42) Very often, mutated seeds from plants edited with the reagents and methods described here are collected for phenotypic characterization. In some cases, pollen from edited plants is used in crosses with other individuals, or mutated individuals are pollinated with pollen of unedited plants or wildtype plants.
(43) There are numerous plant-endogenous targets (i.e., DNA sequence targets) for genome editing. Any defective allele found in elite germplasm can get edited to a non-deleterious version. The methods presented here can be applied to a promoter bashing or fine-tuning approach, to create a range of phenotypes based on promoter alterations of a gene of a certain sequence or gene of interest (Rodriguez-Leal et al., Cell. 2017 Oct. 5; 171(2):470-480).
(44) Editing of coding sequences can be made using the methods disclosed herein to increase the level of preselected amino acids in the encoded polypeptide. For example, the gene encoding the barley high lysine polypeptide (BHL) is derived from barley chymotrypsin inhibitor, U.S. application Ser. No. 08/740,682, filed Nov. 1, 1996, and WO 98/20133, the disclosures of which are herein incorporated by reference. Other proteins include methionine-rich plant proteins such as from sunflower seed (Lilley et al. (1989) Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs, ed. Applewhite (American Oil Chemists Society, Champaign, Ill.), pp. 497-502; herein incorporated by reference); corn (Pedersen et al. (1986) J. Biol. Chem. 261:6279; Kirihara et al. (1988) Gene 71:359; both of which are herein incorporated by reference); and rice (Musumura et al. (1989) Plant Mol. Biol. 12:123, herein incorporated by reference). Other agronomically important genes encode latex, Floury 2, growth factors, seed storage factors, and transcription factors.
(45) The methods disclosed herein can be used to modify herbicide resistance traits including genes coding for resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance, in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides that act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the bar gene); glyphosate (e.g., the EPSPS gene and the GAT gene; see, for example, U.S. Publication No. 20040082770 and WO 03/092360); or other such genes known in the art. The bar gene encodes resistance to the herbicide basta, the nptII gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS-gene mutants encode resistance to the herbicide chlorsulfuron. Additional herbicide resistance traits are described for example in U.S. Patent Application 2016/0208243, herein incorporated by reference.
(46) Sterility genes can also be modified and provide an alternative to physical detasseling. Examples of genes used in such ways include male tissue-preferred genes and genes with male sterility phenotypes such as QM, described in U.S. Pat. No. 5,583,210. Other genes include kinases and those encoding compounds toxic to either male or female gametophytic development. Additional sterility traits are described for example in U.S. Patent Application 2016/0208243, herein incorporated by reference.
(47) Genome editing can also be used to make haploid inducer lines as disclosed in WO2018086623 and U.S. 20190292553.
(48) The quality of grain can be altered by modifying genes encoding traits such as levels and types of oils, saturated and unsaturated, quality and quantity of essential amino acids, and levels of cellulose. In corn, modified hordothionin proteins are described in U.S. Pat. Nos. 5,703,049, 5,885,801, 5,885,802, and 5,990,389.
(49) Commercial traits can also be altered by modifying a gene or that could increase for example, starch for ethanol production, or provide expression of proteins. Another important commercial use of modified plants is the production of polymers and bioplastics such as described in U.S. Pat. No. 5,602,321. Genes such as .beta.-Ketothiolase, PHBase (polyhydroxyburyrate synthase), and acetoacetyl-CoA reductase (see Schubert et al. (1988) J. Bacteriol. 170:5837-5847) facilitate expression of polyhyroxyalkanoates (PHAs).

(50) Exogenous products include plant enzymes and products as well as those from other sources including prokaryotes and other eukaryotes. Such products include enzymes, cofactors, hormones, and the like. The level of proteins, particularly modified proteins having improved amino acid distribution to improve the nutrient value of the plant, can be increased. This is achieved by the expression of such proteins having enhanced amino acid content.
(51) The methods disclosed herein can also be used for modification of native plant gene expression to achieve desirable plant traits. Such traits include, for example, disease resistance, herbicide tolerance, drought tolerance, salt tolerance, insect resistance, resistance against parasitic weeds, improved plant nutritional value, improved forage digestibility, increased grain yield, cytoplasmic male sterility, altered fruit ripening, increased storage life of plants or plant parts, reduced allergen production, and increased or decreased lignin content. Genes capable of conferring these desirable traits are disclosed in U.S. Patent Application 2016/0208243, herein incorporated by reference.
(52) The present disclosure may be used for genomic editing of any plant species, including, but not limited to, monocots and dicots (i.e., *monocotyledonous* and *dicotyledonous*, respectively). Examples of plant species of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (e.g., *B. napus, B. rapa, B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor, Sorghum vulgare*), camelina (*Camelina sativa*), millet (e.g., pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), quinoa (*Chenopodium quinoa*), chicory (*Cichorium intybus*), lettuce (*Lactuca sativa*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense, Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oil palm (*Elaeis guineensis*), poplar (*Populus* spp.), eucalyptus (*Eucalyptus* spp.), oats (*Avena sativa*), barley (*Hordeum vulgare*), vegetables, ornamentals, and conifers.
(53) The embodiments described methods and reagents can have many advantages over other known solutions. The techniques presented generally bypass callus induction or tissue culture that are necessary for alternative or widely practiced genome editing procedures, thus speeding up (i.e., accelerating) and lowering or reducing the cost of the process of producing plants with targeted mutations. Epigenetic resetting (i.e., interference) is also eliminated. The editing can be performed in individuals of an elite genetic background, making lengthy backcrossing schemes unnecessary.
Embodiments
(54) Various embodiments of the compositions, vectors, recombinant DNAs, RNAs, and methods provided herein are set forth in the following set of numbered embodiments.
(55) 1. A composition comprising at least one RNA molecule comprising a cargo segment fused to a meristem transport segment (MTS), wherein the cargo segment comprises one or more guide RNAs for an RNA-guided nuclease or wherein the cargo segment comprises RNA encoding a TALEN or ZFN protein.
(56) 2. The composition according to embodiment 1, wherein the guide RNA is flanked by or comprises processing elements.
(57) 3. The composition according to embodiment 2, wherein the processing elements are direct repeat sequences of the bacterial CRISPR array of the RNA-guided nuclease or are direct repeat sequences that are processed by the RNA-guided nuclease.

(58) 4. The composition according to embodiment 3, wherein the cargo segment comprises a plurality of guide RNAs.
(59) 5. The composition according to embodiments 3 or 4, wherein the guide RNAs and the direct repeat sequences of the bacterial CRISPR array are for a Cas12a or a Cas12j RNA-guided nuclease.
(60) 6. The composition according to embodiment 1, wherein the composition comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS, wherein the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the second RNA molecule, optionally wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases.
(61) 7. The composition according to any one of embodiments 1 to 6, wherein the cargo segment does not contain an RNA-guided nuclease polypeptide-encoding sequence.
(62) 8. The composition according to any one of embodiments 1 to 6, wherein the cargo segment further comprises an RNA-guided nuclease polypeptide-encoding sequence.
(63) 9. The composition according to embodiment 8, wherein RNA-guided nuclease polypeptide-encoding sequence can be translated in a plant cell cytosol.
(64) 10. The composition according to embodiment 8 or 9, wherein the RNA molecule further comprises at least one polyA region, wherein the polyA region is 3′ of the RNA-guided nuclease polypeptide-encoding sequence, and 5′ of the guide RNA and/or wherein the polyA region is at the 3′ end of the RNA molecule.
(65) 11. The composition according to any one of embodiments 1 to 10, wherein the composition comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS, wherein at least the first RNA molecule comprises a cargo sequence further comprising an RNA-guided nuclease polypeptide-encoding sequence, wherein the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the second RNA molecule.
(66) 12. The composition according to embodiment 11, wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases, and optionally wherein the processing elements in the first RNA molecule are not recognized by the RNA-guided nuclease polypeptide encoded by the first RNA molecule.
(67) 13. The composition according to any one of embodiments 1 to 12, wherein the MTS comprises: (i) a Flowering Time (FT)-derived sequence, optionally wherein the FT-derived sequence is SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (ii) a tRNA like sequence (TLS), optionally wherein the TLS sequence comprises SEQ ID NO: 29 or 30, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, MTC fragment thereof, and/or an RNA hairpin comprising a first stem of 8 to 12 nucleotides, at least one variable bulge, a second stem of 4 to 7 nucleotides, and a variable loop; (iii) a GAI sequence, optionally wherein the GAI sequence comprises SEQ ID NO: 26, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof, (iv) a BEL5 sequence optionally wherein the BEL5 sequence comprises SEQ ID NO: 28, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; (v) a CmNACP sequence optionally wherein the CmNACP sequence comprises SEQ ID NO: 25, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; or (vi) a LeT6 sequence optionally wherein the LeT6 sequence comprises SEQ ID NO: 27, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, a MTC fragment thereof.
(68) 14. The composition according to embodiment 13, wherein the MTS comprises a Flowering Time (FT)-derived sequence of SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

21, 22, 23, 24, or a meristem transport-competent (MTC) fragment thereof.
(69) 15. The composition according to any one of embodiments 1 to 14, wherein the MTS is located 3′ of the cargo segment.
(70) 16. The composition according to any of embodiments 1 to 15, further comprising RNase inhibitors.
(71) 17. The composition according to any one of embodiments 1 to 16, wherein the RNA molecule is a substantially purified RNA molecule.
(72) 18. The composition according to any one of embodiments 1 to 17, wherein the RNA molecule is not operably linked to a viral vector RNA and/or associated with a viral protein.
(73) 19. A meristem-delivery vector comprising a cargo segment fused to a meristem transport segment (MTS), wherein the cargo segment comprises one or more guide RNAs for an RNA-guided nuclease.
(74) 20. A recombinant DNA having a sequence capable of producing as a transcript a vector according to embodiment 19, or producing an RNA that can be purified and combined with one additional component to form a composition according to any one of embodiments 1 to 18.
(75) 21. A method of producing a plant with an altered genome comprising (i) contacting a plant with at least a first composition according to any of embodiments 1 to 18, and (ii) retrieving a progeny of the plant, wherein the progeny has an altered genome.
(76) 22. The method according to embodiment 21, wherein contacting comprises phloem loading.
(77) 23. The method according to embodiment 21 or 22, wherein the contacting with the composition occurs at the vegetative stage of the plant life cycle.
(78) 24. The method according to any one of embodiments 21 to 23, wherein contacting comprises contacting the plant with the first composition, and after a time interval contacting the plant with a second composition according to any one of embodiments 1 to 18, wherein the guide RNAs in the cargo segment of the RNA molecule in the first composition are different than the guide RNAs in the second cargo segment of the RNA molecule in the second composition.
(79) 25. The method according to embodiment 24, wherein the time interval is about 18 or 20 to about 24, 30, or 36 hours.
(80) 26. The method according to any one of embodiments 21 to 25, wherein the guide RNA(s) of the RNA molecule are flanked by or comprise processing elements which are processed by the RNA-guided nuclease.
(81) 27. The method according to any one of embodiments 21 to 26, wherein: (i) wherein the RNA molecule does not contain an RNA-guided nuclease polypeptide-encoding sequence; and (ii) wherein the plant comprises a polynucleotide encoding the RNA-guided nuclease, optionally wherein the polynucleotide is integrated into the genome of the plant and/or optionally wherein an active form of the RNA guided nuclease is predominantly localized in meristem tissue of the plant.
(82) 28. The method of embodiment 27, wherein the RNA-guided nuclease is encoded by a DNA molecule, optionally wherein the DNA molecule is integrated into the genome of the plant, optionally wherein the DNA molecule is operably linked to a promoter which is preferentially expressed in target plant cells, and/or optionally wherein the target plant cells are meristem cells.
(83) 29. The method according to any one of embodiments 21 to 28, wherein the composition comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS, wherein the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the second RNA molecule, optionally wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases.
(84) 30. The method according to embodiment 29, wherein the composition comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS, wherein at least the first RNA molecule comprises a cargo sequence further comprising an RNA-guided nuclease polypeptide-encoding sequence, wherein the cargo segment of the first RNA molecule comprises

guide RNAs which are distinct from the guide RNAs of the second RNA molecule.
(85) 31. The method according to embodiment 29, wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases, and optionally wherein the processing elements in the first RNA molecule are not recognized by the RNA-guided nuclease polypeptide encoded by the first RNA molecule.
(86) 32. A plant comprising: (i) an RNA molecule comprising a cargo segment fused to a meristem transport segment, wherein the cargo segment comprises one or more guide RNAs for an RNA-guided nuclease or a vector encoding the RNA molecule or wherein the cargo segment comprises RNA encoding a TALEN or ZFN protein; and, (ii) a DNA molecule or RNA molecule encoding the RNA-guided nuclease.
(87) 33. The plant according to embodiment 32, wherein the cargo segment does not contain a sequence encoding the RNA-guided nuclease.
(88) 34. The plant according to embodiment 32 or 33, wherein the cargo segment comprises a plurality of guide RNAs.
(89) 35. The plant according to any one of embodiments 32 to 34, wherein the guide RNAs and the direct repeat sequences of the bacterial CRISPR array are for a Cas12a or a Cas12j RNA-guided nuclease.
(90) 36. The plant according to any one of embodiments 32 to 35, wherein the plant comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS, wherein the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the second RNA molecule, optionally wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases.
(91) 37. The plant according to any one of embodiments 32, or 34 to 36, wherein the cargo segment contains a sequence encoding a Cas12a or a Cas12j RNA-guided nuclease, optionally wherein the Cas12a RNA-guided nuclease comprises SEQ ID NO: 53 or 55, or optionally wherein the Cas12j RNA-guided nuclease comprises SEQ ID NO: 57, 59, or 61.
(92) 38. The plant according to any one of embodiments 32, or 34 to 36, wherein the cargo segment further comprises an RNA-guided nuclease polypeptide-encoding sequence, optionally wherein a Cas12a or a Cas12j RNA-guided nuclease is encoded.
(93) 39. The plant according to embodiment 37 or 38, wherein RNA-guided nuclease polypeptide-encoding sequence can be translated in a plant cell cytosol.
(94) 40. The plant according to any one of embodiments 37, 38, or 39, the RNA molecule further comprising a polyA region, wherein the polyA region is 3′ of the RNA-guided nuclease polypeptide-encoding sequence, and 5′ of the guide RNA.
(95) 41. The plant according to any one of embodiments 32, or 34 to 40, wherein the composition comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS, wherein at least the first RNA molecule comprises a cargo sequence further comprising an RNA-guided nuclease polypeptide-encoding sequence, wherein the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the second RNA molecule.
(96) 42. The plant according to embodiment 41, wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases, and optionally wherein the processing elements in the first RNA molecule are not recognized by the RNA-guided nuclease polypeptide encoded by the first RNA molecule.
(97) 43. The plant according to any one of embodiments 32 to 42, wherein the MTS comprises: (i) a Flowering Time (FT)-derived sequence, optionally wherein the FT-derived sequence is SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or a meristem transport-competent (MTC) fragment thereof; (ii) a tRNA like sequence (TLS), optionally wherein the TLS sequence comprises SEQ ID NO: 29, SEQ ID NO: 30, a MTC fragment thereof, and/or an RNA

hairpin comprising a first stem of 8 to 12 nucleotides, at least one variable bulge, a second stem of 4 to 7 nucleotides, and a variable loop; (iii) a GAI sequence, optionally wherein the GAI sequence comprises SEQ ID NO: 26, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof, (iv) a BEL5 sequence optionally wherein the BEL5 sequence comprises SEQ ID NO: 28, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof, (v) a CmNACP sequence optionally wherein the CmNACP sequence comprises SEQ ID NO: 25, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, and/or a MTC fragment thereof; or (vi) a LeT6 sequence optionally wherein the LeT6 sequence comprises SEQ ID NO: 27, a meristem transport-competent (MTC) ortholog thereof, a MTC variant thereof, a MTC fragment thereof.

(98) 44. The plant according to embodiment 43, wherein the MTS comprises a Flowering Time (FT)-derived sequence of SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or a meristem transport-competent (MTC) fragment thereof.

(99) 45. The plant according to any one of embodiments 32 to 44, wherein the MTS is located 3′ of the cargo segment.

(100) 46. The plant according to any one of embodiments 32 to 45, wherein an active form of the RNA guided nuclease is predominantly localized in meristem tissue of the plant.

(101) 47. The plant of any one of embodiments 32, or 34 to 46, wherein the RNA-guided nuclease is encoded by a DNA molecule and optionally wherein the DNA molecule is integrated into the genome of the plant.

(102) 48. The plant of embodiment 47, wherein the DNA molecule encoding the RNA-guided nuclease is operably linked to a promoter which is preferentially expressed in target plant cells and optionally wherein the target plant cells are meristem cells.

(103) 49. A plant comprising an altered genome made by the method of any one of embodiments 21 to 31.

(104) 50. The use of the composition of any one of embodiments 1 to 18 to obtain a plant with an altered genome.

EXAMPLES

Example 1—RNA Design

(105) The basic plasmid design to produce the editing message starts with a standard high copy plasmid that contains a multiple cloning sites downstream of the T7 promoter, such as pBluescript™ or pSP73. Each component can be easily introduced using an efficient assembly approach. The design consists of a plant codon optimized Cas12a coding sequence followed by the DR sequence of the Cas12a CRISPR array, in which the DNA-targeting spacer sequences are replaced a guide with soybean phytoene desaturase (PDS) gene as a visual marker (Du et al. J. Biotech 2016, 217:90-97; doi.org/10.1016/j.jbiotec.2015.11.005). The guide RNA region is followed by the an FT sequence derived from Arabidopsis (SEQ ID NO: 1). The DNA vector sequence ends in a unique restriction site to linearize the plasmid for runoff transcription. This arrangement enables production of high quantity editing mRNA.

Example 2—Production of the RNA Composition

(106) To produce the mRNA for plant delivery the production vector above is linearized as template for in vitro transcription to produce tens of micrograms of editing mRNA using a system such as mScript™ (CAMBIO, Cambridge, UK; on the world wide web https internet site “cambio.co.uk/20/431/21/products/t7-mscript-standard-mrna-production-system/”). The product is cleaned up and characterized to make sure it is the expected size and to determine how much mRNA was produced. The purification process includes a DNAase treatment followed by a phenol chloroform extraction then ethanol precipitation and resuspension in RNase free water. RNAase inhibitor is also added (New England Biolabs, Ipswich, MA, USA; on the world wide web https internet site “neb.com/products/m0314-rnase-inhibitor-murine#Product%20Information”) to stabilize the editing mRNA during uptake by the plant.

Example 3—Phloem Loading

(107) The in vitro transcription reaction of Example 2 produces 50 micrograms of editing mRNA. It is suspended in a mix at 0.2 micrograms per microliter (10 micrograms mRNA in 50 microliters of RNase free water) in nuclease-free Eppendorf™ tubes (1.5 mL). These steps produce sufficient material for five replicates. A negative control contains everything but the editing mRNA. The soy plants are at the 2-3 trifoliate stage in small pots. Using sharp, clean & heat sterilized scissors to remove a leaf tip in the second trifoliate of each plant then the leaf tip is cut when submerged in sterile nuclease free water. Very gently the leaf is placed in the RNA solution and the setup stabilized so the plant can absorb the mRNA solution with no undue stress. Uptake of the editing mRNA takes several hours.

Example 4—Phenotyping

(108) The treated leaves are removed from the editing mRNA tubes when the solution is depleted to minimize wounding. In 1-2 weeks for the intended phenotype will appear in new growth. The soy PDS knockout is lethal so the plants will likely not set seed, but the same method can be adapted to make non-lethal mutations that are transmissible through in the germline.

(109) All cited patents and patent publications referred to in this application are incorporated herein by reference in their entirety. All of the materials and methods disclosed and claimed herein can be made and used without undue experimentation as instructed by the above disclosure and illustrated by the examples. Although the materials and methods of this disclosure have been described in terms of embodiments and illustrative examples, it will be apparent to those of skill in the art that substitutions and variations can be applied to the materials and methods described herein without departing from the concept, spirit, and scope of the invention. For instance, while the particular examples provided illustrate the methods and embodiments described herein using a specific plant, the principles in these examples are applicable to any plant of interest. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of the invention as encompassed by the embodiments of the inventions recited herein and the specification and appended claims.

## Claims

1. A composition comprising at least one RNA molecule comprising a cargo segment fused to a meristem transport segment (MTS), wherein the cargo segment comprises one or more guide RNAs for an RNA-guided nuclease, wherein the RNA molecule is a substantially purified RNA molecule.
2. The composition according to claim 1, wherein the guide RNA is flanked by or comprises processing elements.
3. The composition according to claim 2, wherein the processing elements are direct repeat sequences of a bacterial CRISPR array of the RNA-guided nuclease or are direct repeat sequences that are processed by the RNA-guided nuclease.
4. The composition according to claim 3, wherein the cargo segment comprises a plurality of guide RNAs.
5. The composition according to claim 3, wherein the guide RNAs and the direct repeat sequences of the bacterial CRISPR array are for a Cas12a or a Cas12j RNA-guided nuclease.
6. The composition according to claim 1, wherein the composition comprises both a first and a second RNA molecule each comprising a cargo segment fused to an MTS and wherein the cargo segment of the first RNA molecule comprises one or more guide RNAs for an RNA-guided nuclease.
7. The composition according to claim 6, wherein the cargo segment of the first RNA molecule comprises guide RNAs which are distinct from the guide RNAs of the second RNA molecule.
8. The composition according to claim 1, wherein the cargo segment does not contain an RNA-guided nuclease polypeptide-encoding sequence.

9. The composition according to claim 1, wherein the cargo segment further comprises an RNA-guided nuclease polypeptide-encoding sequence.
10. The composition according to claim 6, wherein the cargo segment of the first RNA molecule comprises guide RNAs and wherein the cargo segment of the second RNA molecule comprises an RNA-guided nuclease polypeptide-encoding sequence.
11. The composition according to claim 9, wherein the RNA-guided nuclease polypeptide-encoding sequence can be translated in a plant cell cytosol.
12. The composition according to claim 11, wherein the RNA molecule further comprises a polyA region.
13. The composition according to claim 12, wherein the poly A region is 3′ of the RNA-guided nuclease polypeptide-encoding sequence, and 5′ of the guide RNA.
14. The composition according to claim 7, wherein the guide RNAs of the first and second RNA molecule are flanked by or comprise processing elements which are processed by different RNA-guided nucleases.
15. The composition according to claim 1, wherein the MTS comprises: (i) a Flowering Time (FT)-derived sequence or (ii) a tRNA like sequence (TLS).
16. The composition according to claim 15, wherein the MTS comprises a Flowering Time (FT)-derived sequence of SEQ ID NO: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or a meristem transport-competent (MTC) fragment thereof.
17. The composition according to claim 1, wherein the MTS is located 3′ of the cargo segment.
18. The composition according to claim 1, further comprising an RNase inhibitor.
19. The composition according to claim 1, wherein the RNA molecule is not operably linked to a viral vector RNA and/or associated with a viral protein.
20. A method of producing a plant or plant part with an altered genome comprising: (i) contacting a plant or plant part with at least a first composition according to claim 1; and (ii) retrieving a progeny or descendant of the plant or plant part, wherein the progeny or descendent has an altered genome.
21. The method according to claim 20, wherein contacting comprises phloem loading.
22. The method according to claim 20, wherein the contacting with the composition occurs at the vegetative stage of the plant life cycle.
23. A plant or plant part comprising an altered genome made by the method of claim 20.